UNIVERSITÀ CATTOLICA DEL SACRO CUORE
Sede di Piacenza

Scuola di Dottorato per il Sistema Agro-alimentare

Doctoral School on the Agro-Food System

cycle XXV

S.S.D: AGR/13, CHIM/10, CHIM/01

APPLICATION OF DIFFERENT TECHNIQUES FOR ARSENIC DETERMINATION IN HUMAN FOOD CHAIN: *FROM GROUNDWATER TO DINING TABLE.*

Coordinator: Ch.mo Prof. Romeo Astorri

_____

Candidate: Fontanella Maria Chiara
Matriculation n. : 3810668

Tutor: Dott. Gian Maria Beone
Prof. Ettore Capri

Academic Year 2011/2012

ABSTRACT

The aim of this thesis was to explore new analytical techniques as well as to carry out further characterisations of human health risks, which derive from water pollution, in particular groundwater, and food, in particular rice. The prevention of water pollution is an environmental aspect, that includes monitoring of both natural enrichment and outside pollution with routine analysis but also with new techniques, e.g. the application of passive sampling techniques and advanced technologies.

The diffusive gradients in thin films (DGT) technique with ferrihydrite adsorbent, has been investigated for the accumulation of different species of Arsenic (As), like Inorganic Species (arsenite and arsenate) and Organic Arsenic (dimethylarsinic and monomethylarsenate) in aqueous matrix.

To evaluate the performance of DGT method for accumulation of arsenic species, after deployment in synthetic solutions, DGT devices were carried out on groundwaters collected in six different towns in the North of Italy, where the As concentration is very high. Recently, health effects at arsenic exposures have been observed in areas where levels of inorganic As in drinking water are not excessive. Antimony (Sb) is associated to As in several studies because the physical and chemical properties of these two elements are similar, and it has been recently recognized as water contaminant. In this thesis I reported for the first time detailed performance characteristics of the Fe-oxide gel associated to DGT devices deployed in known aqueous solutions of trivalent and pentavalent Sb. Speciation analysis of Sb(III) and Sb(V) in aqueous samples was performed through extraction and on-line determination of isotope dilution concentration after a chromatographic separation.

Generally rice, unlike food products of terrestrial origin, contains significant amounts of inorganic arsenic. Recently some Government Organizations (e.g. EFSA) debated the possibility to set an upper limit for total and inorganic arsenic in rice. Arsenic speciation was realized in 70 Italian rice samples from different representative cultivation conditions. The most abundant forms in rice were As(III) and DMA(V). After that, it was fundamental to investigate the localization of As in rice grains in different processes (raw, brown and milled rice with or without parboiling technique), because both speciation and distribution throughout the grain are key factors controlling bioavailability of the contaminant. The As distribution in rice grains of two varieties (Gladio and Ronaldo) from different processes, was determined by LA-ICP-MS. The distribution of As varied between the various parts of the grains (exterior, medium and interior part). During parboiling, the partial boiling of food as the first step in the cooking process, arsenic might have released from the

grain to the boiling water. Thus, parboiling of rice grain may reduce the magnitude of arsenic intake in human body. Actually the As content was higher in non-parboiled rice grain than in parboiled rice. The relationship between As intensities and the different parts of rice grain revealed that As levels decreased from the external part towards the middle position, and then the intensity values seem to be similar between medium and internal part in non parboiled products.

TABLE OF CONTENTS

INTRODUCTION

Chapter I. Current status of speciation analysis of Arsenic and Antimony.

1.1. Why it is important to examine the status of groundwater.

The Earth is known as the Blue Planet for the predominance of volumes of water in comparison with land, but it has less than 3% of the available fresh water. More than 2.5% of this small percentage is ice in the Arctic and Antarctic zone. Humanity must rely on 0.5% of fresh water for all human and ecosystem needs (World Business Council for Sustainable Development, 2005).

Some 0,5 percentage of fresh water is stored in the following sectors: $10 * 10^6$ km^3 in deep aquifers, $119 * 10^3$ km^3 in the rain, $91 * 10^3$ km^3 in the lakes, $5 * 10^3$ km^3 in artificial basins and 2.120 km^3 in the rivers, constantly supplied by rain, snow and melted ice (Vörösmarty, 1997, Foster and Chilton, 2003).

The fresh water is used for different reasons: urban use (nutrition, hygiene), agriculture, breeding, industry, tourism, energy source and commerce.

The most important uses, in terms of total extraction, can be identified as a public water supply, for agriculture, industry and energy production.

Between 1998 and 2007 in Europe 21% of water was used for public water supply, 22% on average for agriculture, 12% for industry and 45% for the production of energy (EEA – ETC/WTR, 2010).

Two different trends are observed in Europe during the last 10 to 15 years in the public supply of water: the countries of Eastern and Western Europe had a decrease in consumption, while in the Southern European countries, the domestic use increased by 12 %.

The decrease in consumption is higher in England and Germany, as well as in Eastern Europe (Poland, Bulgaria and Romania) and everything can be attributed to the promotion of water-saving practices (Dworak et al., 2007).

In the South, the observed increase in public supply of water could be attributed to climate change and tourism. The increase in temperature (observed in the Mediterranean area) showed a rise in demand for water for domestic use, for personal hygiene and for outdoor use (gardening, swimming pools) (Cohen, 1987, Downing et al. 2003, Herrington, 1996, Kenneth, 1997). In France, Greece, Italy, Portugal and Spain, the tourism is increased by 90% in the last two decades (Attané and Courbage, 2001, De Stefano, 2004).

In Italy, supplies from groundwater are higher than those from surface waters, with differences at the provincial and sector level.

For example, in the province of Piacenza (288.003 inhabitants) (ISTAT), 20% of surface water and 80% of groundwater supplies the civil sector in the city of Piacenza (102.687 inhabitants) (ISTAT) all withdrawals for civil purposes derived from groundwater (Regione Emilia, 2005).

Any assessment of the availability and sustainability of water use must take into account the amount and use of available fresh water but also its quality. In fact, a poor quality will lower the apparent availability of water.

Groundwater is endangered and polluted in various ways and some of these chemicals may affect human health.

Water is the food that every living being consumes continuously and it is more fundamental than any other food in the human diet. Water also performs the function of cleaning and sanitizing, thus helping to prevent diseases and ensure a better quality of life.

In every case, the water must be healthy because it could be a formidable factor of disease spreading. The increasing production of industrial and urban waste forced to make use of surface and groundwater as receptors of discharges, often contaminated by toxic or carcinogenic substances (such as metals, solvents, pesticides, oils).

At Community level, a series of guidelines were developed to protect the whole water compartment. Directive 2000/60/EC supports the requirement of extended protection by the Community authorities, through national and local authorities on all the different types of water (surface and underground), recognizing the citizens, ideal subjects to be involved to obtain objectives by identifying needs, requests and suggestions of users as priority.

Directive 2006/118/EC recognizes groundwater as fundamental element for the ecosystems and water supply for human consumption.

In Italy the key laws are: national decree DL 31/2001 of Directive 98/38/EC, concerning the quality of water for human consumption, D. Lgs 152/2006 establishing environmental standards for…water quality…and D. Lgs 30/2009, of Directive 2006/118/EC, on the groundwater protection against pollution and deterioration.

At the local level, the "Piano di Gestione del Distretto Idrografico del fiume Po" is very important because objectives and priorities of hydrographic basin are defined. The "Piano di Tutela delle Acque" is a planning document established at regional level and adopted at the provincial level, and it must comply with the instructions of the "Piano di Gestione".

Effective controls on the quantity and quality of the water withdrawn are carried out by different authorities.

The "SIAN – AUSL" (Servizio Igiene Alimenti e Nutrizione) provides a judgment of suitability of water for human consumption, maintaining open channels of communication with municipalities, agencies and distribution services with the "Autorità D'Ambito".

The "Gestore del Servizio Idrico" feed in the water system with the quality characteristics constantly monitored for human consumption, after the collection and treatment of drinking water.

ARPA (Agenzia Regionale Prevenzione e Ambiente) has a monitoring network with the following objectives: (a) to classify surface and groundwater, (b) to check the status of the resources, (c) to verify the water pollution, (d) to define the potential of the aquifers, (e) to identify the main environmental emergencies (nitrates and pesticides), (f) to assess the effectiveness of rehabilitation systems and (g) to support complex plant and animal ecosystems.

Prevention of surface and groundwater pollution is very important from an economic and environmental respect point of view. Natural features of groundwater aquifer or soil might cause the presence of high concentrations of different chemical elements, without outside pollution.

The characterization and the study of the qualitative characteristics of water are part of the concept of sustainable management, thanks to the involvement of the three aspects, environmental, social and economical, that contribute to specific sustainability concept.

Prevention of water pollution is an environmental aspect, that include the monitoring of natural enrichment or outside pollution with routine analysis but also with new technique, for example the application of passive sampling techniques and advanced technologies. This type of monitoring is useful because it is possible to employ accurate and aimed purification technologies without public resources waste.

1.2. Arsenic and Antimony.

Arsenic (As) is a metalloid that occurs in different inorganic and organic forms. The inorganic forms of arsenic are more toxic but, in official food control, only total arsenic content is usually reported, without differentiating the arsenic species. The investigation of total arsenic would lead an overestimation of health risk related to dietary arsenic exposure (EFSA, 2009).

D. Lgs 31/2001 indicates a parameter value of 10 $\mu g \, L^{-1}$ for arsenic in groundwater, the same value reported in D. Lgs 30/2009.

The toxicity of As depends not only on total concentrations, but also on its chemical forms. The inorganic As species have been classified in group 1 as carcinogenic to humans (IARC 1987). Arsenite (As(III)) has higher toxic effects than arsenate (As(V)). Organic form of As, like monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) also exist. Until recently,

methylation of arsenic was considered to be a detoxification process because the toxicity of MMA(V) and DMA(V) was much lower than that of inorganic arsenicals (Del Razo et al. 2001, Eguchi et al. 1997). In contrast to the low toxicity of MMA(V) and DMA(V), several authors (Petrick et al. 2001, Sakurai et al. 2002) reported that MMA(III) and DMA(III) are more toxic than inorganic arsenics.

MMA(III) and DMA(III) have been reported to break down DNA at lower concentrations than inorganic arsenicals or pentavalent methylated arsenicals (Mass et al, 2001, Nesnow et al. 2002). Therefore, due to large differences of toxicity among As(III), As(V) and organic As, an accurate As speciation is essential.

Natural concentrations of arsenic in soil are typically less than 10 mg kg^{-1}, but anthropogenic and natural inputs may raise concentrations substantially. In Bangladesh, for example, there is great concern about the contamination of soil and drinking water that originates from a diffusive source, resulting in toxicity problems on a regional scale, and in serious threats to 85 million people (Hossain, 2006). In the same area with arsenic endemic areas, recent reports showed an increase of As(III) amount in drinking water and the existence of low concentrations of organic arsenicals in drinking water (Harvey et al 2002, Shraim et al. 2002).

The speciation of As is strongly influenced by pH and redox potential (Eh) (Cullen and Reimer, 1989). If the groundwater is under reducing conditions (low Eh) prevails As(III), with high concentrations of iron, manganese, ammonia and phosphate (Katsoyiannis et al 2007).

Because the dominant species of pentavalent arsenic in aqueous solution, $H_2AsO_4^-$, is isoelectronic and similar in volume to phosphate, $H_2PO_4^-$, phosphate transporters can potentially allow the passage of arsenate. This situation is probably true in most organisms including humans (Huang and Lee, 1996). Upon its entry into the cell, also in mammal cells, arsenate is reduced to the trivalent arsenite (Radabaugh and Aposhian, 2000).

Arsenic might cause cancer to the brain, liver, kidney and stomach, it has a great affinity with hydrogen sulphide groups of biomolecules such as glutathione (GSH), fatty acids and cysteine of many enzymes (Aposhian and Aposhian, 2006).

The formation of bonds between sulphuric groups and As(III) causes dangerous effects such as inhibition of various enzymes such as glutathione reductase, glutadione peroxidase, thioredoxin reductase and thioredoxin peroxidase (Schuliga et al., 2002; Wang et al., 1997; Lin et al., 2001; Chang et al., 2003).

As(III) also interact poorly with the surface of many solids, and then As(III) is difficult to remove with conventional methods of treatment (eg. adsorption and precipitation).

Several studies have been published on the oxidation of As(III) via traditional chemical oxidants as chlorine, chlorine dioxide, chlorine amines, ozone, hydrogen peroxide, permanganate ion and ferric ion (Frank and Clifford, 1986; Kim et al., 2000; Pettine et al., 1999; Emett and Khoe, 2001; Johnston and Heijnen, 2001; Bissen and Frimmel, 2003; Lee et al., 2003; Ghurye and Clifford, 2004; Vasudevan et al., 2006; Dodd et al., 2006; Sharma et al., 2007).

The chlorination is effective for the oxidation of As(III), but this technique creates and releases products of synthesis in the tap water. Trihalomethanes are products by this synthesis, which are carcinogenic for rodents (Boorman et al., 1999); NH_2Cl also produces N-nitrosodimetilammine (NDMA), substance suspected to be carcinogenic for humans (Mitch and Sedlak, 2002).

Ozone is able to reduce the levels of trihalomethanes, however, it causes the formation of bromate ion, highly carcinogenic. The ferric ion does not produce bromate ions and products of synthesis are non-toxic (Sharma, 2007 , Sharma, 2002, 2004; Sharma et al., 2005; Yngard et al., 2008).

The knowledge of elements present in the future drinking water is essential to ensure an appropriate choice of the disinfection system.

Ingestion of polluted groundwater is not the only source of arsenic. In the human dietary there are staple foods, like rice, that may represent a hazard for human health. Rice is one of the most important exposure route for arsenic. In comparison with other cereal grains, baseline concentrations of As in rice are approx. 10-fold larger (Williams et al., 2007). Moreover, discovering the location and speciation of arsenic within the edible rice grain is essential to understanding the human health risk (Meharg et al. 2008) and establishing effective strategies to reduce concentrations of this metalloid.

Williams et al. (2006) reported that the dominant species in rice were inorganic (arsenate and arsenite), with dimethylarsenic acid [DMA(V)] being only a minor component. This distribution was almost similar to that found in Italian rice in the present study (70 rice samples came from different areas in the North of Italy), in particularly the most abundant species in Italian rice were As(III) and DMA(V) (Chapter V). Different authors (Meharg et al. 2009, Williams et al. 2005, Norton 2009, Norton 2009, Williams et al. 2006, Zavala et al. 2008, Zhu et al. 2008, Adomako et al. 2009) established that the proportion of inorganic-to-organic arsenic varies geographically and genotypically; rice in the USA contains proportionately more DMA and rice in Asia contains proportionally more inorganic arsenic. Methylated species are taken up rather inefficiently compared with inorganic species but seem to be translocated more efficiently (Raab et al., 2007). Because of the complexity of these processes, the mechanisms responsible for As loading into the rice grains and its speciation and distribution within the grain are not fully understood.

Several studies have analysed arsenic distribution in rice grain by quantifying arsenic in separated

fractions (Sun et al., 2008, Ren et al. 2006, Rahman et al. 2007); all reported that arsenic was most concentrated in the bran, with levels following the pattern bran > wholegrain rice > polished rice. Meharg et al. (2008) showed that whole grain (brown) rice had a higher inorganic arsenic and total arsenic content than polished (white) rice.

Accumulation of As in rice plant tissues and grains was reported resulting from the soils or irrigation waters containing an elevated level of As. Abedin et al. (2002) discovered that As concentrations in rice grains, husks, stalks, and roots were positively correlated with arsenate contents in the irrigation water. The large majority of the information available on As distribution and speciation in rice is related to the analyses of powdered rice grains. In Chapter VI, we reported laser ablation ICP-MS (LA-ICP-MS) results which show a potential analytical technique for As intensity estimation in rice samples and a methodfor rapid, direct analysis of solid samples without dissolution and with minimal sample preparation.

Antimony (Sb) behaves like a metal in most reaction. However, in some reactions, it demonstrates nonmetal characteristics. Antimony may occur at four oxidation states, that is, -3, $+3$, $+4$, and $+5$. It occurs mainly in Sb^{3+} and Sb^{5+} forms in the biological and geochemical environment. It is present in all units of the environment and its natural background in various environmental matrices is highly diversified (Smichowski, 2008). In aqueous solution, antimony exists either in the pentavalent or trivalent oxidation state.

In general, inorganic antimony compounds are more toxic than the organic ones. Sb(III) compounds are ten times more toxic than Sb(V) ones. On the other hand, the toxicity of antimony compounds is approximately ten times lesser than arsenic ones but it depends on their oxidation states and structure. Antimony in the elemental form is more toxic than its salts (Nordberg et al., 2007).

D. Lgs 31/2001 indicates a trigger value of 5 $\mu g\ L^{-1}$ for antimony, the same value reported in D. Lgs 30/2009 for groundwater.

Recent studies show that the concentration of Sb in uncontaminated groundwater is very low, below 1 $\mu g\ L^{-1}$. Antimony concentrations are much higher in natural geothermal systems where they can range from 500 mg L^{-1} up to 10 wt%. (Ritchie, 1961; Weissberg et al., 1979; Kolpakova, 1982; Stauffer and Thompson, 1984). Probably due to its lower abundance and the relative insolubility of most of its compounds, antimony is usually overlooked as an element of environmental concern and its study has been largely neglected.

In terms of hard-soft behaviour (Ahrland, 1968, 1973; Pearson, 1963), Sb(III) is commonly classified as a borderline metal, which makes its interactions with a soft ligand, like SH, and a hard ligand such as –COOH, both possible.

The physical and chemical properties of Sb and As are similar. In the past, these two elements and their compounds were often determined together (Gebel, 1997). As first pointed out by Pauling (1933), the coordination with Sb(V) with oxygen is quite different from that of As(V). Based on its larger ionic radius and lower charge density, this author suggested that antimony should be octahedrally coordinated with oxygen and its compounds, rather than tetrahedrally like As(V) is.

In contrast to arsenate, the entrance route of pentavalent antimony, or antimonate, into the cells has not yet been identified. The uptake mechanism may be different from that of arsenate because the stable form of antimonate in aqueous solutions, $Sb(OH)_6^-$, is not isoelectronic with arsenate/phosphate. Nevertheless, once antimonate is inside the cell, it is also reduced to the trivalent state, antimonite. The process of reducing antimonate to antimonite is very important because, as is the case for arsenic, the trivalent form of antimony is the active and the more toxic state (Zangi and Filella, 2012).

A certain number of studies have been devoted to the chemical solution of Sb(III)-polyaminocarboxylic acids (CDTA, DTPA, EDTA) (Bhat and Iyer, 1965; Bhat et al., 1966; Anderegg and Malik, 1967, 1970a,b; Özer and Bogucki, 1971; Er-kang, 1982). Although significant complexation has been observed at acidic pH values, experimental results show that Sb(III) prefers sulphur as a ligand at low temperatures but that, at higher temperature, it forms mixed ligand complexes containing both sulphur and oxygen group (Krupp, 1988; Sherman et al., 2000).

Sb(III) shows a high affinity for red blood cells and hydrogen sulphide groups of biomolecules of the cells, while the same red blood cells are impermeable to the Sb(V). The primary effects of chronic exposure to antimony in humans are respiratory problems, lung damage, cardiovascular effects, gastrointestinal disorders, and adverse reproductive outcomes (Bhattacharjee et al. 2009).

IARC (International Agency for Research on Cancer) has added Sb in the group of suspected substances to be carcinogenic for human beings (IARC 1989, Gebel, 1997). However, the U.S. Environmental Protection Agency (USEPA, 1999) and German Research Community (DFG, 1994) categorize antimony as a main pollutant but do not indicate it as a carcinogen.

The comprehension of antimony behaviour in aqueous matrix is very important because many studies have been published in which drinking water contamination from bottle materials was investigated. According to estimations, approximately 38% of the total Sb intake of an adult (about 7.4 μg Sb/day) would come from drinking water (Greathouse and Craun, 1978)

The only element which is highly concentrated in PET bottled water was antimony with a 21-fold concentration over glass (Reimann et al. 2010). The higher concentration of antimony in PET bottle water is expected, because antimony trioxide (Sb_2O_3) or its reaction product with ethylene glycol is widely used as a polycondensation catalyst in the manufacturing of PET. The antimony catalyst

offers a high catalytic activity and has a low tendency to catalyse side reactions. In addition, antimony does not engender undesirable colours and the polymerisation catalyst remains in the PET polymer. The Sb concentration of the commercialised PET resin is between 190 and 300 $\mu g\ g^{-1}$. Recently, an Sb(V) complex, Sb(V)-citrate, was identified for the first time in no spiked orange juice contained in poly(ethyleneterephthalate) (PET) bottles (Duh, 2002).

On the contrary, Sb was not detectable in water samples originating directly from the wells or stored in glass bottles. In the freshly bottled samples, the Sb concentration ranged between very low values and it depends on the PET surface/water volume ratio, therefore the storage in smaller bottles results in higher Sb concentration. Moreover, illumination and increased storage temperature augmented the Sb concentration (Keresztes et al. 2009).

All this evidences emphasizes the importance of identifying and quantifying the chemical forms of antimony to provide comprehensive information about its toxicity and human health relevance.

In conclusion, antimony must be considered as the most important inorganic species that may migrate from the PET bottle wall into the beverages.

1.3. Speciation analysis of elements by HPLC-ICP-MS.

Arsenic is a metalloid with organic and inorganic forms. The inorganic forms of As are more toxic, but only the total content of arsenic is reported in official controls on food, without difference between the arsenic species. The analysis of total As concentration in the diet could lead to an overestimation of the risk for human health (EFSA, 2009).

An accurate arsenic speciation is essential to determine its impact on humans through the diet, because of the large toxicity differences between As(III), As(V) and organic species.

Numerous instrumental methods for the speciation of these arsenic species are reported in literature. Most of them are based on chromatographic separation techniques such as High Performance Liquid Chromatography (HPLC) (Gailer and Irgolic, 1996; Teräsahde et al., 1996; Le and Ma, 1997; Dagnac et al., 1999; Kohlmeyer et al., 2002).

The HPLC-ICP-MS technique is the most powerful method for arsenic speciation. The advantages associated with the HPLC-ICP-MS technique include high elemental specificity, the possibility to record real time chromatograms, the ability to separate the signals of interfering species from the peaks of interest, a high linear range, a multi-element capability and the possibility to obtain isotopic information.

However, the use of ICP-MS as a detector for HPLC gives rise to some constraints on the choice of chromatographic conditions concerning the nature and concentration of the buffer salts of the mobile phase and the presence of organic solvents.

Moreover, because of its high sensibility, ICP-MS may suffer from many isobaric interferences caused mainly by phenomena occurring either in the plasma or in the ion extraction device. For example, presence of chlorine in the sample may give rise to the formation of $^{40}Ar^{35}Cl^{+}$ that interferes strongly with the mono-isotopic $^{75}As^{+}$ (Gray, 1986; Hywel Evans and Giglio, 1993).

All the arsenic species of this study have a range of dissociation constants making them suitable for anion exchange column, as they exist in anionic form in alkaline mobile phase (Teräsahde et al., 1996).

Na_2HPO_4 and NaH_2PO_4 are often used as mobile phase for the As species separation, but deposition of salt on the sampling interface causes a rapid degradation and instability of the signal. For this reason, the selected mobile phase used in this study was ammonium dihydrogen phosphate ($NH_4H_2PO_4$), for which less deposit was observed together with a good stability of the signal (Ronkart et al. 2007).

Alternatively, total As concentration could be measured by inductively coupled plasma mass spectrometer (Agilent 7700x, Agilent Technologies, USA) with octapole reaction system (ORS system). ICP–MS has revolutionised quantitative analysis of arsenic in rice graina number of studies have combined the good separation capabilities of HPLC with highly sensitive ICP-MS detection, to identify and quantify arsenic species in mature rice grain, by detecting arsenite, arsenate, and dimethylated arsenic (DMA) with, occasionally, trace amounts of monomethylated arsenic (MMA) (Schoof et al. 1999, Heitkemper et al. 2001, Williams et al. 2005, Sun et al. 2008, Norton 2009, Norton 2009).

A certified Reference Material was used to ensure the accuracy and precision of the analytical procedure (CRM BCR 610, groundwater). The same procedure was applied to check whether the analytical results of As in rice agreed (NIST 1568a, IMEP 107, rice samples).

The first works relating to antimony speciation were published in the early 1980s (Andreae et al., 1983). The speciation of antimony consists in the determination of Sb(III) and Sb(V) and organic antimony compounds.

Antimony and its compounds are often determined in various types of water, such as drinking, mineral, and surface water. However, the information on the content of different forms of antimony is not ample.

Most of the studies reporting HPLC (high-performance liquid chromatography) separations of Sb species are based on anion-exchange chromatographic methods, due to the predominance of Sb

anionic species in aqueous environmental samples (Smichowski et al., 1995; De Gregori et al. 2005; Potin-Gautier et al. 2005; De Gregori et al. 2007; Zheng et al. 2000; Zheng et al. 2001; Hansen and Pergantis, 2006; Ulrich 1998; Sayago et al. 2002; Nash et al. 2006).

The determination of Sb(III) and Sb(V) in aqueous solution is most commonly performed by separation by anion-exchange chromatography (AEC), followed by element specific detection (Hansen and Pergantis, 2007; Miravet et al. 2010).

The main differences in method-development strategies described in the literature are mostly based on the mobile phases employed. In general, the elution of Sb(V) is readily achieved with a variety of mobile phases, while, for Sb(III), a broad tailing chromatographic peak is usually observed. Complexing mobile phases of pH 4.0–5.5 containing tartrate buffers (Zhang X et al. 1998), sodium citrate (Satiroglu, 2000) or EDTA with or without potassium-hydrogen phthalate (KHP) have been also proposed (Krachler and Emons, 2000, 2001; Dodd et al., 1992; Sayago et al., 2000) to solve this problem. These methods usually show a good performance for standard solutions, and, in most cases, they have been applied to Sb speciation in spiked water samples. The importance of complexing ligands in the mobile phase for AEC was established by Lintschinger et al. (1997), who also showed that addition of a strong competing anion, e.g. phthalate, to the EDTA mobile phase improved the chromatographic system by shifting the Sb(III) peak to a shorter retention time and improving its symmetry.

A complexing mobile phase serves to preserve the trivalent oxidation state of Sb during the chromatographic separation, as Sb(III) easily oxidizes to Sb(V) in aqueous solutions (Krachler et al. 2001). Moreover, in most cases, Sb(III) is irreversibly retained on the AEC columns when chelators are not present in the mobile phase (Lintschinger et al. 1997; Hansen and Pergantis, 2007)

As the AEC method using a combination of EDTA and phthalic acid in the mobile phase appears to be the most successful approach for determination of Sb oxidation state so far, it has been extensively applied (Miravet et al. 2010, Chapter IV).

1.4. Advantages of DGT technique for speciation analysis.

In recent experiments (Chapter III and IV), two approaches have been used together to study elements speciation in raw groundwater: HPLC-ICP-MS and Ferrihydrite (FH) Diffusive Gradients in Thin Films (DGT) (DGT Research Ltd, Lancaster, UK). Usually, DGT has been used in parallel with several other speciation and fractionation techniques for a comparison, and to investigate the fractions and species measured.

In Chapter III and IV the commercially available FH-DGT, already characterised for measurements of phosphate and total inorganic arsenic, was evaluated for determination of labile anionic forms like arsenite, As(III), arsenate, As(V), antimonite, Sb(III), antimonate, Sb(V), with some observations about organic arsenic species behaviour.

Organic arsenic species are commonly found in natural waters (Hasegawa et al. 2010) and sediments (Orero Iserte et al. 2004). The two most prevalent, dimethylarsonate (DMA(V)) and monomethylarsinate (MMA(V)) can potentially adsorb to the ferrihydrite binding agent (Lafferty and Loeppert, 2005) used for DGT measurements of inorganic arsenic, and might thus contribute to total arsenic measurements. If total arsenic is determined in DGT-eluates but only the concentration of inorganic arsenic is of interest, there is the profound risk for making overestimations and consequently speciation analysis on the sampled water is needed to confirm that neither DMA(V) or MMA(V) are present at significant levels.

The first scientific work concerning the DGT technique was presented in a letter to Nature in 1994 by Prof. William Davison and Dr. Hao Zhang of Lancaster University, United Kingdom. In this chapter and in the following, DGT technique (Davison & Zhang, 1994; Zhang & Davison, 1995) has been proposed as a tool to assess the real risk of polluted soils and the potential availability of pollutants and a mean by which to measure concentrations of trace metals in natural waters and to estimate their concentrations in soil pore water (Zhang 2002). When exposed to a soil solution, a DGT device provides an indirect measure of the maximum potentially available concentration of pollutants in soil water, and consequently an estimation of potential uptake by plants. This technique can be used to study As speciation in irrigation water and soil solution in experiments of rice growing.

Sampling with DGT offers a wide range of applications. It has been used for many bioavailability and toxicity studies (Røyset et al., 2005; Tusseau-Vuillemin et al., 2004; Ferreira et al., 2008; Diviš et al., 2007), detailed studies to quantify labile organic and inorganic trace metal species (Warnken et al., 2008) and for the evaluation of trace pollution sources in sewer systems (Thomas, 2009). The diversity in applications implies that DGT is a useful tool for researchers from varying disciplines.

DGT has the benefit of eliminating the risk of speciation changes due to transportation and storage of samples prior to preparation and analysis (Lead et al. 1997) and it is designed to accumulate labile species in environmental systems (Zhang and Davison 1995, Zhang and Davison 2000, Davison et al. 2000) as a result of the in situ sampling capabilities.

The technique consists of a diffusive layer (hydrogel) placed on top of a selective binding phase (resin–gel). Both are sealed in a plastic unit. The diffusive layer has a well-defined thickness and typically consists of a polyacrylamide diffusive gel and a protective membrane filter that is exposed

to the bulk solution through an opening/"window" in the sampling device (Figure 1).
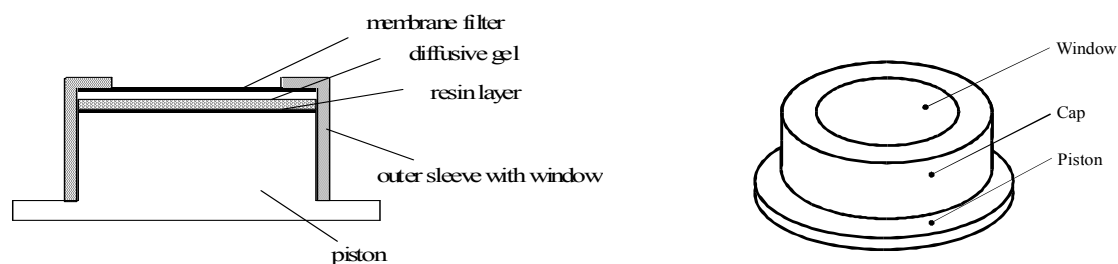

Fig. 1. Schematic representation of a DGT device.

The hydrogel and membrane filter allow free diffusion of analyte species, smaller than the hydrogel pore size, from the bulk solution to the binding layer, where adsorption and accumulation occurs. DGT incorporates ferrihydrite (an iron oxyhydroxide) anion exchanger for determination of labile anionic phosphate (Zhang H et al. 1998) and inorganic As (Panther et al. 2008). Though the differences between the DGTs were often rather small, there were significant differences between the binding layers for mass accumulation, and it was observed that the estimated concentrations of DGT labile metals are dependent on the binding phase used.

The ferrihydrite backed DGT (FH-DGT) device has also been applied to Se investigation in soil (Sogn et al. 2008), to study of P, As, V, W, Mo, Sb, and U microniches in sediments (Stockdale et al. 2008, 2010). Recently, full characterisation was reported for the measurement of As(V), V(V), Se(VI) and Sb(V) (Luo et al. 2010).

Analytes in the sampling medium diffuse through the membrane filter and hydrogel to finally accumulate in the binding layer. Accumulation continues as long as the sampling device is exposed, and after retrieval the analytes are eluted and determined at the laboratory. The knowledge of the accumulated mass and diffusion coefficient of the analyte, as well as the deployment time and temperature, enables calculation of the average concentration during the time of exposure.

It is important to note that the DGT concentrations may not be directly related to the total or dissolved analyte concentrations, since species accumulated by the DGT are dependent on size and lability.

DGT is to a certain extent special because it is designed to:

(a) bind the substances of interest and

(b) accurately control the transport of the substances to the device.

Concentration in solution is calculated using the Fick's first law of diffusion and the measured mass of solutes accumulated on the binding agent after a known deployment time. The Fick's first law of

diffusion (Equation 1) is based on diffusion coefficient (D; $cm^2 s^{-1}$) and the concentration gradient ($\Delta C/dx$; mol cm^{-4}).

$$F = \frac{D \times \Delta C}{dx} \qquad\qquad eq.1$$

If diffusion coefficients of ions in the diffusive gel are the same as in water, the flux is given by Equation 2, where C (mol cm^{-3}) is the bulk concentration of an ion and C' is the concentration at the boundary between the binding-gel and the diffusive gel.

$$F = \frac{D \times (C - C')}{\Delta g + \delta} \qquad\qquad eq.2$$

If the free metal ions reach rapidly the equilibrium with the binding agent and with a large binding constant, C' is effectively zero, providing that the binding agent is not saturated. In well stirred solutions, the boundary layer thickness, δ (cm), is negligibly small compared to the thickness of the diffusive layer, Δg (cm) of ~0.1 cm. Equation (2) then simplifies to Equation (3).

$$F = \frac{D \times C}{\Delta g} \qquad\qquad eq.3$$

Therefore, Eq. (3) can be rearranged to Equation (4).

$$C = \frac{F \times \Delta g}{D} \qquad\qquad eq.4$$

According to the definition of flux F=M/At, where M is the mass of element species diffused through a known area (A) after a given time (t), the concentration in the solution can be calculated using Eq. (5).

$$C = \frac{M \times \Delta g}{D \times A \times t} \qquad\qquad eq.5$$

M can be obtained from the direct measurement of element concentration, C_e, in the oxide-gel layer of total volume of V_{gel} (Eq. (6) using a chromatographic separation techniques such as High Performance Liquid Chromatography (HPLC).

$$C = C_e \times (V_{gel} + V_{acid}) \qquad\qquad eq.1$$

The opportunities associated with the DGT technique make further development desirable in order to increase the number of elements suitable for DGT measurements. In principle the DGT technique is quite simple, but detailed interpretation of the results of DGT-based measurements is associated with a range of uncertainties and questions that need further investigation. Complexed metal ions have slower diffusion rate than the corresponding free metal ion (Scally et al., 2003). Cattani et al. (2009), used the DGT technique for the first time to assess the complexed fraction of an element, like mercury, by humic acids. When DGT is immersed in a solution, it measures: (i) free metal ions, which are usually both the minor and the soluble fraction of trace metals in natural waters due to the complexation to natural inorganic and organic ligands; (ii) metal-ligand complexes which can dissociate within the diffusion time-scale in the diffusive layer; (iii) metal from the exchange reaction of metal-ligand and functional groups of chelating resin at the interface between the binding phase and the diffusive layer (if the stability constant for the metal of the metal-resin is much greater than that of metal-ligand). DGT does not measure inert diffusible complexes which will not contribute to the mass accumulation in the binding phase, or large complexes of metal, such as metal adsorbed to particles and large colloids, which are excluded from the diffusive layer (Li et al., 2005).

Diffusion coefficients are normally determined at room temperature and thereafter adjusted to deployment temperature. This corresponds to a change of 3% per °C. However, Larner et al. (2006) showed that diffusion coefficients of Cd, Pb, Al, Mn, Co, Cu and Zn measured at –1 °C on average differed 12% from the recalculated values determined at 20 °C. Therefore, for accurate measurements in low temperature waters it may be necessary to repeat diffusion coefficient measurements. When high accuracy is necessary, temperature loggers might be needed. The temperature logger is attached in situ close to the DGT devices and is programmed to register the temperature at specified time intervals.

1.5. Principle of isotopic diluition-ICP-MS.

Isotope dilution analysis (IDA) is a well-known analytical technique based on the measurement of isotope ratios in samples where its isotopic composition has been altered by the addition of a known amount of an isotopically enriched element.

The use of IDA for total elemental determinations is well documented in the literature and several reviews and books have been written on this subject (Heumann, 1992 & 1998; Fasset and Paulsen, 1989; De Bievre, 1994). In the last years, we have seen the application of isotope dilution methodologies in some new analytical fields. One of those new fields is elemental speciation, where the aim is the determination of the individual chemical species in which an element is distributed in a given sample.

Isotope dilution analysis relies on the intentional alteration of the isotope abundances of an endogenous element in a given sample by the addition of a known amount of an enriched isotope of the same element (spike). The element to be analysed must have, therefore, at least two stable or long-lived radioactive isotopes able to be measured in a mass spectrometer free of spectral interferences.

For best comprehension, the isotope *a* is the most abundant in the sample whereas the spike is isotopically enriched in the isotope *b*. It is clear that the abundance of the two isotopes and, hence, the isotope ratio in the mixture, will be intermediate between those in the sample and the spike and it will depend both on the amount of spike added and on the initial amount of the element in the sample. Those relationships can be expressed mathematically using a simple isotope dilution equation:

$$c_S = c_{Sp} \frac{m_{Sp}}{m_S} \frac{M_S}{M_{Sp}} \frac{A_{Sp}^b}{A_S^a} \left(\frac{R_m - R_{Sp}}{1 - R_m R_{Sp}} \right) \qquad\qquad Eq.1$$

c_S = concentration of the element in the Sample

c_{Sp} = concentration of the element in the Spike

m_{Sp} = mass taken from the spike in the mixture

m_S = mass taken from the sample in the mixture

M_S= atomic weight of the element in the sample

M_{Sp} = atomic weight of the element in the spike

A_{Sp}^a = isotopic abundance of isotope a in the spike

A_{Sp}^b = isotopic abundance of isotope *b* in the spike

A_S^a = isotopic abundance of isotope *a* in the sample

$A_S^b =$ isotopic abundance of isotope *b* in the sample

R_m = isotopic ratio of isotopes a and *b* in the mixture

For example, when a sample is analysed by ICP MS to calculate the concentration of Sb in the sample (c_S), we just need to know the Sb concentration in the unspiked reference standard, the natural $^{123}Sb/^{121}Sb$ ratio and to know the $^{121}Sb/^{123}Sb$ ratio in the spike, plus the measured $^{121}Sb/^{123}Sb$

ratios in the spiked standard and samples. Therefore, Eq. 1 becomes an Online Isotope Dilution equation (Eq. 2)

$$c_S = c_{St} \frac{(R_{st}R_n - 1)}{(R_mR_n - 1)} \frac{R_{Sp} - R_m}{R_{Sp} - R_{St}} \qquad\qquad Eq.2$$

c_{St}= is the known concentration of Sb in the natural reference standard

R_n = is the known reverse natural ratio of the analyte (123/121, 42.79% / 57.21%)

R_{sp} = is the certified ratio in the spike (121/123, 1.343% / 98.6575%)

R_{st} = is the measured 121/123 Sb ratio in the spiked standard

R_m = is the measured 121/123 Sb ratio in the spiked unknown samples/blanks

As can be observed, in contrast to other calibration strategies such as external calibration or standard additions, in Eq. (1.7), there is no parameter regarding the instrumental sensitivity. Therefore, the first advantage of isotope dilution analysis is that any variation of this parameter due to instrumental instabilities such as signal drift or matrix effects will have no influence on the final value for the element concentration in the sample (c_S).

On the other hand, the uncertainty in the concentration measurement depends only on the uncertainty in the measurement of the isotope ratios R_S, R_{Sp} and R_m,since the elemental atomic weights in the sample and spike (M_S and M_{Sp}) are known and the mass taken from sample and spike (m_S and m_{Sp}) can be gravimetrically determined. In most cases, except for certain elements, which show natural variations in their isotope abundances, R_S is known and this is also the case for R_{Sp} if a certified tracer or spike is used. Therefore, the only parameter that has to be experimentally determined is R_m, and this can be done with high accuracy and precision by using a mass spectrometer. Possible loss of substance of the isotope-diluted sample will have no influence on the final result.

Trace elemental speciation analysis has been usually performed by resorting to hybrid techniques, based on the coupling of an effective separation technique to a sensitive element-specific detector (Sanz-Medel, 1998). The selection of an adequate separation technique is paramount and will depend on the nature of the species to be determined and sample to be analysed, being the most commonly used chromatographic techniques high-performance liquid chromatography (HPLC) or gas chromatography (GC) and other separation techniques such as capillary electrophoresis (CE). HPLC or GC couplings are especially easy, since the gas or liquid flows can be directly introduced into the ICP torch with slight disturbance of the plasma and without any splitting or dilution

process. Due to the multi-element capability and the high sensitivity of the inductively coupled plasma–mass spectrometer (ICP-MS), as well as the possibility of measuring different isotopes of a given element, the coupling of these separation techniques to an ICP-MS has been in the past years one of the most powerful tools for elemental speciation.

The application of isotope dilution analysis for elemental speciation can be performed under two different modes: species-specific and species-unspecific spiking, depending on when and in which chemical form the isotope tracer (spike) is added to the sample.

The species-specific spiking mode requires the use of a spike solution containing the species to be analysed in an isotopically labelled form.

Conversely, in the species-unspecific spiking mode, the addition of the isotope tracer or spike is carried out after the complete separation of the naturally occurring species in the sample has taken place (post-column spiking). This mode is especially useful either when the structure and composition of the sought species is not exactly known or when the corresponding isotopically labelled compounds are not commercially available or cannot be synthesized.

Also, this mode of spiking can only be applied when the ionisation efficiency of the element is independent from the chemical form in which the element is presented to the ion source. Hence, only ICP-MS has been used till now for post-column isotope dilution as the ionisation efficiencies in this ion source can be considered independent from the chemical form of the element (Schwarz and Heumann, 2002). The isotope ratio changes along the peak as the enriched spike is pumped continuously post-column. Only point-by-point isotope ratio measurement is adequate, because the integration of the isotope ratio chromatogram will provide the concentration of the sought species.

Rottmann and Heumann described the first application of species-unspecific isotope dilution analysis in 1994. In this pioneer chapter, an approach based on an on-line isotope dilution technique coupled with HPLC-ICP-MS was developed and applied for the determination of metal complexed with humic substances in river water.

In order to obtain the concentration of the different species in the sample, the continuous addition of the spike solution containing the enriched isotopes is performed by a peristaltic pump in such a way that it is completely and continuously mixed -through a T piece- with the effluent from the column containing the separated species to be determined (Fig. 1).
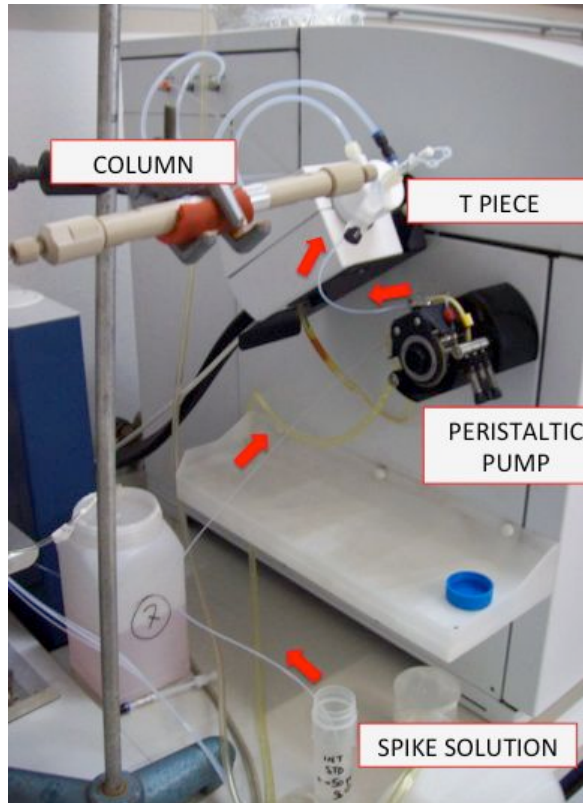
Fig. 1 Representation of mix of spike solution and effluent from the column.

In brief, the basis of this on-line isotope dilution technique relies on the plotting of the mass flow vs. retention time. The various peaks detected have to be integrated and normalised to the injection volume. The mass flow is calculated by measuring the corresponding isotope ratio throughout the whole chromatographic run. If no discrimination of the species during the ionisation processes is assumed, a mass flow profile can therefore be obtained. The equations used for post-column isotope dilution analysis are described below.

$$MF_S = c_{Sp} d_{Sp} f_{Sp} \frac{AW_S}{AW_{Sp}} \frac{A_{Sp}^b}{A_S^a} \left(\frac{R_m - R_{Sp}}{1 - R_m R_S} \right) \qquad\qquad Eq.\,3$$

MF_S= mass flow of the sample eluting from the column

c_{Sp}= concentrations of the element in the spike (ex. 49.4546 ng/g)

a is the most abundant isotope in the sample

b is the most abundant isotope in the spike

d_{Sp}= density of spike solution (ex. 1 g ml^{-1})

f_{Sp}= flow rate of spike solution (ex. 0.04 mL min^{-1})

AW_S and AW_{Sp} = atomic weight of the element in the sample and in the spike

A_S^a = Isotope abundances for isotopes a (121) in the sample (ex. 57,21)

20

A_{Sp}^{b} = Isotope abundances for isotopes *b* (123) in the spike (ex. 98.66)

R_m = the isotope ratio (a/b) (121/123) in the mixture

R_{Sp} = is the isotope ratio (a/b) (121/123, 1.343% /98.6575%) in the spike

R_S = the isotope ratio (b/a) (123/121, 42.79% / 57.21%) in the sample

The process to obtain the mass flow chromatogram requires the use of a spreadsheet software and the availability of the whole chromatogram in table form with at least three columns: time, intensity for isotope *a* and intensity for isotope *b*. For that purpose, intensity data must be expressed in counts s^{-1} (Fig. 2 a). Now, we can calculate the isotope ratio chromatogram (*Rm* vs. time) by dividing the corrected intensities obtained for isotope *a* with those for isotope *b* (Fig. 2 b). Once the isotope ratio chromatogram has been corrected for mass bias, we can apply the isotope dilution Eq. (3) to the whole chromatogram (Fig. 2 b). For that purpose, we need to know accurately the mass flow of the spike ($c_{Sp}d_{Sp}f_{Sp}$) which can be carried out by injecting a standard solution of the element. The integration of the peaks provides the amount of the element in each peak (in ng) which can be related to the injection volume (10 μl in this case). Additionally, the integration of the whole chromatogram provides the total amount of the element.
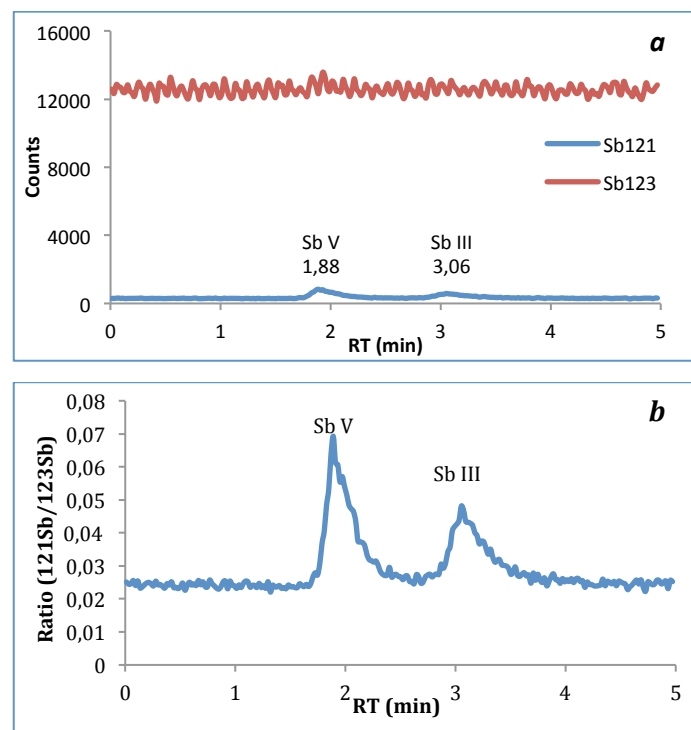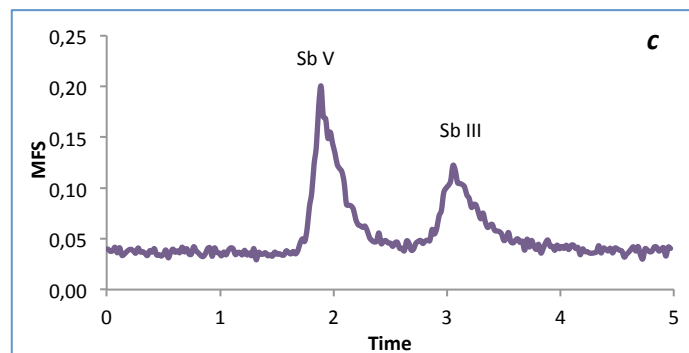
Fig. 2. Chromatogram procedure to obtain mass flow with isotopic dilution equation (Eq. 3).

However, since the quantification parameter is an isotope ratio instead of an absolute intensity, this isotope dilution analysis mode does correct for those errors derived from instrumental instabilities and matrix effects providing accurate and precise determinations of the sought element.

Finally, one of the most important advantages of the use of the species-unspecific spiking mode is the possibility of determining of species of unknown structure and composition by isotope dilution analysis. This is especially important when the synthesis of the isotopically enriched species to be analysed is not possible (Rodríguez-González et al.,2005).

1.6. Employment of Laser Ablation.

Ever since the first research efforts made during the 1980s (Gray, 1985; Arrowsmith and Hughes, 1988), laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has gained growing attention and is now considered an off-the-shelf method for the element and isotope-specific analysis of solid materials (Durrant and Ward, 2005; Pisonero et al. 2009; Fernandez et al. 2007). The LASER (Light Amplification by Stimulated Emission of Radiation) has demonstrated its potential sampling capabilities with various applications over several decades. Its development is closely associated with the ICP-MS (Inductively Coupled Plasma- Mass Spectrometer).

Several research fields have employed LA-ICP-MS as a versatile analytical tool such as: proteomics (Bettmer et al. 2009; Heras et al. 2011; Jimenez et al. 2010) forensic (Castro et al. 2010; Berends-Montero et al. 2006; Arroyo et al. 2010), environmental (Durrant et al. 2005; Arroyo et al. 2009; Brown et al. 2009), geologic (Campbell et al. 2006; Nehring et al. 2008; Liu et al. 2008), archeology and cultural heritage (Giussani et al. 2009; Bartkus et al. 2011; Byrne et al. 2010), clinical and biological (Waentig et al. 2011; Kumtabtim et al. 2011; Kang et al. 2004), among others. Many different types of samples are used in these studies including soils, sediments, rocks, tree rings, hair, teeth, bone, plants, and glasses.

The most striking features of LA-ICP-MS are ease of use, high sensitivity, and a dynamic range covering up to twelve orders of magnitude, allowing for the simultaneous acquisition of major, minor, and trace constituents. Furthermore, little or even no sample preparation is required, making LA-ICP-MS particularly attractive for the analysis of chemically resistant materials such as fluorites or zircons (Jeffries et al. 1998; Kosler et al. 2005). Another important feature is its high spatial resolution (<1 µm) and therefore small material uptake (<0.1 µg s^{-1}), which accounts for the non-destructive sample appearance on the macroscopic scale (>1 mm).

Depending on the LA protocol and sample material chosen, heterogeneous aerosols composed of nanoparticles and larger aggregates are released upon laser exposure, which can limit the accuracy of analyses as a result of varying evaporation and ionization patterns inside the ICP if non-matrix-matched calibration standards are used.

To suppress the occurrence of molecular interferences formed by polyatomic ions and to achieve the optimum ion yield needed for trace element determinations, the inert gases argon and helium are supplied as aerosol carriers.

Considering the above-mentioned heterogeneity and size structure of aerosols emerging from the LA process, indispensable pre-conditions for accurate analyses are (1) a representative aerosol composition, (2) high transport efficiencies, and (3) a complete decomposition of particles that reach the ICP (Garcia et al. 2009). For the purpose of representative sampling, Nd:YAG laser sources emitting nano-second (ns) pulses (5 to 10 ns) in the mid- and far-ultraviolet (UV) spectral range down to 213 nm have been most commonly used.

However, the formation of particles in the micrometer-size range produced in this way has been reported to strongly affect precision and accuracy of Nd:YAG laser-based LA-ICP-MS analyses, since they were found to insufficiently evaporate in the ICP, resulting in spikes and drifting signals (Guillong and Gunther, 2002; Hola et al. 2008).

Because the detection of analyte ions formed inside the ICP can only be accomplished under vacuum conditions, a differentially pumped sampler-skimmer system is arranged in front of the mass analyser, that separates ions according to their mass-to-charge (m/z) ratio. Depending on mass resolution, sensitivity, and precision required, ICP-MS analyses of laser-produced aerosols are carried out by either quadrupole (Q). Today, virtually all ICP-MS instruments can be equipped with detectors covering a linear dynamic range of a maximum of twelve orders of magnitude, permitting the simultaneous acquisition of major and trace elements.

The quantification capabilities of LA-ICP-MS critically depend on the availability of adequate reference standards for calibration that, at best, exactly match the sample composition.

Conceptually, the laser ablation system, CETAC LSX-213 G2 in our study, provides a mean of rapid and direct analysis of solid samples without dissolution and with minimal sample preparation. The laser ablation system features a high-energy laser and computer-controlled sampling methods using the DigiLaz^{TM} G2 Software. The laser ablation system generates particulate aerosols from soil material by an extreme rapid interaction between a high energy UV larger pulse and the sample surface. This process is referred to as ablation. Adjusting laser energy, spot size and pulse frequency using the DigiLaz G2 Software optimizes signal intensity and stability. Ablated material is swept into the ICP-MS by carrier gas (Fig. 1). Typically, a solid sample is placed inside an enclosed chamber (the sample cell) and a laser beam is focused on the surface of the sample. The sample cell is mounted on a computer controlled X-Y-Z translation stage, with a step size of 0.25 μm.

When the laser is fired, a cloud of particles is produced. These particles are removed from the sample cell by a carrier gas, and are swept into the inductively coupled plasma for atomization and ionization and subsequent analysis. Compared with conventional dissolution techniques, laser ablation has many advantages. Most analytical techniques involve removing a portion of the solid sample, which is then dissolved in acid solutions. With this procedure, there is a greater chance of exposure to hazardous materials and there is a risk of introducing contaminants or losing volatile components during sample preparation. For laser ablation, any type of solid sample can be ablated for analysis; there are no sample-size requirements and no sample preparation procedures. In addition, a focused laser beam permits spatial characterization of heterogeneity in solid samples, with typically micron resolution both in terms of lateral and depth conditions.

The LSX-213 G2 employs a specially designed Nd:YAG laser; frequency quintuplicated to the ultraviolet wavelength of 213 nm (Fig. 2). This laser provides a uniforms energy profile ("flat-top profile") across all spot sizes and yields a flat-bottomed crater on the sample. The laser can be operated at a high repetition rate of up to 20 Hz for increased sampling efficiency and better ICP-MS sensitivity.
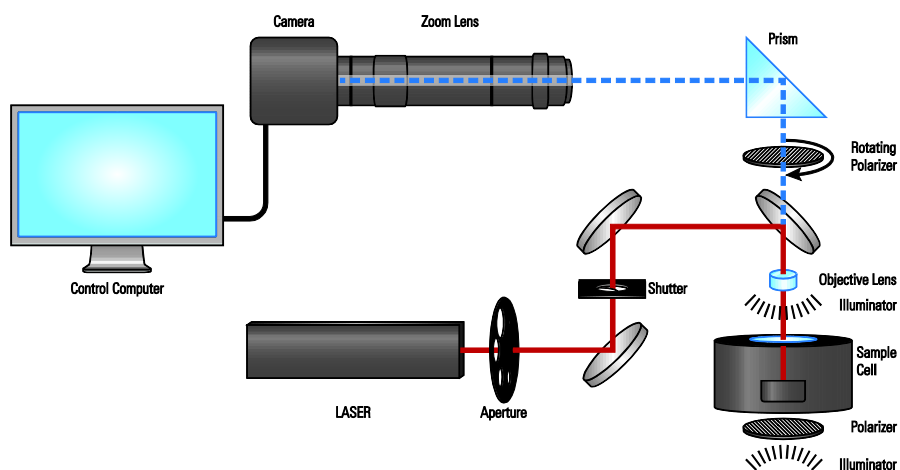
Fig. 1. Schematic diagram of the laser ablation system.

High sensitivity and spatial resolution make LA-ICP-MS an excellent tool for the element- as well as isotope-specific microanalysis of solid materials, offering detection limits at the μg g^{-1} level and below. According to the literature, the interest in LA-ICP-MS is continuously growing because of improved instrumentation and refined quantification strategies.

1.7. References

Abedin MJ, Cotter-Howells JC, Meharg AA. 2002. Arsenic uptake and accumulation in rice (Oryza sativa L.) irrigated with contaminated water. *Plant and Soil*, Vol 240, N 2, 311-319.

Adomako EE, Solaiman ARM, Williams PN, Deacon C, Rahman GKMM, Meharg AA. 2009. Enhanced transfer of arsenic to grain for Bangladesh grown rice compared to US and EU. *Environ Int* 35:476–479

Ahrland S. 1968. Thermodynamics of complex formation between hard and soft acceptors and donors. *Struct. Bond.* 5, 118–149.

Ahrland, S. 1973. Thermodynamics of stepwise formation of metal – ion complexes in aqueous solutions. *Struct. Bond.* 15, 167 – 188.

Anderegg G and Malik S. 1970. Die Komplexbildungstendenz des dreiwertigen Antimons in wässeriger Lösung. *Helv. Chim. Acta.* 53, 577–600.

Anderegg G and Malik S. 1970. Komplexone XLIII. Die Komplexe des dreiwertigen Antimons mit Polyaminocarboxylates. *Helv. Chim. Acta.* 53, 564–569.

Anderegg G and Malik S. 1967. Wasserlösliche Komplexe des dreiwertigen Antimons. *Chimia.* 21, 541–544.

Andreae MO, Byrd TJ, Froelich ON. 1983. Arsenic, antimony, germanium and tin in the Tejo estuary, Portugal: modelling of a polluted estuary. *Environ. Sci. Technol.* 17, 731–737.

Aposhian HV, Aposhian MM. 2006. Arsenic toxicology: five questions. Chem Res Toxicol. 19: 1–15.

Arrowsmith P and S. K. Hughes. 1988. Entrainment and Transport of Laser Ablated Plumes for Subsequent Elemental Analysis. *Appl. Spectrosc.* 42, 1231.

Arroyo L, Trejos T, Gardinali PR and Almirall JR. 2009. Optimization and validation of a Laser Ablation Inductively Coupled Plasma Mass Spectrometry method for the routine analysis of soils and sediments. *Spectrochim. Acta.* Part B, 64, 16.

Arroyo L, Trejos T, Hosick T, Machemer S, Almirall JR and Gardinali PR. 2010. Analysis of Soils and Sediments by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS): An Innovative Tool for Environmental Forensics. *Environ. Forensics.* 11(4), 315.

Attané I, Courbage J, 2001. La démographie en Méditerranée. Situation et projections. Les Fascicules du Plan Bleu, 11. Paris, *Economica*, pp. 249.

Bartkus L, Amarasiriwardena D, Arriaza B, Bellis D and Yanez J. 2011.Exploring lead exposure in ancient Chilean mummies using a single strand of hair by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). *Microchem. J.* 98, 267.

Berends-Montero S, Wiarda W, de Joode P and G. van der Peijl. 2006. Forensic analysis of float glass using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS): validation of a method. *J. Anal. At. Spectrom.* 21, 1185.

Bettmer J, Bayon MM, Encinar JR, Sanchez MLF, M. R. F. de la Campa and Medel AS. 2009.The emerging role of ICP-MS in proteomic analysis. *J. Proteomics.* 72, 989.

Bhat TR, Iyer RK, Shankar J. 1966. Antimony and bismuth chelates of N-hydroxyethyl ethylene diamine triacetic acid. *Z. Anorg. Chem.* 343, 329–336.

Bhat TR, Iyer RK. 1965. Studies on EDTA complexes. V. Antimony(III) and bismuth(III) EDTA systems. *Z. Anorg. Allg. Chem.* 335, 331–336.

Bhattacharjee H, Rosen BP and R Mukhopadhyay. 2009. Aquaglyceroporins and metalloid transport: implications in human diseases. *Handb. Exp. Pharmacol.* 190, 309–325.

Bissen M, Frimmel FH. 2003. Arsenic—a review. Part II: oxidation of arsenic and its removal in water treatment. *Acta Hydrochim Hydrobiol.* 31:97–107.

Boorman GA, Dellarco V, Dunnick JK, Chapin RE, Hunter S, Hauchman F, et al. 1999. Drinking water disinfection byproducts: review and approach to toxicity evaluation. *Environ Health Perspect.* 107(Suppl 1): 207–17.

Brown JC, Jarvis KE, Disch BA, Goddard SL and AS Brown. 2009. Spatial inhomogeneity of metals in particulate matter on ambient air filters determined by LA-ICP-MS and comparison with acid digestion ICP-MS. *J. Environ. Monit.* 11, 2022.

Byrne S, Amarasiriwardena D, Bandak B, Bartkus L, Kane J, Jones J, Yanez J, Arriaza B and L Cornejo. 2010. Were Chinchorros exposed to arsenic? Arsenic determination in Chinchorro mummies' hair by laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). *Microchem. J.* 94, 28.

Campbell IH, Ballard JR, Palin JM, Allen C and A Faunes. 2006. U-Pb Zircon Geochronology of Granitic Rocks from the Chuquicamata-El Abra Porphyry Copper Belt of Northern Chile: Excimer Laser Ablation ICP-MS. *Analysis. Econ. Geol.* 101(7), 1327.

Castro W, Hoogewerff J, Latkoczy C and JR Almirall. 2010. Application of laser ablation (LA-ICP-SF-MS) for the elemental analysis of bone and teeth samples for discrimination purposes. *Forensic Sci. Int.* 195, 17.

Cattani I, Zhang H, Beone GM, Del Re AA, Boccelli R, Trevisan M. 2009. The role of natural purified humic acids in modifying mercury accessibility in water and soil. *J Environ Qual.* 6:493-501.

Chang KN, Lee TC, Tam MF, Chen YC, Lee LW, Lee SY, et al. 2003. Identification of galectin I and thioredoxin peroxidase II as two arsenic-binding proteins in Chinese hamster ovary cells. *Biochem J.* 371:495–503.

Cohen S. 1987. Projected increases in municipal water use in the Great Lakes due to C02- induced climatic change. *Water Resources Bulletin.* 23(1), 91-101.

Cullen WR, Reimer KJ. 1989. Arsenic speciation in the environment. *Chem Rev.* 89: 713–64.

D. Lgs 152/2006, Norme in materia ambientale, Parte Terza, 2006, p. 30-83.

D. Lgs 30/2009, Attuazione della dir 2006/118/CE , relativa alla protezione delle acque sotterranee dall'inquinamento e dal deterioramento.

D. Lgs 31/2001 Attuazione della direttiva 98/38/CE relativa alla qualità delle acque destinate al consumo umano.

Dagnac T, Padro⁄A, Rubio R, Rauret G. 1999. Speciation of arsenic in mussels by the coupled system liquid chromatography UV irradiation hydride generation inductively coupled plasma mass spectrometry. *Talanta.* 48, 763–772.

Davison W, Fones G, Harper M, Teasdale P, Zhang H In J. Buffle, G. Horvai (Eds.). 2000. In Situ Monitoring of Systems: Chemical Analysis and Speciation. *John Wiley &Sons Inc.,* New York, p. 495.

Davison W, Zhang H. 1994. In situ speciation measurements of trace components in natural waters using thin-film gels. *Nature.* 367, 546-548.

De Bievre P. 1994. Isotope-dilution mass spectrometry. *Tech. Instrum. Anal. Chem.* 15, 169 – 183.

De Gregori I, Quiroz W, Pinochet H, Pannier F, Potin-Gautier M. 2005. Simultaneous speciation analysis of Sb(III), Sb(V) and (CH$_3$)$_3$SbCl$_2$ by high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry detection (HPLC-HG-AFS): application to antimony speciation in sea water. *J. Chromatogr.* A 1091, 94.

De Gregori I, Quiroz W, Pinochet H, Pannier F, Potin-Gautier M. 2007. Speciation analysis of antimony in marine biota by HPLC-(UV)-HG-AFS: Extraction procedures and stability of antimony species. *Talanta*. 73, 458.

De Stefano L. 2004. Freshwater and Tourism in the Mediterranean. WWF Mediterranean Programme

Del Razo LM, Quintanilla Vega B, Brambila Colombres E, Calderon Aranda ES, Manno M, Albores A. 2001. Stress proteins induced by arsenic. *Toxicol. Appl. Pharmacol.* 177, 132–148.

Deutsche Forschungsgemeinschaft (DFG), Analysis of Hazardous Substances in Biological Materials, Wiley-VCH, Weinheim, Germany, 1994.

Diviš P, Dočekalová H, Brulík L, Pavliš M, Hekera P. 2007. Use of the diffusive gradients in thin films technique to evaluate (bio) available trace metal concentrations in river water. *Anal. Bioanal. Chem.* 387, 2239-2244.

Dodd M, Grundy SL, Reimer KJ, Cullen WR. 1992. Methylated antimony(V) compounds: Synthesis, hydride generation properties and implications for aquatic speciation. *Appl. Organomet. Chem.* 6, 207.

Dodd MC, Vu ND, Ammann A, Le VC, Kissner R, Pham HV, et al. 2006. Kinetics and mechanistic aspects of As(III) oxidation by aqueous chlorine, chloroamines, and ozone: relevance to drinking water treatment. *Environ Sci Technol.* 40: 3285–92.

Downing TE, Butterfield RE, Edmonds B et al. 2003. Climate Change and the Demand for Water, Research Report, Stockholm Environment Institute Oxford Office, Oxford.

Duh B. 2002. Effect of antimony catalyst on solid-state polycondensation of poly(ethyleneterephthalate). *Polymer.* 43: 3147–54.

Durrant SF and NI Ward. 2005. Recent biological and environmental applications of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *J. Anal. At. Spectrom.* 20, 821.

Dworak T, Berglund M, Laaser C. et al. 2007. EU Water Saving Potential. Retrieved January 2009, from http://ec.europa.eu/environment/water/quantity/pdf/water_saving_1.pdf

EEA – ETC/WTR based on data from Eurostat data table: Annual water abstraction by source and by sector. 2010

EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on Arsenic in Food. *EFSA Journal 2009*. 7(10):1351..

Eguchi N, Kuroda K and G Endo. 1997. Metabolites of arsenic induced tetraploids and mitotic arrest in cultured cells. *Arch. Environ. Contam. Toxicol.* 32. 141–145.

Emett MT, Khoe GH. 2001. Photochemical oxidation of arsenic by oxygen and iron in acidic solutions. *Water Res.* 35: 649–56.

Er-kang W. 1982. Polarographic electrode process of antimony(III) complexes with complexones. *Coll. Czech. Chem. Commun.* 47, 3243–3251.

EU. Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Comm.* L327, 1–73. 2000.

EU. Directive 2006/118 of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. *Off. J. Eur. Comm.* L372, 1–19. 2006

Fasset JD and Paulsen PJ. 1989. Isotope dilution mass spectrometry for accurate elemental analysis. *Anal. Chem.* 61, 623A – 644A.

Fernandez B, Claverie F, Pecheyran C, and OFX Donard. 2007. Direct analysis of solid samples by fs-LA-ICP-MS. *Trends Anal. Chem.* 26, 951.

Ferreira D, Tousset N, Ridame C, Tusseau-Vuillemin MH. 2008. More than inorganic copper is bioavailable to aquatic mosses at environmentally relevant concentrations. *Environ. Toxicol. Chem.* 27, 2108-2116.

Foster SSD & Chilton PJ. 2003. Groundwater: the processes and global significance of aquifer degradation. *Phil. Trans. R. Soc. Lond.* B 358, 1957–1972.

Frank P and D Clifford. 1986. Arsenic(III) oxidation and removal from drinking water. *U.S. Environmental Protection Agency.* EPA-600-52-86/021, pp. 2–86.

Gailer J and Irgolic KJ. 1996. Retention behavior of arsenobetaine, arsenocholine, trimethylarsine oxide and tetramethylarsonium iodide on a styrene divinylbenzene column with benzenesulfonates as ion pairing reagents. *J. Chromatogr. A.* 730, 219–229.

Garcia CC, Lindner H, and Niemax K. 2009. Laser ablation inductively coupled plasma mass spectrometry—current shortcomings, practical suggestions for improving performance, and experiments to guide future development. *J. Anal. At. Spectrom.* 24, 14.

Gebel T. 1997. Aresnic and antimony: comparative approach on mechanistic toxicology. *Chemico-Biological Interactions.* vol. 107, no. 3, pp. 131–144.

Ghurye G, Clifford D and Tripp A. 2004. Iron coagulation and direct microfiltration to remove arsenic from groundwater. *J Am Water Works Assoc.* 96: 84–9.

Giussani B, Monticelli D and Rampazzi L. 2009. Role of laser ablation–inductively coupled plasma–mass spectrometry in cultural heritage research: A review. *Anal. Chim. Acta.* 635, 6.

Gray AL. 1985. Solid sample introduction by laser ablation for inductively coupled plasma source mass spectrometry. *Analyst* (Cambridge, U.K.) 110, 551.

Greathouse DG and Craun GF. 1978. Cardiovascular disease study — occurrence of inorganics in household tap water and relationships to cardiovascular mortality rates. In: Hemphill DD, editor. Trace substances in environmental health XII — 12th annual conference on trace substances in environmental health. p. 31–9. Columbia, Missouri, 6–8 June.

Guillong M and Gunther D. 2002. Effect of particle size distribution on ICP-induced elemental fractionation in laser ablation-inductively coupled plasma-mass spectrometry. *J. Anal. At. Spectrom.* 17, 831.

Hansen HR and Pergantis SA. 2007. Identification of Sb(V) Complexes in Biological and Food Matrixes and Their Stibine Formation Efficiency during Hydride Generation with ICPMS Detection. *Anal. Chem.* 79, 5304–5311.

Hansen R and Pergantis SA. 2006. Detection of antimony species in citrus juices and drinking water stored in PET containers. *J. Anal. At. Spectrom.* 21, 731.

Harvey CF, Swartz CH, Badruzzaman ABM, Keon-Blute N, Yu W, Ali MA, Jay J, Beckie R, Niedan V, Brabander D, Oates PM, Ashfaque KN, Islam S, Hemond HF and MF Ahmed. 2002. Arsenic mobility and groundwater extraction in Bangladesh. *Science.* 298, 1602 – 1606.

Hasegawa H, Rahman MA, Kitahara K, Itaya Y, Maki T, Ueda K. 2010. Seasonal changes of arsenic speciation in lake waters in relation to eutrophication. *Sci. Tot. Environ.* 408, 1684-1690.

Heitkemper DT, Vela NP, Stewart KR, Westphal CS. 2001. Determination of total and speciated arsenicin rice by ion chromatography and inductively coupled plasma mass spectrometry. *J Anal At Spectrom.* 16: 299–306 17.

Heras IL, Palomo M and Madrid Y. 2011. Selenoproteins: the key factor in selenium essentiality. State of the art analytical techniques for selenoproteins studies. *Anal. Bioanal. Chem.* 400, 1717.

Herrington P. 1996. Climate change and the demand for water. HMSO, London.

Heumann KG. 1992. Isotope Dilution mass spectrometry (IDMS) of the elements. *Mass Spectrom. Rev.* 11, 41 – 67.

Heumann KG. 1998. Isotope dilution mass spectrometry, in: F. Adams, R. Gijbels, R. Van Grieken (Eds.), Inorganic Mass Spectrometry, Wiley, New York, pp.301 – 376.

Hola M, Konecna V, Mikuska P, Kaiser J, Palenikova K, Prusa S, Hanzlikova R, and V Kanicky. 2008. Study of aerosols generated by 213 nm laser ablation of cobalt-cemented hard metals. *J. Anal. At. Spectrom.* 23, 1341.

Hossain MF. 2006. Arsenic contamination in Bangladesh - an overview. *Agriculture, Ecosystems & Environmen.* 113, 1-16.

Huang RN and TC Lee. 1996. Cellular uptake of trivalent arsenite and pentavalent arsenate in KB cells cultured in phosphate-free medium. Toxicol. Appl. Pharmacol. 136, 243–249.

Hywel Evans E and Giglio JJ. 1993. Interferences in inductively coupled plasma mass spectrometry a review. *J. Anal. Atom. Spectrom.* 8, 1–18.

International Agency for Research on Cancer (IARC). 1989. IARC Monograph: Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting. *IARC*, Lyon, Vol. 47, p. 291.

ISTAT, http://demo.istat.it/pop2010/index.html

Jeffries TE, Jackson SE, and HP Longerich. 1998. Application of a frequency quintupled Nd:YAG source (λ=213 nm) for laser ablation inductively coupled plasma mass spectrometric analysis of minerals. *J. Anal. At. Spectrom.* 13, 935.

Jimenez MS, Rodriguez L, Gomez MT and JR Castillo. 2010. Metal–protein binding losses in proteomic studies by PAGE–LA-ICP-MS. *Talanta*. 81, 241.

Johnston R and Heijnen H. 2001. Safe water technology for arsenic removal. In: Ahmed MF, Ali MA, Adeel Z, editors. Technologies for arsenic removal from drinking water. p. 1–22.

Kang D, Amarasiriwardena D and AH Goodman. 2004. Application of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to investigate trace metal spatial distributions in human tooth enamel and dentine growth layers and pulp. *Anal. Bioanal. Chem.* 378, 1608.

Katsoyiannis IA, Hug SJ, Ammann A et al. 2007. Arsenic speciation and uranium concentrations in drinking water supply wells in Northern Greece: Correlations with redox indicative parameters and implications for groundwater treatment. *Science of the Total Environment.* 383: 128–140.

Kenneth DF. 1997. Water Resources and Climate Change. Resources for the future Climate. Issues Brief No. 3.

Keresztes S, Tatar E, Mihucz VG, Virag I, Majdik C, Zaray G. 2009. Leaching of antimony from polyethylene terephthalate (PET) bottles into mineral water. *Sci. Total Environ.* 407, 4731-4735.

Kim M, Nriagu J and Haack S. 2000. Carbonate ions and As dissolution by groundwater. *Environ. Sci. Technol.* 34, 3094– 3100.

Kohlmeyer U, Kuballa J, Jantzen E. 2002. Silmultaneous separation of 17 inorganic and organic arsenic compounds in marine biota by means of high performance liquid chromatography inductively cou- pled plasma mass spectrometry. Rapid Comm. *Mass Spectrom.* 16, 965–974.

Kolpakova NN. 1982. Laboratory and field studies of ionic equilibria in the Sb_2S_3–H_2O–H_2S system. *Geochem. Int.* 19, 46–54.

Kosler J, Wiedenbeck M, Wirth R, Hovorka J, Sylvester P, and J Mikova. 2005. Chemical and phase composition of particles produced by laser ablation of silicate glass and zircon—implications for elemental fractionation during ICP-MS analysis. *J. Anal. At. Spectrom.* 20, 402.

Krachler M and H Emons. 2000. Potential of high performance liquid chromatography coupled to flow injection hydride generation atomic absorption spectrometry for the speciation of inorganic and organic antimony compounds. *J. Anal. At. Spectrom.* 15, 281.

Krachler M and H Emons. 2001. Speciation analysis of antimony by high-performance liquid chromatography inductively coupled plasma mass spectrometry using ultrasonic nebulization. *Anal. Chim. Acta.* 429, 125.

Krachler M, Emons H and J Zheng. 2001. Speciation of antimony for the 21st century: promises and pitfalls. *TrAC, Trends Anal. Chem.* 20, 79–90.

Krupp RE. 1988. Solubility of stibnite in hydrogen sulfide solu- tions, speciation, and equilibrium constants, from 25 to 350°C. *Geochim. Cosmochim. Acta.* 52, 3005–3015.

Kumtabtim U, Siripinyanond A, Auray-Blais C, Ntwari A and JS Becker. 2011. Analysis of trace metals in single droplet of urine by laser ablation inductively coupled plasma mass spectrometry. *Int. J. Mass Spectrom.*

Lafferty BJ and RH Loeppert. 2005. Methyl arsenic adsorption and desorption behavior on iron oxides. *Environ. Sci. Technol.* 39, 2120-2127.

Larner BL, Seen AJ, Snape I. 2006. Evaluation of diffusive gradients in thin film (DGT) samplers for measuring contaminants in the Antarctic marine environment. *Chemosphere.* 65, 811-820.

Le XC and M Ma. 1997. Speciation of arsenic compounds by using ion pair chromatography with atomic spectrometry and mass spectrometry detection. *J. Chromatogr. A.* 764, 55–64.

Lead JR, Davison W, Hamilton-Taylor J. 1997. Characterizing Colloidal Material in Natural Waters. *Aquat. Geochem.* 3, 213–232.

Lee Y, Um IK, Yoon J. 2003. Arsenic(III) oxidation by iron(VI) (ferrate) and subsequent removal of arsenic(V) by iron(III) coagulation. *Environ Sci Technol.* 37:5750–6.

Li W, Zhao H, Teasdale PR, John R and Wang F. 2005. Metal speciation measurement by diffusive gradients in thin films technique with different binding phases. *Anal. Chim. Acta.* 533: 193–202.

Lin TF, Wu JK. 2001. Adsorption of arsenite and arsenate within activated alumina grains: equilibrium and kinetics. *Water Res.* 35(8): 2049–57.

Lintschinger J, Koch I, Serves S, Feldmann J and WR Cullen. 1997. Determination of antimony species with high-performance liquid chromatography using element specific detection. *J. Anal. Chem.* 359, 484–491.

Liu Y, Hu Z, Gao S, Gunther D, Xu J, Gao C and H Chen. 2008. In situ analysis of major and trace elements of anhydrous minerals by LA-ICP-MS without applying an internal standard. *Chem. Geol.* 257, 34.

Luo J, Zhang H, Santner J, Davison W. 2010. Performance characteristics of diffusive gradients in thin films equipped with a binding gel layer containing precipitated ferrihydrite for measuring arsenic(V), selenium(VI), vanadium(V), and antimony(V). *Anal. Chem.* 82, 8903-8909.

Mass MJ, Tennant A, Roop BC, Cullen WR, Styblo M, Thomas DJ, Kligerman, A.D., 2001. Methylated trivalent arsenic species are genotoxic. *Chem. Res. Toxicol.* 14, 355–361.

Meharg AA, Sun G, Williams PN, Adomako E, Deacon C, Zhu YG, Feldmann J, Raab A. 2008. Inorganic arsenic levels in baby rice are of concern. *Environ Pollut.* 152: 746–749.

Meharg AA, Williams PN, Adomako E, Lawgali YY, Deacon C, Villada A, Cambell RCJ, Sun G, Zhu YG, Feldmann J, Raab A, Zhao F, Islam R, Hossain S, Yanai J. 2009. Geographical Variation in Total and Inorganic Arsenic Content of Polished (White) Rice. *Environ Sci Technol.* 43:1612–1617.

Miravet R, Hernandez-Nataren E, Sahuquillo A, Rubioand R and JF Lopez-Sanchez. 2010. Speciation of antimony in environmental matrices by coupled techniques. *TrAC, Trends Anal. Chem.* 29, 28–39.

Mitch WA, Sedlak DL. 2002. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol.* 36:588–95.

Nash MJ, Maskall JE and SJ Hill. 2006. Developments with anion exchange stationary phases for HPLC-ICP-MS analysis of antimony species. *Analyst (Cambridge, UK).* 131, 724.

Nehring F, Jacob DE, Barth MG and SF Foley. 2008. Laser-ablation ICP-MS analysis of siliceous rock glasses fused on an iridium strip heater using MgO dilution. *Microchim. Acta.* 160, 153.

Nesnow S, Roop BC, Lambert G, Kadiiska M, Mason RP, Cullen WR and MJ Mass. 2002. DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. *Chem. Res. Toxicol.* 15, 1627–1634.

Nordberg GF, Flower BA, Nordberg M, and Friberg L. 2007. Handbook of the Toxicology of Metals, Academic Press, London, UK, 3rd edition.

Norton GJ, Duan G, Dasgupta T, Islam MR, Lei M, Zhu Y, Deacon CM, Moran AC, Islam S, Zhao F, Stroud JL, Mcgrath SP, Feldmann J, Price AH, Meharg AA. 2009. Environmental and Genetic Control of Arsenic Accumulation and Speciation in Rice Grain: Comparing a Range of Common Cultivars Grown in Contaminated Sites Across Bangladesh, China, and India. *Environ Sci Technol.* 43:8381–8386.

Norton GJ, Islam MR, Deacon CM, Zhao F, Stroud JL, Mcgrath SP, Islam S, Jahiruddin M, Feldmann J, Price AH, Meharg AA. 2009. Identification of Low Inorganic and Total Grain Arsenic Rice Cultivars from Bangladesh. *Environ Sci Technol.* 43:6070–6075.

Orero Iserte L, Roig-Navarro AF, Hernández F. 2004. Simultaneous determination of arsenic and selenium species in phosphoric acid extracts of sediment samples by HPLC-ICP-MS. *Anal. Chim. Acta.* 527, 97-104.

Özer UY, Bogucki RF. 1971. Equilibrium studies of antimony(III) chelates in aqueous solution. *J. Inorg. Nucl. Chem.* 33, 4143 – 4153.

Panther JG, Stillwell KP, Powell KJ, Downard AJ. 2008. Development and application of the diffusive gradients in thin films technique for the measurement of total dissolved inorganic arsenic in waters. *Anal. Chim. Acta.* 622, 133-142.

Pauling L. 1933. The formulas of antimonic acid and the antimonates. *J. Am. Chem. Soc.* 55, 1895–1900.

Pearson RG. 1963. Hard and soft acids and bases. *J. Am. Chem. Soc.* 85, 3533–3539.

Petrick JS, Jagadish B, Mash EA and HV Aposhian. 2001. Monomethylarsonous acid (MMA(III)) and arsenite: LD(50) in hamsters and in vitro inhibition of pyruvate dehydrogenase. *Chem. Res. Toxicol.* 14, 651 – 656.

Pettine M, Campanella L, Millero FJ. 1999. Arsenite oxidation by H_2O_2 in aqueous solutions. *Geochim Cosmochim Acta.* 63:2727–35.

Pisonero J, Fernandez B, and D Gunther. 2009. Critical revision of GD-MS, LA-ICP-MS and SIMS as inorganic mass spectrometric techniques for direct solid analysis. *J. Anal. At. Spectrom.* 24, 1145.

Potin-Gautier M, Pannier F, Quiroz W, Pinochet H and I De Gregori. 2005. Antimony speciation analysis in sediment reference materials using high-performance liquid chromatography coupled to hydride generation atomic fluorescence spectrometry. *Anal. Chim. Acta.* 553, 214.

Provincia di Parma (Servizio Amb., Difesa del Suolo e Tutela del territorio), Piano Territoriale di Coordinamento Provinciale - PTCP, A. Quadro conoscitivo, A.1 Approfondimento in materia di tutela delle acque, Elaborato 1, Analisi e dettagli tecnici, 2008.

Provincia di Piacenza, Piano regionale di Tutela delle Acque nella Provincia di Piacenza, 2007.

Raab A, Williams PN, Meharg A, Feldmann J. 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. *Environmental Chemistry.* 4: 197–203.

Radabaugh TR and HV Aposhian. 2000. Enzymatic reduction of arsenic compounds in mammalian systems: reduction of arsenate to arsenite by human liver arsenate reductase, Chem. Res. Toxicol. 13, 26–30.

Rahman MA, Hasegawa H, Rahman MM, Rahman MA, Miah MAM. 2007. Arsenic accumulation in duckweed (Spirodela polyrhiza L.): A good option for phytoremediation. *Chemosphere.* 69:942–948.

Regione Emilia Romagna, Piano di Tutela delle acque della Regione Emilia Romagna, 2005.

Regione Emilia Romagna. 2005. Piano di Tutela delle Acque. pp 371

Reimann C, Birke M, Filzmoser P. 2010. Bottled drinking water: water contamination from bottle materials (glass, hard PET, soft PET), the influence of colour and acidification. *Appl Geochem.* 25(7): 1030–1046.

Ren XL, Liu QL, Wu DX, Shu Q. 2006.Variations in Concentration and Distribution of Health-Related Elements Affected by Environmental and Genotypic Differences in Rice Grains. *Rice Sci.* 13:170–178

Ritchie JA. 1961. Arsenic and antimony in some New Zealand thermal waters. *N. Z. J. Sci.* 4, 218–229.

Rodríguez-González P, Marchante Gayón JM, García Alonso JI and A Sanz-Medel. 2005. Isotope dilution analysis for elemental speciation: A tutorial review. *Spectrochim. Acta.* B 60, 151.

Ronkart SN, Laurent V, Carbonnelle P, Mabon N, Copin A. 2007. Speciation of five arsenic species (arsenite, arsenate, MMAA(V), DMAA(V) and AsBet) in different kind of water by HPLC-ICP-MS. *J. P. Chemosphere.* 66 (4), 738-745.

Rottmann L and KG Heumann. 1994. Development of an on-line isotope dilution technique with HPLC/ICP-MS for the accurate determina- tion of elemental species. *J. Anal. Chem.* 350, 221 – 227.

Røyset O, Rosseland BO, Kristensen T, Kroglund F, Garmo ØA, Steinnes E. 2005. Diffusive gradients in thin films sampler predicts stress in brown trout (Salmo trutta L.) exposed to aluminum in acid fresh waters. *Environ. Sci. Technol.* 2005, 39, 1167- 1174.

Sakurai T, Qu W, Sakurai MH and MP Waalkes. 2002. A major human arsenic metabolite, dimethylarsinic acid, requires reduced glutathione to induce apoptosis. *Chem. Res. Toxicol.* 15, 629–637.

Sanz-Medel A. 1998. Trace element analytical speciation in biological systems: importance, challenges and trends. *Spectrochim. Acta.* Part B 53, 197 – 211.

Satiroglu N, Bektas S, Genc O, Hazer H. 2000. HPLC/Hydride Generation AAS Coupling for the Speciation of Sb(III) and Sb(V) in Wastewaters. *Turk. J. Chem.* 24, 371.

Sayago A, Beltrán R, Gómez-Ariza JL. 2000. Hydride generation atomic fluorescence spectrometry (HG-AFS) as a sensitive detector for Sb(III) and Sb(V) speciation in water. *J. Anal. At. Spectrom.* 15, 423.

Sayago A, Beltrán R, Recamales MAF, Gómez-Ariza JL. 2002. Optimization of an HPLC-HG-AFS method for screening Sb(V), Sb(III), and Me_3SbBr_2 in water samples. *J. Anal. At. Spectrom.* 17, 1400.

Scally S, Davison W, Zhang H. 2003. In situ measurements of dissociation kinetics and labilities of metal complexes in solution using DGT. *Environ. Sci. Technol.* 37, 1379-1384.

Schoof RA, Yost LJ, Eickhoff J, Crecelius EA, Cragin DW, Meacher DM, Menzel DB. 1999.A Market Basket Survey of Inorganic Arsenic in Food. *Food Chem Toxicol.* 37:839–846.

Schuliga M, Chouchane S, Snow ET. 2002. Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentration of inorganic arsenic. *Toxicol Sci.* 70: 183–92.

Schwarz A and Heumann KG. 2002. Two-dimensional on-line detection of brominated and iodinated volatile organic compounds by ECD and ICP-MS after GC separation. *Anal. Bioanal. Chem.* 374, 212 – 219.

Sharma VK, Burnett CR, Yngard R, Cabelli DE. 2005. Iron(VI) and iron(V) oxidation of copper (I)cyanide. *Environ Sci Technol.* 39: 3849–55.

Sharma VK, Dutta PK, Ray AK. 2007. Review of kinetics of chemical and photocatalytical oxidation of arsenic(III) as influenced by pH. *J Environ Sci Health.* Part A. 42: 997–1004.

Sharma VK, Kazama F, Jiangyong H, Ray AK. 2005. Ferrates as environmentally-friendly oxidants and disinfectants. *J Water Health.* 3: 42–58.

Sharma VK. 2002. Potassium ferrate(VI): an environmentally friendly oxidant. *Adv Environ Res.* 6:143–56.

Sharma VK. 2004. Use of iron(VI) and iron(V) in water and wastewater treatment. *Water Sci Technol.* 49:69–74.

Sherman DM, Ragnarsdottir KV, Oelkers EH. 2000. Antimony transport in hydrothermal solutions: and EXAFS study of antimony(V) complexation in alkaline sulfide and sulfide– chloride brines at temperatures from 25 °C to 300 °C at Psat. *Chem. Geol.* 167, 161–167.

Shraim A, Sekaran NC, Anuradha CD and S Hirano. 2002. Speciation of arsenic in tube-well water samples collected from West Bengal, India, by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *Appl. Organomet. Chem.* 16, 202–209.

Smichowski P, Madrid Y, Calle-Guntiñas MB, Cámara C. 1995 Separation and determination of antimony(III) and antimony(V) species by high-performance liquid chromatography with hydride generation atomic absorption spectrometric and inductively coupled plasma mass spectrometric detection. *J. Anal. At. Spectrom.* 10, 815.

Smichowski P. 2008. Antimony in the environment as a global pollutant: a review on analytical methodologies for its determination in atmospheric aerosols. *Talanta.* vol. 75, no. 1, pp. 2–14.

Sogn TA, Eich-Greatorex S, Røyset O, Øgaard AF, Almås ÅR. 2008. Use of diffusive gradients in thin films to predict potentially bioavailable selenium in soil. *Comm. Soil Sci. Plant Anal.* 39, 587-602.

Stauffer RE, Thompson JM. 1984. Arsenic and antimony in geothermal waters of Yellowstone National Park, Wyoming, USA. *Geochim. Cosmochim. Acta.* 48, 2547–2561.

Stockdale A, Davison W, Zhang H. 2008. High-resolution two-dimensional quantitative analysis of phosphorus, vanadium and arsenic, and qualitative analysis of sulfide, in a freshwater sediment. *Environ. Chem.* 5, 143-149.

Stockdale A, Davison W, Zhang H. 2010. 2D simultaneous measurement of the oxyanions of P, V, As, Mo, Sb, W and U. *J. Environ.* Monit. 12, 981.

Sun GX, Williams PN, Carey AM, Zhu YG, Deacon C, Raab A, Feldmann J, Islam RM, Meharg AA. 2008. Inorganic Arsenic in Rice Bran and Its Products Are an Order of Magnitude Higher than in Bulk Grain. *Environ Sci Technol.* 42:7542–7546.

Teräsahde P, Pantsar-Kallio M, Manninen PKG. 1996. Silmutaneous determination of arsenic species by ion chromatography inductively coupled plasma mass spectrometry. *J. Chromatogr.* A. 750, 83–88.

Thomas P. 2009. Metals pollution tracing in the sewerage network using the diffusive gradients in thin films technique. *Water Sci. Technol.* 60, 65-70.

Tusseau-Vuillemin M-H, Gilbin R, Bakkaus E, Garric J. 2004. Performance of diffusion gradient in thin films to evaluate the toxic fraction of copper to Daphnia magna. *Environ. Toxicol. Chem.* 23, 2154-2161.

Ulrich N. 1998. *Anal. Chim. Acta.* 359, 245.

US Environmental Protection Agency, Integrated Pisk Information System (IRS) on Antymony, National Centem for Environmental Assessement, Office of Research and Developments, Washington, DC, 1999.

Vasudevan S, Mohan S, Sozhan G, Raghavendran NS, Murugan CV. 2006. Studies of oxidation of As(III) to As(V) by in-situ-generated hypochlorite. *Ind Eng Chem Res.* 45: 7729–32.

Vörösmarty CJ et al. 1997. The Storage and Aging of Continental Runoff in Large Reservoir Systems of the World. *Ambio.* Vol.26 n°4, pp. 210 – 219.

Waentig LN, Jakubowski and PH Roos. 2011. Multi-parametric analysis of cytochrome P450 expression in rat liver microsomes by LA-ICP-MS. *J. Anal. At. Spectrom.* 26, 310.

Wang TS, Shu YF, Liu YC, Jan KY, Huang H. 1997. Glutathione peroxidase and catalase modulate the genotoxicity of arsenic. *Toxicology.* 121:229–37.

Warnken KW, Davison W, Zhang H. 2008. Interpretation of in situ speciation measurements of inorganic and organically complexed trace metals in freshwater by DGT. *Environ. Sci. Technol.* 42, 6903-6909.

Weissberg BG, Browne PRL, Seward TM. 1979. Ore metals in active geothermal systems. In: Barnes, H.L. (Ed.), Geochemistry of Hydrothermal Ore Deposits. Wiley, New York, pp. 738 – 780.

Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J, Meharg AA. 2006. Increase in Rice Grain Arsenic for Regions of Bangladesh Irrigating Paddies with Elevated Arsenic in Groundwaters. *Environ Sci Technol.* 40:4903–4908.

Williams PN, Price AH, Raab A, Hossain SA, Feldmann J, Meharg AA. 2005. Variation in Arsenic Speciation and Concentration in Paddy Rice Related to Dietary Exposure. *Environ Sci Technol.* 39:5531–5540.

Williams PN, Raab A, Feldmann J, Meharg AA. 2007. Market basket survey shows elevated levels of As in South Central US processed rice compared to California: consequences for human dietary exposure. *Environmental Science Technology.* 41: 2178–2183.

World Business Council for Sustainable Development. 2005. Facts and Trends. Water. Version 2.

World Health Organization, Antimony in Drinking-Water, WHO/ SDE/WSH/03.04/74, Geneva, 2003.

Yngard RA, Sharma VK, Philips J, Zboril R. 2008. Ferrate(VI) oxidation of weak acid dissociable cyanides. *Environ Sci Technol.* 42:3005–10.

Zangi R and M Filella. 2012. Transport routes of metalloids into and out of the cell: A review of the current knowledge. Chemico-Biological Interactions. 197, 47–57

Zavala YJ, Gerads R, Gürleyük H, Duxbury JM. 2008. Arsenic in Rice: II. Arsenic Speciation in USA Grain and Implications for Human Health. *Environ Sci Technol.* 42:3861–3866

Zhang H and W. Davison. 1995. Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution. *Anal. Chem.* 67, 3391.

Zhang H and W. Davison. 2000. Direct In Situ Measurements of Labile Inorganic and Organically Bound Metal Species in Synthetic Solutions and Natural Waters Using Diffusive Gradients in Thin Films. *Anal. Chem.* 72, pp 4447–445.

Zhang H, Davison W, Gadi R, Kobayashi T. 1998. In situ measurement of dissolved phosphorus in natural waters using DGT. *Anal. Chim. Acta* 370, 29-38.

Zhang H, Davison W, Mortimer RJG, Krom MD, Hayes PJ, Davies IM. 2002. Localised remobilization of metals in a marine sediment. *Sci. Total Environ.* 296: 175–187.

Zhang X, Cornelis R and L Mees. 1998. Speciation of antimony(III) and antimony(V) species by using high-performance liquid chromatography coupled to hydride generation atomic absorption spectrometry. *J. Anal. At. Spectrom.* 13, 205.

Zheng J, Iijima A and N. Furuta. 2001. Complexation effect of antimony compounds with citric acid and its application to the speciation of antimony(III) and antimony(V) using HPLC-ICP-MS. *J. Anal. At. Spectrom.* 16, 812.

Zheng J, Ohata M and N. Furuta. 2000. Antimony Speciation in Environmental Samples by Using High-Performance Liquid Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry. *Anal. Sci.* 16, 75.

Zhu YG, Sun GX, Lei M, Teng M, Liu YX, Chen NC, Wang LH, Carey AM, Deacon C, Raab A, Meharg AA, Williams PN. 2008. High Percentage Inorganic Arsenic Content of Mining Impacted and Nonimpacted Chinese Rice. *Environ Sci Technol.* 42:5008–5013.

PhD thesis project

Development of the research project

The aim of this thesis was in general to explore health risks in human food chain, from water to foodstuff. In particulary, we had investigated the distribution of Arsenic and its chemical forms in groundwater and rice. Millions of people are threatened by exposure to inorganic As from drinking water and another importa pathway of human As uptake is rice consumption. Moreover, the information on the content of different speciation forms of elements is not ample. Actually International and national laws classify the harmful elements based on the total content.

It is important to focus the attention on the importance of groundwater because it reprents the main sorce of drinking water and the knowledge of chemical composition is necessary for risk assessment and for appropriate choice of removal technology.

Chronic exposure to Arsenic can lead to cancerous health hazards and other public health problems. We had taken adventages of binding capacity of fehhydrite and chromatographic separation of HPLC to obtain a characterisation of inorganic and organic Arsenic species in groundwater. Our results has confirmed that DGT is able to maintain the Arsenic species without risk of changes for speciation and its application avoids an overestimation of risk for human health.

The physical and chemical qualities of Antimony (Sb) and Arsenic are similar. In the past, these two elements and their compounds were often determined together. It should be noted that this similarity in chemical properties has been assumed for many years without a rigorous study.

The biochemical significance and toxicological behavior of Sb coordination complexes is however unknown, as their detection in real samples has been prevented partly due to naturally low Sb levels and partly due to limitations of the analytical techniques applied in antimony speciation analysis. Sb speciation in water samples is of general interest for human health assessment. The comprehension of Sb behaviour in aqueous matrix is very important, as several studies demonstrated that bottle materials may contaminate drinking water. Thus, it is imperative that new advanced analytical techniques are developed to overcome the existing severe limitations.

In this paper, we report the first investigation of inorganic antimony behaviour, especially Sb(III), in water samples. Speciation analysis of Sb(III) and Sb(V) in aqueous samples was performed through DGT resin extraction and on-line isotope dilution concentration determination after a chromatographic separation by HPLC-ICP-MS.

The risk for human health doesn't come only from total concentration and species of elements present not only in the water, but also from in foodstuffs, such as rice. Generally rice contains significant amounts of inorganic As but little information about speciation of As(III) and As(V) in rice is currently available in the literature. In our study, we investigated different types of rice grains

from different areas of Northern Italy. Our data showed the influences of As(III) and DMA(V) on the As concentration in rice grain and the relevance of As(III) and As(V) speciation for assessing As toxicity to human health.

After having determined the concentration of the As species in rice, it is fundamental to investigate the element distribution in the different parts of rice grain obtained from different processes. LASER (Light Amplification by Stimulated Emission of Radiation)-ICP-MS emerges as a potential analytical tool for the estimation of As localisation in rice samples. The purpose of this study was to evaluate As distribution within the rice grains (exterior, medium, interior part) of different varieties (Gladio and Ronaldo) obtained from different processes (raw, brown and milled rice with or without parboiling technique) with direct determination of LA-ICP-MS. The distribution of As varied in the different parts of the grains. The relationship between As intensities and several parts of grain in rice revealed that As levels decreased from the external part towards the middle position, and then the intensity values appeared to be similar between medium and internal part in non parboiled products. Arsenic content was higher in non-parboiled rice grain than in parboiled rice.

Chapter II – Water: one, none, one hundred thousand uses… and then?

E. Capri, M.C. Fontanella

Acqua: uno, nessuno, centomila usi… e poi?

Title
Water: one, none, one
hundred thousand uses…
and then?

Key words
Groundwater, surface water,
quantity and chemical status,
virtual water, sustainability

Parole chiave
Quantità e qualità dell'acqua,
acqua di superficie, acqua di falda,
acqua virtuale, sostenibilità

Centro di Ricerca Opera;
Università Cattolica del Sacro Cuore,
Piacenza

Indirizzo per la corrispondenza:
Maria Chiara Fontanella, PhD Student
Istituto di Chimica Agraria e Ambientale
Università Cattolica del Sacro Cuore
via Emilia Parmense, 84
29122 Piacenza - Italy
E-mail: MariaChiara.Fontanella@unicatt.it

Summary
Water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such (Dir 2000/60/EC). Characteristics and uses of water depend on different activities, agricultural, industrial and civil, and country income. Water is an element always present in citizens life but water is also used to make every product on Earth, and so all business, and all sector, depend on it in some way, so daily custom of citizens can influence the consumption of water, precious water for physiological intake and domestic use but also for aquatic and terrestrial ecosystems. Sustainable measures must be developed and existing initiative must be improved. It is useful develop a planning of prevention and safeguard measures between citizens, water management service, administration and health authorities because the time of natural recharge of water is so long.

Riassunto
L'acqua non è un prodotto commerciale al pari di altri, bensì un patrimonio che va protetto, difeso e trattato come tale (DIR 2000/60/EC). Lo sfruttamento e le caratteristiche della risorsa acqua sono differenti a seconda del settore di utilizzo, agricolo, industriale e domestico, e dello sviluppo economico del paese. L'acqua è un elemento presente tal quale nella vita quotidiana dei cittadini, ma è anche un elemento essenziale per la realizzazione di tutti i prodotti, che quotidianamente vengono mangiati, indossati e utilizzati; in questo modo le abitudini quotidiane dei cittadini possono influenzare fortemente il consumo di risorse idriche preziose non solo per gli esseri umani e per le loro attività domestiche ed economiche ma per mantenere in funzione gli ecosistemi acquatici e terrestri ad esse associati. È quindi necessario elaborare misure di sostenibilità e migliorare quelle esistenti per tutelare l'acqua nella sua totalità tramite una programmazione condivisa e partecipata delle misure di prevenzione e salvaguardia tra i cittadini, i gestori del servizio idrico, le autorità amministrative, le autorità sanitarie, visti i tempi necessari per la formazione e il ricambio naturale delle acque.

1

Introduzione

Si tende a considerare l'acqua e i suoi molteplici usi attraverso comparti stagni: acqua destinata all'agricoltura, all'industria e al settore civile e anche all'interno di quest'ultimo si tende a privilegiare divisioni tra acqua destinata all'igiene personale ed uso domestico, da impiegare nella cucina e come fonte alimentare. L'acqua in realtà è un elemento unico, impiegato nelle attività sopra elencate grazie alle caratteristiche uniche che possiede l'acqua dolce, presente in modo fruibile sottoforma di fiumi, laghi, falde e sorgenti. Ma prima di tutto questo, l'acqua è essenziale per la vita di tutti gli ecosistemi. Gli ecosistemi acquatici sono richiesti da specie animali e vegetali come habitat. Essi forniscono beni e servizi, necessari per attività socio-economiche e possono giocare un ruolo nella prevenzione del rischio. Le attività antropogeniche possono mettere sotto pressione gli ecosistemi acquatici, alcune volte danneggiando altre volte distruggendo, insieme alla popolazione animale e vegetale, beni e servizi. Le pressioni antropogeniche sugli ecosistemi acquatici sono di diversi tipi: crescita del carico dei sedimenti, inquinamento, frammentazione del flusso (es. dighe), specie invasive, uso eccessivo. L'inquinamento potrebbe derivare da sorgenti puntuali (es. sversamenti accidentali) o da fonti diffuse (es. fertilizzanti agricoli, contaminazione dei suoli da sversamenti domestici e industriali). Certe infrastrutture o lavori possono avere effetti positivi, come il trattamento delle acque reflue o attività di riabilitazione della flora originale (1).

Misure di sostenibilità sono necessarie anche attraverso il miglioramento di quelle già presenti, per tutelare il comparto acqua nella sua totalità tramite una programmazione condivisa delle misure di prevenzione e salvaguardia, visti i tempi necessari per la formazione e il ricambio naturale delle acque.

La quantità dell'acqua

Nel mondo

La Terra, anche se soprannominata Pianeta Azzurro, per la prevalenza di volumi d'acqua rispetto alle terre emerse, ha meno del 3% dell'acqua disponibile dolce, di questa esigua percentuale oltre il 2,5% è sottoforma di ghiaccio in Artico ed Antartico non fruibile dall'uomo. L'umanità deve contare sullo 0,5% per tutte le necessità di acqua dolce sia umane che a livello di ecosistema (Fig. 1) (2).

Lo 0,5% di acqua dolce è stoccato nei seguenti comparti: $10*10^6$ km³ in acquiferi profondi, $119*10^3$ km³ netti nella pioggia, $91*10^3$ km³ nei laghi, $5*10^3$ km³ nei bacini artificiali e 2 120 km³ nei fiumi, costantemente riforniti da piogge, con neve e ghiaccio sciolto (3, 4).

In teoria le acque superficiali e sotterranee sono risorse naturali rinnovabili e l'uomo ha sempre sfruttato tale risorsa dando per scontato la sua perenne disponibilità. Fonti d'acqua sufficientemente ampie sono ancora disponibili a

Figura 1 - Acqua dolce disponibile (percentuale) a livello globale (1).
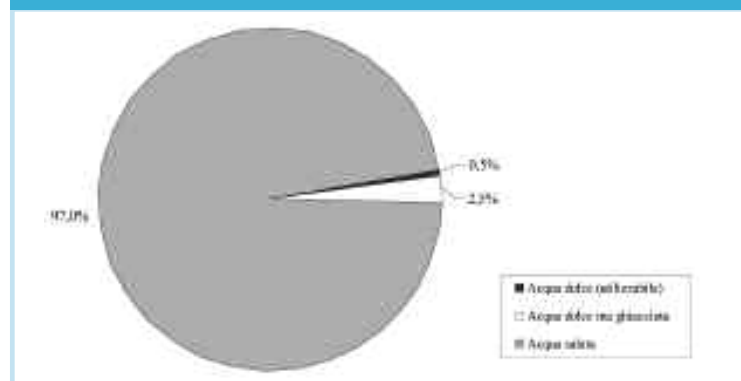
livello globale, ma a livello regionale i fabbisogni non coincidono con la reale disponibilità, cioè l'impiego di acqua è maggiore rispetto al quantitativo idoneo per mantenere un adeguato sostentamento della risorsa.

Nel 2002, in occasione della stesura del United Nations Environmental Programme è stato quantificato il livello di stress idrico per ogni stato, confrontando la situazione reale risalente all'anno 1995 e come essa evolverà nell'anno 2025, mostrando una non edificante evoluzione. Nel 2025, la maggior parte degli stati, che avevano nel 1995 una percentuale di prelievo di acqua rispetto alle reali risorse disponibili tra il 10 e il 20%, vedranno lievitare tale percentuale dal 20 al 40% (U.S.A, Francia, alcuni paesi dell'Africa come Mauritania e Sudan e buona parte dell'Asia sud - orientale), mentre alcuni stati il cui stress idrico era già critico vedranno aumentare la percentuale di prelievo oltre il 40% delle risorse disponibili come il Marocco, Algeria e Tunisia ma anche il Sud – Africa e l'India. (Fig. 2) (5).

I motivi, per cui viene sfruttata l'acqua dolce sono molto differenti: turismo, industria, agricoltura, allevamento, impiego urbano (igiene, alimentazione), fonte energetica, commercio. I più importanti usi, in termini di estrazione totale, possono essere identificati come fornitura di acqua pub-

Figura 2 - Stress idrico globale, ammontare di acqua prelevata rispetto alle risorse disponibili, anno 1995 - 2025. Blu = prelievo inferiore al 10% rispetto alla naturale disponibilità di acqua, Verde = prelievo tra il 10 e il 20%, Giallo = prelievo tra il 20 e il 40 % e Rosso = prelievo oltre il 40% (4)
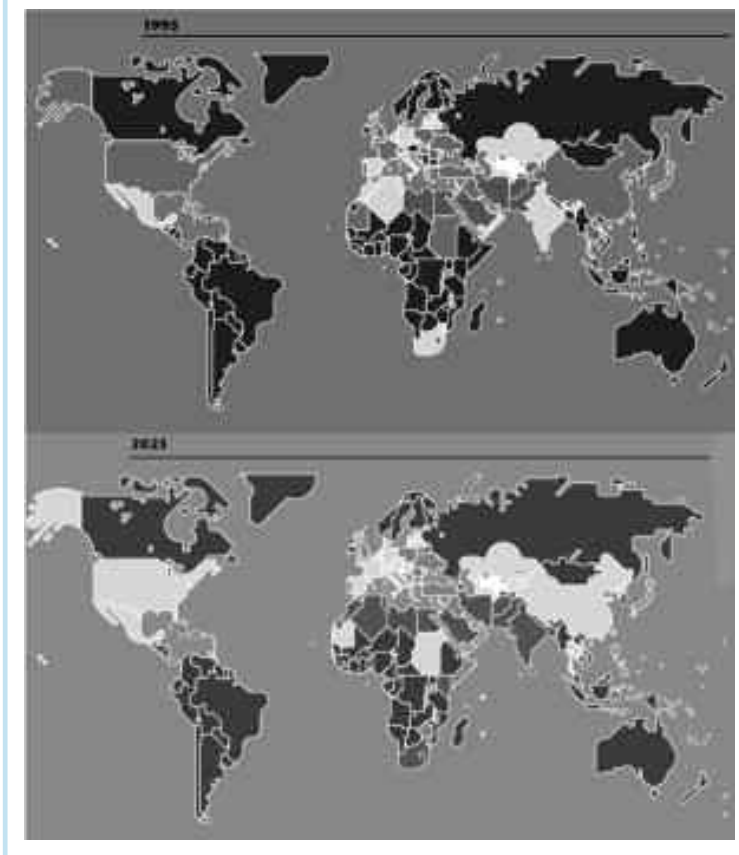
blica a scopo domestico, per l'agricoltura, industria ed energia. L'accesso a fonti di acqua dolce salubre è necessario per le persone, per le loro famiglie e per gli edifici pubblici, essa viene sfruttata per il consumo e scopi igienici, in rile-

vanti quantità e qualità. Lo sfruttamento della risorsa acqua avviene in modo differente dal settore di utilizzo e dallo sviluppo economico del paese. Infatti l'accesso ad una fonte di acqua salubre, oltre ad avere impatti sulla salute umana,

3

influenza fortemente la condizione sociale, costituendo così un fattore di disuguaglianza (1). Nei paesi a medio e basso reddito la percentuale maggiore dell'acqua viene impiegata a scopo agricolo, mentre nei paesi a reddito elevato l'impiego maggiore è nel settore industriale. Globalmente le percentuali di acqua impiegate possono essere così ripartire: 70% agricoltura, 22% industria e 8% per uso domestico (6). Si stima che tra 20 anni l'agricoltura sarà ancora il settore che richiederà il maggior quantitativo di acqua, mentre il prelievo di acqua per usi domestici subirà un incremento tale da superare quello per scopi industriali.

Più la popolazione diventa ricca, più gli standard di vita crescono e con essi cambia l'impiego di acqua a scopo domestico. In un paese industrializzato i consumi di acqua in ambiente domestico si suddividono nel seguente modo: 5% per pulire casa, 10% per cucinare e bere, 20% per fare il bucato, 35% per la pulizia personale, 30% come acqua di scarico (7).

In Europa

In Europa il 20,7% in media di tutta l'acqua è stato impiegato per la fornitura di acqua pubblica tra il 1997 e il 2005, il 23,6 %in media per l'agricoltura, 12,3% per l'industria e il 43,4% per la produzione di energia (8).

L'uso di acqua a scopo agricolo è cresciuto di circa il 6% nel Sud Europa, mentre nel resto dell'Europa è possibile osservare una riduzione di tale percentuale (92% per l'Est Europa e il 56% per l'Europa dell'Ovest). La riduzione è guidata principalmente dal declino di attività agricole in Bulgaria e Romania, nei rimanenti paesi dell'Europa dell'Est l'area totale irrigabile è diminuita di circa il 20%. In Bulgaria il cambiamento nella struttura dei campi dovuto all'instabilità dei prezzi dei prodotti agricoli e l'irregolarità nella fornitura di acqua ha contribuito all'abbandono dei sistemi di irrigazione (9). L'impiego di acqua per l'irrigazione è diminuito nell'Europa dell'Ovest (Nord e Centrale) del 56%. Questa decrescita è principalmente guidata da Danimarca, Germania, Paesi Bassi e Inghilterra, mentre è stato osservato che in Austria e in Belgio si sta realizzando un andamento contrario. La decrescita generale può essere attribuita parzialmente alla decrescita nell'area irrigabile (Germania, Paesi Bassi, Finlandia) e in parte a un più efficiente uso dell'acqua nei paesi dove l'area irrigabile sta attualmente crescendo (Danimarca, Svezia, Inghilterra). Invece l'uso di acqua è cresciuto di circa il 6% nel Sud Europa. In Turchia, l'uso di acqua per l'irrigazione è aumentato di un terzo rispetto ai livelli del 1990.

L'aumento della percentuale dell'acqua per l'irrigazione nel Sud Europa è solo un quinto dell'aumento di percentuale della terra irrigabile durante gli ultimi 17 anni, tutto ciò può essere attribuito alle tecnologie per il risparmio dell'acqua e ad un generale accrescimento nell'efficienza (10, 11, 12). Inoltre l'uso di acqua riciclata e la desalinizzazione si stanno diffondendo (soprattutto in Spagna) (13). Mentre la principale fonte per l'irrigazione è l'acqua di superficie, estrazione da acqua sotterranea dovrebbe essere aggiunta al quantitativo riportato in grafico per l'irrigazione nei paesi del Sud Europa (es. Italia) (13). Impiego di acqua per l'industria manifatturiera sostanzialmente decresce per tutta Europa: 10% di riduzione nei paesi dell'Europea dell'Ovest (centro e nord); 19% di riduzione nei paesi del Sud Europa e più del 79% di riduzione nei paesi dell'est. Questa decrescita generale può essere attribuita alla transizione verso nuove tipologie di industrie dotate di una tecnologia, che permette un uso più efficiente dell'acqua. Le informazioni provenienti da Inghilterra, Francia e Spagna permettono di sapere che il 30-40% delle industrie hanno migliorato le proprie tecnologie a tale riguardo (14). Inoltre l'aumento dell'impiego di acque grigie e il riutilizzo delle acque per l'industria può avere causato una riduzione

(15). Nel caso dell'estrazione di acqua per la fornitura pubblica due differenti andamenti sono osservati in Europa durante gli ultimi 10-15 anni: i paesi dell'Est e dell'Ovest Europa hanno avuto una riduzione generale, mentre nei paesi del Sud Europa l'uso domestico è aumentato del 15% in Turchia oltre il 53%. La decrescita è molta pronunciata in Inghilterra e in Germania, così come nei paesi dell'Est (Polonia, Bulgaria e Romania) e tutto ciò può essere attribuito alla promozione di pratiche per il risparmio idrico (10). In particolare nei paesi ad est, le nuove condizioni economiche hanno fatto scattare un aumento del prezzo da parte delle compagnie responsabili della fornitura di acqua. Questo ha portato ad un consumo minore di acqua, in ambito domestico ed industriale, che è connesso con la distribuzione pubblica (16). Comunque la rete di distribuzione in questi paesi è obsoleta e le perdite di acqua nei sistemi di distribuzione richiede grandi estrazioni di acqua per mantenere la fornitura (17). Nei paesi più a Sud, l'osservato aumento nella fornitura pubblica di acqua potrebbe essere attribuito al cambiamento climatico e al turismo. L'aumento della temperatura (osservato nell'area Mediterranea) ha mostrato un aumento della domanda di acqua ad uso domestico per l'igiene personale e per usi esterni (giardinag-

gio, piscine) (18-21). In Francia, Grecia, Italia, Portogallo e Spagna il turismo è aumentato del 90% nelle ultime due decadi (11, 22).

In Italia

In Italia, il maggior quantitativo di acqua viene usato per scopo agricolo, seguito da quello industriale e domestico, in linea con l'andamento globale di sfruttamento della risorsa (Fig. 3) (23).
In Emilia Romagna, settima regione più popolata d'Italia (24), gli approvvigionamenti da acqua di falda risultano preminenti rispetto a quelli da acque superficiali, costituendo quasi il 60% dei prelievi complessivi, con una notevole diversificazione a livello provinciale e per settore. I prelievi da falda so-

no considerevoli per il comparto irriguo nelle città di Piacenza, Parma e Reggio Emilia. L'impiego di acque superficiali risulta significativo solo nelle province di Ferrara e Ravenna. Si evidenzia come le cinque province centro occidentali, da Piacenza a Bologna, il ricorso ad acque di falda avvenga mediamente per il 45% delle necessità complessive, mentre per le 4 province più orientali tale percentuale scende notevolmente (Fig. 4) (25). Nella città di Parma, sede dell'incontro "*Acqua e vita: sicurezza, disponibilità e salute*" durante l'annuale settimana dedicata alla prevenzione dell'obesità e per un corretto stile di vita, il settore civile è coperto completamente con la fornitura di acque sotterranee e anche la quasi totalità del settore industriale (Fig. 5).
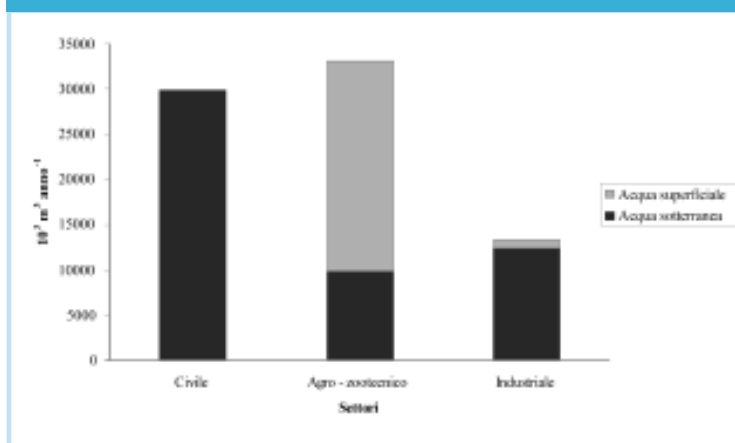
Figura 3 - Prelievi di acqua dolce (10^9 m^3 anno^{-1}) suddivisi per il settore civile, agricolo, industriale in Italia (anno 2000) (23)
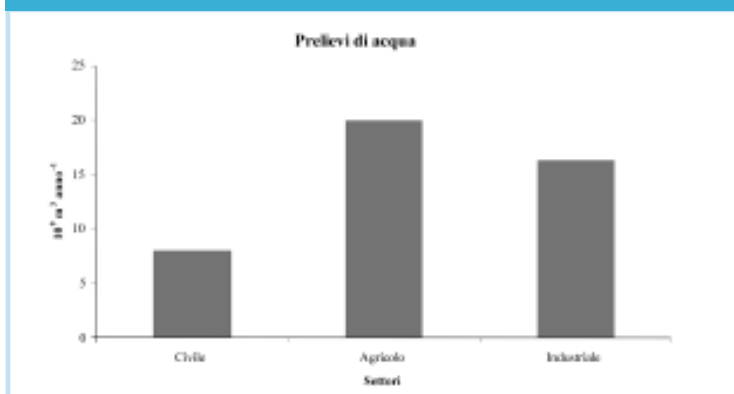
Figura 4 - Prelievi di acqua superficiale e sotterranea (Mm³ anno⁻¹) nella Regione Emilia Romagna per il settore civile, agro zootecnico e industriale
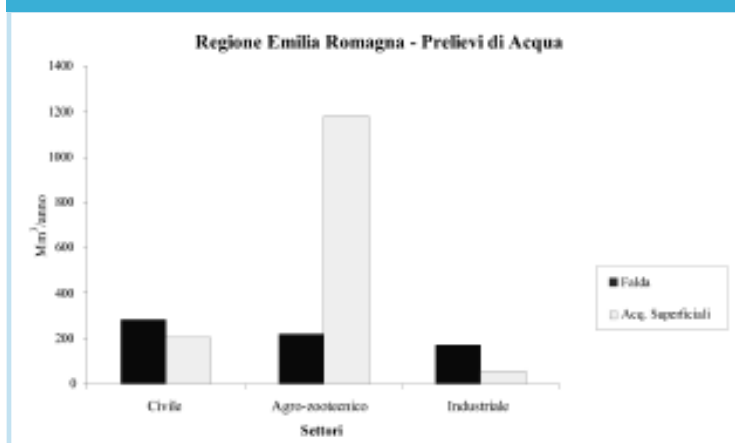
Figura 5 - Prelievi di Acqua superficiale e sotterranea (10³ m³ anno⁻¹) nella città di Parma

Acqua Virtuale

L'acqua è un elemento presente tal quale nella vita quotidiana dei cittadini, ma è un elemento essenziale per la realizzazione di tutti i prodotti, che quotidianamente vengono mangiati, indossati e utilizzati. Tale concetto può essere riassunto attraverso il termine *"Virtual Water"* o *"Acqua Virtuale"*, cioè tutta quell'acqua che non solo è contenuta fisicamente nel prodotto ma che è stata impiegata in ogni fase della sua produzione. L'Acqua Virtuale viene per questo suddivisa in Acqua Virtuale Verde, Blu e Grigia (26).

L'Acqua Virtuale Verde (Green Virtual Water) rappresenta il quantitativo di acqua piovana evaporata durante il ciclo produttivo delle colture, includendo anche la traspirazione delle piante e altre forme di evaporazione.

L'Acqua Virtuale Blu rappresenta il quantitativo di acqua superficiale o sotterranea evaporata durante il processo produttivo. Per i prodotti agricoli si prende in considerazione l'acqua di irrigazione evaporata dal terreno, dai canali di irrigazione e dalle riserve artificiali. Per i prodotti industriali e domestici si considera il quantitativo di acqua che non viene reintrodotta nel sistema idrico di provenienza.

L'Acqua Virtuale Grigia rappresenta il volume d'acqua necessario per diluire gli agenti inquinanti immessi nel sistema idrico durante il processo produttivo.

Da queste definizioni è facile dedurre che i prodotti di allevamento (carne, latte, uova e derivati) posseggono un contenuto di acqua virtuale maggiore rispetto ai prodotti coltivati. Lo stesso prodotto può presentare un valore di acqua diverso a seconda del luogo di

provenienza a causa delle condizioni climatiche, dalle tecniche agricole e dalla resa dei raccolti. Per esempio una colazione composta da una tazza di caffè (125 ml) con latte (200 ml), una fetta i pane (30 g) e un'arancia (100 g) porta ad un consumo di acqua virtuale pari a 140 + 200 + 40 + 50 = 420 litri di acqua virtuale (27).

Il concetto dell'acqua virtuale focalizza l'attenzione su due concetti fondamentali: la realizzazione di ogni prodotto, oltre a quello alimentare, necessita di acqua per prendere forma, in quanto l'impiego di acqua è fondamentale per lo sviluppo di ogni settore e prodotto; il consumo di acqua virtuale dipende fortemente dalle decisioni della singola persona. Le abitudini alimentari possono influenzare fortemente il consumo di risorse idriche, per una dieta principalmente vegetariana il consumo d'acqua giornaliero varia da 1500 a 2600 l invece nel caso di una dieta ricca di carne il consumo varia da 4 000 a 5 400 l.

La qualità dell'acqua

Qualsiasi valutazione della disponibilità, quindi della sostenibilità, dell'uso dell'acqua deve prendere in considerazione non solo, quanta acqua è disponibile e come viene impiegata ma anche la sua qualità. Infatti una qualità alterata renderà inferiore la disponibilità apparente dell'acqua. Un determinato grado di qualità dell'acqua viene richiesto per i diversi usi (acqua potabile – DL 31/2001) (28). Inoltre una qualità minima è richiesta per mantenere in funzione l'ecosistema acquatico e quello terrestre ad esso associato, distinguendo anche le diverse origini dell'acqua (acque sotterranee – DL 30/2009) (29).

Per garantire una buona qualità all'acqua potabile è preferibile un approccio preventivo e collaborativo, per assicurare che i responsabili di diversi settori all'interno del ciclo dell'acqua possano essere coinvolti nella gestione della qualità. La consultazione con le autorità saranno sempre più necessarie, i consumatori stessi spesso giocano un ruolo importante nel prelievo, nello stoccaggio e nel trattamento dell'acqua. Le loro azioni possono aiutare ad assicurare salubrità all'acqua, che essi consumano, ma possono anche contribuire a contaminare l'acqua consumata da altri. I consumatori devono assicurarsi che le loro azioni non abbiano un impatto negativo sulla qualità delle acque. La direttiva 2000/60/EC (30) sottoscrive la necessità di tutela estesa da parte delle autorità comunitarie, passando alle autorità nazionali e infine locali su tutti i diversi tipi di acqua (superficiale e sotterranea), riconosce nei cittadini, soggetti ideali da coinvolgere per giungere al pie-

no ottenimento degli obiettivi posti, individuando anche bisogni, istanze e proposte degli utenti finali come prioritarie.

Le acque sotterranee, fonte principale locale di approvvigionamento a scopo urbano, sono minacciate ed inquinate in vari modi. Ci possono essere molti elementi chimici presenti nell'acqua, ma solo alcuni possono influire sulla salute. Nitrati e pesticidi sono causa di importanti problemi, in quanto potenzialmente pericolosi per salute umana. Per esempio, il nitrato si trova normalmente nell'ambiente ed è un importante nutriente per la pianta ma può raggiungere acque superficiali e sotterranee a causa delle attività agricole, dello smaltimento delle acque di scarico e dell'ossidazione di prodotti di scarto di origine animale e umana e per questo può cambiare velocemente la sua concentrazione. Negli esseri umani, circa il 25% del nitrato ingerito entra in circolo nella saliva, e il 20% di esso viene convertito in nitrito dall'azione dei batteri della bocca. In una persona adulta, si può verificare la sintesi endogena di nitrato, la quale può arrivare a 62 mg di ione nitrato per giorno nelle urine. La metemoglobinemia è una conseguenza della reazione tra nitrito ed emoglobina nei globuli rossi del sangue, che causa la formazione di metemoglobina, la quale lega saldamente l'ossigeno, impedendo il

rilascio e causando un blocco nel trasporto dell'ossigeno. Alti livelli di metemoglobinemia (> del 10%) possono causare la cianosi riportata anche come sindrome del bambino blu. In studi epidemiologici sulla metemoglobinemia, il 97% dei casi si verificano a concentrazioni maggiori di 44,3 mg/L con sintomi clinici associati ad alte concentrazioni (gli individui affetti avevano meno di 3 mesi di età). Il rischio della metemoglobinemia aumenta in presenza di simultanee infezioni intestinali (31).

Non ci sono evidenze significative tra l'assunzione di nitrato attraverso l'acqua potabile e il rischio di formazione delle cancro nel tratto gastrico. I mezzi più appropriati per controllare la concentrazione di nitrati, soprattutto nelle acque sotterranee, derivano dal controllo delle contaminazioni (32).

Il cromo è un elemento ampiamente distribuito nella crosta terrestre. Il cibo sembra essere la sua maggior fonte di introduzione nell'organismo. Ma solo il cromo VI è stato classificato come elemento carcinogenico (31).

Il fluoro è anch'esso un elemento normalmente presente nella crosta terrestre. È riconosciuto l'effetto benefico del fluoro contro le carie dentali, ma un elevata introduzione di fluoruro può provocare effetti sui tessuti scheletrici (ossa e denti). La fluorosi dello scheletro (cambiamento e frattura delle ossa) può essere osservata quando l'acqua potabile contiene 3-6 mg per litro, con alte ingestioni di acqua (31).

Il cadmio viene rilasciato nell'ambiente tramite acque di scarico e da fonti diffuse ed è dovuto all'uso dei fertilizzanti e all'inquinamento dell'aria, anche se la principale fonte di esposizione rimane l'alimentazione. Il cadmio viene accumulato principalmente nei reni e ha un periodo lungo di persistenza ma non dimostra segni di carcinogenicità (31).

L'arsenico è normalmente presente nell'acqua a concentrazioni inferiori a 1-2 μgr L^{-1}, la concentrazione può aumentare nelle acque sotterranee, dove sono presenti depositi sedimentari di origine vulcanica. L'As non è stato dimostrato essere un elemento essenziale negli esseri umani. Segni di arsenicalismo cronico, includendo lesioni dermali, neuropatie, cancro alla pelle, alla vescica e ai polmoni sono state osservate in popolazioni soggette all'ingestione quotidiana di acqua potabile contaminata (31, 33).

La disinfezione è di importanza inquestionabile nella fornitura di acqua potabile sicura. La distruzione dei patogeni di tipo microbiologico è essenziale ed è molto comune impiegare l'uso di agenti chimici come il cloro. La disinfezione è effettivamente una barriera verso molti patogeni (soprattutto batteri) nel trattamento dell'acqua potabile e deve essere usata per l'acqua di superficie e per quella sotterranea, soggetta a contaminazione fecale. L'uso di disinfettanti chimici nel trattamento dell'acqua causa la formazione di prodotti secondari. Comunque, il rischio derivante da questi prodotti secondari è nettamente più basso in confronto con i rischi associati ad un disinfezione inadeguata. I trialometani (bromoformio, bromodiclorometano, dibromoclorometano, cloroformio) si formano come risultano della clorazione della sostanza organica presente naturalmente nelle acque di origine. L'andamento e il grado di formazione dei trialometani crescono in funzione del cloro, della concentrazione di acidi umici, temperatura, pH e della concentrazione dello ione bromuro. Il cloroformio è il più comune trialometano nelle acque potabili clorate. La maggior parte dei trialometani vengono trasferiti all'aria come risultato della loro volatilità.

Il cloroformio e il bromodiclorometano vengono classificati come possibili carcinogenici per gli esseri umani, mentre il bromoformio e il dibromoclorometano sono classificati come non carcinogenici per l'uomo.

La quantità quanto la qualità vanno di pari passo per assicurare caratteristiche ottimali dell'acqua, poiché il rischio di trasferimento diretto di malattie da persona a persona o da alimenti contaminati è più elevato quando dalla penuria

di acqua deriva da prassi igieniche insufficienti.

Sostenibilità

Sostenibilità è diventato chiaramente un termine popolare in politica ambientale e nel mondo della ricerca come si può intuire dalla familiarità delle espressioni "Sviluppo sostenibile", "Sostenibilità Ecologica". In anni recenti, l'attenzione della comunità politica e scientifica è stata focalizzata sul concetto di sostenibilità globale (34). World Resources Institute (35) considera lo sviluppo sostenibile come "una strategia di sviluppo nella quale vengono gestiti tutti i beni – risorse naturali ed umane, così come i beni fisici e finanziari". Sostenibilità è un concetto nascente che ha stimolato un importante gruppo di lavoro e di riflessione su varie tematiche come lo sviluppo economico, produzione agricola, equità sociale e la biodiversità. Sostenibilità è il termine che lega sviluppo e ambiente, originariamente esso si applicava in contesti forestali, di prelievo di acqua sotterranea o di pesca, includendo solo il concetto di quantità. Anche nel rispetto di un concetto quantitativo di prelievo o sfruttamento, non è detto che un sistema possa essere definito sostenibile. Lo sviluppo sostenibile richiede sostenibilità sociale così come sostenibilità economica ed ambientale. I fattori chiave che governano una tale prospettiva di sviluppo sono la povertà, l'inquinamento, la partecipazione, la politica, il mercato (good governance) insieme alla prevenzione e alla gestione dei disastri. Uno degli aspetti fondamentali elencati precedentemente, che influenza la sostenibilità, è la partecipazione. La partecipazione è un processo attraverso il quale gli stakeholders possono influenzare e suddividere le responsabilità tra lo sviluppo di iniziative e le risorse usate attraverso un impegno attivo durante la presa di decisioni. Tra gli stakeholders vengono inclusi anche i cittadini che beneficiano dello sviluppo, compreso l'apparto governativo, il settore privato e la società civile (incluse le università e gli istituti di ricerca, unione dei lavoratori, organizzazioni religiose, parti politiche, i media, le fondazioni, servizi sociali e le organizzazioni non governative) a livello locale, distrettuale e nazionale.

Gleick et al. (36) approfondisce la definizione dell'uso sostenibile dell'acqua: l'uso dell'acqua, che sostiene l'abilità della società umana nel tollerare e nel fiorire nell'indefinito futuro, non deve pregiudicare l'integrità del ciclo idrogeologico o dei sistemi ecologici che dipendono da esso. Un uso sostenibile delle risorse idriche significa soddisfare il fabbisogno attuale, senza compromettere la capacità per le future generazioni di soddisfare il proprio. Gleick et al. (36) fornisce criteri espliciti e obiettivi per la sostenibilità delle risorse di acqua dolce (Tab. 1) (36).

Questi criteri sono alla base di una "visione" alternativa per la gestione dell'acqua futura e sono in grado di offrire diversi consigli per azioni legislative e non governative nel futuro (36).

Attualmente sono stati compiuti diversi passi per perseguire l'obiettivo di uso sostenibile delle risorse idriche, in ambito europeo, nazionale, distrettuale, regionale e locale. La sostenibilità oggi può essere identificata nelle seguenti azioni:

- il riconoscimento a livello europeo dell'acqua come patrimonio;
- obiettivo di qualità dei corpi idrici (Buono stato nel 2015 stabilito dalla direttiva 2000/60/EC) (30);
- distinzione delle caratteristiche qualitative delle acque a seconda della loro destinazione (acque potabili, acque reflue) ma anche come valore intrinseco del corpo idrico in tutela dell'ecosistema globale;
- controlli efficaci sulla qualità e quantità dell'acqua prelevata dalle diverse autorità (ARPA, Gestore del Servizio Idrico, AUSL);
- comunicazione del rischio tra le autorità responsabili della salute del cittadino;
- iniziale applicazione di tecnologie efficaci per il risparmio idrico in ambito domestico, agricolo, industriale;

Tabella 1 - Criteri di sostenibilità per le risorse di acqua dolce (36)	
Criterio 1	I fabbisogni di acqua dovranno essere garantiti per tutti gli esseri umani per preservare la salute umana.
Criterio 2	Le esigenze di acqua dovranno essere garantite per ripristinare e assicurare la salute degli ecosistemi. La gestione dovrà seguire un modello adattabile, dove le decisioni dovranno essere revisionate frequentemente sulla base delle ultime informazioni.
Criterio 3	Gli standard minimi di qualità dell'acqua devono essere cautelati, anche se essi dipendono dal luogo e da come l'acqua viene impiegata.
Criterio 4	Le azioni umane (prelievi eccessivi, contaminazione degli acquiferi) non dovranno danneggiare la capacità di rinnovamento a lungo termine delle riserve di acqua.
Criterio 5	I dati sulla disponibilità, l'uso e la qualità dovranno essere raccolti e resi disponibili a tutte le parti coinvolte; meccanismi istituzionali dovranno essere avviati per prevenire e risolvere i conflitti sull'acqua.
Criterio 6	La pianificazione e le prese di posizione dovranno essere democratiche, assicurando la rappresentanza di tutte le parti coinvolte.

- applicazione di tecnologie di potabilizzazione delle acque sotterranee (clorazione e denitrificazione).

Grazie allo spirito innovatore introdotto in Europa dalla direttiva 2000/60/EC (30), la gestione dell'acqua può e deve migliorare attraverso:

- riduzione degli inquinanti di origine agricola, tramite l'applicazione a livello nazionale e locale della direttiva 2009/128/EC sull'utilizzo sostenibile dei pesticidi;
- riduzione della pressione quantitativa sulle acque sotterranee con un parallelo miglioramento della qualità delle acque superficiali, nel rispetto del deflusso minimo vitale (decreto 28 luglio 2004), e dei bacini artificiali;
- applicazione di nuove tecniche, di origine biologica, per la potabilizzazione o immobilizzazione dei prodotti chimici;
- applicazione sempre più mirata di tecnologie avanzate per il risparmio idrico in ambito domestico, agricolo, industriale;
- utilizzo di acque grigie, le quali possono essere impiegate direttamente come fonte di irrigazione;
- tavolo di lavoro permanente con le autorità responsabili della salute del cittadino e della qualità degli ecosistemi con agricoltori, industriali e università;
- informazione, coinvolgimento e sensibilizzazione dei cittadini della gestione dell'acqua.

Bibliografia

1. Schenk C, Roquier B, Soutter M, et al. A System Model for Water Management. Environmental Management 2009; 43: 458-69.
2. World Business Council for Sustainable Development. Facts and Trends. Water. Version 2, 2005.
3. Vörösmarty CJ, et al. The Storage and Aging of Continental Runoff in Large Reservoir Systems of the World. Ambio 1997; 26 (4): 210-9.
4. Foster SSD, Chilton PJ. Groundwater: the processes and global significance of aquifer degradation. Phil Trans R Soc Lond B 2003; 358: 1957-72.
5. United Nations Environment Programme. "Fresh Water Stress" graph in the series of "Virtual Water Graphics", 2002.
6. UNESCO. "Water for People, Water for Life", United Nations World Water Development Report, 2003.
7. Environmental Canada. "Wise Water Use", 2003. http://www.ec.gc.ca/eau-water/default.asp?lang=En&n=CC09EDA0-1
8. EEA – ETC/WTR based on data from Eurostat data table: Annual water abstraction by source and by sector, 2009.

9. Penov I. The use of irrigation water during transition in Bulgaria's Plovdiv region. CEESA Discussion Paper No 7/2002 UNEP: United Nations Environment Programme, 2002.
10. Dworak T, Berglund M, Laaser C, et al. EU Water Saving Potential, 2007. http://ec.europa.eu/environment/water/quantity/pdf/water_saving_1.pdf
11. Attané I, Courbage J. La démographie en Méditerranée. Situation et projections. Les Fascicules du Plan Bleu, 11. Paris, Economica, 2001: 249.
12. Massarutto A. Water pricing, the Common Agricultural policy and irrigation water use, draft report, Udine, Italy, 2001.
13. OECD. Environmental Performance of Agriculture in OECD countries since 1990, Paris, France, 2008.
14. Institut Català d'Energia-ICAEN. Gestió de l'aigua a la Indústria. Estalvi i Depuració, 1999.
15. UNEP
16. Dalmas L, Reynaud A. Residential Water Demand in the Slovak Republic, LERNA CEA-INRA-UT1, 2003.
17. EEA. Environmental issue report No 19, Sustainable water use in Europe Part 2: Demand management, EEA, Copenhagen, 2001.
18. Cohen S. Projected increases in municipal water use in the Great Lakes due to CO2- induced climatic change. Water Resources Bulletin 1987; 23 (1): 91-101.
19. Downing TE, Butterfield RE, Edmonds B, et al. Climate Change and the Demand for Water, Research Report, Stockholm Environment Institute Oxford Office, Oxford, 2003.
20. Herrington P. Climate change and the demand for water. HMSO, London, 1996.
21. Kenneth DF. Water Resources and Climate Change. Resources for the future Climate Issues Brief No. 3, 1997.
22. De Stefano L. Freshwater and Tourism in the Mediterranean. WWF Mediterranean Programme, 2004.
23. FAO. Aquastat. 2003. http://www.fao.org/nr/water/aquastat/main/index.stm
24. ISTAT, http://demo.istat.it/pop2010/index.html
25. Regione Emilia Romagna. Piano di Tutela delle Acque, 2005: 371
26. Allan JA. Virtual water: A strategic resource global solutions to regional deficits. Ground Water 1998; 36 (4): 545-6.
27. Hoekstra AY, A K. Globalisation of Water: sharing the planet's freshwater resources. Wiley - Blackwell, 2008: 232.
28. Decreto Legislativo 2 febbraio 2001, n.31. Attuazione della direttiva 98/83/CE relative alla qualità delle acque destinate al consumo umano.
29. Decreto Legislativo 16 marzo 2009, n. 30. Attuazione della direttiva 2006/118/CE, relativa alla protezione delle acque sotterranee dall'inquinamento e dal deterioramento.
30. Direttiva 2000/60/CE del 23 ottobre 2000. Direttiva del Parlamento europeo e del Consiglio che istituisce un quadro per l'azione comunitaria in materia di acque.
31. WHO. Guidelines for Drinking – water Quality. Third Edition, 2008.
32. Schmoll O, Howard G, Chilton J, et al. Protecting Groundwater for Health. Managing the Quality of Drinking Water Sources. World Health Organization, IWA Publishing, London, UK, 2006: 199-270.
33. IPCS. Arsenic and arsenic compounds. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 224), 2001.
34. Clark W. Unpublished notes and documents on the Sustainable Development of the biosphere Project. International Institute for Applied Systems Analysis, Laxenburg, Austria, 1986.
35. Repetto R (ed). The global possible. Yale University Press, New Haven, Connecticut, 1985.
36. Gleick P, Loh P, Gomez S, et al. California water 2020: a sustainable vision. Pacific Institute Report, Pacific Institute for Studies in Development, Environment, and Security. Oakland, California, USA, 1995.

11

Chapter III. Determination of Arsenic Species in Diffusive Gradients in Thin Films (DGT) device.

ABSTRACT

The diffusive gradients in thin films (DGT) technique, utilizing a ferrihydrite adsorbent, has been investigated for the accumulation of different species of Arsenic, like Inorganic Species (arsenite and arsenate) and Organic Arsenic (dimethylarsinic and monomethylarsenate). The results, obtained by application of HPLC-ICP-MS, has confirmed that DGT is a reliable method for pre-concentration of Total Dissolved As and it is able to maintain the Arsenic species. It has been shown that MMA(V) and, under some conditions, DMA(V) can accumulate on the ferrihydrite adsorbent and will therefore contribute to the total As measured by DGT sampling. To evaluate the performance of DGT method for accumulation of arsenic species, DGT devices were carried out on groundwater collected in six different towns where the As concentration in groundwater is very high. The total arsenic concentration was represented by inorganic forms. Further investigations are necessary to study probable interactions of organic As species to colloids and seasonal variations of groundwater conditions.

Key words: Ferrihydrite, Arsenate, Arsenite, Monomethylarsonic Acid, Dimethylarsinic Acid, Diffusion coefficients, Groundwater.

INTRODUCTION

Arsenic (As) is a ubiquitous element found in the atmosphere, soils and rocks, natural waters and organisms. It is mobilised through a combination of natural processes such as weathering reactions, biological activity and volcanic emissions as well as through a range of anthropogenic activities. Most environmental As problems are the result of mobilisation under natural conditions.
Of the various sources of As in the environment, drinking water probably poses the greatest threat to human health. A big percentage of tap and drinking water comes from groundwater because the surface water is often polluted by agricultural and industrial substances. In Europe, particularly in Italy, during the last years the presence of arsenic in groundwater was reported from many provinces in the North of Italy.
Arsenic is human carcinogen and it exists under different chemical species in groundwater.

The WHO guideline value for As in drinking water was provisionally reduced in 1993 from 50 to 10 μg L^{-1}. The new recommended value was based on the increasing awareness of the toxicity of As, particularly its carcinogenicity, and on the ability to measure it quantitatively (WHO, 1993).

Inorganic arsenite and arsenate are predominant, but low amounts of organic species may also be found, like monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)). The toxicity of As in the environment depends upon speciation. The general order of toxicity for As is given as: organoarsenicals (e.g. methylated species) < arsenates (As(V)) < arsenites (As(III)) (Yamauchi and Fowler 1994). Another important characteristic is that As(V) species are less mobile than As(III) species in many environmental systems (Bhattacharya et al. 2002).

It is useful to measure different types of species because the efficiency of As removal from drinking water depends on its speciation. The knowledge of As speciation is necessary for risk assessment and for appropriate choice of arsenic removal technology.

As species are very unstable in natural water; their distribution depends on the redox condition, pH, DOC and the presence of precipitating elements (Smedley and Kinniburgh 2002). As(III) (arsenite) is thermodynamically stable in anoxic environments and As(V) (arsenate) in oxygenated environments.

Redox potential (Eh) and pH are the most important factors controlling As speciation. Under oxidising conditions, $H_2AsO_4^-$ is dominant at low pH (less than about pH 6.9), whilst at higher pH, $HAsO_4^{2-}$ becomes dominant ($H_3AsO_4^0$ and AsO_4^{3-} may be present in extremely acidic and alkaline conditions respectively). Under reducing conditions at pH less than about pH 9.2, the uncharged arsenite species $H_3AsO_3^0$ will predominate (Brookins, 1988; Yan et al., 2000).

In the present study, two approaches are combined to study As speciation in raw groundwater: (a) Ferrihydrite (FH) Diffusive Gradients in Thin Films (DGT), (b) HPLC-ICP-MS, technique with excellent separation and high sensitivity.

As a result of the in situ sampling capabilities, DGT has the advantage of obtaining time-weighted average concentrations of analytes, eliminating the risk of speciation changes due to transportation and storage of samples prior to preparation and analysis (Lead et al. 2002), its preconcentration effect can lower effective detection limits for trace species and it is designed to accumulate labile species in environmental systems (Zhang and Davison 1995, Zhang and Davison 2000, Davison et al. 2000).

The use of DGT technique is a well established approach to the in-situ determination of dynamic trace element concentrations (Warnken et al. 2007). The DGT plastic sampling unit holds a binding layer covered by a diffusion gel and a membrane filter that is exposed to the target solution. Analytes diffuse through the diffusive layer (i.e. the diffusive gel and membrane filter) and

accumulate in the binding layer to an analyte specific adsorbent. After deployment the binding layer is extracted from the sampling unit and subjected to suitable analysis.

A DGT device with a layer of iron (Fe) oxide gel (impreganted with ferrihydrite) was developed for dissolved reactive phosphorous measurements (Zhang et al. 1998) and it has lately also been applied for the determination of labile oxyanions, including arsenic (Osterlund et al. 2010; Luo et al. 2010). Both arsenite and arsenate adsorb to the ferrihydrite binding layer, and methods to measure total inorganic arsenic (Panther et al. 2008 b) as well as the individual species have been developed (Panther et al. 2008 b).

Methylated arsenic species are formed by microorganisms (Cullen and Reimer, 1989), and therefore the speciation changes in response to biological activity, resulting in seasonal variation (Hasegawa et al. 2010; Hasegawa et al. 2009; Anderson and Bruland, 1991). Although it is known that organic arsenic species also adsorb to ferric oxides (Lafferty and Loeppert, 2005), the effects on DGT sampling have not yet been investigated.

This work aims to investigate the simultaneously adsorption and accumulation of the two inorganic arsenic species, As(III) and As(V), and organic species, DMA(V) and MMA(V), by ferrihydrite DGT. Furthermore, limitations for using such DGT devices to retrieve arsenic speciation information from natural water bodies are identified.

MATERIALS AND METHODS

Following the procedure of Huang for quantitative chemical extraction for As in food, like rice grain (Huang et al. 2010), it is possible to apply this technique for digestion FH DGT resin layer favoured the matrix free from interferences in the ICP-MS measurement.

Solutions

Stock solutions of arsenic species (1000 mg l^{-1} for As) were prepared by As_2O_3 (ArsenicIII Oxide) and $Na_2HAsO_4 \ast 7H_2O$ (sodium arsenate dibasic heptahydrate) and of DMA(V) and MMA(V) from dimethylarsinic acid (($CH_3)_2As(O)OH$) and disodium methylarsenate ($Na_2CH_3AsO_3$).

The standard solutions of arsenic species were used by diluting the corresponding stock solutions. Standard solutions of As were prepared by dilution of a multielement standard (100 mg L^{-1}) obtained from CPI International (Amsterdam, The Netherlands) for total concentration analysis.

All reagents were of analytical grade. Ultra-pure water was prepared by a Milli-Q system (18MΩ-cm resistance, Millipore® system, Millipore, Bedford, MA).

Instrumentation for Arsenic speciation ad total concentration analysis

The concentration of total As in resin layers is determined by Inductively Coupled Plasma Mass Spectrometer (Agilent 7700x, Agilent Technologies, USA) with Octopole Reaction System (ORS system). Speciation of As has been performed by HPLC on a anion exchange column with a mobile phase (0.64 mL min^{-1}). The mobile phase is made of 13.2 mM $NH_4H_2PO_4$ at pH 6.

Certified standard materials are used to ensure the accuracy and precision of analytical procedure (Certified Reference Material BCR 610 - Groundwater).

Figure 3.1 shows the mass chromatogram for the separation of As(III), DMA(V), MMA(V) and As(V) standards in aqueous solution.

ICP-MS may suffer from many isobaric interferences caused mainly by phenomena occurring either in the plasma or in the ion extraction device. For example, presence of chlorine in the sample may give rise to the formation of $^{40}Ar^{35}Cl^+$ that interferes strongly with the mono-isotopic $^{75}As^+$ (Gray, 1986; Hywel Evans and Giglio, 1993).

All measurements were performed under the operating conditions given in Table 3.1.

All experiments was carried out in plastic containers, cleaned with HNO_3 1% and rinsed with Milli-Q Water. All arsenic solutions, applied in tests, contained 0,01 mol L^{-1} di $NaNO_3$ at pH 5.
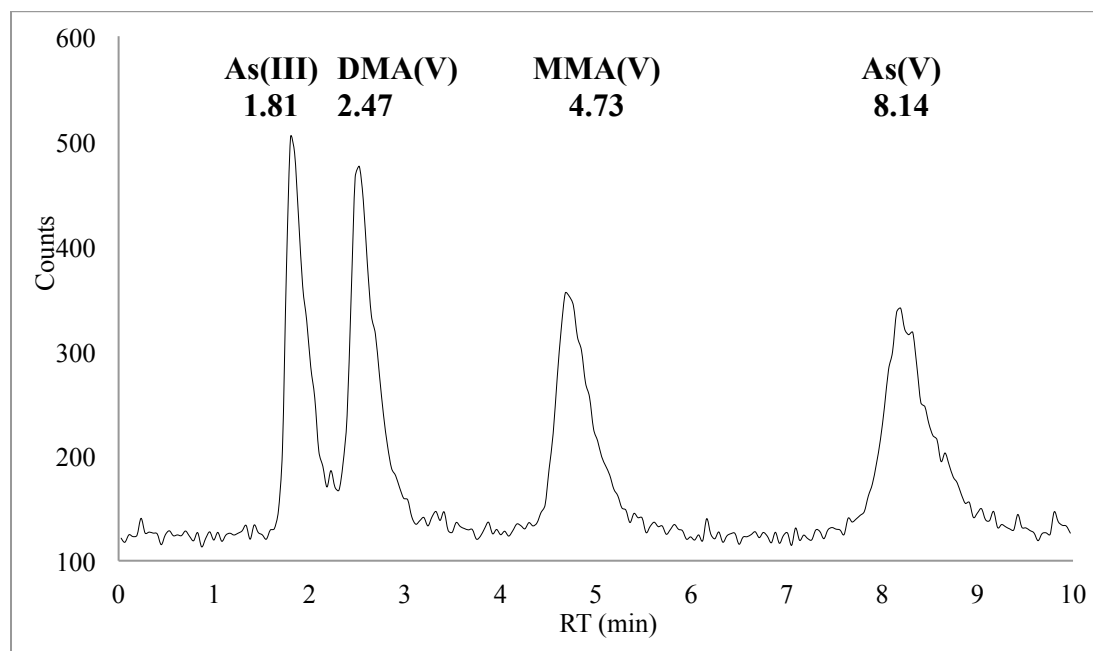
Fig. 3.1. HPLC-ICP-MS chromatogram of inorganic species (As(III) and As(V)) and organic species (DMA(V) and MMA(V)) of arsenic with different retention time (min). The concentrations of each species in resin are 2.78 – 2.81 – 2.90 – 3.19 ng cm^{-3}.

Table 3.1. Instrumental operating conditions of HPLC–ICP-MS

CHROMATOGRAPHIC CONDITIONS	
Column:	Agilent, As speciation column for Drinkihg Water (4.6 mm × 150 mm i.d)
Column material:	(polyetheretherketone) PEEK1
Eluent:	13.2 mM $NH_4H_2PO_4$
Flow rate:	1 mL min^{-1}
Injection volume:	10 μL
Column temperature	room temperature
Acquisition time	660 sec

ICP-MS CONDITIONS	
Rf power:	1550 W
RF Matching:	1.8 V
Carrier gas flow rate:	Ar 0.95 L min^{-1}
Dilution Mode:	ON
Dilution Gas:	Ar 0,25 L min^{-1}
Sampling depth:	8 mm
S/C Temp:	2 °C
Reaction mode:	OFF
Measured m/z:	As: 75, Cl: 35

Quadrupole bias:	-3 V

Diffusive Gradient in Thin Films (DGT) application

DGT units, with Fe-oxide resin gel, were exposed directly to solutions of inorganic and organic arsenic (pH 5, 0.01 M NaNO$_3$). After 24 h on the stirrer, DGT units were taken out of the solution and the surface was rinsed with MilliQ water. After that, the resin gel could be retrieved and the Fe-oxide gel placed in a clean sample tube. All experiments were carried out at 24.0 ± 0,5 °C.

After that, the Fe-oxide gels are placed in a plastic flask (digiTUBES 50 mL) with 15 mL of 0.28 M of HNO$_3$. They are mineralized at 95° C for 90 min in a heating block system (DIGIPREP, Scp Science, Quebec, Canada).

The digested DGT gel solutions were filtered by using 0.45 μm filter (digiFILTER) after appropriate dilution with Milli- Water.

Testing DGT performance in laboratory

The amount of As absorbed by the resin was analysed, and determined after deploying of two DGT units for 24h in a solution of inorganic arsenic (As(III) and As(V)). The time-averaged concentration of a specie in a solution, C, was then calculated using the DGT equation (1),

$$C_{DGT} = \frac{M * \Delta g}{t * A * D} \tag{1}$$

Δg = the thickness of the diffusive gel (cm),

t = the deployment time (s)

A = the surface area of the diffusive gel exposed to the bulk solution (cm^2)

D = the diffusion coefficient of analyte in the diffusive gel (cm^2 s^{-1}),

C_{DGT} was compared with the immersion solution concentration analysed in the samples taken during the experiment. There was a difference between the concentrations within 10%.

A solution of organic arsenic (DMA(V) and MMA(V)) was made for testing DGT performances, even if the diffusion coefficient of organic arsenic species was not known before the deployment of DGT devices for measurement of DMA(V) and MMA(V) diffusion coefficients.

Measurement of As Diffusion Coefficients using DGT devices

Diffusion coefficients of As(V) and As(III) were measured using DGT devices. Ten DGT devices were deployed in the As solution for times ranging from 3 to 24h. At each retrieval time, 2 DGT

devices were removed providing duplicate samples. The diffusion coefficients were calculated using Eq. (2).

$$D = \frac{slope * \Delta g}{C * A}$$ (2)

Where D is the diffusion coefficient, *slope* is the slope of a linear plot of measured mass of As diffused through the diffusive gel (and membrane filter) vs. time, *A* is the exposed area of diffusive gel/membrane filter, *Δg* is the combined thickness of the diffusive gel and membrane filter (i.e. 0.091 cm), and *C* is the concentration of As initially present in the source compartment of the diffusion cell.

At the start and end of the experiment and at each retrieval time, the concentration of As in the source compartment was measured to confirm that the As species concentration did not change significantly over the experimental time. This procedure was also made to measure and control changes in pH values (Fig. 3.2) and temperature (24°C ± 0.5).

Organic species and inorganic species were put together in As solution.
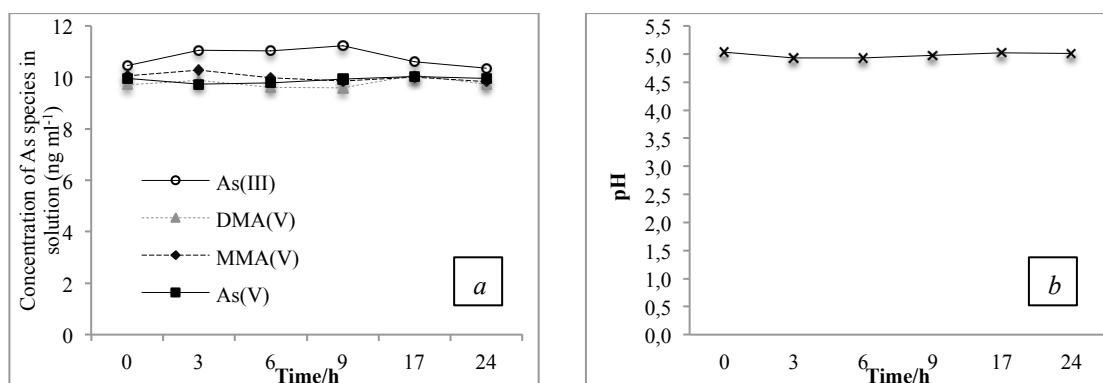
Fig. 3.2. Trend of As species concentrations during the experiment (*a*) and pH (*b*).

Kinetics of Binding and Elution Efficiency

Triplicate precipitated Fe – oxide gel discs were loaded with a known amount of As(III), As(V), DMA(V) and MMA(V) by shaking them for various times from 0.5 min to 24h in 10 mL of 50 μg L^{-1} of each arsenic species with a matrix of 0.01 M of NaNO$_3$. Elution efficiency of these species was obtained by eluting loaded Fe-oxide gel discs in HNO$_3$ 0.28 M at 95°C for 90 min in heating block system (DIGIPREP, Scp Science, Quebec, Canada) in plastic flasks (digiTUBES 50 mL). The elution and the immersion solutions were analysed by HPLC-ICP-MS with appropriate dilution.

Ionic strength and pH influence

To test the effects of ionic strength and pH, triplicate FH DGTs, were deployed for 24h in various well-stirred solutions at known constant temperatures.

To study the effect of ionic strength, a range of $NaNO_3$ concentrations, from 0 to 100 mM, was prepared in 2.5 L of solution (pH 5) containing each species at 50 μg L^{-1}.

For pH influence, 2.5 L containing 0.01 M of $NaNO_3$ were prepared at different pHs adjusted with HNO_3 and NaOH prior to spiking with As species stock solutions to 50 μg L^{-1}.

Field measurements

Sampling was conducted in six different towns in Cremona province (Fig. 3.3), where the As pollution of groundwater is a great problem. Groundwater samples were collected and stored in the freezer (- 20°C) until use. Water chemistry data of six points are presented in Table 3.2.

Tab. 3.2. Water chemistry of sampled water in Cremona province. Data obtained by environmental monitoring programs or by chemistry analysis of groundwater samples. NA: not available.

Parameters	CORTE DE FRATI	DOSIMO	GRONTARDO	OLMENETA	PESCAROLO	POZZAGLIO
pH	7.91 – 7.7	8.1	8.1 – 7.8	7.8- 7.6	7.8	7.8 – 7.86
conductivity µs/cm	540 - 349	470	513 - 348	424 - 328	460	321 - 469
alkalinity	NA	51	NA	NA	58	NA
NH^+_4 (ammonium ions)	1.24 – 0.86	1.57	1.68 – 1.18	0.86	2.5	1.4 – 1.06
NO^{-2} (nitrite ions) (mg L^{-1})	<0.02	NA	<0.01	<2	NA	<0,01
NO^{-3} (nitrate ions) (mg L^{-1})	2.9 - <1	NA	<1	<1	NA	<0.1
PO_3^{-4} (phosphate ions) (mg L^{-1})	0,538	0.63	0.37	0.59	1.3	0.3
SO_2^{-4} (sulfate ions) (mg L^{-1})	0.4 - <1	NA	2	<1	NA	0.4
Mg (mg L^{-1})	11.97	12.16	11.71	11.90	11.83	12.39
K (mg L^{-1})	0.515	0.542	0.995	0.589	1.161	0.911
Na (mg L^{-1})	13.93	14.18	17.55	11.63	13.74	14.47
P (mg L^{-1})	0.236	0.256	0.261	0.269	0.542	0.214
Ca (mg L^{-1})	80.54	77.88	81.40	71.61	88.25	69.33
As (µg L^{-1})	32.8	34.8	37.0	29.1	182.2	24.8
Se (µg L^{-1})	0.34	0.37	0.33	0.40	0.33	0.26
Pb (µg L^{-1})	<0.01	0.20	0.09	0.01	0.35	<0.01
Cd (µg L^{-1})	0.01	0.05	0.04	0.01	0.01	0.03
Cu (µg L^{-1})	1.6	2.1	2.1	4.6	1.0	0.9
Ni (µg L^{-1})	0.6	0.5	1.0	0.4	0.5	0.3
Be (µg L^{-1})	0.012	0.004	0.011	0.018	0.007	0.004
Cr (µg L^{-1})	0.05	0.19	0.36	0.09	0.07	0.11
Fe (µg L^{-1})	158.6	96.86	79.36	150.3	647.7	69.35
Zn (µg L^{-1})	0.27	1.18	1.13	0.36	0.69	0.44

Fig. 3.3. Sampling area in Cremona province.

Approximately 2 L was collected, filtered by nylon filter membranes 0.45 μm and used for DGT deployments in laboratory. DGT devices were exposed to groundwater samples for 24h, with two devices in each container. Continuous flow conditions were maintained using magnetic stirrer. Each device was analysed for arsenic speciation by HPLC-ICP-MS.

RESULTS AND DISCUSSION

DGT Blanks and Detection limit

The As blank also determines the minimum mass of As that can be reliably quantified after accumulation in the DGT devices; the actual detection limit of the method will however depend on the instrumental technique used to analyze the eluent.

Blank concentrations were assessed by measuring the mass of metal present in ferrihydrite gels after they had been loaded into DGT devices, left assembled for a time typical of deployed devices, and then removed from the assembly.

The measured masses were converted to a blank concentration assuming a deployment time of 24 h at 24 °C with a 0.78 mm thick diffusive gel and a 0.13 mm filter. Method detection limits (MDL), calculated as three times the standard deviation of the blank, are 0.24 ng mL^{-1} for As(III), 0.37 ng mL^{-1} for As(V), 0.08 ng mL^{-1} for DMA(V) and 0.17 ng mL^{-1} for MMA(V). Moreover the blank concentrations were detected with application of HPLC-ICP-MS. MDLs calculated with application of DGT devices, are quite low respect the method detection limits calculated during the evaluation of arsenic species concentration in aqueous solution. For the last situation, the MDLs are 0.20 ng mL^{-1} for As(III), 1.22 ng mL^{-1} for As(V), 1.18 ng mL^{-1} for DMA(V) and 0.42 ng mL^{-1} for MMA(V).

The MDL for As, calculated with DGT application, is lower than the measured concentration range of groundwater samples.

Elution Efficiency and Kinetics of Binding

Accurate calculation of environmental concentrations from DGT deployments depends on quantitative recovery of the element from the binding gel.

For quantitative application of DGT, the elution efficiency of the analyte from the adsorbent is required. The elution efficiency is expressed as a percentage of the initial mass of analyte loaded on the adsorbent and is commonly determined over a range of mass loadings (Zhang and Davison, 1995; Dahlqvist et al. 2002; Alfaro et al. 2000).

The elution efficiencies were obtained for four different chemical forms using the same extraction procedure (Table 3.3).

Tab. 3.3. Elution efficiencies of As(III), DMA(V), MMA(V) and As(V) determined for Fe-oxide gels using 0,28 M HNO$_3$. The error limits are standard deviations calculated for 9replicates.

	As(III)	DMA(V)	MMA(V)	As(V)
0,28 M HNO₃	0.90 ± 0.08	0.96 ± 0.05	0.97 ± 0.07	0.91 ± 0.06

The mass measured after 60 min was, within the experimental error, the same as at 24 h for As(V), 89% for MMA(V), 82% for As(III) and 76% for DMA(V) (Fig. 3.4). It provides direct evidence that binding of the target analyte to the Fe-oxide gels within DGT is sufficiently fast to ensure that its concentration at the diffusive gel/binding gel interface is effectively zero.

Even if Ona-Nguema et al. (2005) and Wilkie et al. (1996) reported that As(III) was partially converted to As(V) on the iron-hydroxide surface, in our experiment the conversion between inorganic species had not occurred
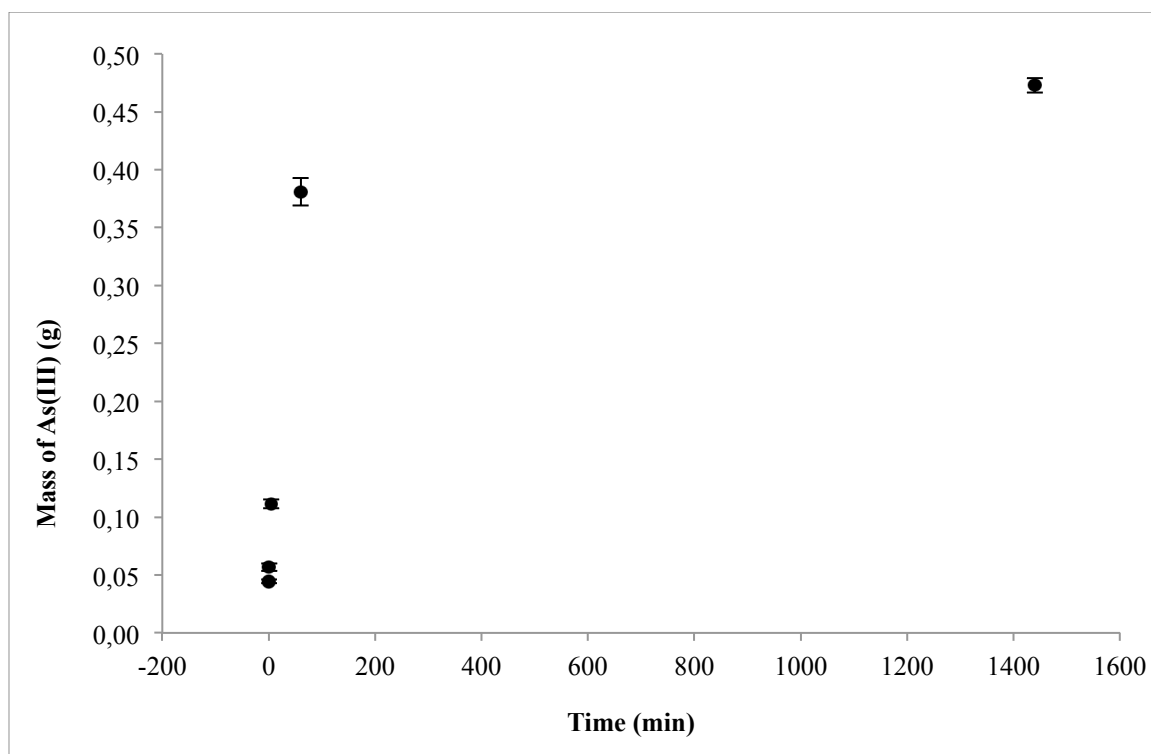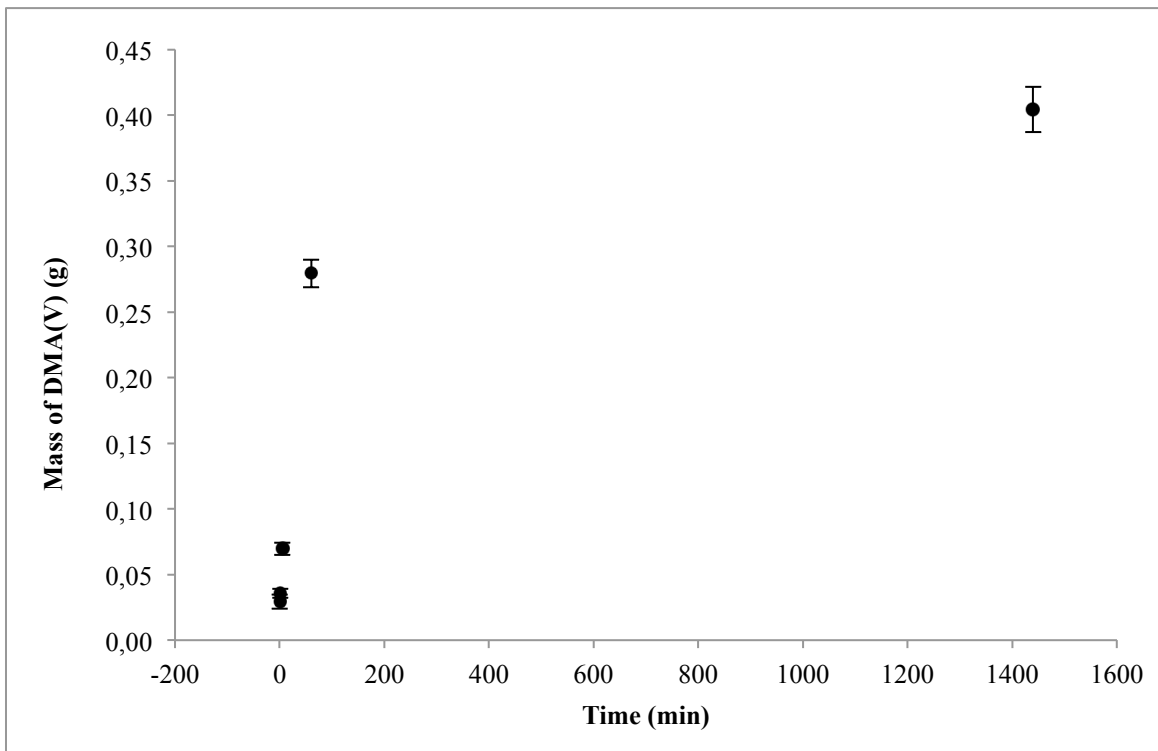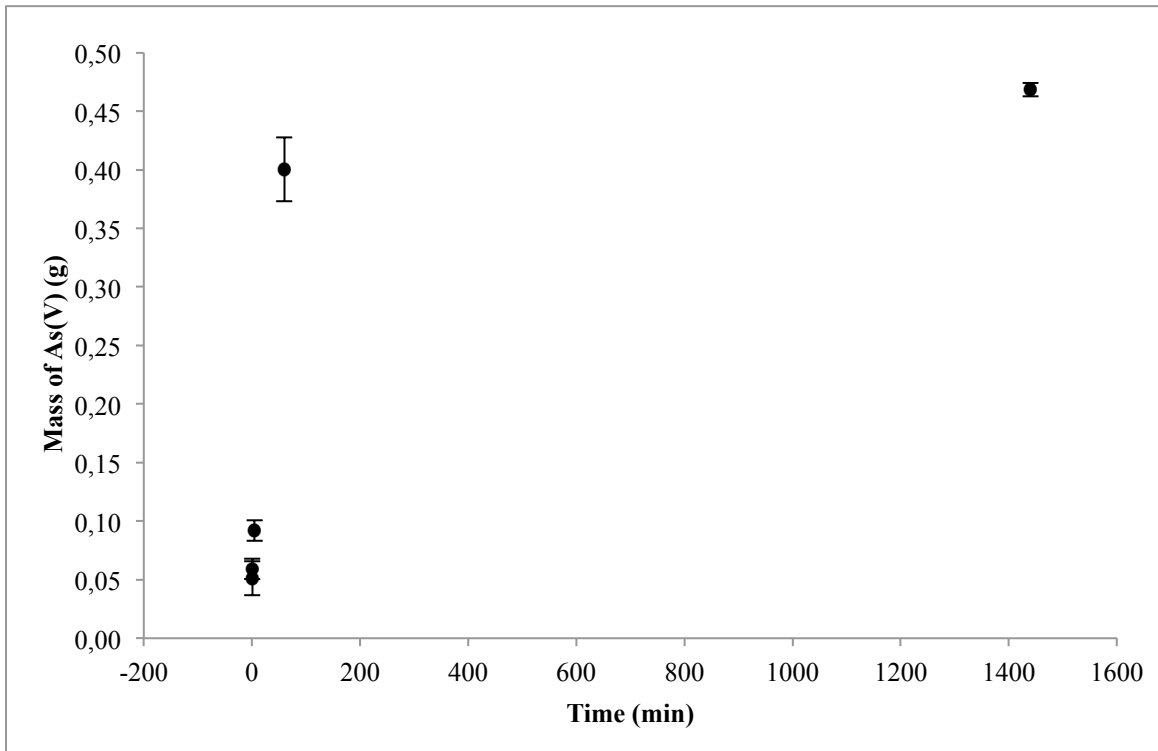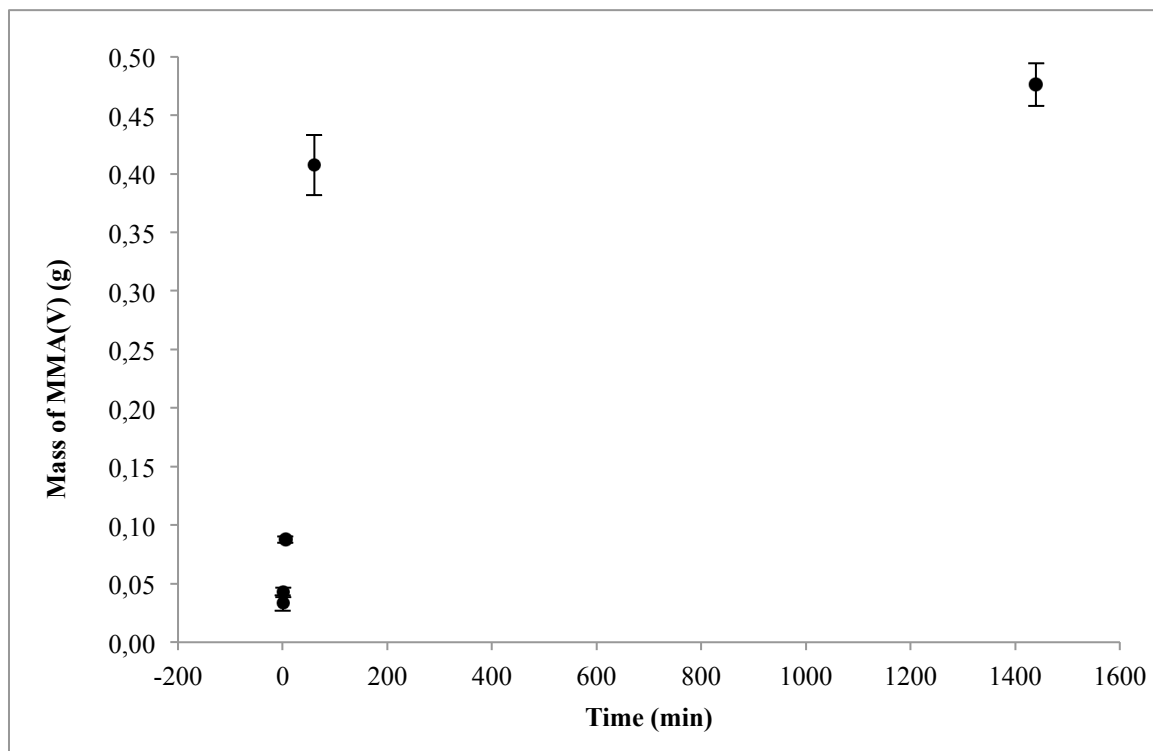
Fig. 3.4. Mass of As species accumulated by ferrihydrite gels placed in solutions containing 50 μ g L^{-1} As species for different times. Error bars are calculated from the standard deviation of replicates (n =3).

Ionic strength and pH influence

Panther et al. (2008) confirmed in their article that the diffusion coefficients measured at pH 5.0 for As can be used when applying DGT over the pH range 3–7, because the results were within experimental uncertainty for inorganic species. The uptake of As species by the iron hydroxide adsorbent is known to be rapid and quantitative between pH 3 and 7. They had shown that diffusion coefficient of As(III) and As(V) are unaffected by pH over the range 3-7 (Panther et al. 2008).

Any differences in the masses of As accumulated can be attributed to changes in diffusion coefficients.

The charge state of As(III) is essentially constant (neutral) between pH 3 and 7 (Jain and Ali, 2000). The pH-dependence of As(V) distribution is a consequence of the increase in the $[H_3AsO_4]/[H_2AsO_4^-]$ ratio from pH 5 to pH 3. At pH 5, >99% of As(V) exists as $H_2AsO_4^-$, whereas at pH 3.0, As(V) is distributed between $H_2AsO_4^-$ (83%) and H_3AsO_4 (17%), and at pH 7 the calculated amounts are 44% $HAsO_4^{2-}$ and 56% $H_2AsO_4^-$ (Pettit and Powell, 2005).

The As diffusion coefficients were calculated from the mass of As accumulated, assuming that the uptake of inorganic As species by the iron hydroxide adsorbent is unaffected by the added cations and anions (Panther et al. 2008).

The consistent performance of DGT containing ferrihydrite gels across the pH range from 5 to 9

suggests that accumulated mass are not appreciably affected by pH, especially for inorganic species (Fig. 3.5).

The ratio of DGT measured to solution concentrations of As species was not very reproducible for deployment solutions without added $NaNO_3$ or with low concentration (0.001 M) (Fig. 3.6).

Similar poor reproducibility has been observed previously when measuring cations at such low ionic strengths ($\sim$1 μM), where the negligible screening accentuates effects associated with charges within the gel, including Donnan portioning (Yezek and van Leeuwen, 2004; Zhang and Davison, 1999).

With increasing concentrations of $NaNO_3$ in solution, the concentrations measured by DGT were the same as those in solution, with generally good reproducibility.
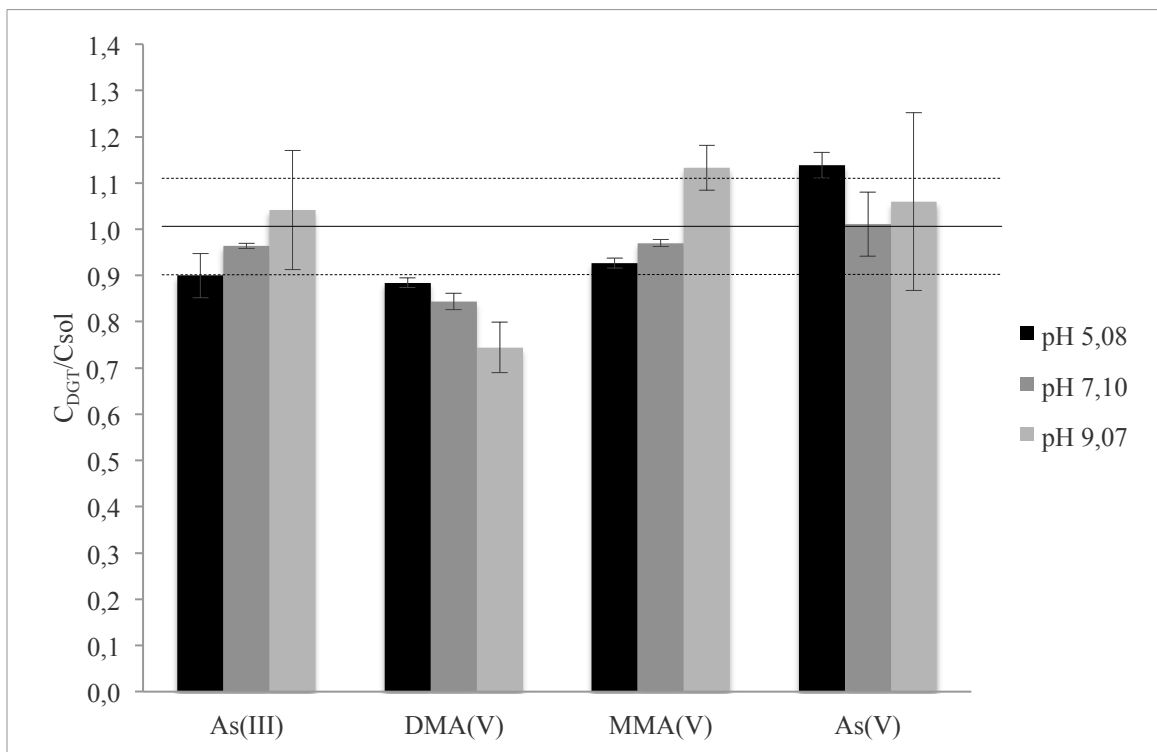
Fig. 3.5. Effect of pH on the ratio of concentrations of As species measured by DGT, C_{DGT}, to deployment solution concentrations, C_{sol}. Error bars represent the standard deviation of three replicates. The solid horizontal line and dotted horizontal lines represent target values of 1 ± 0.1.
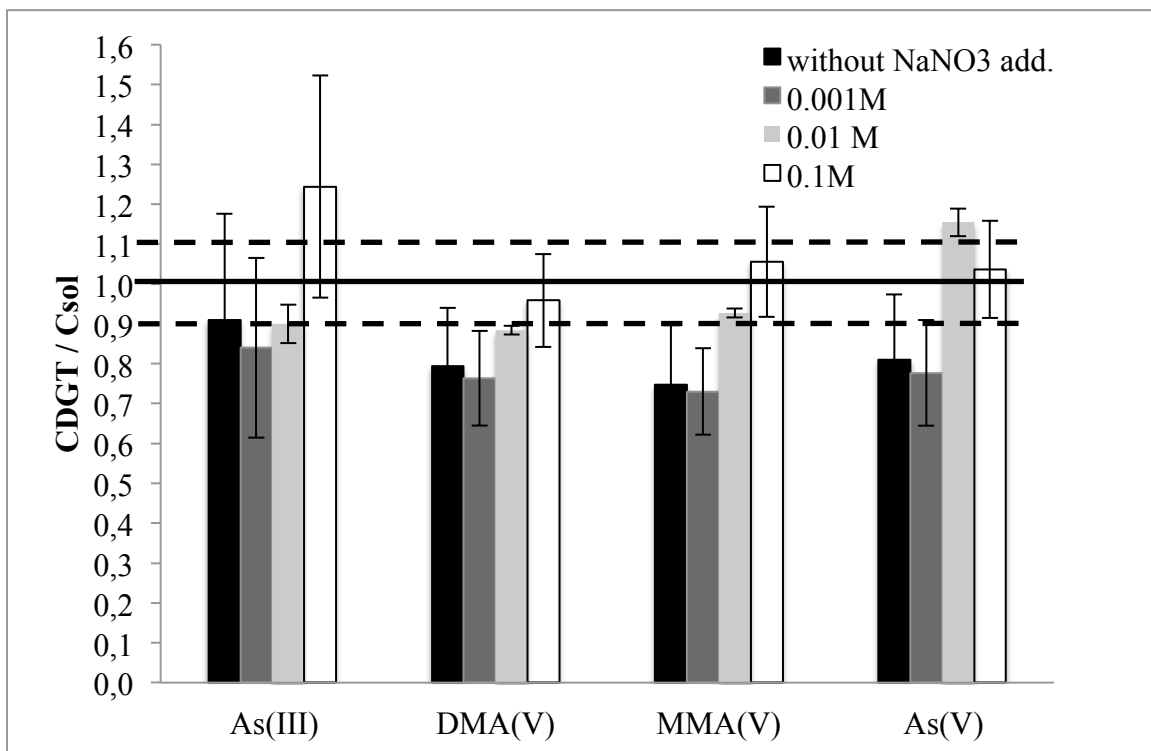
Fig. 3.6. Effect of concentration of supporting electrolyte, NaNO₃, on the ratio of concentrations measured by DGT containing ferrihydrite gels, C_{DGT}, to deployment solution concentrations of As species, C_{sol}.

The inorganic arsenic species and MMA(V) were almost completely adsorbed in all solutions.

On the contrary, DMA(V) behaved erratically between matrices showing low uptake in the synthetic solutions, expecialy without NaNO₃ addition and 0.001 M NaNO₃ and with different pH. The same DMA(V) behaviour had been also observed by Osterlund et al. (2012). Moreover, diminished uptake of DMA(V) on ferrihydrite is in agreement with results reported by Lafferty and Loeppert (2005), which indicated weaker adsorption of DMA(V), compared with inorganic arsenic and MMA(V). The 84 % of DMA(V) was adsorbed in a 0.01M matrix at pH 7.10, but this figure decreased as the pH increase from 7 to 9.

The adsorption efficiency of MMA(V) is higher compared with DMA(V) under all studied conditions. It can also be seen that pH has a larger effect on the adsorption than ionic straight. Lafferty and Loeppert (2005) suggested that the decreased adsorption of DMA(V) to ferrihydrite they observed between pH 7 and 8, was attributable to the point of zero charge (PZC) of the two-line ferrihydrite at pH 8; the negatively charged surface at pH values above the PZC prevented DMA(V) adsorption.

Testing DGT performance in laboratory

To be sure that procedure of arsenic species extraction was correct and reproducible, two different solutions, one with inorganic species and one with organic species, were been used in the recovery test.

Before starting the test, arsenic solutions were prepared and left to equilibrate overnight. The C_{DGT} were calculated with application of particular diffusion coefficients for As(III) ($5.95*10^{-6}\pm 0.30$ cm^2 s^{-1}), As(V) ($4.90*10^{-6}\pm 0.05$ cm^2 s^{-1}) and total As ($5.45*10^{-6}$ cm^2 s^{-1}) in the diffusive gel, founded by Panther et al. (2008) in their performance test.

During the performance test of DGTs, FH gel discs were exposed directly to a solution of inorganic As with a known concentration (pH 5 and 0.01 M NaNO$_3$) and after 24 h on the stirrer, about 100% of total arsenic, derived from the sum of As(III) and As(V) concentrations, was recovered by the adsorbent (Tab. 3.4).

Table 3.4. Concentrations of Inorganic As, divided by As(III) and As(V) with the use of FH DGT.

	C_{DGT} (ng cm^{-3})	SD
As(III)	9.92	± 0.04
As(V)	6.90	± 0.27
Σ Inorg. As (As(III)+As(V))	17.11	± 0.28
Inorg As in solution	16.93*	± 1.03
Recovery %	101	± 1.65

* Concentration of Inoganic As in solution (ng ml^{-1})

Moreover FH gel discs were put in a solution of organic As (DMA(V) and MMA(V)) with a known concentration, and after the same exposition period, it was possible to show an adsorption of two organic species in HPLC-ICP-MS chromatogram after the elution with nitric acid. After this application the calculation of C_{DGT} was impossible because the diffusive coefficients of DMA(V) and MMA(V) were unknown.

Measurement of As Diffusion Coefficients using DGT devices

The As(V) and As(III) diffusion coefficients, measured using DGT devices, are presented in Table 3.5.

Table 3.5. Diffusion coefficients 10^{-6} cm^2 s^{-1} measured using DGT devices at pH 5. Valid at 24.0 ± 0.5 °C. Uncertainty is the S.D. of the mean from n replicate determinations.

As species	Diffusion coefficient (D) (10^{-6} cm^2 s^{-1})	
	DGT devices	Literature
As(III)	5.40 ± 0.2 (n=2)	5.95 ± 0.30 (Panther 2008)
		4.90 ± 0.05 (Panther 2008)
		5.25 (Luo 2010)
As(V)	4.39 ± 0.07 (n=2)	$4.4/4.2$ (Sogn 2008)
		6.01 ± 0.15 (Fitz 2003)
		5.26 ± 0.28 (Osterlunf 2010)

The diffusion coefficients of inorganic species, based on slope in linear plot, were determined on DGT devices method with application of Eq. 2. The results of DGT devices for As(III) and As(V) are illustrated in Fig. 3.7 a & b.
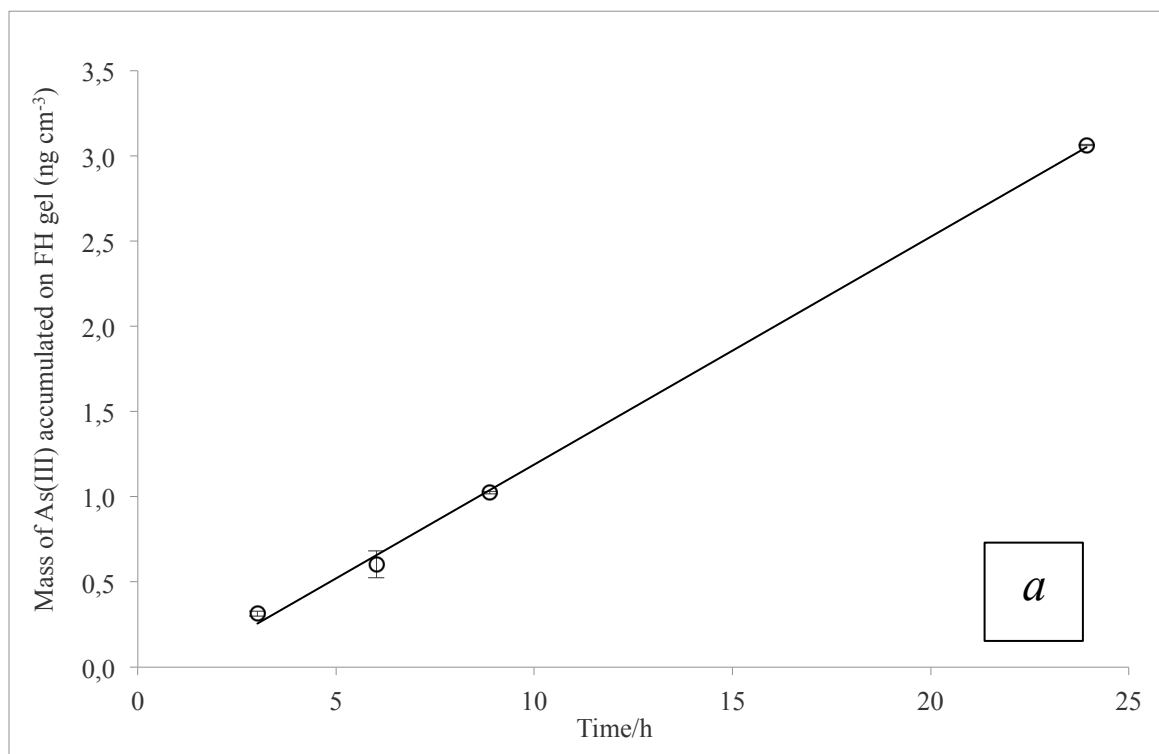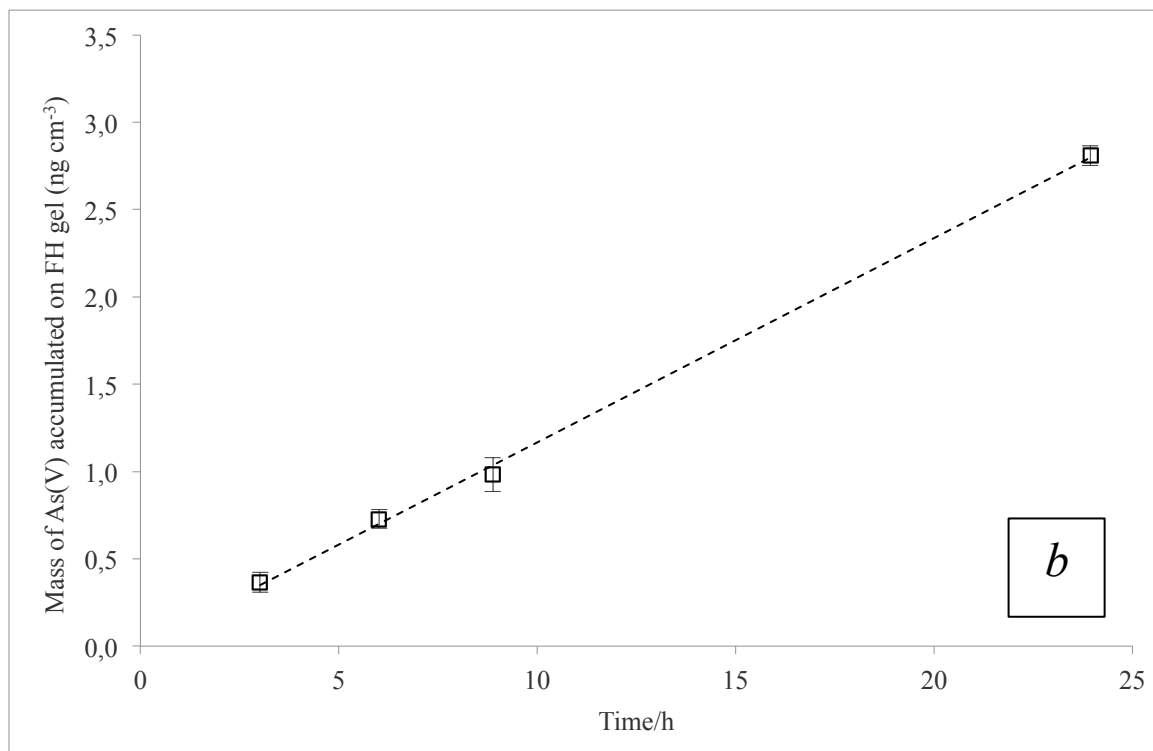
Fig. 3.7. Plots of measured mass of As (III) (*a*) and As(V) (*b*) vs. time.

Detailed studies of the As speciation method were made at pH 5.0, where As(III) and As(V) exist as H_3AsO_3 and $H_2AsO_4^-$, respectively (Pettit and Powell, 2005). The diffusion coefficient of As(V) (4.39 ± 0.07) is ~19% lower than the diffusion coefficient of As(III) (5.40 ± 0.2) This is attributed to more extensive hydration of charged $H_2AsO_4^-$ than of neutral H_3AsO_3, a factor that increases the hydrodynamic size of the former species (Panther et al 2008). In this study, diffusion coefficient ratio for As(III) and As(V) species trought the polyacrylamide diffusive gel is 1.23, very similar to the reported ratio of Panther et al. (2008).

Published diffusion coefficients for As through polyacrylamide gels are limited. For example, Fitz et al. (2003) measured the diffusion coefficient of As(V) (as $HAsO_4^{2-}$) using a diffusion cell and obtained a value of $(5.69 \pm 0.14) \times 10^{-6}$ $cm^2\ s^{-1}$. In different articles, diffusion coefficients could be seemed or not to our results but differences in solution composition or oxidation of As(V) to As(III) in the experiments may be relevant factors.

It was also possible to measure diffusion coefficient of organic species (DMA(V) and MMA(V)) with the employment of DGT devices (Table 3.6 and Fig. 3.8). These new diffusion coefficients are useful to calculate the C_{DGT} of monomethylarsonic acid dimethylarsinic acid in the initial performance test. Also in this case, about 100% of total arsenic, derived from the sum of DMA(V) and MMA(V) concentrations, was recovered by the adsorbent (Tab. 3.7).

Table 3.6. Diffusion coefficients 10^{-6} cm^2 s^{-1} measured using DGT devices at pH 5. Valid at 24 ± 0.5 °C. Uncertainty is the S.D. of the mean from n replicate determinations.

As species	Diffusion coefficient (D) (10^{-6} cm^2 s^{-1})	
	DGT devices	Literature (Österlund et al. 2012)
DMA(V)	4.48 ± 0.06 (n=2)	4.67 ± 0.13 (pH=4; 0.01M)
		1.81 ± 0.05 (pH=6; 0.01M)
MMA(V)	4.34 ± 0.2 (n=2)	5.53 ± 0.46 (pH=4; 0.01M)
		4.49 ± 0.19 (pH=6; 0.01M)
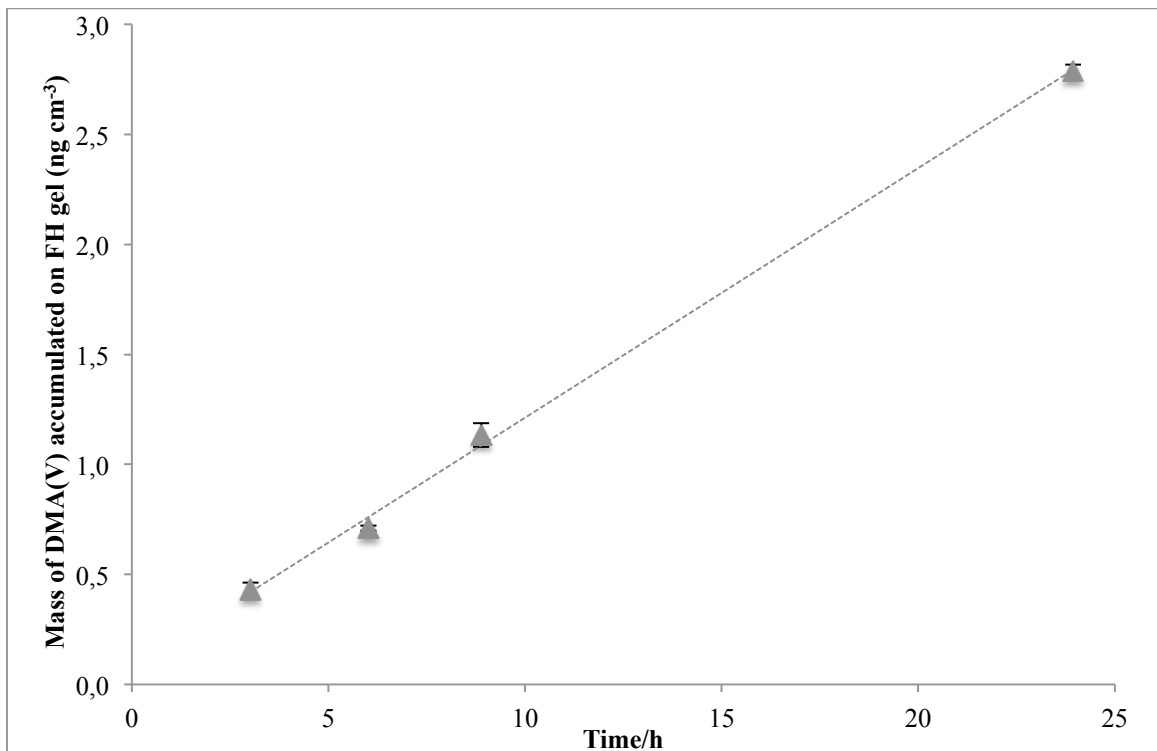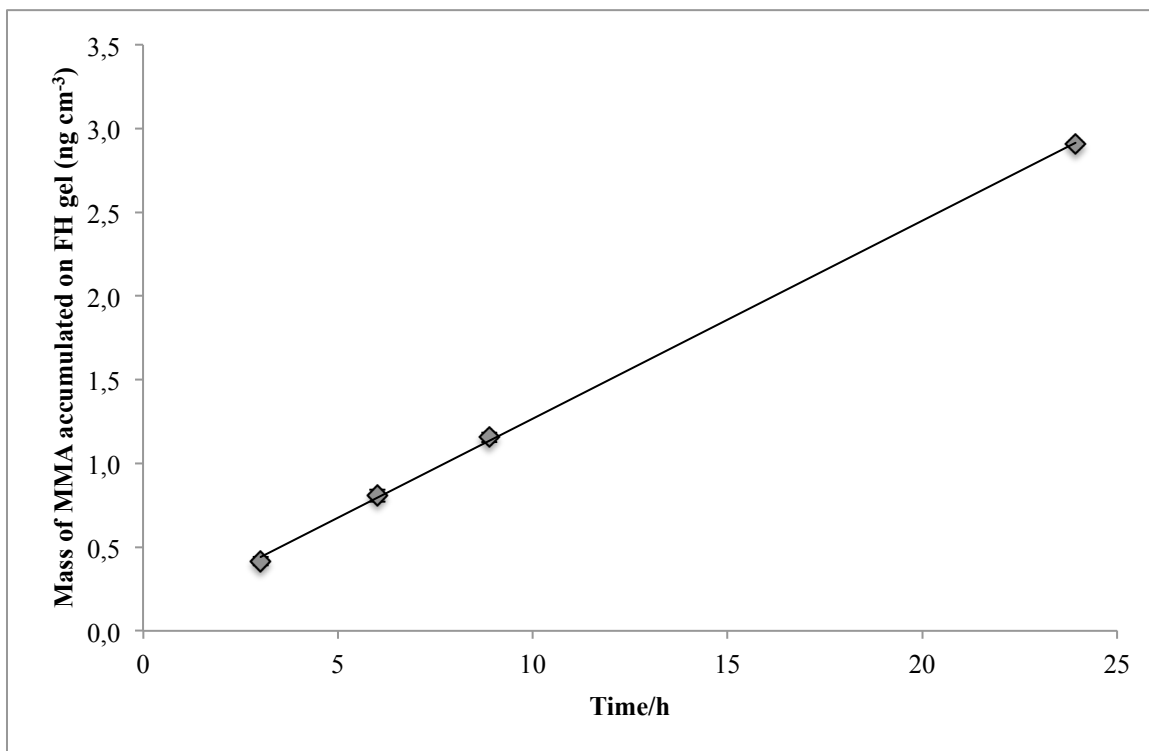
Fig. 3.8. Plots of measured mass of DMA(V) (a) and MMA(V) (b) vs. time.

Table 3.7. Concentrations of Organic As, divided by DMA(V) and MMA(V) with the use of FH DGT.

	C_{DGT} (ng cm^{-3})	SD
DMA(V)	10.07	± 0.24
MMA(V)	9.13	± 0.43
Σ Orga. As (DMA(V)+MMA(V))	19.21	± 0.67
Org. As in solution	19.54*	± 0.06
Recovery %	98	3

*Concentration of Organic As in solution (ng ml^{-1})

Even if in most environmental situations, the contribution of methylated As species to the total As pool is negligible (Smedley, 2002), Zhang et al (2007) reported that methylated arsenic binds to iron-hydroxides, and actually DGT method can distinguish between inorganic and organic As species with use of FH gel discs (Fig. 3.9).

When deploying DGT in water containing As(V) and As(III), the total mass of As accumulated by DGT is a weighted average which depends on the concentrations of each species in solution and its diffusion coefficients. This raises the question of what diffusion coefficient to use when calculating the concentration of total As for water with unknown As speciation. In Panther et al. (2008), appears that the use of the average of the As(V) and As(III) diffusion coefficient is probably the best approach with problems of under/overestimation of As concentration. It is important that the level of uncertainty is acceptable for a field method.

Scally et al. (2000) also identified possible bias errors associated with uncertainties of temperature and gel layer thickness of ±1% and ±1.3%, respectively.
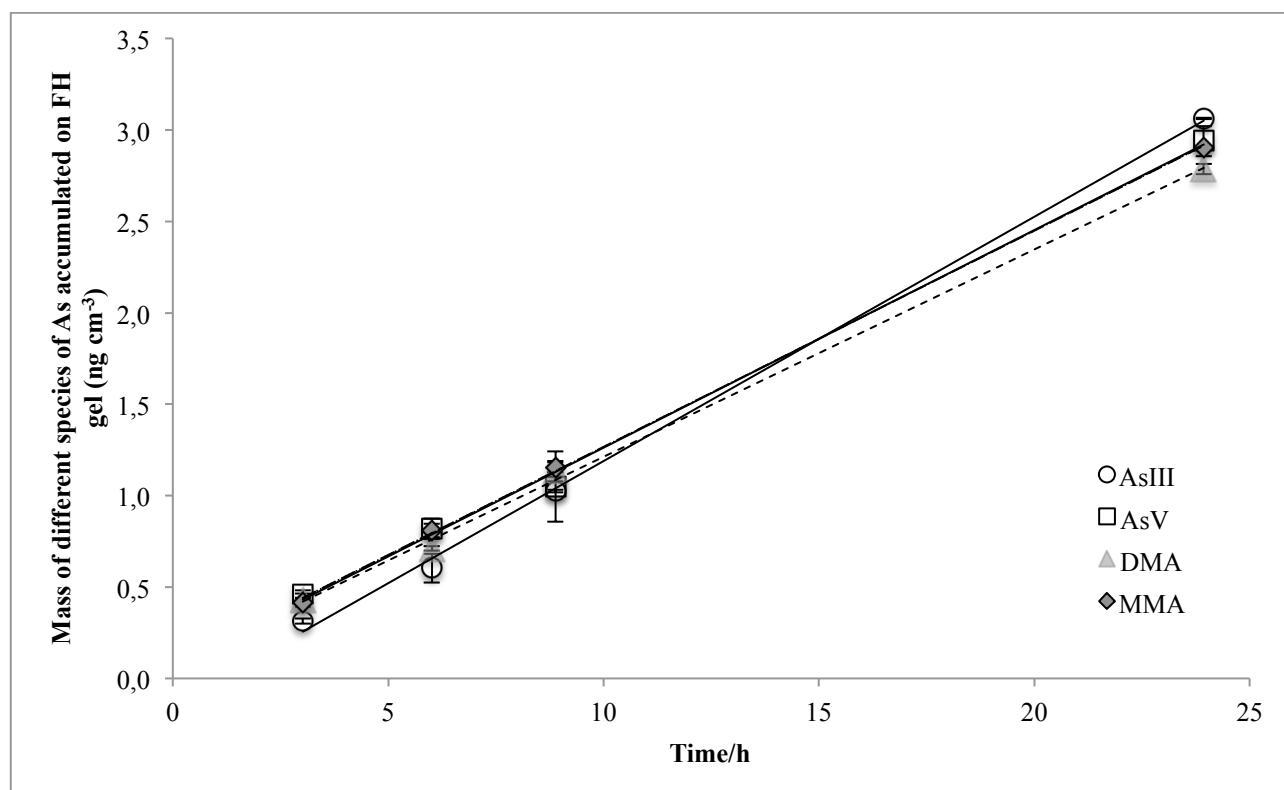

Fig. 3.9. Plots of measured mass of all arsenic species (As(III), As(V), DMA(V), MMA(V)).

DGT deployment

The As(III) and As(V) diffusion coefficients through a polyacrylamide diffusive gel were previously shown to be unaffected by the presence of major environmental ions at concentrations 4-fold higher than those used in the present measurements (Panther et al. 2008). To evaluate the performance of DGT method for accumulation of arsenic species, DGT devices were carried out in the laboratory in water collected in six different towns where the As concentration in groundwater is very high. Analyses were performed on the filtered (0.45 μm) water samples as well as on DGT eluates after 24h deployment and the As concentrations in the water samples were measured without the addition of any reagent (Tab. 3.8).

Tab. 3.8. Concentrations of Inorganic As, distinguishing between As(III) and As(V) with the use of FH DGT.

	PESCAROLO	POZZAGLIO	GRONTARDO
[As(III)] HPLC	97.29 ± 2.86	2.27 ± 0.12	4.34 ± 0.50
[As(III)] DGT	100.76 ± 3.94	2.60 ± 0,45	4.43 ± 0.22
Recovery (%)	104 %	109 %	102 %
[As(V)] HPLC	56.25 ± 2.47	16.52 ± 0.56	23.55 ± 0.67
[As(V)] DGT	56.76 ± 1.41	13.36 ± 1.91	19.70 ± 0.18
Recovery (%)	101 %	81 %	84 %

	CORTE	OLMENETA	DOSIMO
[As(III)] HPLC	1.79 ± 0.18	2.09 ± 0.43	2.34 ± 0.05
[As(III)] DGT	1.78 ± 0.03	2.10 ± 0.05	2.34 ± 0.03
Recovery (%)	99%	100%	100 %
[As(V)] HPLC	17.65 ± 0.23	22.28 ± 0.29	23.26 ± 2.38
[As(V)] DGT	21.67 ± 0.79	21.70 ± 1.00	23.77 ± 1.05
Recovery (%)	123 %	97 %	102 %
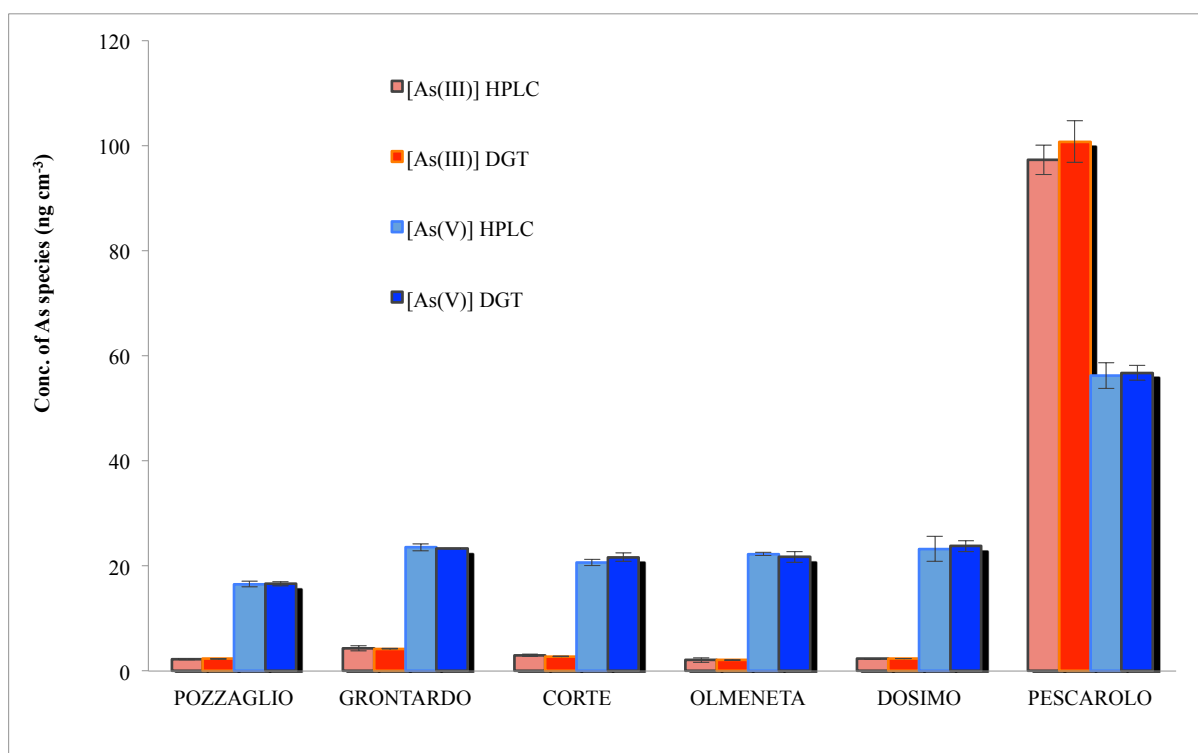
Fig. 3.10. Concentrations of As species in different groundwaters, measured with application of DGT and in water (HPLC).

Anions and cations, at higher concentrations, in natural water, have a negligible effect on the diffusion of As(V) and As(III), and their uptake by the iron-hydroxide adsorbent.

The percentage of As(III) ranged from 9 to 16% of the total concentration in five points of sampling. Only in one sampling point, the concentration of As(III) was about 65% the total concentration.

Marks of DMA(V) concentrations were not found in groundwater samples. It is possible to assume that the colloids (e.g. fulvic acids) were present in low concentration, because normally they perform absorption action to As(III) and formation of soluble As - colloid complexes is expected to decrease the amount of As accumulated by DGT, in this cases the As species concentration had a good recovery in deployed DGT probes.

CONCLUSIONS

It has been shown from laboratory experiments and field measurements that the ferrihydrite-DGT is suitable for simultaneous determination of labile arsenic, over a wide pH range.

This work establishes that it is possible to use Diffusive Gradient in Thin Films in the identification of As species. The presence of inorganic species has been well identified in DGT resin gel with the use of HPLC-ICP-MS. The DGT method can be used in the quantitative identification of organic species like DMA(V) and MMA(V).

Compared with other sampling methods, the major advantages of DGT include its ability to preconcentrate the analyte (leading to lower detection limits, after extended deployment times, than for direct measurements).

Panther et al. (2008) noted that longer deployment times, higher concentrations of competing ions, or a change in pH could affect the reliability of the DGT measurement. Therefore diffusion coefficients in the protective membrane filter and diffusive gel were estimated at several pH values using a direct uptake to DGT devices. When diffusive gels were equilibrated in solutions of As species at different ionic strengths and pH, the concentrations measured in the gels and in solution were not significantly different (Williams, personal communication), indicating no measurable charge effect, Donnan partitioning, or specific binding.

In groundwater samples, the presence of anions and cations, at concentrations up to 4 fold higher than those expected in unpolluted natural water, has no significant effect on the accumulation of As by DGT.

The results of Luo et al. (2010) suggest that for conditions likely to be encountered in natural water, possible competition effects between the oxyanions are likely to be negligible. Ferrihydrite exhibits a higher affinity for MMA(V) than for DMA(V) and Osterlund et al. (2012) noted that DMA(V) was shown to be the arsenic species most affected by competition from other anions, such as sulfate and phosphate.

We have shown that MMA(V) and, under some conditions, DMA(V) can accumulate on the ferrihydrite adsorbent and can therefore contribute to the total As measured by DGT sampling. Probably, DMA(V) could increase the total concentration of arsenic, but it is necessary to perform further investigations about seasonal variations of arsenic speciation in groundwater.

DGT results were generally lower than the corresponding filtered (<0.45 μm) concentrations, which is normal when complexation with colloids could be expected. The application of the appropriate pore size DGT may allow this detection in water with humic acids. Actually As is reported to form soluble complexes with natural organic matter such as fulvic acid (Redman et al. 2002; Lin et al. 2004; Ritter et al. 2006). Fulvic acids also adsorb to a variety of iron-hydroxides and can inhibit the adsorption of As(V) and As(III) at the iron-hydroxide surface (Simeoni et al. 2003; Grafe et al. 2001; Redman et al. 2002).

The ability of DGT to accumulate inorganic As species in a predictable manner, opens a range of possibilities for its use in environmental analyses. The potential applications include utilizing the pre-concentration ability of DGT to obtain measurements of lower concentrations of As than in the absence of a pre-concentration step, using DGT as a means of avoiding the difficulties of preservation of sample characteristics during storage, and exploitation of the time-averaging aspect of DGT deployments.

To facilitate accurate interpretation of DGT results under these conditions it is recommended to also carry out arsenic speciation analysis on the water in question.

REFERENCES

Alfaro-De la Torre MC, Beaulieu PY and A Tessier. 2000. In situ measurement of trace metals in lakewater using the dialysis and DGT techniques. *Anal. Chim. Acta.* 418, 53.

Anderson LCD and KW Bruland. 1991. Biogeochemistry of arsenic in natural waters: the importance of methylated species. *Environ. Sci. Technol.* 25, 420.

Bhattacharya P, Frisbie SH, Smith E, et al. 2002. Arsenic in the environment: a global perspective. In: Sarkar, B. (Ed.), Handbook of Heavy Metals in the Environment. Marcell Dekker Inc., New York, pp. 147–215.

Brookins DG. 1988. Eh-pH Diagrams for Geochemistry. Springer-Verlag, Berlin.

Cullen WR and KJ Reimer. 1989. Arsenic speciation in the environment. *Chem. Rev.* 89, 713.

Dahlqvist R, Zhang H, Ingri J and W Davison. 2002. Performance of the diffusive gradients in thin films technique for measuring Ca and Mg in freshwater. *Anal. Chim. Acta.* 460, 247.

Davison W, Fones G, Harper M, Teasdale P, Zhang H In J. Buffle, G. Horvai (Eds.). 2000. In Situ Monitoring of Systems: Chemical Analysis and Speciation. *John Wiley &Sons Inc.,* New York, p. 495.

Fitz Wj, Wenzel Ww, Zhang H, Nurmi J, Stipek K, Fischerova Z, Schweiger P, Kollensperger G, Ma Lq and G Stingeder. 2003. Rhizosphere Characteristics of the Arsenic Hyperaccumulator Pteris vittata L. and Monitoring of Phytoremoval Efficiency. *Environ. Sci. Technol.* 37, 5008-5014.

Grafe M, Eick MJ and PR Grossl. 2001. Adsorption of Arsenate (V) and Arsenite (III) on Goethite in the Presence and Absence of Dissolved Organic Carbon. *Soil Sci. Soc. Am. J.* 65, 1680.

Hasegawa H, Rahman MA, Kitahara K, Itaya Y, Maki T and K Ueda. 2010. Seasonal changes of arsenic speciation in lake waters in relation to eutrophication. *Sci. Total Environ.* 408, 1684.

Hasegawa H, Rahman MA, Matsuda T, Kitahara T, Maki T and K Ueda. 2009. Effect of eutrophication on the distribution of arsenic species in eutrophic and mesotrophic lakes. *Sci. Total Environ.* 407, 1418.

Huang JH, G Ilgenb and P Fecher. 2010. Quantitative chemical extraction for arsenic speciation in rice grains. *J. Anal. At. Spectrom.* 25, 800–802.

Jain CK and I Ali. 2000. Arsenic: occurrence, toxicity and speciation techniques. *Water Res*. 34, 4304–4312.

Lafferty BJ and RH Loeppert. 2005. Methyl arsenic adsorption and desorption behavior on iron oxides. *Environ. Sci. Technol.* 39, 2120.

Lead JR, Davison W, Hamilton-Taylor J and J Buffle. 1997. Characterizing Colloidal Material in Natural Waters. *Aquat. Geochem.* 3, 213–232.

Lin HT, Wang MC and GC Li. 2004. Complexation of arsenate with humic substance in water extract of compost. *Chemosphere.* 56, 1105.

Luo J, Zhang H, Santner J, and W Davison. 2010. Performance Characteristics of Diffusive Gradients in ThinFilmsEquipped with a Binding Gel Layer Containing Precipitated Ferrihydrite for Measuring Arsenic(V), Selenium(VI), Vanadium(V), and Antimony(V). *Anal. Chem. 82* (21), pp 8903–8909.

Ona-Nguema G, Morin G, Juillot F, Calas G and GE Brown. 2005. EXAFS Analysis of Arsenite Adsorption onto Two-Line Ferrihydrite, Hematite, Goethite, and Lepidocrocite. *Environ. Sci. Technol.* 39, 9147.

Österlund H, Chlotb S, Faarinena M, Widerlundb A, Rodushkina I, Ingri J and DC Baxtera. 2010. Simultaneous measurements of As, Mo, Sb, V and W using a ferrihydrite diffusive gradients in thinfilms (DGT) device. *Analytica Chimica Acta.* 682, 59–65.

Österlund H, Faarinen M, Ingri J and DC Baxter. 2012. Contribution of organic arsenic species to total arsenic measurements using ferrihydrite-backed diffusive gradients in thin films (DGT). *Environmental Chemistry.* 9(1) 55-62.

Panther JG, Stillwell KP, Powell KJ and AJ Downard. 2008. Development and application of the diffusive gradients in thin films technique for the measurement of total dissolved inorganic arsenic in waters. *Analytica Chimica Acta.* 622, 133–142.

Panther JG, Teasdale PR, Bennett WW, Welsh DT and H Zhao. 2011. Comparing dissolved reactive phosphorus measured by DGT with ferrihydrite and titanium dioxide adsorbents: anionic interferences, adsorbent capacity and deployment time. *Anal. Chim. Acta.* 698, 20.

Pettit L and HKJ Powell. IUPAC Stabilty Constants Database; Academic Software: Oxford. U.K. 2005.

Redman AD, Macalady DL and D Ahmann. 2002. Natural Organic Matter Affects Arsenic Speciation and Sorption onto Hematite. *Environ. Sci. Technol.* 36, 2889.

Ritter K, Aiken GR, Ranville JF, Bauer M and DL Macalady. 2006. Evidence for the Aquatic Binding of Arsenate by Natural Organic Matter−Suspended Fe(III). *Environ. Sci. Technol.* 40, 5380.

Simeoni MA, Batts BD and C McRae. 2003. Effect of groundwater fulvic acid on the adsorption of arsenate by ferrihydrite and gibbsite. *Appl. Geochem.* 18, 1507.

Smedley PL and DG Kinniburgh. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* 17, 517.

Sogn TA, Eich-Greatorex S, Øgaard Røyset O, Almås AF and AR Comm. 2008. Use of Diffusive Gradients in Thin Films to Predict Potentially Bioavailable Selenium in Soil. *Soil Sci. Plant Anal.* 39, 587–602.

Warnken KW, Zhang H and W Davison. 2007. In situ monitoring and dynamic speciation measurements in solution using DGT, in Passive Sampling Techniques in Environmental Monitoring (Eds R. Green- wood, Q. Mills, B. Vrana).

WHO, 1993. Guidelines for drinking-water quality. Volume 1: Recommendations, 2nd ed. WHO, Geneva.

Wilkie JA and JG Hering. 1996. Adsorption of arsenic onto hydrous ferric oxide: effects of adsorbate/adsorbent ratios and co-occurring solutes. *Colloids Surf.* A 107, 97.

Yamauchi H and BA Fowler. 1994. Toxicity and metabolism of inorganic and methylated arsenicals.In: Nriagu, J.O. (Ed.), Arsenic in the Environment. Part II;Human Health and Ecosystem Effects. Wiley, New York.

Yan XP, Kerrich R and MJ Hendry. 2000. Distribution of arsenic(III), arsenic(V) and total inorganic arsenic in pore-waters from a thick till and clay-rich aquitard sequence, Saskatchewan, Canada. *Geochim. Cosmochim. Acta.* 64, 2637–2648.

Yezek LP and HP van Leeuwen. 2004. An electrokinetic characterization of low charge density cross-linked polyacrylamide gels. *J. Colloid Interface Sci.* 278, 243– 250.

Zhang H, Davison W, Gadi R and T Kobayashi. 1998. In situ measurement of dissolved phosphorus in natural waters using DGT. *Anal. Chim. Acta.* 370, 29.

Zhang H and W Davison. 2000. Direct In Situ Measurements of Labile Inorganic and Organically Bound Metal Species in Synthetic Solutions and Natural Waters Using Diffusive Gradients in Thin Films. *Anal. Chem.* 72, 4447–445.

Zhang H and W Davison. 1995. Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution. *Anal. Chem.* 67, 3391.

Zhang JS, Stanforth RS and SO Pehkonen. 2007. Effect of replacing a hydroxyl group with a methyl group on arsenic (V) species adsorption on goethite (α-FeOOH). *Colloid Interface Sci.* 306, 16.

Zhang H and W Davison. 1999. Diffusional characteristics of hydrogels used in DGT and DET techniques. *Anal. Chim. Acta.* 398, 329–340.

Chapter IV. Speciation analysis of Antimony by Diffusive Gradients in Thin Films (DGT) ID ICP MS.

ABSTRACT

Measurements of inorganic species of antimony (Sb) require a binding agent, like Fe-oxide gel in DGT, EDTA, for preservation of different species, HPLC ICP MS, like analytical technique with anionic exchange column. Here, we report for the first time detailed performance characteristics of the Fe-oxide gel and associated DGT devices obtained by deployment in known solutions of trivalent and pentavalent Sb. The diffusion coefficients for each chemical forms were determined using an independent diffusion cell. Theoretical responses were obtained with studies in different ionic strength (from deionized water to 0.1 M $NaNO_3$) and pH (5-9). The potential capability for simultaneously measuring labile forms of antimony in acidic to neutral, fresh to brackish waters was demonstrated. Speciation analysis of Sb(III) and Sb(V) in aqueous samples was performed through extraction from DGT resin after deployment and on-line isotope dilution concentration determination after a chromatographic separation. The separation of Sb(III) and Sb(V) was achieved using an anion exchange column and 10 mmol L^{-1} EDTA and 1 mmol L^{-1} phthalic acid at pH 4.5 as a mobile phase.

The eluent from the HPLC was mixed with an enriched ^{123}Sb spike solution that was pumped by a peristaltic pump with a constant flow rate (0.04 mL min^{-1}) in a three-way valve.

Keywords: *trivalent and pentavalent antimony, Diffusive Gradient in Thin Film, HPLC-ICP-MS, water, isotopic dilution.*

INTRODUCTION

Antimony (Sb) is considered to be a nonessential element in plants, animals or humans (Iffland, 1988; Filella et al. 2002). Sb and its compounds have been listed as priority pollutants by the US Environmental Protection Agency of the United States and the European Union (USEPA, 1979; Dir 98/83/EC).

Antimony belongs to the subgroup 15 of the table of the elements. Its concentrations are much higher in natural geothermal systems, where they can range from 500 mg L^{-1} up to 10 wt %. (Ritchie, 1961; Weissberg et al., 1979; Kolpakova, 1982; Stauffer and Thompson, 1984). Probably due to its lower abundance and the relative insolubility of most of its compounds, this is usually overlooked as an element of environmental concern and its study has been largely neglected.

Most of the analytical methods for antimony assessment are based on the determination of total antimony concentrations. However, it is widely accepted that the impact of a toxic element on the environment cannot be assessed by measuring only its total concentration, and environmental risks linked to the presence of antimony depend to a great extent on the chemical form present.

The majority of speciation analysis techniques consist of three steps: sample preparation, separation of different species, and determination of the separated species. Sample preparation is one of the most critical steps of the analytical speciation procedure,

The development of highly sensitive coupled techniques to identify and/or to quantify one or more Sb species has opened up an increasingly attractive research area for elucidating the fate of Sb among the different environmental compartments. Most commonly employed analytical techniques for the separation and detection of antimony species are the on-line coupling of high-performance liquid chromatography to powerful element specific detectors like ICP-MS (Lintschinger et al. 1997; Lintschinger et al. 1998; Lindemann et al. 2000; Zheng et al. 2000; Zheng et al. 2001; Krachler and Emons, 2001). Much attention has been focused on chromatographic methods, especially HPLC, with different separation mechanisms and different detection methods. The coupling of chromatographic techniques with element-specific detection allows simultaneous separation and determination of Sb(III) and Sb(V) in one step.

In antimony compounds, the most common oxidation states are 5, 3, and -3. It exists mainly as Sb(III) and Sb(V) in environmental, biological, and geochemical samples.

However, as with other elements, the toxicological and physiological behaviour of antimony depends on its oxidation state, the presence of binding partners and potential ligands, and on the solubility of the Sb compound. Elemental Sb is more toxic than its salts and generally trivalent Sb compounds exert a 10 times higher acute toxicity than pentavalent Sb species.

The physical and chemical qualities of Sb and As are similar. In the past, these two elements and their compounds were often determined together. As first pointed out by Pauling (1933), the coordination of Sb(V) with oxygen is quite different from that of As(V). Based on its larger ionic radius and lower charge density, this author suggested that antimony should be octahedrally coordinated with oxygen and its compounds rather than tetrahedrally like As(V) is. Moreover, there is evidence that antimony (in contrast to arsenic) is not detoxified via methylation in mammals, but it still remains unclear which mechanism is responsible for antimony's genotoxicity. Coupled techniques based on the combination of a separation method with a suitable element adsorption system have become reliable in speciation analysis to discriminate specific forms of an element.

We report the first investigation of use of Fe-oxide gels in Diffusive Gradient in Thin Films (DGT) for incorporation of inorganic chemical forms of antimony, especially Sb(III), coupled with HPLC-ICP-MS.

An important feature of an HPLC-ICP-MS system is the capability of the system to perform isotope ratio measurements and consequently isotope dilution mass spectrometry due to the mass-specific detection system (Hill et al.1993). The quantification is based on the determination of the isotope ratio of at least two isotopes of an element in the sample after spiking with an enriched analyte isotope. Since only isotope ratios instead of absolute intensities are used for the concentration calculation, variations in the intensities due to matrix effects have less influence on the accuracy of analytical results compared with conventional calibration strategies.

In this work we developed an analytical chemical procedure based on the above capability of using HPLC-ID-ICP-MS to separate and determine Sb(III) and Sb(V) in aqueous samples and Fe-oxide resins from DGT devices application.

The DGT technique is based on a simple device, which accumulates solutes on a binding agent after passage through a hydrogel, which acts as a well-defined diffusion layer. It relies on the establishment of a steady-state concentration gradient from solution to the binding agent (Davison and Zhang, 1994). Concentration in solution is calculated using Fick's first law of diffusion and the measured mass of solutes accumulated on the binding agent after a known deployment time. These applications are a promising preservation procedure and they have the benefit of eliminating the risk of speciation changes due to transportation and storage of water samples prior to preparation and analysis. Actually the other difficulty encountered with Sb speciation analysis is the oxidation of Sb(III) to Sb(V).

Use of DGTs is useful because species stability in the samples is an important issue, since natural environmental samples are not usually analysed immediately after sampling and long-term storage can produce a significant alteration of the chemical species.

New data on the diffusion coefficients of Sb(V) and the first data on diffusion coefficient for Sb(III) in the diffusive gels are provided to allow accurate calculation of DGT measured concentrations.

MATERIALS AND METHODS

Solutions

Stock solutions of antimony species (1000 mg L^{-1} for Sb) were prepared by antimony (III) potassium tartrate hemihydrate ($C_4H_4KO_7Sb \cdot \frac{1}{2}H_2O$), potassium hexahydroxoantimonate ($KSb(OH)_6$) from Carlo Erba Reagents. The standard solutions of antimony species were used by

diluting the corresponding stock solutions. Solutions of Sb(III) prepared from potassium antimonyl tartrate have been reported to be stable for long periods (Al-Sibaai and Fogg, 1973; Andreae et al., 1981; de la Calle-Guntiñas et al., 1992).

All reagents were of analytical grade. Ultra-pure water was prepared by a Milli-Q system (18MΩ-cm resistance, Millipore® system, Millipore, Bedford, MA).

A mixed standard solution was prepared by diluting stock solutions of antimony species.

Mobile phase

The mobile phase used in this technique was a 10 mM ethylenediaminetetraacetic acid disodium salt dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) (Sigma Aldrich Co) and 2 mM potassium hydrogen phtalate ($C_8H_5KO_4$) (Carlo Erba). Use of 10 mM EDTA decreases the oxidation state of Sb(III).

The main differences in method-development strategies described in the literature are mostly based on the mobile phases employed. In general, the elution of Sb(V) is readily achieved with a variety of mobile phases, while, for Sb(III), a broad tailing chromatographic peak is usually observed.

Complexing mobile phases containing tartrate buffers (Zhang et al. 1998) or EDTA with or without potassium-hydrogen phthalate (KHP) have been also proposed (Krachler et al. 2000; Dodd et al. 1992; Krachler and Emons, et al. 2001; Sayago et al. 2000) to solve the problem.

The importance of complexing ligands in the mobile phase was established by Lintschinger et al. (1997), who also showed that adding a strong competing anion, e.g. phthalate, to the EDTA mobile phase improved the chromatographic system by shifting the Sb(III) peak to a shorter retention time and improving its symmetry. A complexing mobile phase serves to preserve the trivalent oxidation state of antimony during the chromatographic separation, as Sb(III) easily oxidizes to Sb(V) in aqueous solutions (Krachler M, H. Emons. 2001).

Instrumentations

The concentration of total Sb in resin layers of DGT and standard water samples is determined by ICP-MS (Agilent 7700x, Agilent Technologies, USA) with Octopole Reaction System (ORS system).

Speciation of Sb has been performed by HPLC on an anion exchange column with hydrophilic polymethacrylete as basin resin. A HPLC and an ICP-MS were connected with PEEK tube.

All of measurements were performed under the operating conditions given in Table 4.2.

Isotopic Diluition

^{123}Sb-enriched standard solution (ISC Science, Oviedo, Spain) (Tab. 4.1) was used for determination of antimony species concentration with application of specie–unspecific post column isotope equation, and for determination of total antimony concentration with application of online isotope equation (see Introduction chapter).

The continuous addition (0.04 mL min^{-1}) of the enriched spike (50.1401 ng g^{-1}) was achieved by joining the spike solution line, pumped by peristaltic pump equipped with online ISTD addition kit with the HPLC effluent (1 mL min^{-1}) via a three-way valve. The mixed solution passed directly to the nebulizer of the ICP- MS. The "spectrum mode" of the ICP-MS software was used for the data acquisition, where ^{121}Sb and ^{123}Sb isotopes were monitored. The integration time for each Sb isotope was 0.1 sec per point.

A 10 µl aliquot of extraction solution was injected into the HPLC machine using an autosampler. From the measured intensities of the corresponding isotopes ^{121}Sb and ^{123}Sb the isotope ratio R123/121 for every measured time was calculated. The isotope ratio chromatograms were converted into mass flow chromatograms by calculating the absolute amount of Sb for each R123/121 value on the chromatogram by applying post-column isotope dilution equations described in the Introduction chapter. The exact concentrations of Sb(III) and Sb(V) were determined by integration of the areas under the peaks after background subtraction.

Figure 4.1 shows the mass chromatogram for the separation of Sb(III) (antimony potassium tartrate hemihydrate) and Sb(V) (potassium hexahydroxoantimonate) standards in aqueous solution.

Standard reference material 1643e (Trace Elements in Water) were used in this work to validate the post column isotope dilution equation (Table 4.3).

The total Sb concentration in the samples and in reference material was determined using ICP-MS by applying an external calibration technique using enriched standard solution and indium as internal standard like control.

Table 4.1. Characteristics of [123]Sb-enriched standard solution (ISC Science, Oviedo, Spain).

[123]Sb-enriched standard solution product details:	
Chemical species:	Antimony nitrate
Isotope:	[123]Sb (98.66 %)
Form:	2 mL in HNO_3 (2%)
Isotope abundance (%) of [121]Sb	1.343 %
Isotope abundance (%) of [123]Sb	98.657 %
Concentration:	8.831 ± 0.196 ($\mu g \, g^{-1}$ as Sb)

Table 4.2. Instrumental operating conditions of HPLC–ICP-MS.

CHROMATOGRAPHIC CONDITIONS	
Column:	Agilent, As speciation column for Drinkihg Water (4.6 mm × 150 mm i.d)
Column material:	(polyetheretherketone) PEEK1
Eluent:	10 mM Na_2EDTA; 1 mM of KHP
Flow rate:	1 ml min^{-1}
Spike	Sb standard solution enriched in [123]Sb (ISC Science, Oviedo Spain)
Flow rate spike	0.04 mL min^{-1}
Injection volume:	10 μL
Column temperature	room temperature
Acquisition time	300 sec

ICP-MS CONDITIONS	
Rf power:	1550 W
RF Matching:	1.8 V
Carrier gas flow rate:	Ar 1.05 L min^{-1}
Dilution Mode:	ON
Dilution Gas:	Ar 0.2 L min^{-1}
Sampling depth:	9 mm
S/C Temp:	2 °C
Reaction mode:	OFF
Measured m/z:	Sb 121; Sb 123
Quadrupole bias:	-4 V
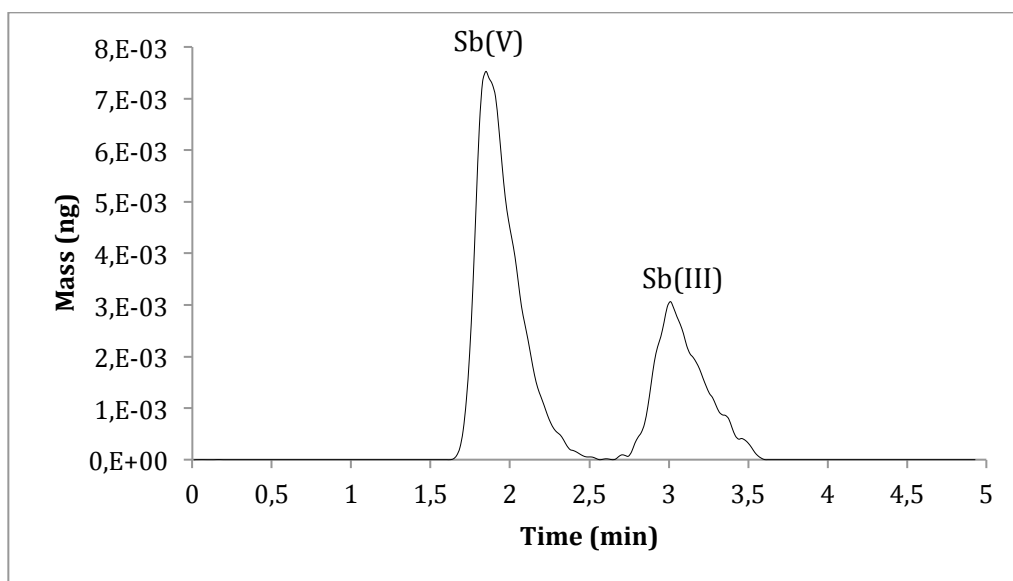
Fig. 4.1. Anion-exchange chromatograms converted to mass via isotope dilution: 10 ng mL^{-1} of Sb(III) and Sb(V) in water (pH =5 and 0.01 M NaNO$_3$).

Table 4.3. Comparison between antimony determined concentration and certified value of SRM 1643e.

	STANDARD REFERENCE MATERIAL 1643e	
	Determined	Certified
Sb	59.13 ± 0.69	58.30 ± 0.61

Diffusive Gradient in Thin Films (DGT) application

DGT units, with Fe-oxide gel, were exposed directly to a solution of inorganic Sb with a known concentration (pH 5, 0.01 M $NaNO_3$). After 24 h on the stirrer, DGT units were taken out of the solution and the surfaces were rinsed with MilliQ water. After that, the resin gel could be retrieved and the Fe-oxide gel placed in a clean sample tube.

The Fe-oxide gels are placed in a plastic flask (digiTUBES 50 mL) with 10 mL of 50 mM of Ethylenediaminetetraacetic acid disodium salt dihydrate. After that, they are mineralized at 95° C for 90 min in a heating block system (DIGIPREP, Scp Science, Quebec, Canada). The digested DGT gel solutions were filtered by using 0.45 μm filter (digiFILTER) after appropriate dilution with Milli- Water.

Testing DGT performance in laboratory

The amount of antimony absorbed by the resin was analysed, and determined after deploying of three DGT units for 24h in a solution of Sb(V). The time-averaged concentration of a specie in a solution, C, was then calculated using the DGT equation (1),

$$C_{DGT} = \frac{M * \Delta g}{t * A * D} \tag{1}$$

Δg = the thickness of the diffusive gel (cm),

t = the deployment time (s)

A = the surface area of the diffusive gel exposed to the bulk solution (cm^2)

D = the diffusion coefficient of analyte in the diffusive gel ($cm^2 \, s^{-1}$),

C_{DGT} was compared with the immersion solution concentration analysed in the samples taken during the experiment. There was a difference between the concentrations within 10%.

Measurement of Sb Diffusion Coefficients using DGT devices

Diffusion coefficients of Sb(V) and Sb(III) were measured using DGT devices. Ten DGT devices were deployed in the Sb solution for times ranging from 3 to 24 h. At each retrieval time, 2 DGT

devices were removed providing duplicate samples. The diffusion coefficients were calculated using Eq. (2).

$$D = \frac{slope * \Delta g}{C * A}$$
(2)

Where D is the diffusion coefficient, Slope is the slope of a linear plot of measured mass of Sb diffused through the diffusive gel (and membrane filter) vs. time, *A* is the exposed area of diffusive gel/membrane filter, *Δg* is the combined thickness of the diffusive gel and membrane filter (i.e. 0.091 cm), and *C* is the concentration of Sb initially present in the source compartment of the diffusion cell. At the start and end of experiment and at each retrieval time, the concentration of Sb in the source compartment was measured to confirm that the Sb species concentration did not change significantly over the experimental time.

This procedure was also made to measure and control changes in pH values and temperature (24°C ± 0.5).

Kinetics of Binding and Elution Efficiency

Triplicate precipitated Fe- oxide gel discs (3.14 cm^2 diameter, 0.60 mm of thickness) were loaded with a known amount of Sb(V) and Sb(III) by shaking them for various times from 0.5 min to 24h in 10 mL of 50 μg L^{-1} of each antimony species with a matrix of 0.01 M of NaNO$_3$. Elution efficiency of these species was obtained by eluting loaded Fe-oxide gel discs in 50 mM of Ethylenediaminetetraacetic acid disodium salt dihydrate at 95°C for 90 min in heating block system (DIGIPREP, Scp Science, Quebec, Canada) in plastic flasks (digiTUBES 50 mL). The elution and the immersion solutions were analysed by HPLC-ICP-MS with appropriate dilution.

Ionic strength and pH influence

To test the effects of ionic strength and pH, triplicate FH DGTs (0.60 mm Fe-oxide gel, 0.78 mm open pore diffusive gel), were deployed for 24h in various well-stirred solutions at known constant temperatures.

To study the effect of ionic strength, a range of NaNO$_3$ concentrations, from 0 to 1 mM, was prepared in 2.5 L of solution (pH 5.13 ± 0.11) containing each species at 50 μg L^{-1}.

For pH influence, 2.5 L containing 0.01 M were prepared at different pHs adjusted with diluted NaOH prior to spiking with Sb species stock solutions to 50 μg L^{-1}.

RESULTS AND DISCUSSION

The quantification of Sb species was done by using HPLC-ID-ICP-MS with a species-unspecific

spike. This kind of quantification was chosen to overcome the problems encountered with external calibration such as matrix effect or standard addition method where the added species often shows different behaviour from the species to be analyzed.

In this case, the spike must be added after the complete separation of different species and this was achieved as previously discussed, and the isotope ratio must be followed over the whole chromatographic peak.

Following the post column ID calculation, the isotope ratio chromatograms were converted into mass flow chromatograms. To obtain the concentrations of different species in the sample, the various peaks detected were integrated after background subtraction then normalized to the injection.

The detection limits were evaluated by making 6 repetitive injections of 50 ng mL^{-1} for each Sb(III) and Sb(V) references prepared in aqueous solutions and were calculated from the standard deviations of the peak areas (3σ) in the mass chromatograms.

They were found to be 4.1 ng mL^{-1} and 7.3 ng mL^{-1} for Sb(V) and Sb(III), respectively. These detection limits are sufficiently low for the speciation of inorganic Sb species in environmental samples. The precision, evaluated by using RSD with 50 ng mL^{-1} calibration solutions, was 2.4% and 5.3% (n=6) for Sb(V) and Sb(III), respectively, in aqueous solutions.

The recovery of Sb, using the 10mM Na$_2$EDTA and 1 mM KHP like eluent in chromatographic condition and applying peak integration with post column ID equation, was in good agreement with the certified result of reference materials.

DGT Blanks and Detection limit

The measured masses were converted to a blank concentration assuming a deployment time of 24 h at 24 °C with a 0.78 mm thick diffusive gel and a 0.13 mm filter. Method detection limits (MDL), calculated as three times the standard deviation of the blank, are 0.23 ng mL^{-1} for Sb(V) and 0.41 ng mL^{-1} for Sb(III). The blank concentrations were detected with application of HPLC-ICP-MS. MDLs calculated with application of DGT devices, are quite low respect the method detection limits calculated during the evaluation of antimony species concentration in aqueous solution. For the last situation, the MDLs are 0.45 ng mL^{-1} for Sb(V) and 0.95 ng mL^{-1} for Sb(III).

Elution Efficiency and Kinetics of Binding

Accurate calculation of environmental concentrations from DGT deployments depends on quantitative recovery of the element from the binding gel.

In this study, less concentrated acid (50 mM Na_2EDTA), as used for elution and preservation of antimony species from ferrihydrite, were tested.

In Table 4.4 the elution efficiencies are reported for two different chemical forms using the same extraction procedure.

Within 30 min of immersing Fe-oxide gels in solutions containing the Sb(V) and Sb(III), the measured mass was within 50% of its final value for Sb(V) and 70% for Sb(III) (Fig. 4.2). The mass measured after 60 min was, within the experimental error, the same as at 24 h for Sb(III) and 70% for Sb(V). Our results are quite different from the flux measured by Luo et al. (2010), where the flux corresponding to the measured mass of Sb(V) in precipitated ferrihydrite was two times greater than our flux after 60 minutes. However, it provides direct evidence that binding of the target analyte to the Fe-oxide gels within DGT is sufficiently fast to ensure that its concentration at the diffusive gel/binding gel interface is effectively zero.

Table 4.4. Elution efficiencies of Sb(III) and Sb(V) determined for Fe-oxide gels using 10 mM $EDTA_3$. The error limits are standard deviations calculated for 10 replicates.

	Sb(III)	Sb(V)
10 mM EDTA	1.03 ± 0.11	1.03 ± 0.05
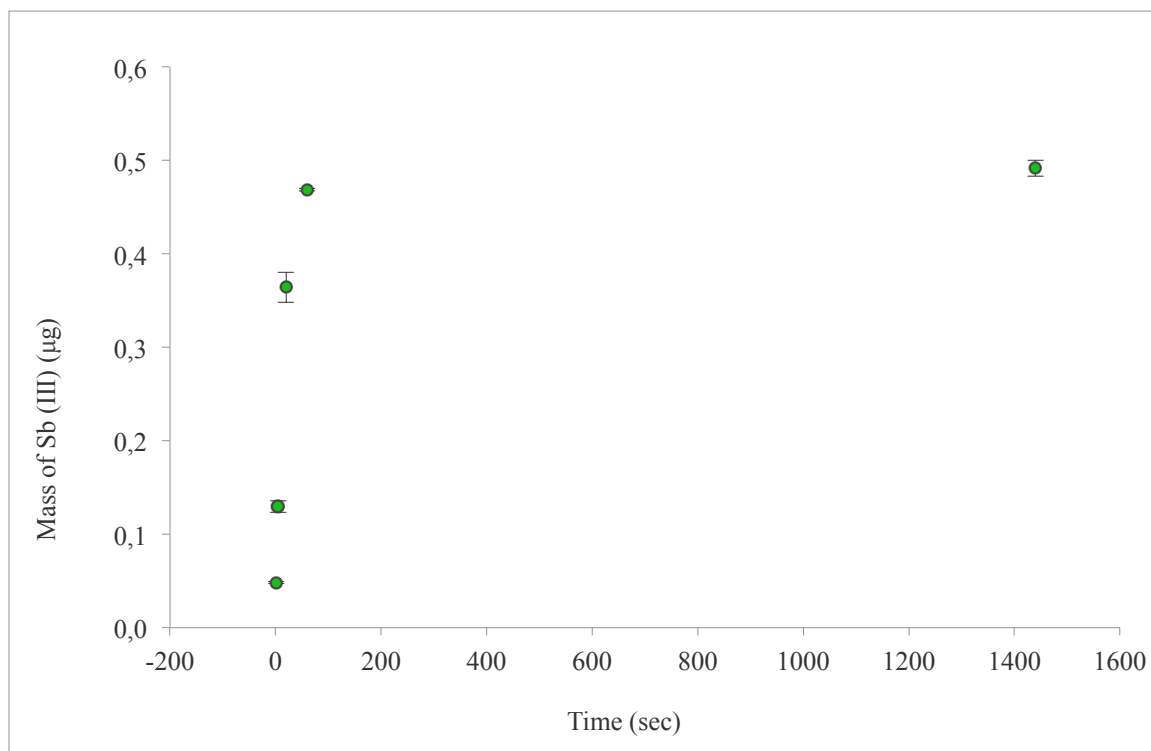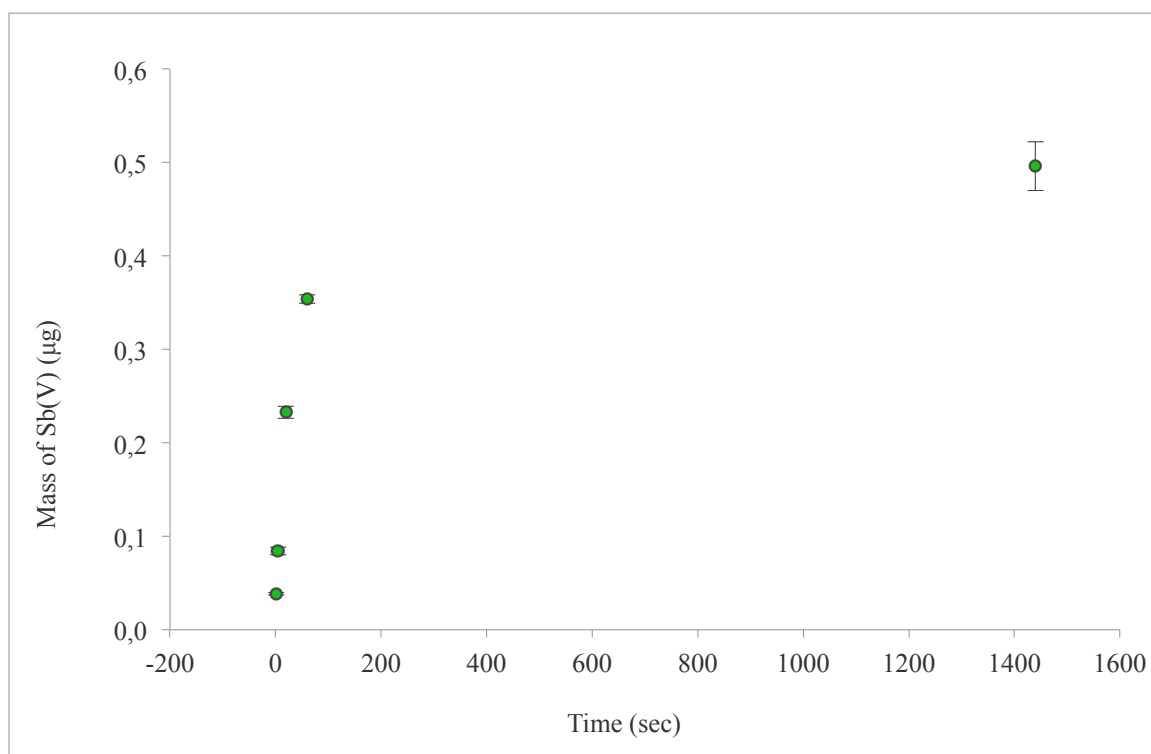
Fig. 4.2. Mass of Sb(III) and Sb(V) accumulated by Fe-oxide gels placed in solutions containing 50 Sb for different times. Error bars are calculated from the standard deviation of replicates (n=3).

Testing DGT performance in laboratory

To be sure that procedure antimony species extraction was correct and reproducible, a solution of Sb(V) was used in the recovery test. Before starting the test, antimony solutions were prepared and left to equilibrate overnight. The C_{DGT} were calculated with application of only diffusion coefficient of Sb(V) ($5.46*10^{-6}$ cm^2 s^{-1}) in the diffusive gel, founded by Luo et al. (2010) using DGT devices. During the performance test of DGTs, FH gel discs were exposed directly to a solution of Sb(V) with a known concentration (pH 5 and 0.01 M NaNO$_3$) and after 24 h on the stirrer, about 100% of Sb(V) was recovered by the adsorbent (Tab. 4.5).

Table 4.5. Concentrations of Sb(V) with the use of FH DGT.

	C_{DGT} (ng cm^{-3})	SD
Sb(V)	10.53	± 0.40
Inorg Sb in solution	11.46*	± 0.93
Recovery %	92 %	

* Concentration of Inoganic Sb in solution (ng mL^{-1})

Measurement of As Diffusion Coefficients using DGT devices

The Sb(V) and Sb(III) diffusion coefficients, measured using DGT devices, are presented in Table 4.6.

Table 4.6. Diffusion coefficients 10^{-6} cm^2 s^{-1} measured using DGT devices at pH 5. Valid at 24 ± 0.5 °C. Uncertainty is the S.D. of the mean from n replicate determinations.

| Sb species | Diffusion coefficient (D) (10^{-6} cm^2 s^{-1}) | |
	DGT devices	Literature
Sb(III)	$7.60 \pm 0,05$	
Sb(V)	$5.23 \pm 0,02$	5.46 (Luo et al. 2010)

The diffusion coefficients of inorganic species, based on slope in linear plot, were determined on DGT devices method with application of Eq. 2. The results of DGT devices for Sb(III) and Sb(V) are illustrated in Fig. 4.3 a & b.
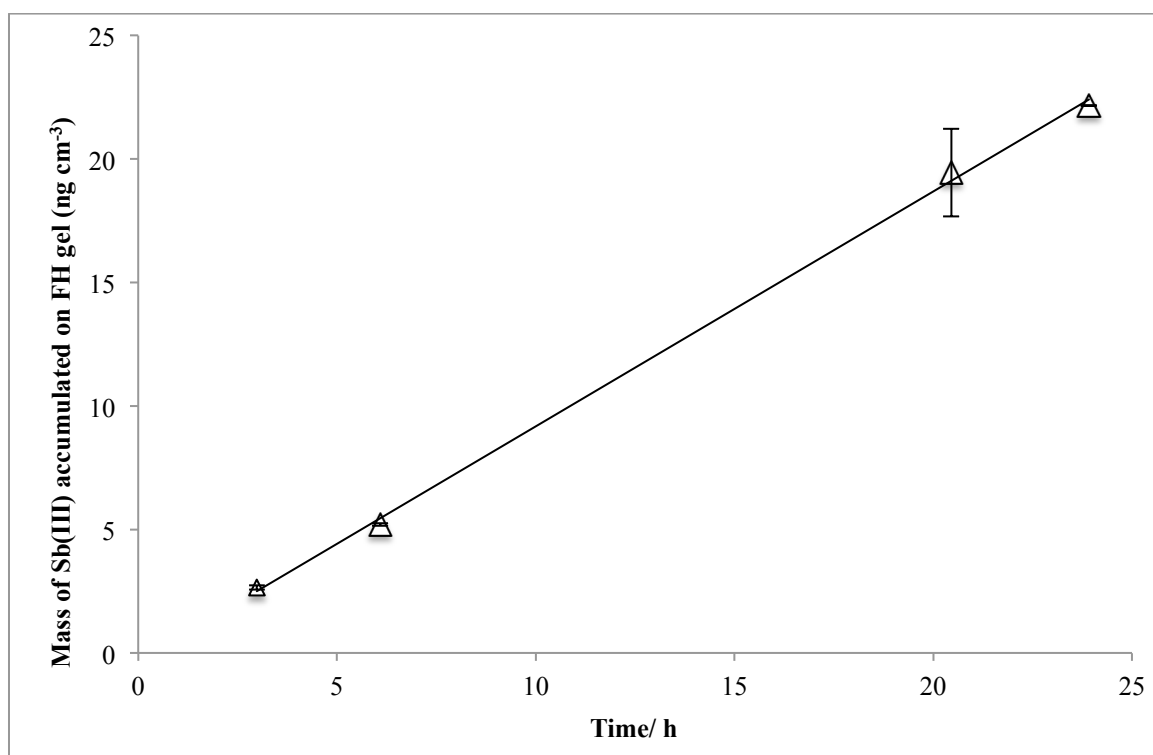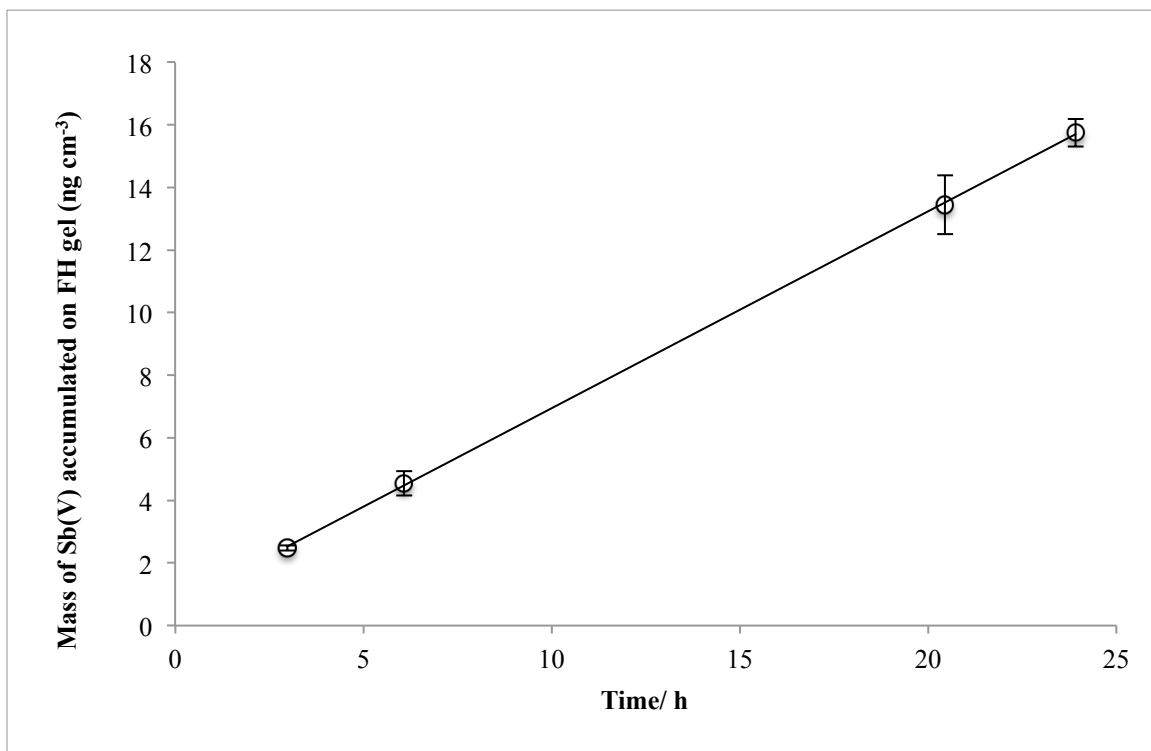
Fig. 4.3. Plots of measured mass of Sb(III) (a) and Sb(V) (b) vs. time.
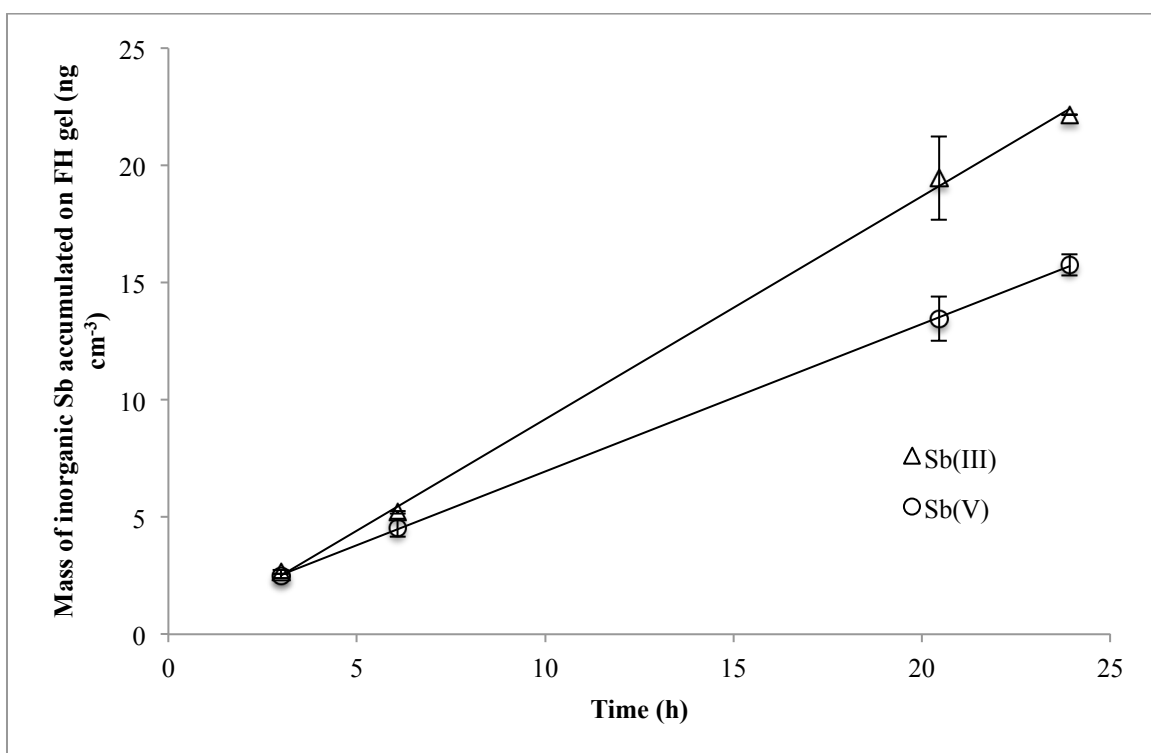
Fig. 4.4. Plots of measured mass of inorganic species of antimony.

The diffusion coefficient of Sb(V) is ~ 30 % lower than the diffusion coefficient of Sb(III) (Fig. 4.4).

Antimony exists primarily as Sb(V) in oxic systems and Sb(III) in anoxic systems. Both Sb(III) and Sb(V) ions hydrolyse easily in aqueous solution, thus making it difficult to keep antimony ions stable in solution except in highly acidic media. In the pH range of 2 to 11, Sb(III) should form a neutral complex, $Sb(OH)_3$, whereas Sb(V) should exist as a negatively charged complex, $Sb(OH)_6^-$ (Filella et al. 2002, Buschmann and Sigg 2004, Watkins et al. 2006).

Ionic Strength and pH

The ratio of DGT measured to solution concentrations of Sb(III) and Sb(V) was not very reproducible for deployment solutions without added $NaNO_3$ (Fig. 4.5). The measured ratios were within the acceptable limits (1.0 ± 0.1) for DGT with 0.01M.
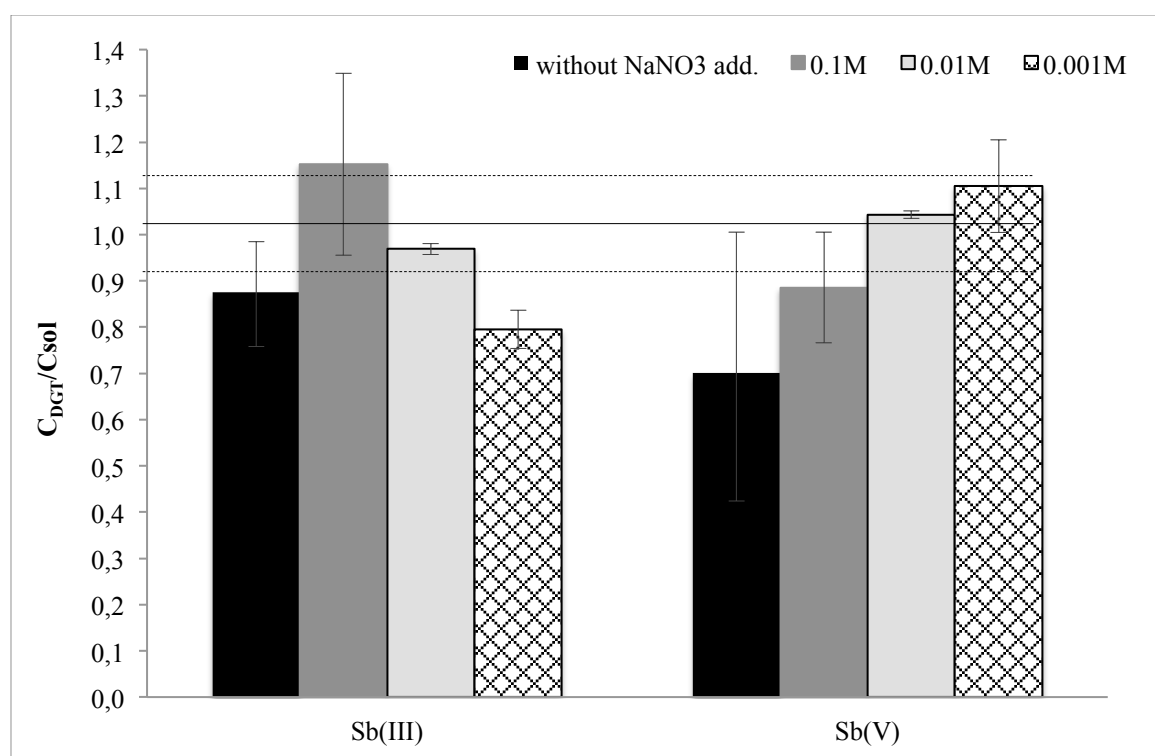
Fig. 4.5. Effect of concentration of supporting electrolyte, $NaNO_3$, on the ratio of concentrations measured by DGT containing Fe-oxide gels, C_{DGT}, to deployment solution concentrations of Sb(III) and Sb(V), C_{sol}. Error bars represent the standard deviation of three replicates. The solid horizontal line and dotted horizontal lines represent target values of 1 ± 0.1.

The effect of pH on the ratio of concentrations of Sb(III) and Sb(V) measured by DGT to those in solution for Fe-oxide gels is shown in Fig. 4.6. The overall performance of devices with the two different species was similar. For pH in the range of 4.98 - 9.01, the ratio for each chemical forms was between 0.9 and 1.1, which is acceptable performance for DGT measurements.

The proportion of Sb(III) measured by DGT at pH 7,17 is less than 1 (Fig. 4.6).

As the ferrihydrite surface is positively charged below pH 5.5, the binding of positively charged species can be expected to be weak. When the charge on the surface of ferrihydrite gels becomes negative, there is likely to be much weaker adsorption of oxyanions, as explained in the previous section.

At higher pH, measurements of Sb, expecially Sb(V), by DGT agreed well with the solution concentrations. The same behaviour was observed in Luo et al. 2010, where the acceptable measurement of Sb(V) at pH 7.82 indicates their reasonably strong adsorption to PF (precipitated ferrihydrite) gels, consistent with a positively charged oxide surface, albeit of lesser magnitude (Luo et al. 2010).
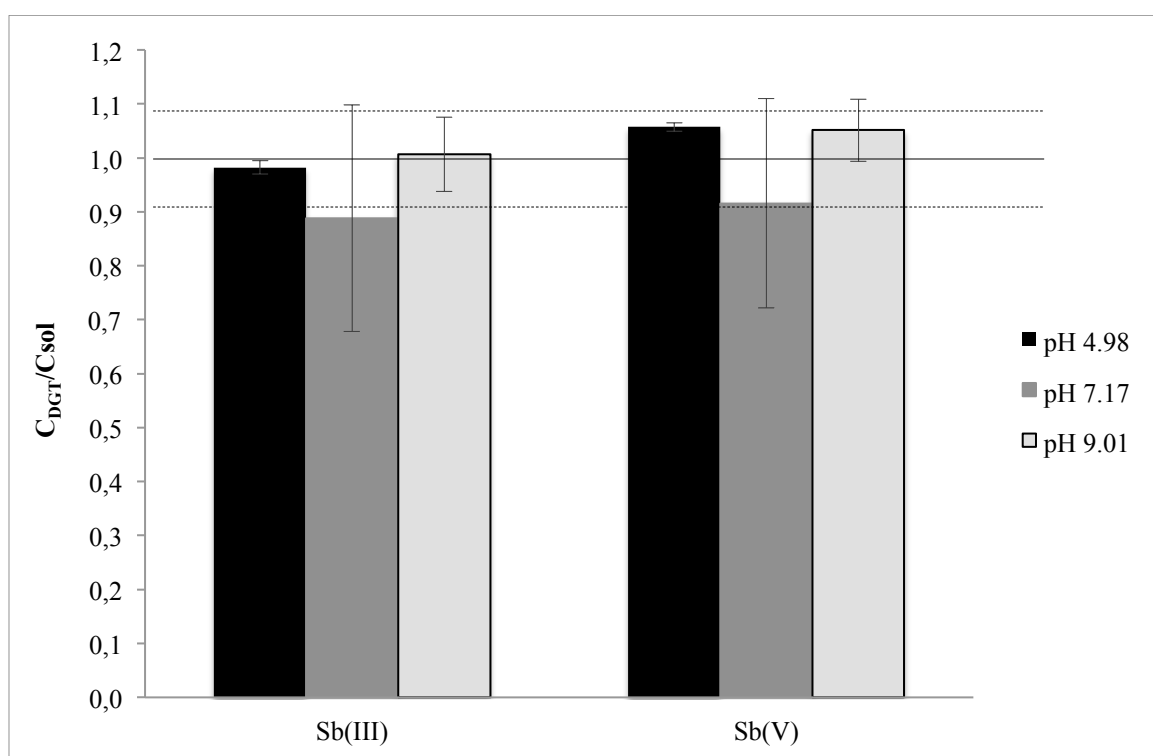
Fig. 4.6. Effect of pH on the ratio of concentrations of Sb(III) and Sb(V), measured by DGT, C_{DGT}, to deployment solution concentrations, C_{sol}. These measurements were performed in the presence of 0,01 M $NaNO_3$. Error bars represent the standard deviation of three replicates. The solid horizontal line and dotted horizontal lines represent target values of 1 ± 0.1.

CONCLUSIONS

This chapter reports the application of HPLC species-unspecific spike isotope dilution ICP-MS to separate and quantify inorganic Sb species in aqueous solution with use of Diffusive Gradient in Thin Films. The Fe-oxide gel has already been used in DGT to provide, with analysis by HPLC-ICP-MS, the possibility to accumulate and determine the inorganic specie, Sb(V), but also Sb(III). The measured value for the diffusion coefficient of Sb(V) in the hydrogel at pH 5 of $5.23 \pm 0.02 \times 10^{-6}$ cm^2 s^{-1} shows sufficient agreement with other measurement in previous work.

However, measured value of $7.60 \pm 0.05 \times 10^{-6}$ for Sb(III), is, as yet, uncorroborated by other workers. The antimony species could be measured quantitatively throughout the pH range tested of 4.98-9.01 without diminution of performance.

The combination of HPLC-ICP-MS, as analytical technique, Fe-oxide DGT, as adsorption system in aqueous solution, and EDTA, for preservation of different species represent an excellent point of departure for antimony investigation in environmental.

Future applications could include the study and the identification of methylated antimony species in some environmental compartments because little information is known about these chemistry solutions. Moreover information on antimony interactions with natural organic matter is very scarse and does not allow any rigorous conclusion to be drawn regarding its role in antimony fate in natural aquatic systems.

REFERENCES

Al-Sibaai AA,and AG Fogg. 1973. Stability of dilute standard solutions of antimony, arsenic, iron and rhenium used in colorimetry. *Analyst.* 98, 732–738.

Andreae MO, Asmode ́ JF, Foster P and L Van't dack. 1981. Determination of antimony(III), antimony(V), and methylanti- mony species in natural waters by atomic absorption spectrometry with hydride generation. *Anal. Chem.* 53, 1766–1771.

Council of European Union, Council Directive 98/83/EC of 3 November 1998, Quality of Water Intended for Human Consumption. *Off. J.* L 330, 32–54.

de la Calle Guntiñas MB, Madrid Y and C Cámara. 1992. Stability study of total antimony, Sb(III) and Sb(V) at the trace level. Fresenius J. Anal. Chem. 344, 27–29.

Dodd M, Grundy SL, Reimer KJ and WR Cullen. 1992. Methylated antimony(V) compounds: Synthesis, hydride generation properties and implications for aquatic speciation. *Appl. Organomet. Chem.* 6, 207.

Filella M, Belzile N and Y Chen. 2002. Antimony in the environment: a review focused on natural waters. I. Occurrence. Earth- Sci. Rev. 57, 125–176. Iffland R. 1988. Antimony, in: Handbooks on Toxicity of Inorganic Com- pounds, Marcel Dekker, New York, (Chapter 7), p. 67.

Gomez Ariza JL, Morales E, Sanchez-Rodas D and I Giraldes. 2000. Stability of chemical species in environmental matrices. *Trends Anal. Chem.* 19, 200.

Hill S, Bloxham M and P Worsfold. 1993. Chromatography coupled with inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry. A review. *J Anal Atomic Spectrom.* 8:499–515.

Kolpakova NN. 1982. Laboratory and field studies of ionic equilibria in the Sb2S3–H2O–H2S system. *Geochem. Int.* 19, 46–54.

Krachler M and H Emons. 2000. Urinary antimony speciation by HPLC-ICP-MS. *J. Anal. At. Spectrom.* 15, 281.

Krachler M, H. Emons. 2001. Speciation analysis of antimony by high-performance liquid chromatography inductively coupled plasma mass spectrometry using ultrasonic nebulization. *Anal. Chim. Acta.* 429, 125.

Krachler M and H Emons. 2001. Speciation of antimony for the 21st century: promises and pitfalls. *Anal. Chim. Acta* 429, 125.

Lindemann T, Prange A, Dannecker W and B Neidhart. 2000. Stability studies of arsenic, selenium, antimony and tellurium species in water, urine, fish and soil extracts using HPLC/ICP-MS. *J. Anal. Chem.* 368, 214.

Lintschinger J, Koch I, Serves S, Feldmann J and WR Cullen. 1997. Determination of antimony species with high-performance liquid chromatography using element specific detection. *J. Anal. Chem.* 359, 484.

Lintschinger J, Schramel O and A Kettrup. 1998. The analysis of antimony species by using ESI-MS and HPLC-ICP-MSJ. *Anal. Chem.* 361, 96.

Miravet R, Lopez-Sanchez JF and R Rubio. 2004. New considerations about the separation and quantification of antimony species by ion chromatography. *J. Anal. Chromatogr.* 1052, 121.

Pauling L. 1933. The formulas of antimonic acid and the antimonates. *J. Am. Chem. Soc.* 55, 1895–1900.

Ritchie JA. 1961. Arsenic and antimony in some New Zealand thermal waters. *N. Z. J. Sci.* 4, 218–229.

Sayago A, Beltran R and JL Gomez-Ariza. 2000. Hydride generation atomic fluorescence spectrometry (HG-AFS) as a sensitive detector for Sb(III) and Sb(V) speciation in water. *J. Anal. At. Spectrom.* 15, 423.

Stauffer RE and JM Thompson. 1984. Arsenic and antimony in geothermal waters of Yellowstone National Park, Wyoming, USA. *Geochim. Cosmochim. Acta.* 48, 2547–2561.

United States Environmental Protection Agency USEPA, Water Related fate of the 129 Priority Pollutants, vol. 1,

Washington, DC, 1979.

Weissberg BG, Browne PRL and TM Seward. 1979. Ore metals in active geothermal systems. In: Barnes, H.L. (Ed.), Geochem- istry of Hydrothermal Ore Deposits. Wiley, New York, pp. 738 – 780.

Zhang X, Cornelis R and L Mees. 1998. Speciation of antimony(III) and antimony(V) species by using high-performance liquid chromatography coupled to hydride generation atomic absorption spectrometry. *J. Anal. At. Spectrom*. 13, 205.

Zheng J, Lijima A and N Furuta. 2001. Complexation effect of antimony compounds with citric acid and its application to the speciation of antimony(III) and antimony(V) using HPLC-ICP-MS. *J. Anal. At. Spectrom.* 16, 812.

Zheng J, Ohata M and N Furuta. 2000. Antimony Speciation in Environmental Samples by Using High-Performance Liquid Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry. *Anal. Sci.* 16, 75.

Zheng J, Ohata M and N Furuta. 2000. Studies on the speciation of inorganic and organic antimony compounds in airborne particulate matter by HPLC-ICP-MS. *Analyst*. 125, 1025.

Chapter V. Survey of Total Arsenic and Arsenic Species in Italian Rice

ABSTRACT

Arsenic toxicity depends on the chemical form. Inorganic arsenic is more toxic than organic arsenic and trivalentarsenite is more toxic than pentavalent and zero-valent arsenic. Generally rice, unlike food products of terrestrial origin, contains significant amounts of inorganic arsenic. Recently some Government Organizations (e.g. EFSA) debated the possibility to set an upper limit for total and inorganic arsenic in rice.

Arsenic speciation was realized in 70 Italian rice samples from different representative cultivation conditions. The adopted method was effective in preserving the arsenic species and suitable for routine analysis of large numbers of samples. The HPLC-ICP-MS technique was used to measure the different arsenic species [arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V))], which were separated using an anion-exchange column. The sum of As(III) and As(V) is known as inorganic arsenic. Total As concentration in samples was determined directly by ICP–MS. Certified reference material, NIST 1568a rice flour and IMEP 107, for total and inorganic As in rice, were included for quality assurance.

The most abundant species in rice were As(III) and DMA(V). Total arsenic levels in the 70 Italian rice samples averaged 0.16 $\mu g\ g^{-1}$ (range 0.06-0.60 $\mu g\ g^{-1}$); inorganic arsenic averaged 0.10 $\mu g\ g^{-1}$ (range 0.04-0.16 $\mu g\ g^{-1}$). The percentage of inorganic arsenic tended to decrease with increasing the total arsenic.

Keywords: Arsenic speciation, rice, nitric acid extraction, inorganic arsenic, organic arsenic.

INTRODUCTION

For many years contaminated drinking water has been assigned as the main source for human exposure to inorganic arsenic, a causal agent for various cancers (Chen et al. 1992) and cardiovascular disease (Chen et al., 2011). More recently, however, rice and rice-based products have been identified as significant dietary sources of inorganic arsenic (Meharg et al., 2008; Signes-Pastor et al., 2009). For populations not exposed to arsenic-contaminated drinking water, food is now considered the major contributor to the intake of inorganic arsenic (EFSA, 2009). Rice, compared to other staple cereal crops, is an important foodstuff for 3 billion people and it is the

most important exposure route for arsenic (As), a non-threshold class 1 carcinogen. Foods, as opposed to drinking water, may contain several As species with different levels of toxicity. The toxicity of As depends not only on total concentrations, but also on its chemical forms. In general, the toxicity of As increases with decreasing oxidation states. In general, inorganic As species are more toxic than organic ones. Arsenite (As(III)) has higher toxic effects than arsenate (As(V)). Speciation of As in rice grain were usually categorised as inorganic and organic As (Heitkemper et al. 2001; Williams et al., 2005).

Little information about speciation of As(III) and As(V) in rice is currently available in the literature. Therefore, due to large differences of toxicity among As(III), As(V) and organic As, accurate As speciation is indispensable to assess the impact of As toxicity in rice on human health. However, since the toxicity of As(III) is higher than As(V) to human, comprehensive understands of the potential As toxicity in rice grain require analytical methods capable of distinguishing As(III) from As(V).

A successful extraction is necessary to break As(III)-thiolate complexes and avoid any As redox transformation caused by an extraction matrix, e.g. extractants and thiolate compounds released from rice grains. A substantial time length seems to be necessary to break As(III)-thiolate complexes with dilute HNO_3 (Heitkemper, 2001). Nitric acid was the most suitable extractant for identification of different species in Arsenic in rice. The extraction with H_2O_2 usually led to complete oxidation of As (III) (Huang et al. 2012). Monomethylarsonic acid and dimethylarsinic acid were affected very little during most extraction procedures.

The aim of the present work was to apply recent extraction method for As speciation in rice grain (Huang et al. 2010). The increasing concern about dietary intake of inorganic As, combined with probable future legislative regulations, highlights the need for a selective, sensitive and robust method for determining the inorganic As content of foods. In our study, we investigated different types of rice grains from different areas of Northern Italy. Our data showed the influences of As(III) and DMA(V) on the As concentration in rice grain and the relevance of As(III) and As(V) speciation for assessing As toxicity to human health.

MATERIALS AND METHODS

Reagents and materials

Stock solutions of arsenic species (1000 mg L^{-1} of As) were prepared by As_2O_3 (ArsenicIII Oxide) and $Na_2HAsO_4*7H_2O$ (sodium arsenate dibasic heptahydrate) and of DMA(V) and MMA(V) from dimethylarsinic acid ((CH_3)$_2$As(O)OH) and disodium methylarsenate ($Na_2CH_3AsO_3$).

The standard solutions of arsenic species were obtained by diluting the corresponding stock solutions.

Standard solutions of total arsenic were prepared by dilution of a multielement standard (100 mg L[1]) obtained from CPI International (Amsterdam, The Netherlands).

Ultra-pure water was prepared by a Milli-Q system (18MΩ cm resistance, Millipore® system, Millipore, Bedford, MA). Nitric acid in analytical grade was obtained from Carlo Erba Reagents.

Sample preparation

The white rice grain samples were milled with a blender, which was cleaned after milling of each sample to avoid cross-contamination. The resulting milling temperature was never high, thus the influence of milling on As speciation was estimated negligible due to the low milling temperature and high stability of As species in rice grain (Huang et al. 2010).

For digestion, approx. 1.5 g of pulverized rice grains were mineralized using HNO_3 0.28 M at 95°C for 90 min in a heating block system (DIGIPREP) in 50 ml polypropylene tubes (digiTUBES, SCP Science). The digested grain solutions were filtered by using 0.45 μm filter (digiFILTER, SCP Science) after appropriate dilution with Milli- Q Water.

The extraction method allows as recovery for quantitative analyses and preserves As(III) and As(V) during extraction (Huang et al. 2010).

Certificated standard materials were used to ensure the precision of analytical procedure (NIST 1568a rice flour and IMEP 107, for total and inorganic As in rice) and they were obtained as fine powders and used as experimental standards without further treatments.

Instrumentation

The concentration of Total Arsenic in rice grain were determined by Inductively Coupled Plasma Mass Spectrometer (Agilent 7700x, Agilent Technologies, USA) with Octopole Reaction System (ORS system).

Speciation of arsenic species was performed by HPLC on an anion exchange column with a mobile phase (1 mL min^{-1}). The mobile phase was made of 13.2 mM $NH_4H_2PO_4$ at pH 6. The ion intensity at m/z 75 ($^{75}As^+$) was monitored without reaction mode with carrier gas (Argon) flow rate of 0.95 L min^{-1}. Clorine ($^{35}Cl^+$) was also monitored because chlorine matrices lead to $^{40}Ar^{35}Cl^+$ interferences. Arsenic speciation in extracts was conducted immediately after extraction. Identification of As species was confirmed by spiking real extracts with a mixture of standard solutions.

Figure 5.1 shows the mass chromatogram for the separation of As(III), DMA(V), MMA(V) and As(V) standards in aqueous solution.

Statistics

We computed correlations between total grain As and the concentrations and proportion of As species to test the dependence of As species on each other in rice grain. Furthermore, we calculated correlations between the concentrations and proportions of each As species with the concentrations of other trace element, like Cd, in rice grain. T-test was performed to check if our results of As speciation and total grain As in NIST-CRM-1568a and IMEP-107 differed significantly from the certificated values and the values in literatures.
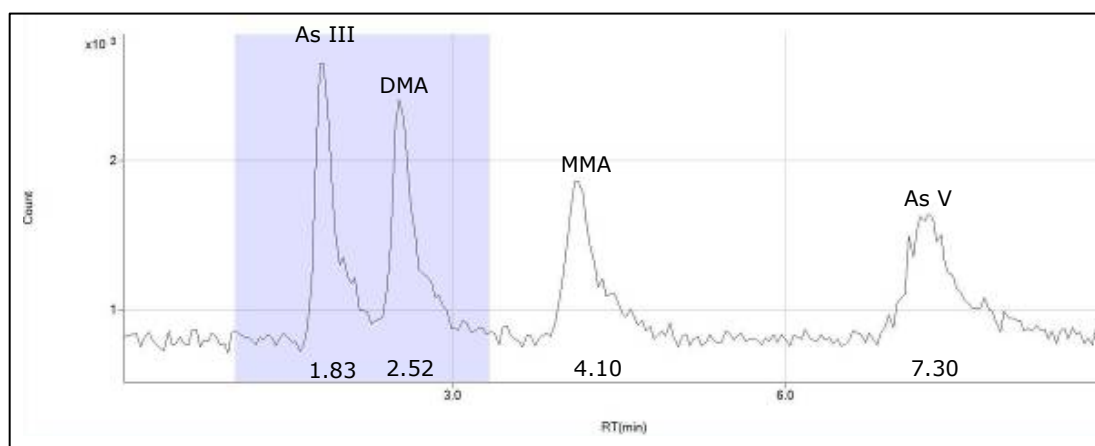
Fig. 5.1 HPLC-ICP-MS chromatogram of inorganic As species (As(III) and As(V)) and organic species (DMA(V) and MMA(V)) with different retention time (min).

RESULTS AND DISCUSSION

The extraction method based on 0.28 M nitric acid at 95°C (Huang 2010) has been verified with 70 samples of different type of rice grain (Aiace, Arborio, Augusto, Balilla, Brio, Carnaroli, Centauro, Creso, Ellebi, Galileo, Gladio, Karnak. Libero, Noto, Nembo, Roma, S.Andrea, Scudo, Selenio, Ulisse, Volano) from different areas of North of Italy (West and East Ticino River of Pavia, Milano – Lodi, Novara, Vercelli). We have also examined the stability and recoveries of As species in two certified rice materials NIST CRM 1568a and IMEP 107.

Method validation with certified materials NIST CRM 1568a and IMEP 107.

To check whether our analytical results of As in rice agreed with SRM samples, the SRM rice samples were analysed with each batch of rice after undergoing the same digestion procedure as the samples. The observed values are in good agreement with the certified values of SRM samples (Tab. 5.1 and 5.2).

The rice flour NIST CRM 1568a is generally adopted as a standard for controlling the quality of applied analytical method (Guzmán Mar et al. 2009, Huang et al. 2010, Kolhmeyer et al. 2003, Narukawa et al. 2008, Sanz et al. 2005, Zhu Sun et al. 2008). The concentrations of As(III), As(V), MMA(V) and DMA(V) and Total As recovery determined from our and others extraction methods in the literature and are listed in Table 5.1. Our results determined in NIST CRM 1568a are 63 ± 10 µg As(III) kg^{-1}, 43 ± 1 µg As(V) kg^{-1}, 166 ± 10 µg DMA(V) kg^{-1} and under the MDL for MMA(V). The sum of As species was 272 ± 19 µg As kg^{-1}, which is in good agreement with the certified value (290 ± 30 µg As kg^{-1}, n = 5) (the accuracy test corresponds), with a recovery of 94 ± 6 %.

The total As, measured by ICP-MS, was 312 ± 11 µg kg^{-1}, which is in good agreement with the certified value (the accuracy test corresponds), with a recovery of 108 ± 4 %.

The range of concentrations of As(III), As(V), DMA(V) and MMA(V) in NIST CRM 1568a that were determined are 52-90, 21-45, 132-200 µg As kg^{-1}, respectively.

IMEP 107 was taken for the 7th inter-laboratory comparison of total and inorganic As in rice organised by the European Union-Reference Laboratory for heavy metals in feed and food. Only inorganic and total As concentrations were assigned by six and seven well established laboratories in IMEP 107 to be 107 ± 14 and 172 ± 18 µg As kg^{-1}, respectively. We determined the concentrations of inorganic and sum of As species in IMEP to be 104 ± 9 µg kg^{-1} and 160 ± 5 µg kg^{-1}, well agreeable with the certified values within uncertainty ranges (the accuracy test is respected for each results), with a recovery of 98 ± 9% and 93 ± 3% respectively. For the last type of material, the total As, measured by ICP MS, was 169 ± 8 µg kg^{-1}, with a recovery of 98 ± 5%.

Huang et al. (2012) published further values of As species in IMEP 107. They find 88-94 µg As(III) kg^{-1}, 8,5-9,7µg As(V) kg^{-1} and 48-51 µg DMA(V) kg^{-1} (n=2). In our case, we find similar results: 93 ± 12 µg As(III) kg^{-1}; 18 ± 8 µg As(V) kg^{-1}; 55 ± 5 µg DMA(V) kg^{-1}.

Table 5.1a. Comparison of the results (As speciation) of standard reference material for rice flour, NIST SRM 1568a from this work with those published by others.

Certified Values (µg kg⁻¹)	n	As(III) (µg kg⁻¹)	As(V) (µg kg⁻¹)	Total Inorganic As (µg kg⁻¹)	DMA(V) (µg kg⁻¹)	Species Sum (µg kg⁻¹)	Extraction efficiency (µg kg⁻¹)	% of DMA (V)	% of inorganic As	Reference
290 ± 30	3	63 ± 10	43 ± 1	106 ± 9	166 ± 10	272 ± 19	94	61	39	This study
	16			80 ± 14	160 ± 24	242 ± 40	83 ± 12	54	26	Williams et al. (2005)
	6			97 ± 10	132 ± 10	245 ± 10	84 ± 3	51 ± 3	33 ± 2	Williams et al. (2006)
	3	77 2			163 ± 32	240	83	56	26	Smith et al. (2006)
	3	90 ± 4	22 ± 1	112	162 ± 10	285	98	56	38	Ohno et al. (2007)
	3	68 ± 4	21 ± 1	89	135 ± 4	232 ± 5	80	47	31	Sanz et al. (2007)
	3	52 ± 1	44 ± 2	96 ± 2	173 ± 2	281 ± 2	96 ± 1			Narukawa et al. (2008)
	-	53 ± 1	45 ± 1	98 ± 1	175 ± 2	286 ± 3	99			Narukawa et al. (2010)
	4			91 ± 11	166 ±13	257 ± 6	89	57	31	Rahman et al. (2011)
	3	74.4 ± 0.09	35.1 ± 0.9		158 ± 2	278 ± 3	93			Tsai et al. (2011)
	3			79 ± 7	200 ± 12	280 ± 3	94			Raber et al. (2012)
	5	74 ± 7	30 ± 3	104.4 ± 7	165 ± 7	285 ± 10	98 ± 3			Huang et al. (2012)

Table 5.1b. Comparison of the results (As speciation) of standard reference material for rice grain, IMEP 107 (JRC Scientific and Technical Reports), from this work with those published by others.

Certified Values ($\mu g\ kg^{-1}$)		n	As(III) ($\mu g\ kg^{-1}$)	As(V) ($\mu g\ kg^{-1}$)	Total Inorganic As ($\mu g\ kg^{-1}$)	DM (V) ($\mu g\ kg^{-1}$)	Species Sum ($\mu g\ kg^{-1}$)	Extraction efficiency ($\mu g\ kg^{-1}$)	% of DMA (V)	% of inorganic As	Reference
Tot As	Inorganic As										
172 ± 18	107 ± 14	3	93 ± 12	18	104 ± 9	55 ± 5	160 ± 5	93	35	65	This study
		2	91	9	100	50	151	88			Huang et al. (2012)

Speciation of arsenite and arsenate in rice grain

The total Arsenic content ranged in our rice grain from 56 to 586 µg kg^{-1}. The mean As concentration in the 70 Italian rice samples was 164 µg kg^{-1}; inorganic arsenic averaged 0.10 µg g^{-1} (range 39 - 158 µg kg^{-1}).

The most abundant species in rice were As(III) and DMA(V). The portion of As(III) and DMA(V) has a mean value of 64 % and 31 %, respectively.

Conversely, As(V) is usually the minor component with an average of 9 % of total grain As.

Arsenite is the predominate inorganic As in all rice samples and accounts for an average of 92 % of inorganic As.

The predominance of As(III) in rice in past studies is generally in agreement with our finding.

In the earlier studies, ranges of the proportion of As(III) in inorganic As were found 74-93% (n=3) (Guzmán Mar et al. 2009), 90-100% (n=3) (Sanz et al. 2005) and 61-80% (n=6) (Narukawa and Chiba, 2010). The recovery of total grain As in Guzmán Mar et al. (2009) and Sanz et al. (2005) were all ≥ 90%. In comparison, total arsenic recoveries in Zhu et al. (2008) were low in average, probably leading to lower As(III) to inorganic As ratios than what found in other studies.

The predominance of As(III) in rice grain can be supported by plenty of information from the literature.

In the porewater of paddy soil, As(III) is usually the dominat arsenic species expecially during the flooded irrigation period (Takahashi et al. 2004). Translocation of As(III) from porewater to rice plant via nodulin 26-like intrinsic proteins aquaporin in one of the most important pathways for As to enter rice plants (Ma et al. 2008).

Dimethylarsinic acid is often the second important component (Fig. 5.3). The relevance of DMA(V) is manifested by its presence in around 30% of total grain As on average. In some cases, DMA(V) became the dominant As species.

The relationship between total rice arsenic and inorganic arsenic levels in rice revealed that inorganic arsenic levels increased linearly between 200 and 300 µg kg^{-1} of total arsenic, and then plateauded at an inorganic arsenic concentration of around 140 µg kg^{-1}. Although the plot of total arsenic against percentage inorganic arsenic showed more scatter there was a trend that in rice samples with high total arsenic concentrations the percentage inorganic arsenic content decreases (Fig. 5.2). This is in agreement with findings for baby rice samples in Meharg et al. (2008).
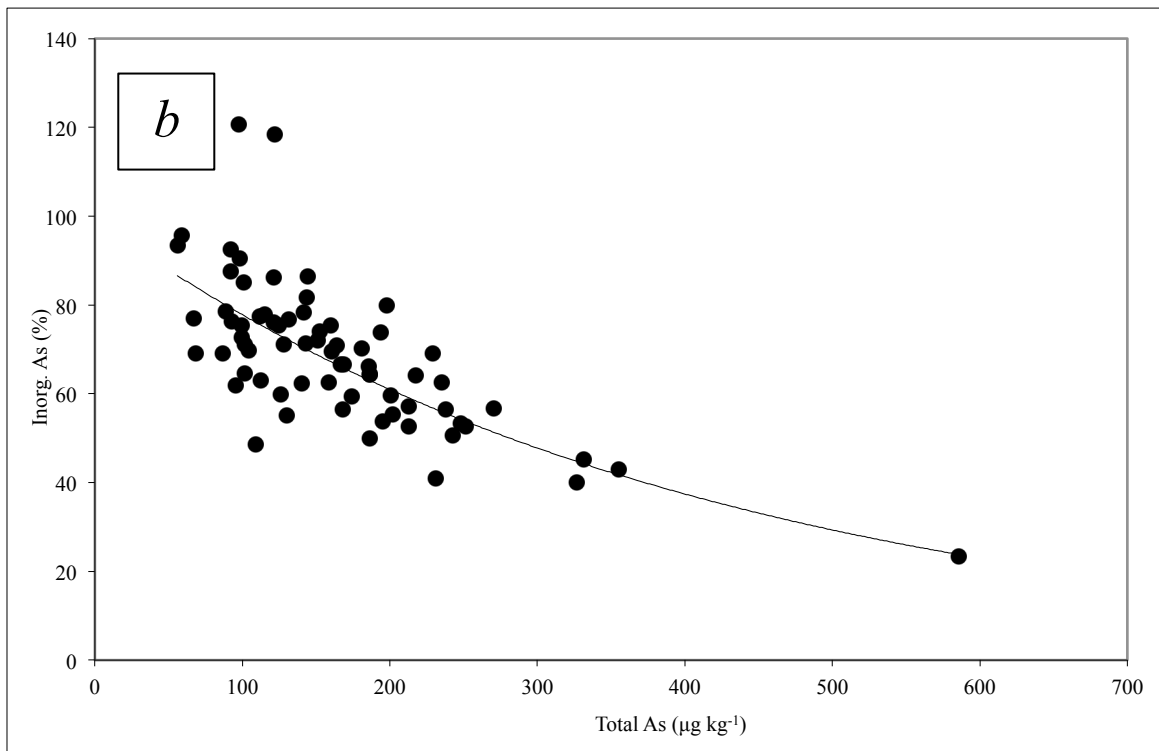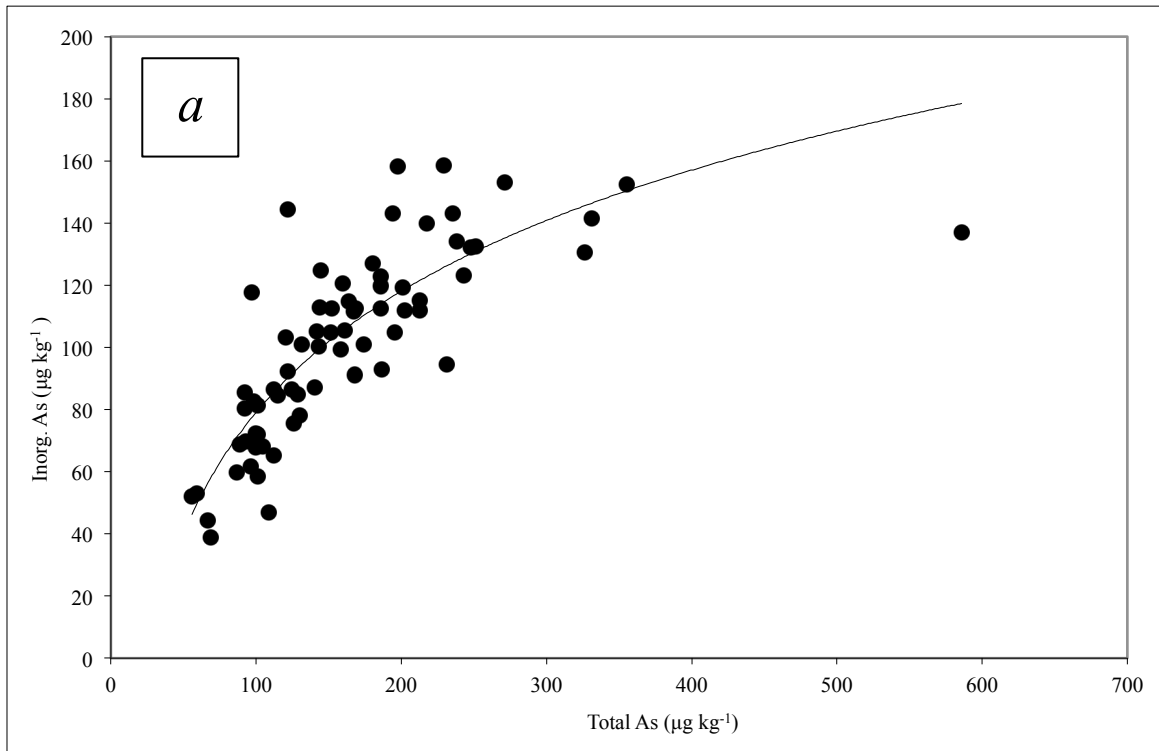
Fig. 5.2. Relationship between inorganic arsenic content (*a*) and percentage inorganic arsenic content (*b*) against total arsenic content in rice grains. (A: $y_A = 56.235 Ln(x) - 179.89$, $R^2 = 0.6842$; B: $y_B = 99.286 e^{-0.0024x}$, $R^2 = 0.6319$)

Zavala et al. (2008) and Huang et al. (2012) categorised rice into DMA (DMA > inorganic As concentrations) and inorganic As types (inorganic As > DMA concentrations). Similar categorisation is applicable to the data obtained from our rice samples by plotting As(III), DMA(V)

109

and As(V) concentrations against total grain As concentrations (Fig. 5.4). The values of MMA(V) are not reported in the graph because less than MDL.

The inorganic As rice type can be regarded as the As(III) type due to the high predominance of As(III) in inorganic fraction.

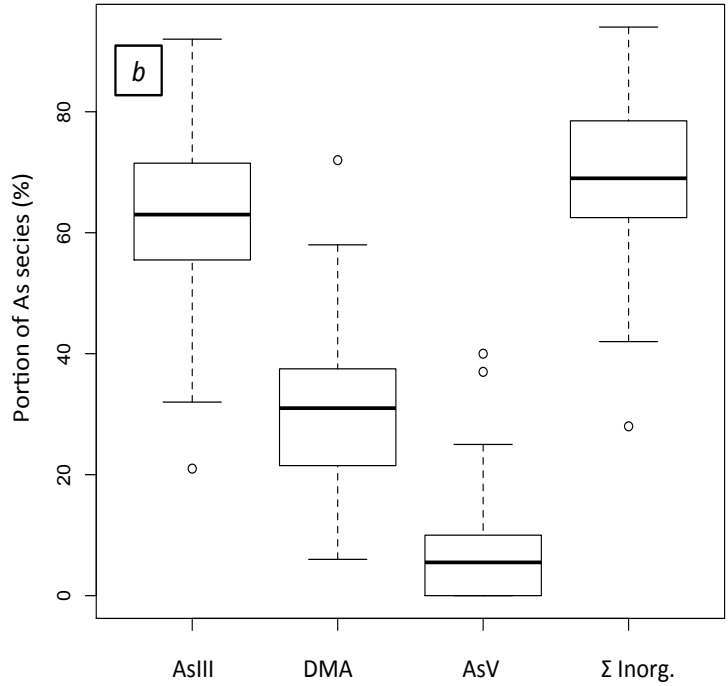The concentrations of DMA(V) increased more strongly with increasing total grain As concentrations in the DMA(V) than the As(III) type rice. Conversely, the concentrations of As(III) and As(V) increased more strongly with increasing total grain As concentrations in the As(III) than the DMA(V) type rice.
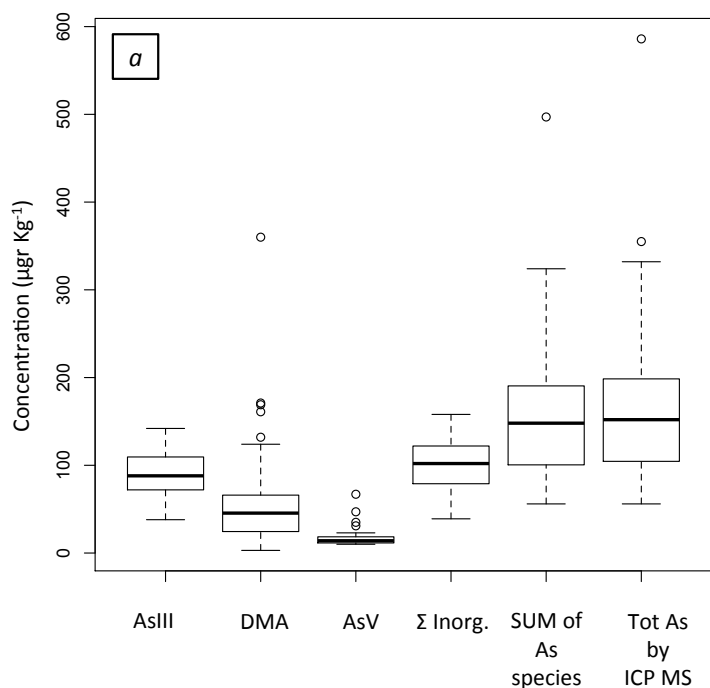
Fig. 5.3. Distribution of concentrations (*a*) and proportions (*b*) of each arsenic species in rice grain. The box represents data between 25th and 75th percentages. The Whiskers (error bars) above and below the box indicate the 95th and 5th percentiles, and spots above and below them represent outliers. The square inside the box represents the median and the dash line represents the mean value. The sample number is 70.
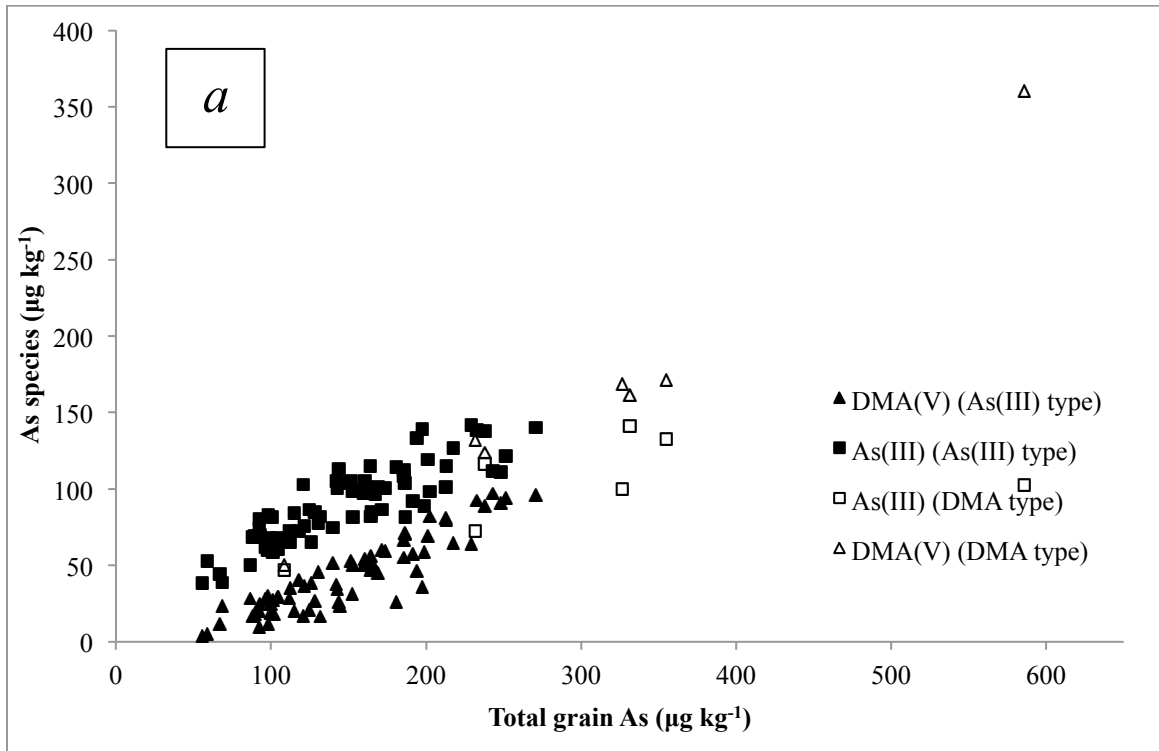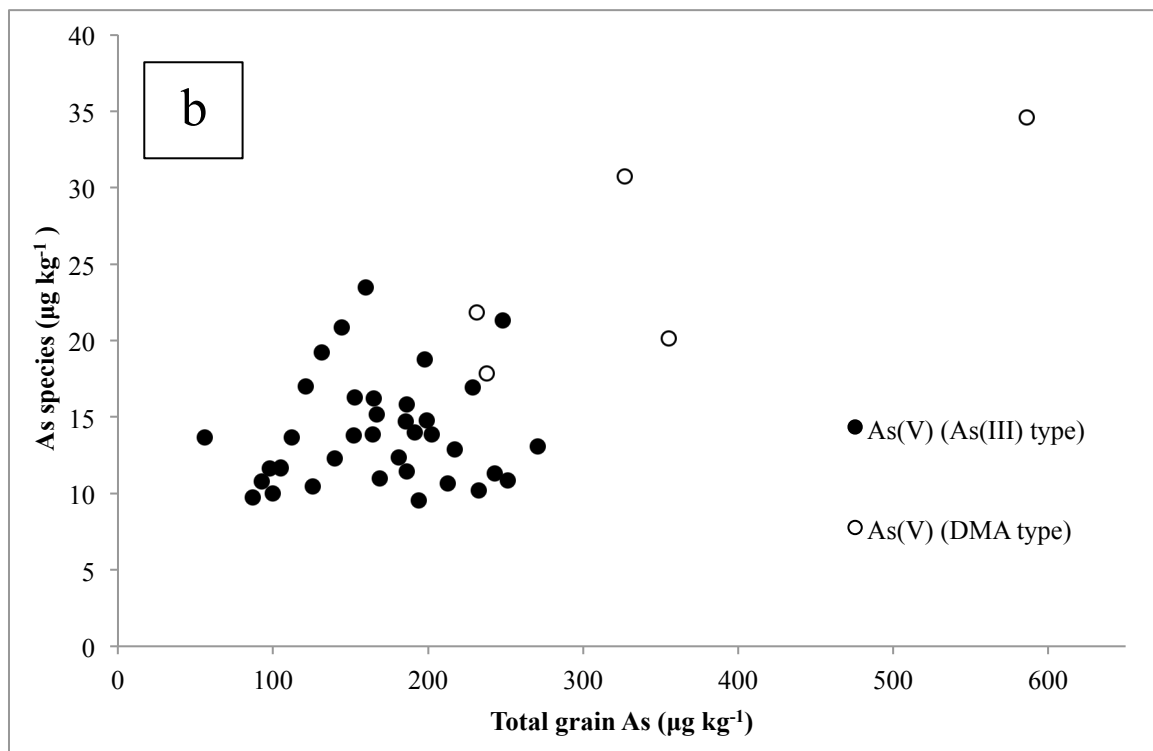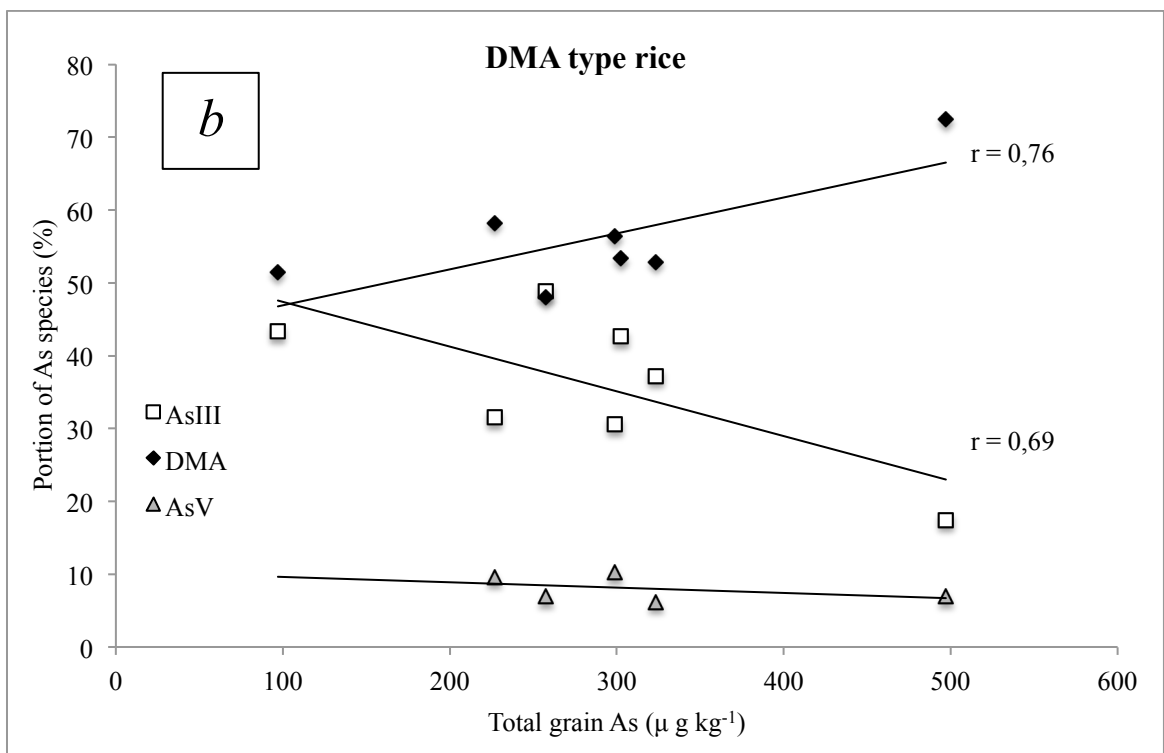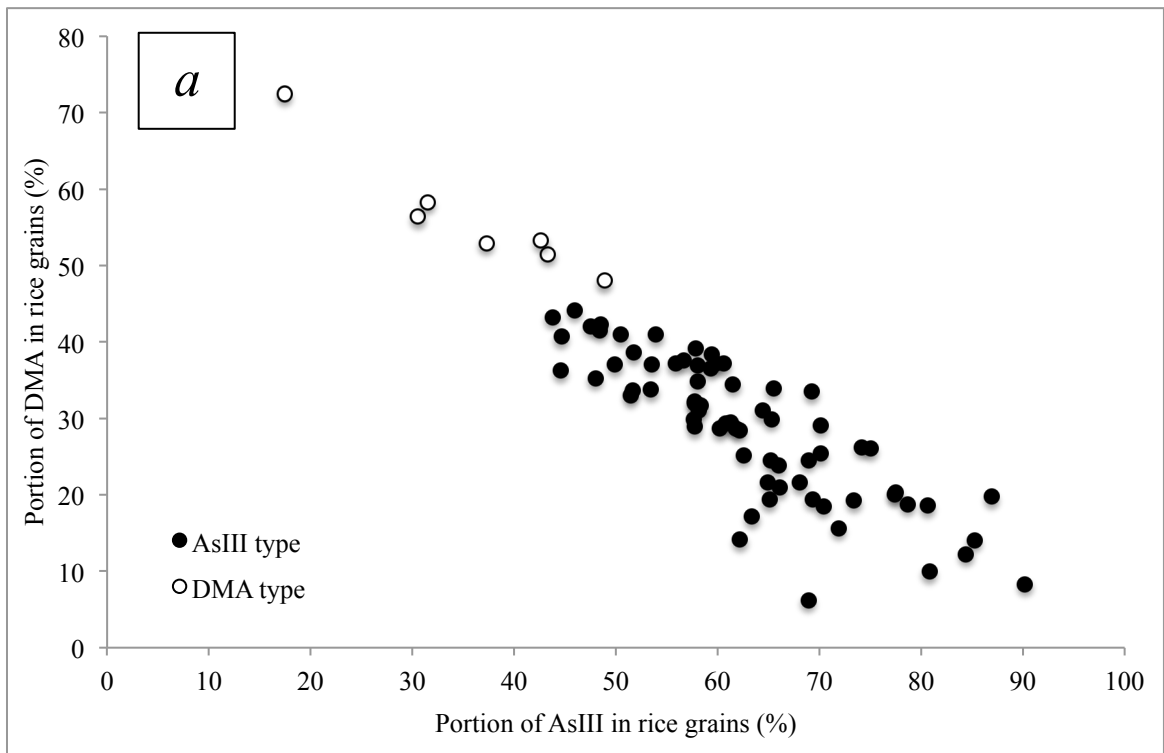
Fig. 5.4. Concentrations of arsenite (As(III)), dimethylarsinic acid (DMA(V)) (*a*) and arsenate (As(V)) (*b*) in relationship with total grain arsenic concentrations in the As(III) and DMA type rice. The number of rice samples is 70.

Our results furthermore indicate the strong negative correlation between As(III) and DMA(V) proportions in rice grain (Fig. 5.5a), reflecting the dominance of either As(III) or DMA(V) in rice grain. For DMA type rice, the DMA(V) concentration has a stronger linear correlation with the total grain As concentration than for As(III) type rice. Our data also show strong positive correlations of total grain As with DMA(V) proportion ($r = 0.76$, $p = 0.049$) (Fig. 5.5b), suggesting the DMA type rice may increase the proportion of methyl As with increasing total grain As concentrations. Obviously, in DMA type rice the portion of As(III) shows a negative tendency ($r = -0.69$, $p = 0.084$). It is possible to observe the detoxification mechanism in Fig. 5.5c, where there is a significant negative correlation ($r = -0.53$, $p < 0.01$) between total grain As with As(III) portion in the As(III) type rice. A possible explanation is that the As(III) type rice detoxifies As mainly by reducing As to As(III) which is then accumulated in As(III)–thiolate complexes (Huang et al. 2012). Instead a positive correlation exists between DMA(V) and total grain As ($r = 0.60$, $p < 0.01$). Therefore, different correlation between DMA(V) and As(III) proportion in the As(III) type rice remains as an open question.
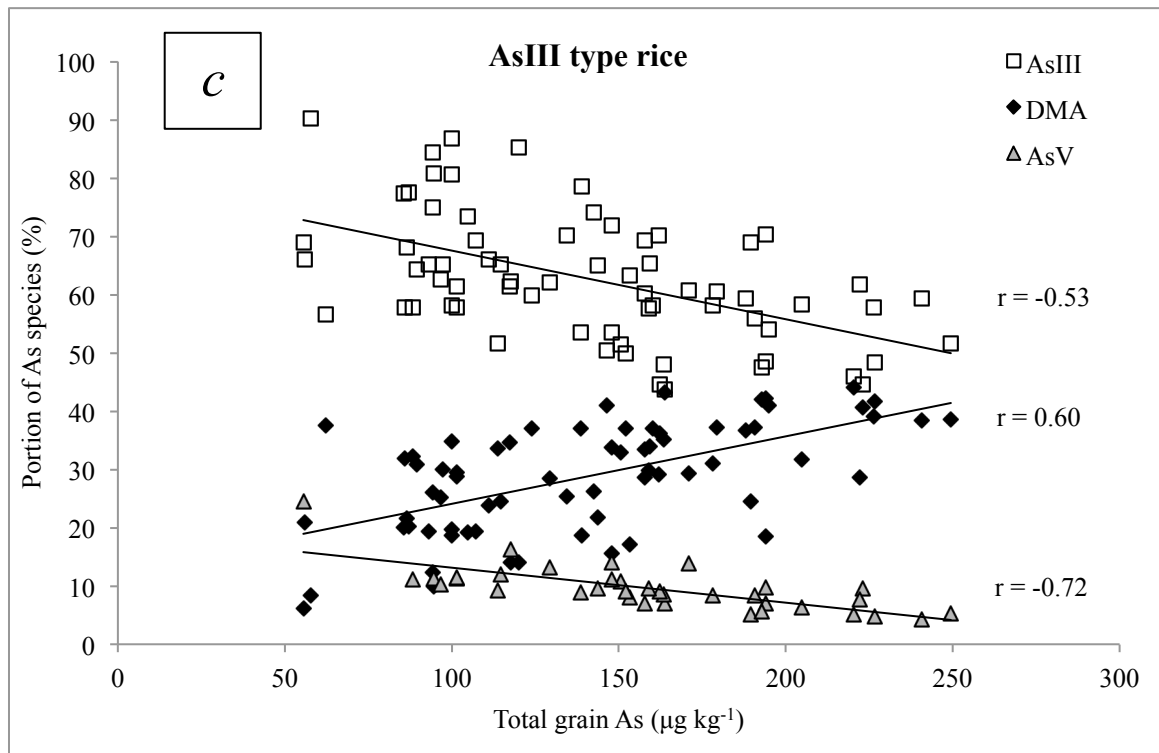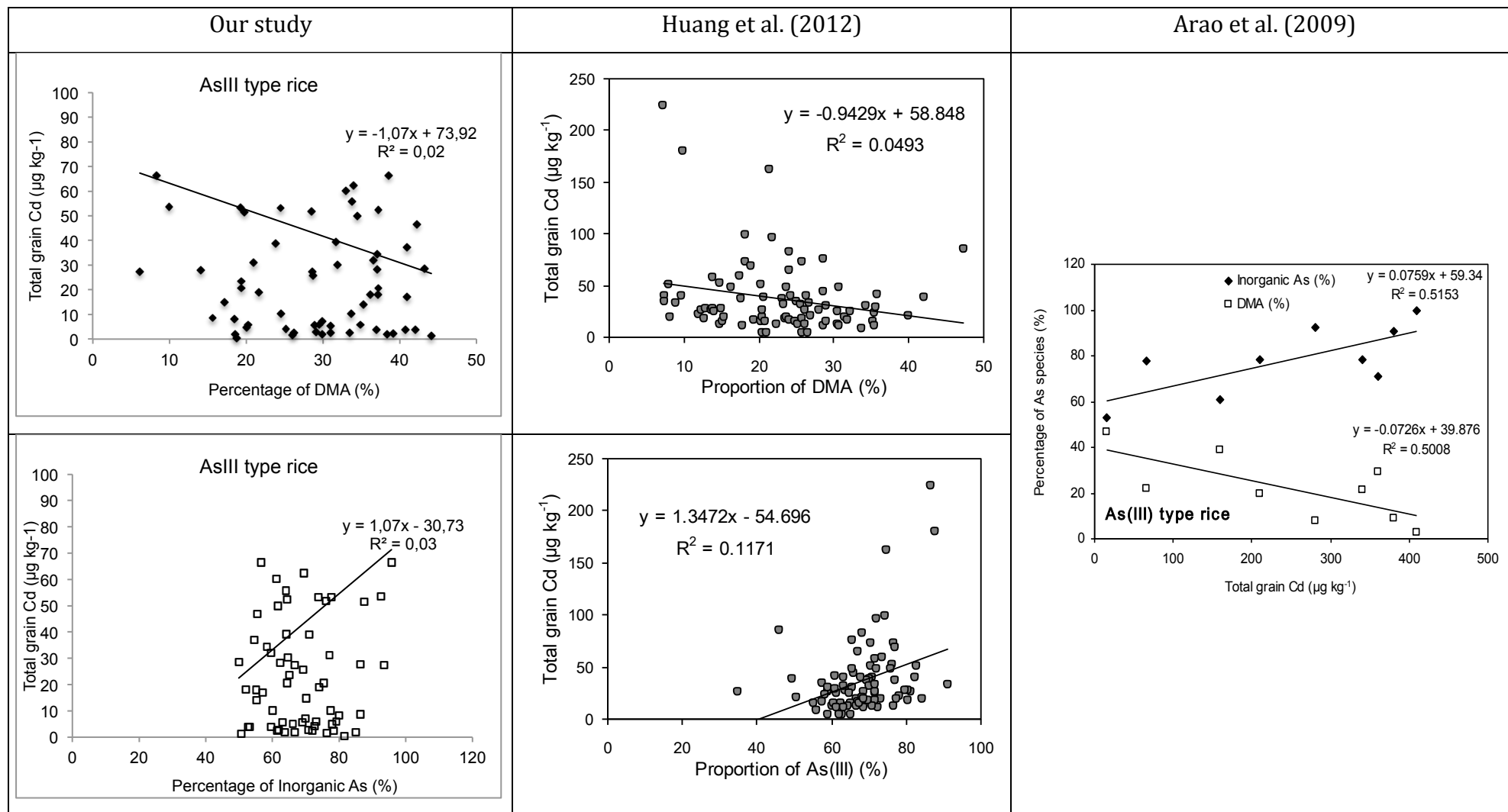
Figure a: Scatter plot showing Portion of DMA in rice grains (%) versus Portion of AsIII in rice grains (%). Legend: ● AsIII type, ○ DMA type.

Figure b: DMA type rice. Scatter plot showing Portion of As species (%) versus Total grain As (µ g kg⁻¹). Legend: □ AsIII, ◆ DMA, △ AsV. r = 0,76; r = 0,69.

113

Fig. 5.5. Plotted regression with correlation coefficients: (*a*) proportions of arsenite versus dimethylarsinic acid. (n = 70), (*b*) proportions of As(III), As(V) and DMA(V) versus total grain arsenic in the DMA type rice (n = 7), (*c*) proportions of As(III), As(V) and DMA(V) versus total grain arsenic in the As(III) type rice (n = 63).

Arao et al. (2009) found correlations between Cd concentrations and the proportion of DMA(V) (r = -0.69, p = 0.007) and As(III) (r = 0.69, p = 0.006) in rice grain from rice growing in different water regimes. There were similar correlations between Cd concentrations and DMA(V) (r = -0.22, p = 0.041) and As(III) percentage (r = 0.34, p = 0.007) in As(III) type rice in Huang et al. (2012) (Tab. 5.3). In our study for the sample pool of As(III) type rice, the regression coefficients between DMA(V) proportion and As(III) proportion with Cd concentration are plotted but they do not exbit significative correlations (Tab. 5.3).

Our study	Huang et al. (2012)	Arao et al. (2009)

AsIII type rice (top-left chart)

$y = -1{,}07x + 73{,}92$
$R^2 = 0{,}02$

X-axis: Percentage of DMA (%)
Y-axis: Total grain Cd (µg kg-1)

AsIII type rice (bottom-left chart)

$y = 1{,}07x - 30{,}73$
$R^2 = 0{,}03$

X-axis: Percentage of Inorganic As (%)
Y-axis: Total grain Cd (µg kg-1)

Huang et al. (2012) (top-center chart)

$y = -0.9429x + 58.848$
$R^2 = 0.0493$

X-axis: Proportion of DMA (%)
Y-axis: Total grain Cd (µg kg^{-1})

Huang et al. (2012) (bottom-center chart)

$y = 1.3472x - 54.696$
$R^2 = 0.1171$

X-axis: Proportion of As(III) (%)
Y-axis: Total grain Cd (µg kg^{-1})

Arao et al. (2009) (right chart)

Inorganic As (%)
DMA (%)

$y = 0.0759x + 59.34$
$R^2 = 0.5153$

$y = -0.0726x + 39.876$
$R^2 = 0.5008$

As(III) type rice

X-axis: Total grain Cd (µg kg^{-1})
Y-axis: Percentage of As species (%)

Tab. 5.3. Plotted regressions between the proportion of DMA(V) or inorganic As (in %) and total grain Cd in this study (n = 70) with correlations of Huang et al (2012) and Arao et al (2009).

115

The 70 different rice samples came from different areas of the north Italy, Pavia (West and East Ticino river, Milano-Lodi, Novara, Vercelli). Williams et al. (2006) reported that the dominant species in rice were inorganic (arsenate and arsenite), with dimethylarsenic acid [DMA(V)] being only a minor component. The maximum As concentration in rice was found in the sampling area of Pavia (North Italy, Fig. 5.6).
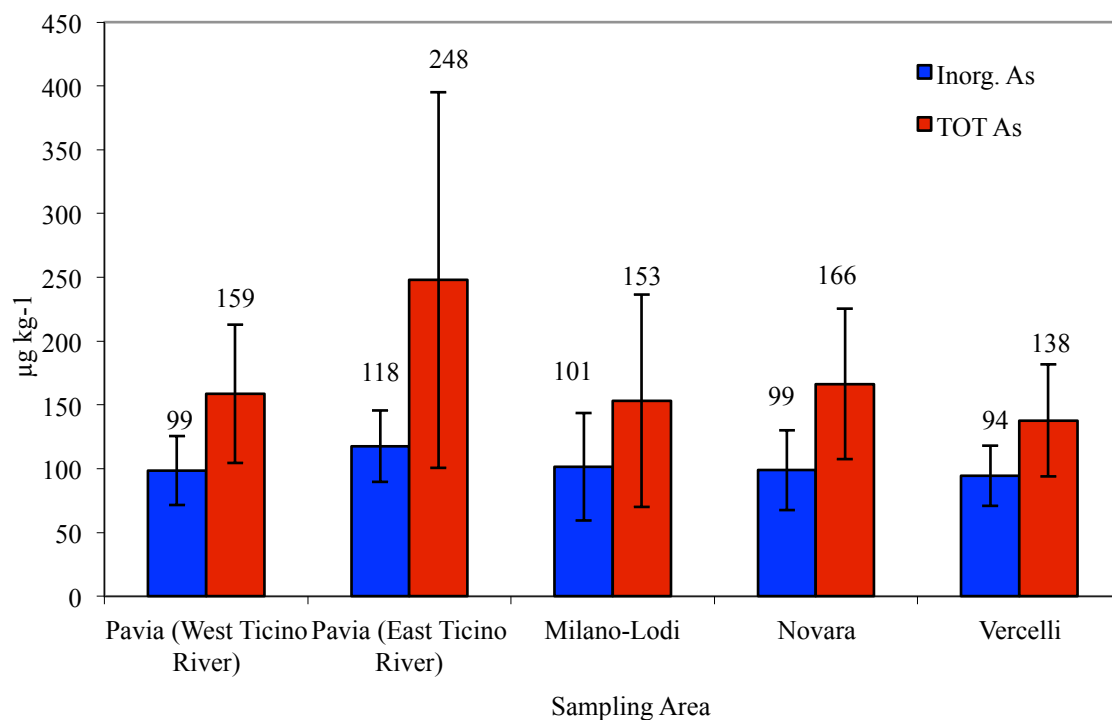

Fig. 5.6. Mean As content in rice grain from different sampling area in the North of Italy (Error bars are SD).

Differences of As concentration between white and brown rice

In this study, we analysed some rice samples (n = 18) with different treatments, polished (white) and unpolished (brown), and in Fig. 5.7 we reported the distribution of Inorganic and Total As between two different process of the same rice cultivar. The concentrations of total and inorganic As were greater in brown rice processing. Laser Ablation ICP-MS revealed that As was located in the outer grain of brown rice (Chapter VI), in line with the findings of Meharg et al. (2008). Meharg et al. (2008) showed that brown rice had a higher proportion of inorganic arsenic present than white rice. The lower As concentration in white rice compared to brown rice is possibly due to the removal of the outer bran layer of rice grain during polishing to make the grain colour white (Ahmed et al. 2011, Norton 2009). The order of arsenic accumulation in above ground tissues of rice plant was: straw > husk > brown rice grain > polish rice grain.
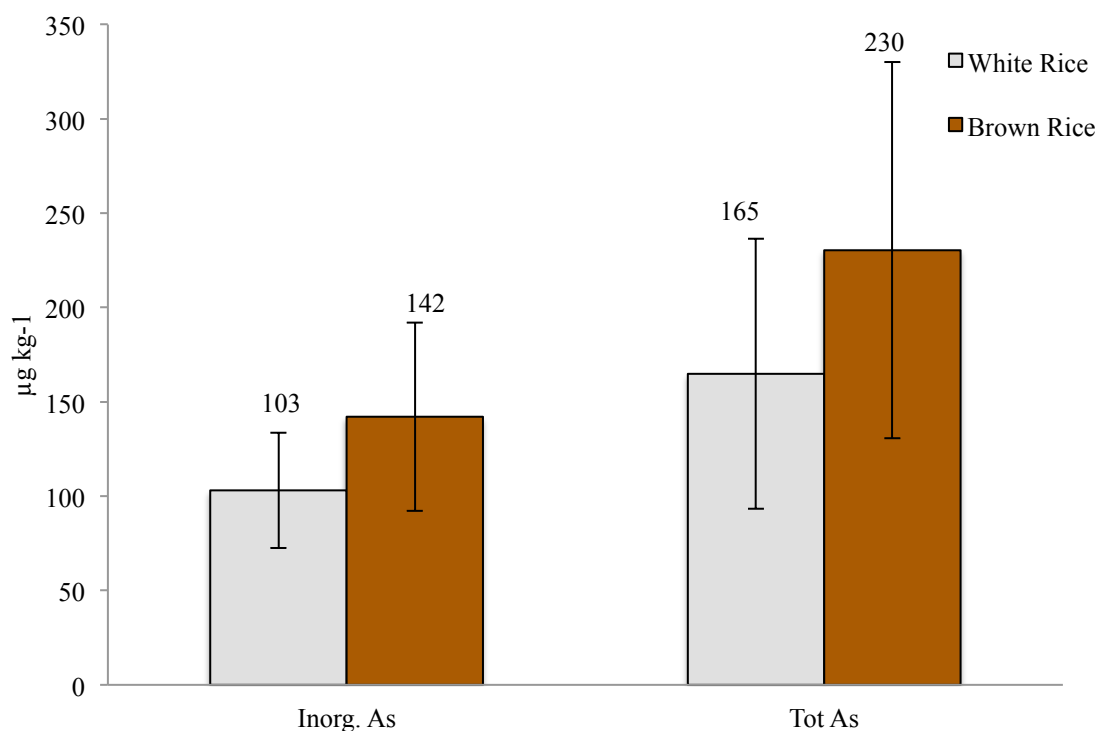
116

Fig. 5.6. Distribution of Inorganic and Total As between white and brown rice (n = 18) (Error bars are SD).

CONCLUSION

Health effects resulting from inorganic As exposure have focused on drinking water as the main arsenic source. Rahman et al. (2011) reveal that rice can also be a most important contributor to the exposure of inorganic As, without considering the As intake from other food sources such as vegetables.

The study shows that inorganic As was the main species present in rice samples and a low amount of DMA(V) was also detected.

The As(III) is the predominant species compared to As(V) in rice grains, probably because Arsenate is reduced to arsenite within the rice root (Xu et al., 2008; Zhao et al., 2009), which then enters the xylem via a silicic acid/arsenite effluxer (Ma et al., 2008; Zhao et al., 2009).

General validation of extraction methods based on 0.28 M nitric acid at 95 °C to different type of rice grain has been strengthened in this study by comparable results of As(III) and As(V) speciation in NIST CRM 1568a and IMEP-107 with either certificated or literature values.

REFERENCES

Ahmed ZU, Panaullah GM, Gauch HJr, McCouch SR, Tyagi W, Kabir MS and JM Duxbury. 2011. Genotype and environment effects on rice (Oryza sativa L.) grain arsenic concentration in Bangladesh. Plant Soil, 338, 367−382.

Arao T, Kawasaki A, Baba K, Mori S and S Matsumoto. 2009. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. Environmental Science & Technology, 43, 9361–9367.

Chen CJ, Chen CW, Wu MM and TL Kuo. 1992. Cancer Potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking-water. British Journal of Cancer, 66(5), 888–892.

Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, et al. 2011. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: Prospective cohort study. British Medical Journal, 342, d2431.

EFSA Panel on Contaminants in the Food Chain (CONTAM). 2009. Scientific Opinion on Arsenic in Food, 199.

Guzmán Mar JL, Reyes LH, Mizanur Rahman GM and HM Skip Kingston. 2009. Simultaneous extraction of arsenic and selenium species from rice products by microwave-assisted enzymatic extraction and analysis by ion chromatography-inductively coupled plasma-mass spectrometry. Journal of Agricultural and Food Chemistry, 57, 3005–3013.

Heitkemper DT, Vela NP, Stewart KR and CS Westphal. 2001. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. Journal of Analytical Atomic Spectrometry 16: 299–306.

Huang JH, Fecher P, Ilgen G, Hu KN and J Yang. 2012. Speciation of arsenite and arsenate in rice grain – Verification of nitric acid based extraction method and mass sample survey. Food Chemistry. Volume 130, Issue 2, Pages 453–459.

Huang JH, Ilgen G and P Fecher. 2010. Quantitative chemical extraction for arsenic speciation in rice grains. Journal of Analytical Atomic Spectrometry (6) 25: 749 – 904.

Kohlmeyer U, Jantzen E and J Kuballa. 2003. Benefits of high resolution IC-ICP-MS for the routine analysis of inorganic and organic arsenic species in food products of marine and terrestrial origin. Analytical Bioanalytical Chemistry, 377, 6–13.

Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath SP and FJ Zhao. 2008. Transporters of arsenite in rice and their role in As accumulation in rice grain. Proc Natl Acad Sci USA 105: 9931–9935.

Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath SP, et al. 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences of the United States of America, 105, 9931–9935.

Meharg AA, Deacon C, Campbell RCJ, Carey A, Williams PN, Feldmann J, et al. 2008. Inorganic arsenic levels in rice milk exceed EU and US drinking water standards. Journal of Environmental Monitoring, 10(4), 428–431.

Meharg AA, Sun GX, Williams PN, Adamako E, Deacon C, Zhu YG, Feldmann J,and A Raab. 2008. Inorganic arsenic levels in baby rice are of concern. Environmental Pollution.

Narukawa T and K Chiba. 2010. Heat assisted aqueous extraction of rice flour for arsenic speciation analysis. Journal of Agricultural and Food Chemistry, 58, 8183–8188.

Narukawa T, Inagaki K, Kuroiwa T and K Chiba. 2008. The extraction and speciation of arsenic in rice flour by HPLC-ICP-MS. Talanta, 77, 427–432.

Ohno K, Yanase T, Matsuo Y, Kimura T, Rahman MH, Magara Y and Y Matsiu. 2007. Arsenic intake via water and food by a population living in an arsenic-affected area of Bangladesh. Sci. Total Environ. 381:68–76.

Raber G, Stock N, Hanel P, Murko M, Navratilova J and KA Francesconi. 2012. An improved HPLC–ICPMS method for determining inorganic arsenic in food: Application to rice, wheat and tuna fish. Food Chemistry. Vol. 134, Issue 1, Pages 524–532.

Rahman MM, Asaduzzaman M and R Naidu. 2011. Arsenic exposure from rice and water sources in the Noakhali district of Bangladesh. Water Qual Expo Health 3:1–10.

Sanz E, Muñoz R and C Cámara. 2005. A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. Analytica Chimica Acta, 535, 227–235.

Sanz E, Munoz-Olivas R, Camara C, Sengupta MK and S Ahamed. 2007. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 42, 1695-1705.

Signes-Pastor AJ, Deacon C, Jenkins RO, Haris PI, Carbonell-Barrachina AA and AA Meharg. 2009. Arsenic speciation in Japanese rice drinks and condiments. Journal of Environmental Monitoring, 11(11), 1930–1934.

Smith NM, Lee R, Heitkemper DT, De Nicola Caggerky K, Hauqe A and AK Henderson. 2006. Inorganic arsenic in cooked rice and vegetables from Bangladeshi households. Sci. Total Environ. 270, 294– 301.

Tsai CY and Jiang. 2011. Microwave-assisted extraction and ion chromatography dynamic reaction cell inductively coupled plasma mass spectrometry for the speci- ation analysis of arsenic and selenium in cereals. Anal. Sci. 27, 271–276.

Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J and AA Meharg. 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ. Sci. Technol. 40, 4903–4908.

Williams PN, Price AH, Raab A., et al. 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environmental Science and Technology, 39, 5531–5540.

Xu XY, Mcgrath SP, Meharg AA and FJ Zhao. 2008. Growingrice aerobically markedly decreases arsenic accumulation. Environ. Sci. Technol. 2008, 42, 5574–5579.

Zavala YJ, Gerads R, Gürleyüket H,and JM Duxbury. 2008. Arsenic in rice. II. Arsenic speciation in USA grain and implication for human health. Environmental Science & Technology, 42, 3861–3866.

Zhao FJ, Ma JF, Meharg AA and SP McGrath. 2009. Arsenic uptake and metabolism in plants. New Phytol 181: 777–794.

Zhu YG, Sun GX, Lei M, Teng M, Liu YX, Chen NC, et al. 2008. High percentage inorganic arsenic content of mining impacted and nonimpacted Chinese rice. Environmental Science & Technology, 42, 5008–5013.

Chapter VI. Investigation of Arsenic distribution inside rice grain using laser ablation inductively coupled plasma mass spectrometry.

ABSTRACT

Arsenic (As) contamination of rice grains has been recognized as a major concern for human health. Here, we investigated the localization of As in rice grains because these are key factors controlling bioavailability of contaminants. Arsenic distribution in rice grains of two varieties, Gladio and Ronaldo, were determined. The distribution of As varied between the various parts of the grains (exterior, medium and interior part).

A method for direct determination of As by LA-ICP-MS in rice samples collected in several post harvest treatments was developed. Once the As concentrations were measured with adequate accuracy and precision, a method for its direct determination by LA-ICP-MS was developed. The final optimized conditions of laser ablation parameters were: laser energy (100%), spot size (100 μm) and repetition rate (10 Hz) and ^{13}C intensity was used as internal standard. As distribution profiles in rice grain cross-sections were also obtained. By these results, LA-ICP-MS emerges as a potential analytical tool for As localisation in rice samples. Minimum amount of sample required, bioimaging capability, high analytical throughput, and minimization of waste generation are the major analytical features of this approach. Arsenic content was higher in non-parboiled rice grain than that of parboiled rice. The relationship between arsenic intensities, corrected with ^{13}C intensities, and several parts of grain in rice revealed that arsenic levels decreased from the external part towards the middle position, and then the intensity values seem to be similar between medium and internal part in non parboiled products.

Keywords: *Arsenic, rice, parboiled and non-parboiled, Laser Ablation, ICP-MS.*

INTRODUCTION

Limited literatures are found on arsenic accumulation in different fractions of rice grain.

Majority of the people of Bangladesh and West Bengal, India, parboil raw rice before cooking though, the people of some other countries like Thailand, Japan and China cook rice without parboiling. Moreover, rice is milled to remove the husk (hull) before cooking. Some times, the bran polish (the outer thin layer of milled rice) becomes detached from the rice grain during

milling. Thus, the total arsenic in raw rice grain does not correspond to the definite amount of arsenic retained in cooked rice.

The traditional parboiling process involves soaking rough rice overnight or longer in water at ambient temperature, followed by boiling or steaming the steeped rice at 100°C to gelatinize the starch, while the grain expands until the hull's lemma and palea start to separate (Gariboldi, 1984; Bhattacharya, 1985; Pillaiyar, 1988). The parboiled rice is then cooled and sun-dried before storage or milling. Modern methods involve the use of a hot-water soak at 60°C (below the starch gelatinization temperature) for a few hours to reduce the incidence of aflatoxin contamination during the soaking step. Leaching of nutrients during soaking aggravates the contamination, with the practice of recycling the soak water. The parboiled product has a cream to yellow colour depending on the intensity of heat treatment. Parboiling gelatinizes the starch granules and hardens the endosperm, making it translucent. Despite the degradation of thiamine, parboiled milled rice had a higher vitamin content than raw milled rices in all parboiling procedures tested (Padua and Juliano, 1974). Although parboiled grains are harder than raw rice, they are also susceptible to fissuring during drying, particularly below 18 % moisture when free water becomes scarce in the grain. (FAO, 1993).

The LASER (Light Amplification by Stimulated Emission of Radiation) has demonstrated its potential sampling capabilities with various applications over several decades. Its development is closely associated with the ICP-MS (Inductively Coupled Plasma- Mass Spectrometer). Conceptually, LSX-213 G2 laser ablation system (CETAC Techologies, Nebraska, USA) provides a means of rapid, direct analysis of solid samples without dissolution and with minimal sample preparation. The laser ablation system features a high-energy laser and computer-controlled sampling methods using the DigiLaz^{TM} G2 Software. The laser ablation system generates particulate aerosols from solid material by an extreme rapid interaction between a high energy UV larger pulse and the sample surface. This process is referred to as ablation. Adjusting laser energy, spot size and pulse frequency using the DigiLaz G2 Software optimizes signal intensity and stability. Ablated material is swept into the ICP-MS by carrier gas (Fig. 6.1). Typically, a solid sample is placed inside an enclosed chambers (the sample cell) and a laser beam is focused on the surface of the sample. The sample cell is mounted on a computer controlled X-Y-Z translation stage, with a step size of 0.25 μm.

When the laser is fired, a cloud of particles is produced. These particles are removed from the sample cell by a carrier gas, and are swept into the ICP plasma for atomization and ionization and subsequent analysis. Compared with conventional dissolution techniques, laser ablation has many advantages. Most analytical techniques involve removing a portion of the solid sample,

which is then dissolved in acid solutions. With this procedure, there is a greater chance of exposure to hazardous materials and there is a risk of introducing contaminants or losing volatile components during sample preparation. For laser ablation, any type of solid sample can be ablated for analysis; there are no sample-size requirements and no sample preparation procedures. In addition, a focused laser beam permits spatial characterization of heterogeneity in solid samples, with typically micron resolution both in terms of lateral and depth conditions.

The LSX-213 G2 employs a specially designed Nd:YAG laser; frequency quintuplicated to the ultraviolet wavelength of 213 nm (Fig. 6.2). This laser provides a uniforms energy profile ("flat-top profile") across all spot sizes and yields a flat-bottomed crater on the sample. The laser can be operated at a high repetition rate of up to 20 Hz for increased sampling efficiency and better ICP-MS sensitivity.
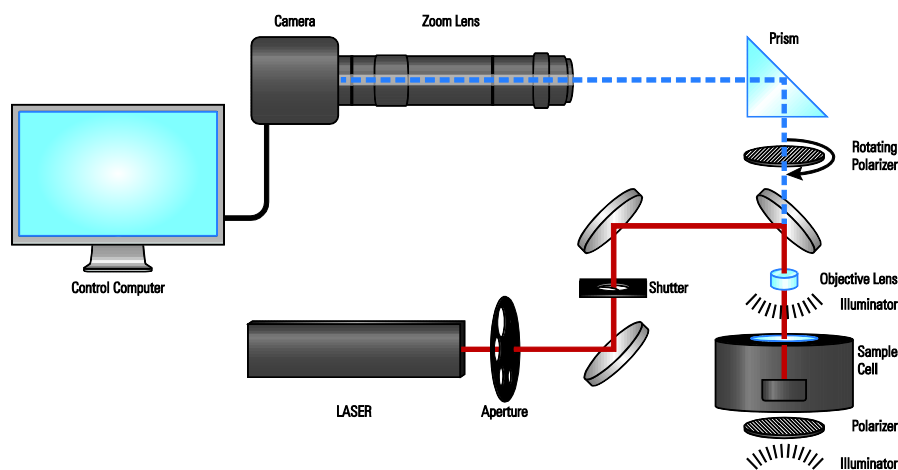
Fig. 6.1. Schematic diagram of the laser ablation system.
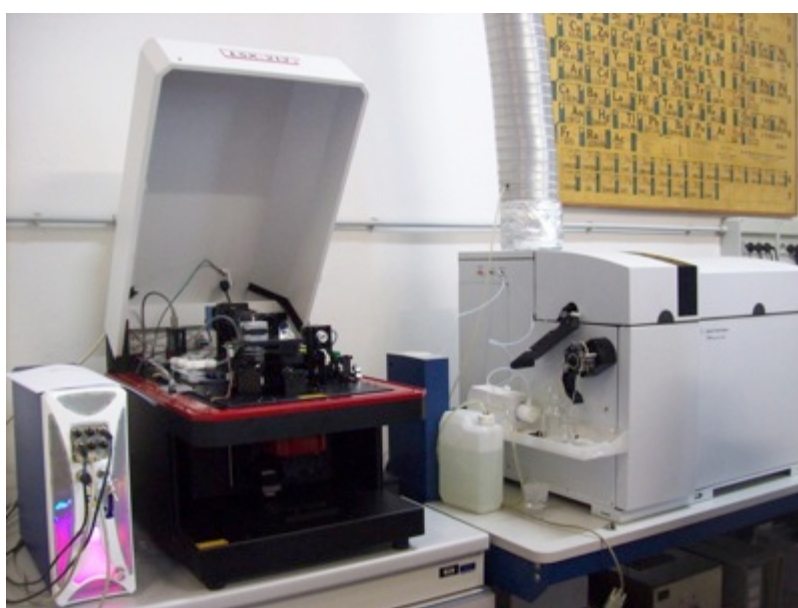
Fig. 6.2. LSX-213 G2 Laser Ablation System associated with ICP MS (7700x, Agilent Technologies).

Several research fields have employed LA-ICP-MS as a versatile analytical tool such as: proteomics (Bettmer et al. 2009; Heras et al. 2011; Jimenez et al. 2010) forensic (Castro et al. 2010; Berends-Montero et al. 2006; Arroyo et al. 2010), environmental (Durrant et al. 2005; Arroyo et al. 2009; Brown et al. 2009), geologic (Campbell IH et al. 2006; Nehring F et al. 2008; Liu Y et al. 2008), archeology and cultural heritage (Giussani B et al. 2009; Bartkus L et al. 2011; Byrne L et al. 2010), clinical and biological (Waentig L et al. 2011; Kumtabtim U et al. 2011; Kang D et al. 2004), among others. Many different types of samples are used in these studies including soils, sediments, rocks, tree rings, hair, teeth, bone, plants, and glasses.

The purpose of this study was to evaluate Arsenic distribution within the rice grains (exterior, medium, interior part) of different varieties (Gladio and Ronaldo) from different processes (raw, brown and milled rice with or without parboiling technique) with direct determination of LA-ICP-MS.

MATERIAL AND METHOD

The rice grains samples were collected from two different varieties, Gladio and Ronaldo, treated with different transformation processes with or without parboiling technique. Three grains of rice with similar diameters were prepared for each transformation process and for each variety. Cross-sections of rice grains were also prepared to assess the arsenic distribution profile along the grain with LA-ICP-MS (Fig. 6.3).
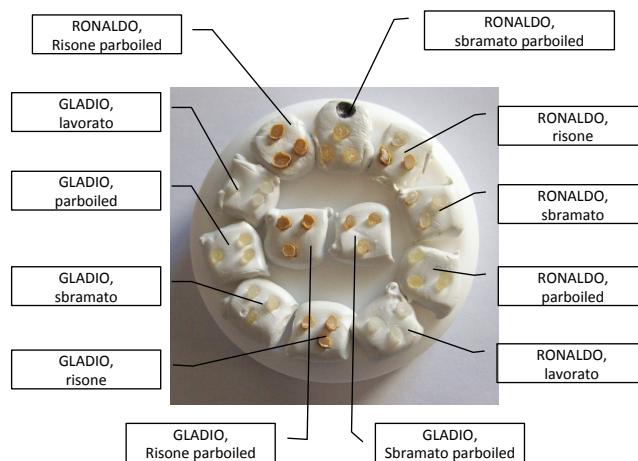

Fig. 6.3 Rice grains sample support usually placed into the sample cell on sample holder.

Instrumentation

The LA-ICP-MS experiments were performed using a Nd:YAG deep UV (213 nm) laser ablation system (CETAC technologies, Nebraska, USA) coupled to an ICP-MS (Agilent 7700x, Agilent Technologies, USA). The detailed experimental conditions are described in the Table 6.1. The inert gas used to transport the sample aerosol from the ablation chamber to the ICP-MS was a mixture containing argon and helium.

Table 6.1. Operational parameters for ICP-MS and LA-ICP-MS determinations

LA-ICP-MS instrumental parameters (LSX-213 G2)	
Parameters	
Laser warm-up time (s)	40
Laser output (%)	60
Repetition rate (Hz)	10
Spot size (μm)	100
Energy delivered (%)	100
Fluence (J cm^{-2})	7.2
ICP-MS instrumental parameters (Agilent 7700x)	
Parameters	
RF power (kW)	1.45
Lens voltage (V)	5.5
Argon nebulizer flow rate (L min^{-1})	0.5
Argon auxiliary flow rate (L min^{-1})	1.2
Argon coolant flow rate (L min^{-1})	15
Dwell time (ms)	100
Isotopes monitored (m/z)	^{75}As ^{13}C

RESULTS AND DISCUSSION

The application of Laser Ablation is based on single point selection. Actually, it is possible to set the location of the spot on the sample image (Fig. 6.5). These laser ablation conditions were employed to achieve a good spatial resolution and satisfactory analytical signals in order to build the elemental bio-image of Arsenic distribution. In Fig. 6.4 there are typical analytical signals obtained for one ablation spot on a rice grain surface (from Parboiled Gladio) under optimized conditions (Energy: 100%).
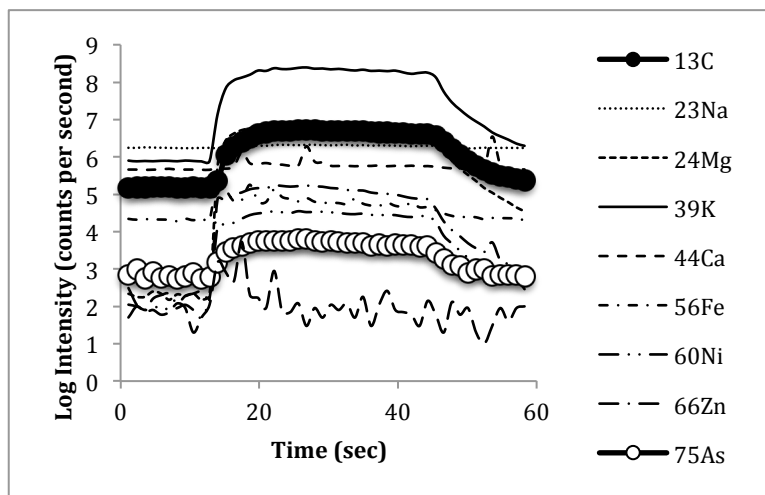
Fig. 6.4 Typical LA-ICP-MS analytical signals obtained in a laser scan of a single spot on a rice grain surface (from Parboiled Gladio) under optimized conditions (Energy: 100%).
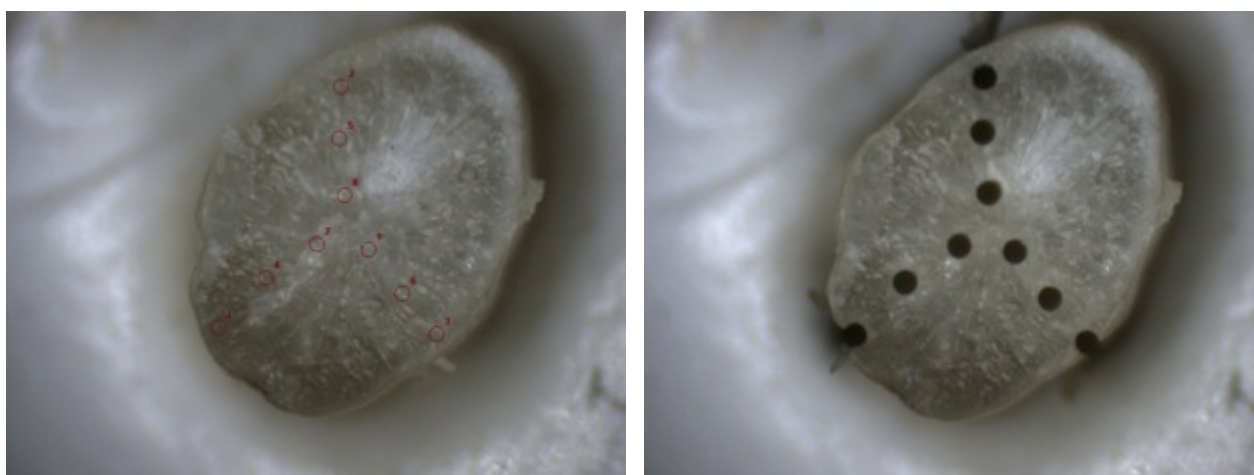

Fig. 6.5 A grid in the rice grains cross-section to cover all different zone of the organism: exterior, medium, and interior part.

It wasn't possible to convert the sample analytical signals to concentration by the use of a single point calibration with the cereal grain CRM signals because there are only CRM (certified reference material) powders for rice analyses. Semi-quantitative data were obtained with approach of intensities (cps) comparison.

The Fig. 6.6 presents the results obtained for all collected samples. The internal standardization is a good analytical strategy in order to correct drifts occurred during the signals acquisition and sampling variations as well as transport effects of the sample aerosol from the ablation chamber to the ICP-MS torch. The ^{13}C satisfied the core requirements of a good candidate of internal standard which include: must be in the same concentration in all samples, must correct fluctuations during the signal acquisition, then it must have a similar behaviour with the analyte

(this is observed by the very comparable analytical signals shapes for both the ^{13}C and ^{75}As as can be seen in Fig. 6.4).

Guerra et al. (2011) pointed out for the use of ^{13}C as a suitable internal standard for Pb determination in lichen samples with application of several statistical parameters on different calibration strategies.

Because of the complexity of post harvest treatments the mechanisms responsible for As loading into the rice grains and its speciation and distribution within the grain are not fully understood.

For example, the large majority of the information available on As distribution and speciation in rice is related to the analyses of powdered rice grains. Only few studies have focused on the in situ speciation and distribution of As in rice grains (Meharg et al., 2008, Lombi et al. 2009). Meharg et al. 2008 reported synchrotron μ-X-ray fluorescence (μ-XRF) results showing that As was mainly localized in the outer regions of the grains (i.e. aleurone/pericarp or outer parts of the endosperm).

In Fig. 6.6, it can be seen that the ratio between the As isotope signals is in relation to ^{13}C intensity obtained with LA - ICP-MS and follows the As isotopic abundance distribution (LOD from 249 cps to 548 cps). As expected, Arsenic concentrationin rice samples decreases in the following way: rough rice > brown rice > milled rice. Furthermore it is possible to note how the signal intensity decreases from the outside towards in three post-harvest processes of rice. This type of distribution is very pronounced in the Ronaldo variety.
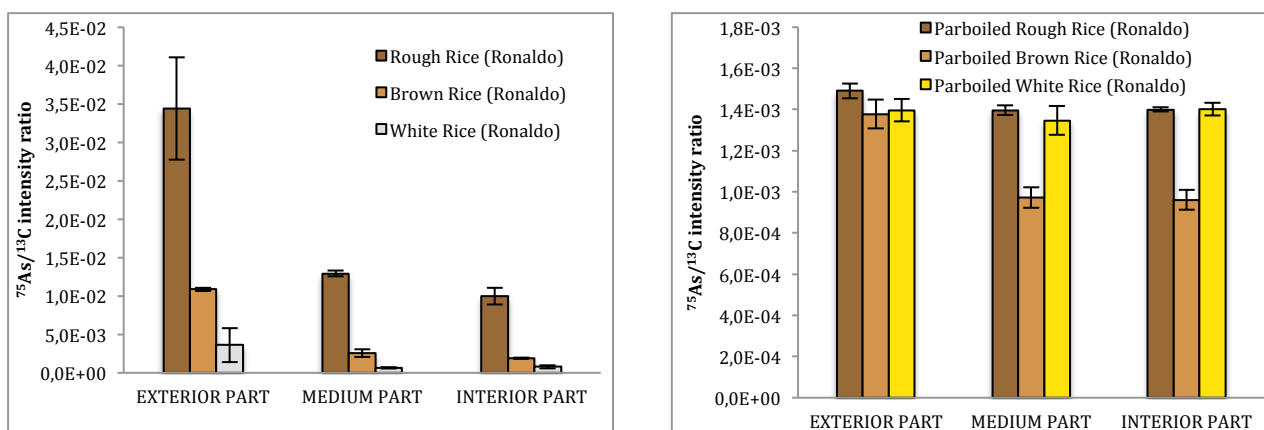

127
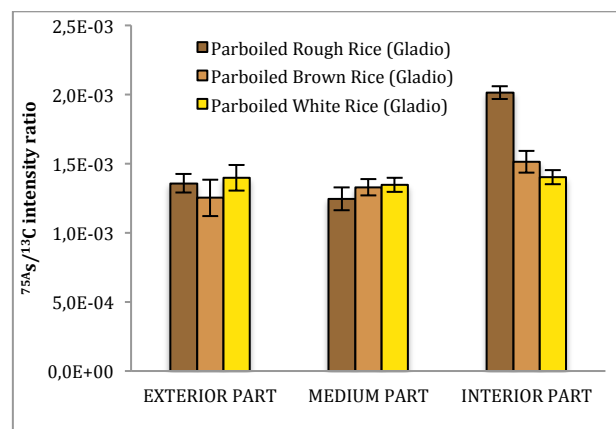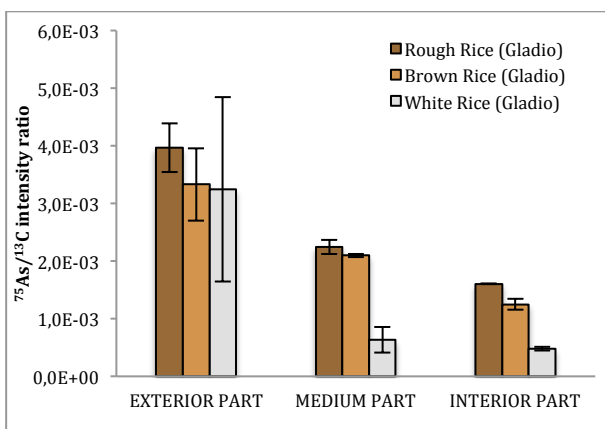
Fig. 6.6. ^{75}As isotopes intensities (counts per second, cps) obtained with LA-ICP-MS using ^{13}C as internal standard in different areas (exterior, medium and interior) of rice grain in two type of variety of rice (GLADIO, RONALDO).

Rahman et al. (2007) reported that fractions of non-parboiled rice contained higher amount of arsenic compared to those of parboiled rice suggesting that parboiling of raw rice may results in the decrease of arsenic concentrations in rice fractions. During parboiling, arsenic might have released from straw and rice grain to the boiling water and the discarding of boiling water may result in the decrease of its concentrations rice. Thus, parboiling of rice grain before cooking may reduce the magnitude of arsenic intake in human body.

During the processing of raw rice for human consumption, some fractions of rice such as husk and bran-polish are removed which contain a significant amount of arsenic. Arsenic concentration in polish rice is also reduced due to parboiling of the raw rice before milling. The same conclusions can be observed in the graphs of Fig. 6.7. The Ronaldo variety shows divergence between parboiled and non-parboiled rice intensities ratio, especially in rough and brown products. The differences between milled parboiled and milled non-parboiled rice are not very obvious because, in both cases, the arsenic data came from grains from which husk, germs, bran layers have been substantially removed. Earlier reports confirmed that rice bran contains greater concentrations of As than the polished rice (Ren et al., 2006; Rahman et al., 2007; Sun et al., 2008).
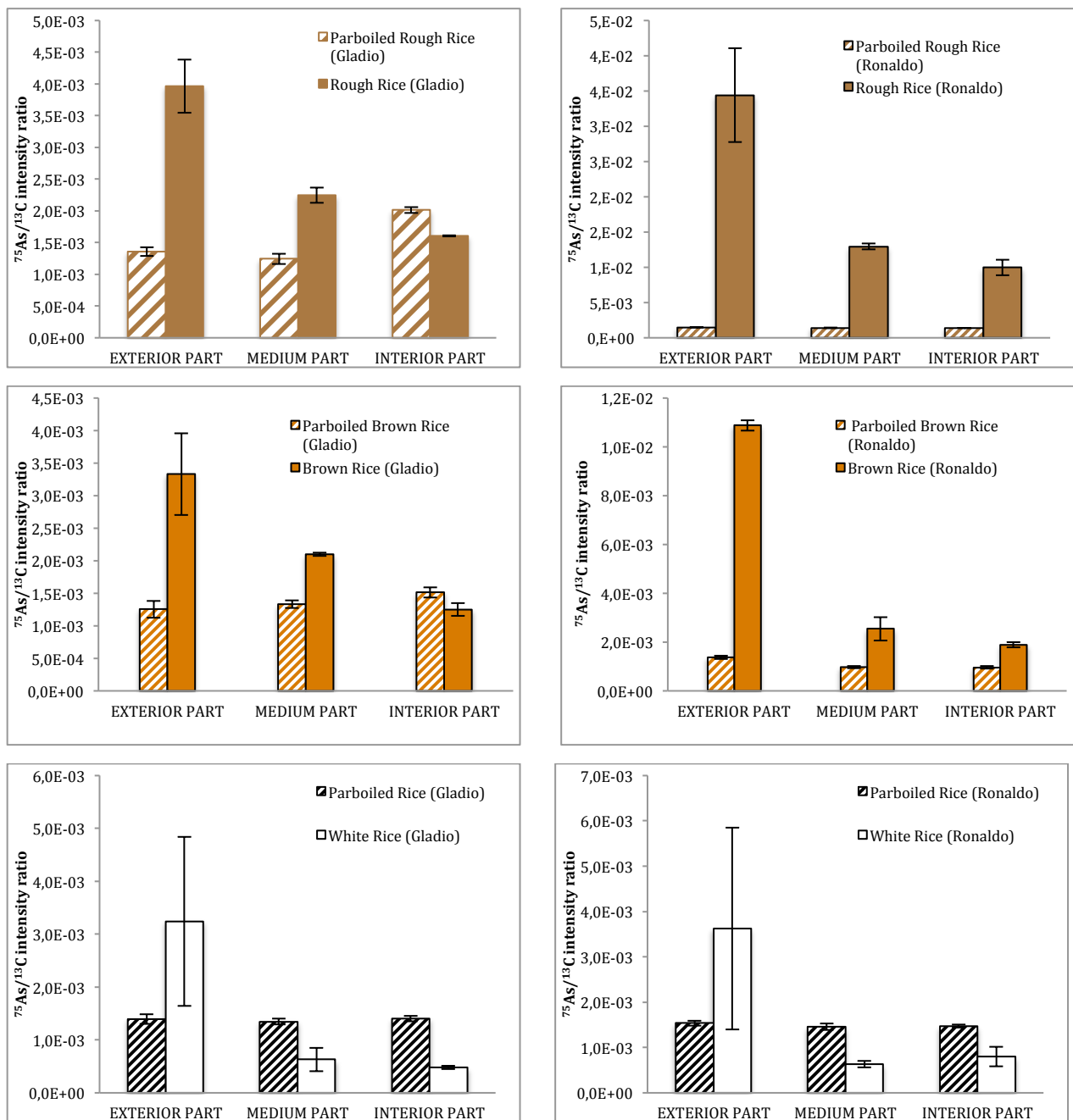
Fig. 6.7. Comparison of ratio intensities in different parts of grain (external, medium, internal part) between parboiled and non-parboiled rice products in Gladio and Ronaldo varieties.

Lombi et al. (2009) also observed a dramatic decrease in As concentration from the husk surrounding the grain to the grain itself. Therefore, the larger concentrations present in the bran fraction compared with the polished rice may have two possible causes. First, there could be a physiological barrier in the unloading and uploading process responsible for the transfer of As from the maternal tissues to the filial tissues. Second, As, as with many other elements, could accumulate preferentially in the protein-rich aleurone and embryo tissues although, because of the lateral resolution of the XRF technique, an accumulation in the outer parts of the endosperm

cannot be excluded. Several arsenic intensities in the external part of rice grains are probably due to arsenic accumulation in a small area on the surface of grain (Meharg et al. 2008).

The relationship between arsenic intensities, corrected with ^{13}C intensities, and several parts of grain in rice revealed that arsenic levels decreased from the external part towards the middle position, and then the intensity values seem to be similar between medium and internal part in non parboiled products (Fig. 6.8). Instead, the histograms of arsenic intensities against various zones of grain in parboiled products don't show any trend between these two variables (Fig. 6.8).
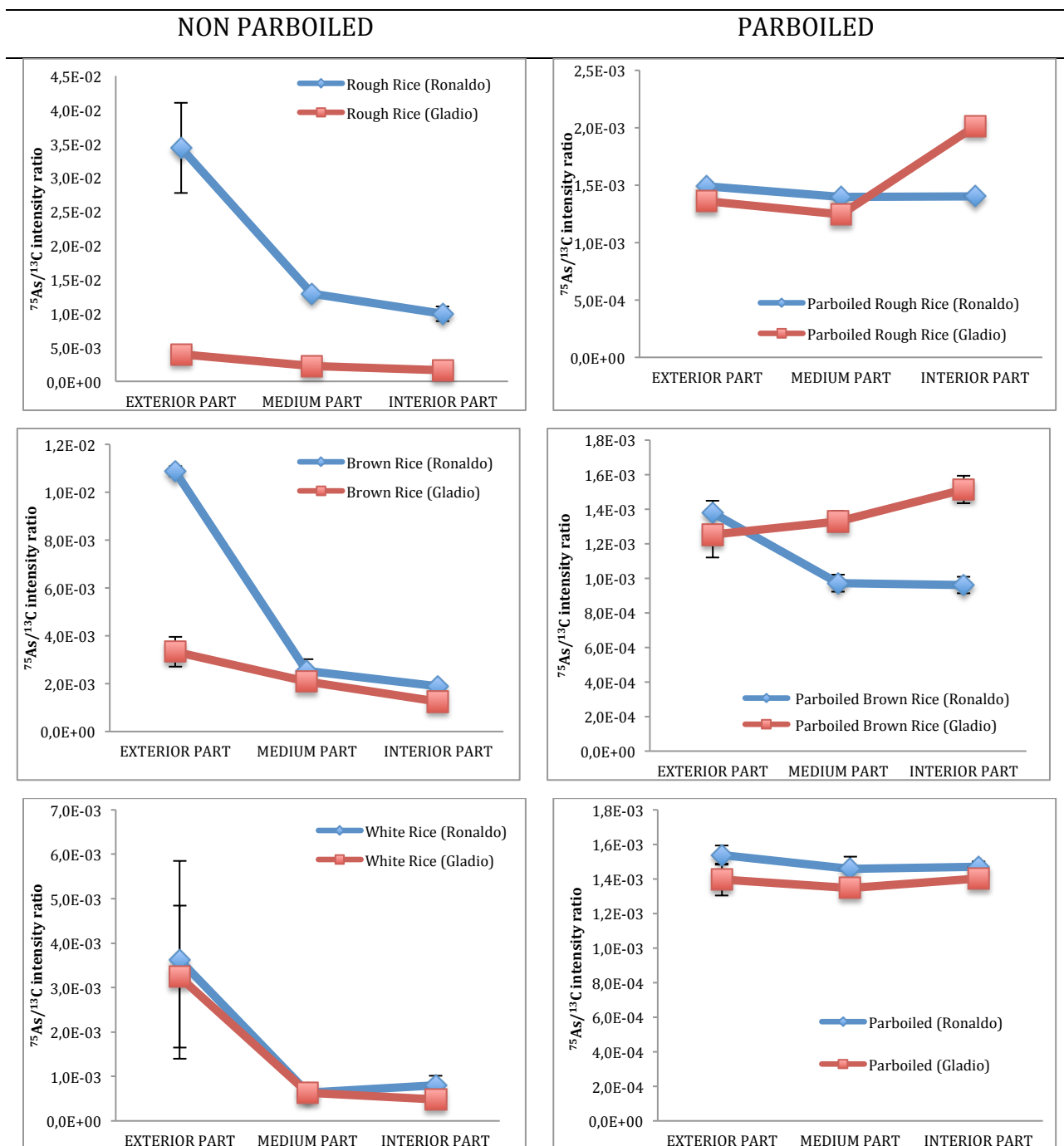

Fig. 6.8. The relationship between ratio intensities and different sections between two rice varieties (Gladio and Ronaldo).

CONCLUSIONS

LA-ICP-MS emerges as a potential analytical technique for As intensity estimation in rice samples. The minimum amount of sample required (few mg in a laser shot) and minimization of waste generation are significant analytical advantages of this approach.

The effectiveness of arsenic reduction in a parboiling process suggests that consuming parboiled rice may be a rapid and cost-effective means to improve the amount of safety rice intake. Further research is required to optimise the arsenic localisation inside the grain under different post harvest treatments with different varieties. Actually, Rahman et al. (2007) revealed that As uptake into plant might differ with the varieties of same species.

REFERENCES

Arroyo L, Trejos T, Gardinali PR and JR Almirall. 2009. Optimization and validation of a Laser Ablation Inductively Coupled Plasma Mass Spectrometry method for the routine analysis of soils and sediments. *Spectrochim. Acta.* Part B, 64, 16.

Arroyo L, Trejos T, Hosick T, Machemer S, Almirall JR and PR Gardinali. 2010. Analysis of Soils and Sediments by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS): An Innovative Tool for Environmental Forensics Environ. *Forensics.* 11(4), 315.

Bartkus L, Amarasiriwardena D, Arriaza B, Bellis D and J Yanez. 2011.Exploring lead exposure in ancient Chilean mummies using a single strand of hair by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). *Microchem. J.* 98, 267.

Berends-Montero S, Wiarda W, de Joode P and G van der Peijl. 2006. Forensic analysis of float glass using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS): validation of a method. *J. Anal. At. Spectrom.* 21, 1185.

Bettmer J, Bayon MM, Encinar JR, Sanchez MLF, de la Campa MRF and AS Medel. 2009.The emerging role of ICP-MS in proteomic analysis. *J. Proteomics.* 72, 989. 39.

Bhattacharya KR. 1985. Parboiling of rice. In B.O. Juliano, ed. Rice chemistry and technology, 2nd ed, p. 289-348. St Paul, MN, USA, Am. Assoc. Cereal Chem.

Brown JC, Jarvis KE, Disch BA, Goddard SL and AS Brown. 2009. Spatial inhomogeneity of metals in particulate matter on ambient air filters determined by LA-ICP-MS and comparison with acid digestion ICP-MS. *J. Environ. Monit.* 11, 2022.

Byrne S, Amarasiriwardena D, Bandak B, Bartkus L, Kane J, Jones J, Yanez J, Arriaza B and L Cornejo. 2010. Were Chinchorros exposed to arsenic? Arsenic determination in Chinchorro mummies' hair by laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). *Microchem. J.* 94, 28.

Campbell IH, Ballard JR, Palin JM, Allen C and A Faunes. 2006. U-Pb Zircon Geochronology of Granitic Rocks from the Chuquicamata-El Abra Porphyry Copper Belt of Northern Chile: Excimer Laser Ablation ICP-MS Analysis. *Econ. Geol.* 101(7), 1327.

Castro W, Hoogewerff J, Latkoczy C and J R Almirall. 2010. Application of laser ablation (LA-ICP-SF-MS) for the elemental analysis of bone and teeth samples for discrimination purposes. *Forensic Sci. Int.* 195, 17.

Durrant SF and NI Ward. 2005. Recent biological and environmental applications of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *J. Anal. At. Spectrom.* 20, 821.

FAO. 1993. Rice in human nutrition, published with the collaboration of the international rice research institute food and agriculture organization of the united nations. Rome.

Gariboldi, F. 1984. Rice parboiling. FAO Agric. Serv. Bull. 56. Rome, FAO. 73 PP.

Giussani B, Monticelli D and L Rampazzi. 2009. Role of laser ablation–inductively coupled plasma–mass spectrometry in cultural heritage research: A review. *Anal. Chim. Acta.* 635, 6.

Guerra MBB, Amarasiriwardena D, Schaefer CEGR, Pereira CD, Spielmann AA, Nóbrega JA and ER Pereira-Filho. 2011. Biomonitoring of lead in Antarctic lichens using laser ablation inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom. 26,*2238.

Hattendorf B, Latkoczy C and D Gunther. 2003. Peer Reviewed: Laser Ablation-ICPMS. It's the aerosol size that really matters in this high-throughput technique for ultratrace analysis of solids. *Anal. Chem.* 15 (1), 341A.

Heras IL, Palomo M and Y Madrid. 2011. Selenoproteins: the key factor in selenium essentiality. State of the art

analytical techniques for selenoprotein studies. *Anal. Bioanal. Chem.* 400, 1717.

Jimenez MS, Rodriguez L, Gomez MT and JR Castillo. 2010. Metal–protein binding losses in proteomic studies by PAGE–LA-ICP-MS. *Talanta.* 81, 241.

Kang D, Amarasiriwardena D and AH Goodman. 2004. Application of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to investigate trace metal spatial distributions in human tooth enamel and dentine growth layers and pulp. *Anal. Bioanal. Chem.* 378, 1608.

Kumtabtim U, Siripinyanond A, Auray-Blais C, Ntwari A and JS Becker. 2011. Analysis of trace metals in single droplet of urine by laser ablation inductively coupled plasma mass spectrometry. *Int. J. Mass Spectrom.* 2011.

Liu Y, Hu Z, Gao S, Gunther D, Xu J, Gao C and H Chen. 2008. In situ analysis of major and trace elements of anhydrous minerals by LA-ICP-MS without applying an internal standard. *Chem. Geol.* 257, 34.

Lombi E, Scheckel KG, Pallon J, Carey AM, Zhu YG and AA Meharg. 2009. Speciation and distribution of arsenic and localization of nutrients in rice grains. 2009. *New Phytologist.* 184: 193–201.

Nehring F, Jacob DE, Barth MG and SF Foley. 2008. Laser-ablation ICP-MS analysis of siliceous rock glasses fused on an iridium strip heater using MgO dilution. *Microchim. Acta.* 160, 153.

Padua AB and BO Juliano. 1974. Effect of parboiling on thiarnin, protein and fat of rice. *J. Sci. Food Agric.* 25: 697-701.

Pillaiyar P. 1988. Rice postproduction manual. New Delhi, Wiley Eastern Ltd. pp 437.

Rahman MA, Hasegawa H, Rahman MM, Rahman MA and MA Miah. 2007. Accumulation of arsenic in tissues of rice plant (Oryza sativa L.) and its distribution in fractions of rice grain. *Chemosphere.* 69: 942–948.

Rahman MA, Hasegawa H, Rahman MM, Rahman MA and MAM Miah. 2007. Accumulation of arsenic in tissues of rice plant (Oryza sativa L.) and its distribution in fractions of rice grain. *Chemosphere.* 69 (6), 942–948.

Ren XL, Liu QL, Wu DX and QY Shu. 2006. Variations in concentration and distribution of health-related elements affected by environmental and genotypic differences in rice grains. *Rice Science.* 13: 170–178.

Sun GX, Williams PN, Carey AM, Zhu YG, Deacon C, Raab A, Feldmann J, Islam RM and AA Meharg. 2008. Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. *Environmental Science Technology.* 42: 7542–7546.

Waentig L, Jakubowski N and PH Roos. 2011. Multi-parametric analysis of cytochrome P450 expression in rat liver microsomes by LA-ICP-MS. *J. Anal. At. Spectrom.* 26, 310.

CONCLUSION

Chapter VII. Conclusion and Future Prespectives.

Arsenic is one of the most studied elements for human health implications. The total concentration of this element is usually reported without differentiating the arsenic species, but its toxicity depends on the chemical forms.

DGT technique, a tool to assess the potential availability of pollutants, is suitable for simultaneous determination of labile Arsenic in groundwater, over a wide pH range. Moreover organic arsenic species, like DMA(V) and MMA(V), are identified in DGT resin and they could contribute to total As concentration.

In groundwater samples, the presence of anions and cations, at concentrations up to 4 fold higher than those expected in unpolluted natural water, has no significant effect on the accumulation of As by DGT. It is essential to monitor the drinking water resources, like groundwater, and it is necessary to perform further investigations about seasonal variations of arsenic speciation in groundwater for future applications. The use of these devices could be very useful to obtain measurements of lower concentrations, taking advantage of the pre-concentration ability of DGT. Moreover, the detection of different arsenic species may be possible with the application of the appropriate pore size DGT in a water sample containing humic acids. It is necessary to investigate the behaviour of different chemical forms of arsenic with natural organic matter such as fulvic acid, because different authors (Simeoni et al. 2003; Grafe et al. 2001; Redman et al. 2002) have reported that fulvic acids can inhibit the adsorption of As(V) and As(III) at the iron-hydroxide surface. This type of deepening may help to improve the application of the DGT devices in different matrices such as soil.

For many years contaminated water has been assigned as the main source for human exposure to inorganic arsenic. However, a recent epidemiological research indicated that inorganic As has detrimental human health effects at exposures much lower than previously thought, and in areas where levels of inorganic As in drinking water are not excessive (Navas-acien et al. 2008). More recently, however, rice and rice-based products have been identified as significant dietary sources of inorganic arsenic (Meharg et al., 2008; Signes-Pastor et al., 2009). Our study, based on different types of rice grains from different areas of Northern Italy, shows that inorganic As is the main species present in rice samples and a low amount of DMA(V) is also detected. A general validation of extraction methods based on 0.28 M nitric acid at 95 °C for different type of rice has been strengthened in this study by results for As(III) and As(V) speciation comparable to NIST CRM 1568a and IMEP-107 (certificated or literature values).

Development of quantification methods of arsenic species is very important for human health risk, expecially the distinction between inorganic species. Actually, in the uptake of As by the human body, As(V) is more readily absorbed than As(III), but recent reports from arsenic endemic areas in West Bengal, India, and Bangladesh showed less amount of As(V) compared to As(III) in drinking water (Harvey et al. 2002, Shraim et al. 2002). Moreover in humans pentavalent arsenicals are reduced to trivalency and the formation of methylated trivalent arsenicals may account for the toxicity and carcinogenicity of inorganic arsenic. Thus, methylation of arsenic has been considered as bioactivation process rather than detoxification process. MMA(III) and DMA(III) have been reported to break down DNA at lower concentrations than inorganic arsenicals or pentavalent methylated arsenicals (Mass et al. 2001, Nesnow et al. 2002).

The arsenic content changes between different process in the same rice, brown and white rice. In our samples, the concentrations of total and inorganic As are greater in brown rice. These results agree with Meharg et al. (2008), who showed that brown rice had a higher proportion of inorganic arsenic than white rice. Our observations show the potential risk to human health from the consumption of rice but it will be necessary to investigate the factors, which may influence the accumulation of the element, such as irrigation system, variety and type of soil. The storage of As in rice plant tissues and grains was recently reported as a result of soils or irrigation waters containing elevated levels of As. Abedin et al. (2002) discovered that As concentrations in rice grains, husks, stalks, and roots were positively correlated with arsenate contents in the irrigation water.

About different As distribution in rice, the localization in the outer grain of brown rice was confirmed by Laser Ablation ICP−MS. The application of the LA-ICP-MS (Nd:YAG laser) allowed to know that arsenic distribution in rice samples of two varieties (Gladio and Ronaldo) decreases in the following way: rough rice > brown rice > milled rice. During the processing of raw rice for human consumption, some fractions of rice, such as husk and bran-polish, which contain a significant amount of arsenic, are removed. Arsenic concentration in polished rice is also reduced due to parboiling of the raw rice before milling. The Ronaldo rice variety shows divergences between parboiled and non-parboiled rice intensities ratio, especially in rough and brown products.

Ablation spots on a rice grain surface under optimized conditions (Energy: 100%), demonstrate that arsenic levels decreased from the external part towards the middle position, and then the intensity values seem to be similar between medium and internal part in non parboiled products. This outcome highlights that rice processes can influence human intake of arsenic. The

effectiveness of arsenic reduction in a parboiling process suggests that consuming parboiled rice may be a rapid and cost-effective means to improve the safety of rice intake. Further research is required to optimise the arsenic localisation inside the grain under different post harvest treatments and in different varieties.

LA-ICP-MS, in association with traditional detection technique, could be a potential analytical technique for estimation of As intensity in rice samples, because this instrument allows the ablation of any type of solid sample for analysis; there is no need of sample-size requirements and no sample preparation procedures. In addition, a focused laser beam permits spatial characterization of heterogeneity in solid samples, with typically micron resolution both in terms of lateral and depth conditions without changes of matrices.

Investigation of antimony is enclosed in this research in association with study of arsenic distribution, because the physical and chemical qualities of Sb and As are similar, and it has been recently recognized as water contaminant. The comprehension of antimony behaviour in aqueous matrix is very important because many studies were published in which drinking water contamination from bottle materials was investigated. The first important objectives were identifying and quantifying the chemical forms of antimony, to provide comprehensive information about its toxicity and human health relevance. We report the application of HPLC species- unspecific spike isotope dilution ICP-MS to separate and quantify inorganic Sb species (Sb(V) and Sb(III)) in aqueous solution using the diffusive gradient in thin films technique. The toxicity of antimony compounds is approximately ten times lesser than for arsenic, even if it depends on the oxidation states and structure. This analytical technique combined with Fe-oxide DGT, as adsorption system in aqueous solution, and EDTA, for preservation of different species, represent an excellent point of departure for antimony investigation in the environment. Analysis of Sb species was suitable for the application of Isotope Dilution Analysis (IDA), a well-known analytical technique based on the measurement of isotope ratios in samples, without uncertainties about instrumental instabilities such as signal drift or matrix effects. Future applications could include the study and the identification of methylated antimony species in some environmental compartments, as little information is known about the behaviour of these species in solution. Moreover, information on antimony interactions with natural organic matter is very scarce and does not allow any rigorous conclusion to be drawn regarding its role in the fate of this element in natural aquatic systems.

REFERENCES

Abedin MJ, Cotter-Howells JC, Meharg AA. 2002. Arsenic uptake and accumulation in rice (Oryza sativa L.) irrigated with contaminated water. *Plant and Soil*, Vol 240, N 2, 311-319.

Grafe M, Eick MJ and PR Grossl. 2001. Adsorption of Arsenate (V) and Arsenite (III) on Goethite in the Presence and Absence of Dissolved Organic Carbon. *Soil Sci. Soc. Am. J.* 65, 1680.

Harvey CF, Swartz CH, Badruzzaman ABM, Keon-Blute N, Yu W, Ali MA, Jay J, Beckie R, Niedan V, Brabander D, Oates PM, Ashfaque KN, Islam S, Hemond HF and MF Ahmed. 2002. Arsenic mobility and groundwater extraction in Bangladesh. *Science.* 298, 1602 – 1606.

Mass MJ, Tennant A, Roop BC, Cullen WR, Styblo M, Thomas DJ and AD Kligerman. 2001. Methylated trivalent arsenic species are genotoxic. *Chem. Res. Toxicol.* 14, 355–361.

Meharg AA, Sun G, Williams PN, Adomako E, Deacon C, Zhu YG, Feldmann J, Raab A. 2008. Inorganic arsenic levels in baby rice are of concern. *Environ Pollut.* 152: 746–749.

Navas-Acien A, Silbergeld EK, Pastor-Barriuso R and E Guallar. 2008. Arsenic exposure and prevalence of type 2 diabetes in US adults. Jama-Journal of the American Medical Association, 300(7), 814–822.

Nesnow S, Roop BC, Lambert G, Kadiiska M, Mason RP, Cullen WR and MJ Mass. 2002. DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. *Chem. Res. Toxicol.* 15, 1627–1634.

Redman AD, Macalady DL and D Ahmann. 2002. Natural Organic Matter Affects Arsenic Speciation and Sorption onto Hematite. *Environ. Sci. Technol.* 36, 2889.

Shraim A, Sekaran NC, Anuradha CD and S Hirano. 2002. Speciation of arsenic in tube-well water samples collected from West Bengal, India, by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *Appl. Organomet. Chem.* 16, 202–209.

Signes-Pastor AJ, Deacon C, Jenkins RO, Haris PI, Carbonell-Barrachina AA and AA Meharg. 2009. Arsenic speciation in Japanese rice drinks and condiments. Journal of Environmental Monitoring, 11(11), 1930–1934.

Simeoni MA, Batts BD and C McRae. 2003. Effect of groundwater fulvic acid on the adsorption of arsenate by ferrihydrite and gibbsite. *Appl. Geochem.* 18, 1507.

Acknowledgements