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Soil contamination with arsenic - risk element transfer from soil to plant.
Bachelor Thesis

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2020

I declare that the Bachelor Thesis Soil contamination with Arsenic - risk element transfer from soil to plant is my own work and all the sources I cited in it are listed in Bibliography.

Prague, Czech Republic

Signature

Acknowledgement

I wish to express my sincere gratitude to my supervisor, Mr. doc. Ing. Ondřej Drábek, Ph.D. and to my advisor, RNDr. Václav Tejnecký, Ph.D., for their continuing guidance, support, patience, recommendations and advice. I would also like to thank Ing. Kateřina Vejvodová for her kind review and thorough comments. And of course, I am so very thankful for the love and support of my family and friends.

Abstract.

Soil contamination with toxic risk elements (RE) is recognized as a global problem that can cause serious damage to both environment and human health. Arsenic is one of the RE that can cause serious health risks even when present in small amounts.

The contaminant can reach the soil through lithogenic (geological; parent material; volcanic activity; trace elements) and anthropogenic sources (industrial waste; mining activities; agri-chemicals). The main natural source in soils is weathering from parent materials. Arsenic also occurs as an impurity in mineral ores and as a result of mining and other industrial operations. Total As soil concentrations in solid soil phase range between 0,1 - 55 mg kg⁻¹ in uncontaminated soils. As (III) and As(V) are major species found in most soil samples, however As(III) is 60 times more toxic than As(V). Arsenic can cause acute and chronic poisoning for humans. If ingested, the absorbed As accumulates mainly in the liver, kidneys, heart and lungs. The contamination pathways can be from As dust, contaminated water or also As that has accumulated in plant parts.

There are some plants that rapidly reduce As(V) to As(III) inside cells, however, the amount of As accumulated and also the plant part in which it is accumulated varies greatly between species. Generally, leafy vegetables tend to be “accumulators” and fruity vegetables tend to be “excluders”. From cereals the highest accumulation rate is for rice. Rice is one of the most important staples in many countries, therefore there have been several studies focusing on reducing and preventing the As accumulation in rice.

In general, there are different ways to prevent As accumulation in crops, such as water regime management, mitigation, amendment and fertilization practices. There are also possible ways of remediation of As contaminated soils, which include methylation, use of soil fungal species and hyperaccumulating ferns (*Pteris vittata* and *Pityrogramma calomelanos*).

Key words: Soil contamination, Arsenic, Toxicity, As accumulating plants

Contents

1. Introduction	1
2. Objectives of work	1
3. Literature review	2
3.1. Soil Contamination	2
3.1.1. Sources	2
3.1.1.1. Lithogenic sources	2
3.1.1.2. Anthropogenic sources	3
3.1.2. RE fate in soil	4
3.1.3. Toxicity	4
3.2. Arsenic	5
3.2.1. Arsenic an element	5
3.2.2. Arsenic in soil	5
3.2.2.1. Microbial Methylation.....	6
3.2.2.2. Arsenic contaminated sites	7
3.2.3. Concentrations	9
3.2.3.1. Arsenic toxicity	9
3.2.3.1.1. Effects on human body	10
3.2.3.2. Methods of determination	11
3.2.3.3. Arsenic Speciation	12
3.2.3.3.1. Stability of species	13
3.2.3.3.2. Combinations of techniques	14
3.2.3.3.3. Arsenic solid phase speciation	14
3.3. Arsenic and plants interactions	15
3.3.1. Arsenic phytotoxicity	15
3.3.2. The soil-plant barrier concept	16
3.3.3. Arsenic accumulation mechanisms in plants	16
3.3.3.1. Arsenic biotransformations In Plants	18
3.3.3.2. Arsenic and Iron interactions	18
3.3.3.3. Arsenate-phosphate antagonism	19
3.3.3.4. Long-Distance Transport of Arsenic	20
3.3.4. Arsenic uptake in different plant species	20
3.3.4.1. Hyperaccumulating plants	22
3.3.5. Crops contaminated with Arsenic	23

3.3.5.1. Rice	24
3.3.5.2. Crop contamination prevention	25
4. Conclusion	27

1. Introduction

Soil is a basic component of ecosystems and is one of the most vulnerable to contamination and degradation through accidental or deliberate mismanagement (Allen, 1995). Soil contamination has severely increased over the last years, especially due to pesticides and Risk Elements (RE) from industrial wastes and human activities (Lai et al., 2011).

Naidu et al. (2006) emphasized that Arsenic (As) is the only known human carcinogen for which there is adequate evidence of carcinogenic risk by both inhalation and ingestion. While As is released to the environment from natural sources such as wind-blown dust and volcanoes, releases from anthropogenic sources far exceed those from natural sources. Nriagu et al. (2007) wrote that contamination of the environment with As from both anthropogenic and natural sources has occurred in many parts of the world and is recognized as a global problem. Long-term cumulative exposure to As in these contaminated environments should be a matter of public health concern and scientific interest.

Soil and water are the main sources of human exposure to As at any given location, either by consumption, inhalation, or direct skin contact. Because As accumulates in vegetables, fruits, and other plants that grow in contaminated soils, another important pathway of exposure is the transfer of As within the food chain (Moreno-Jiménez et al., 2012).

That is why soil contamination with As, its causes, types, uptake in plants, and influence on human health should be researched and widely discussed in order to raise awareness of the importance of this topic.

2. Objectives of work

- To research and describe the soil contamination focusing on soil contaminated with arsenic- its types and influence on the environment and human health.
- To review As transfer from soil to plants as this is one of the many ways the contaminant can enter the human and animal bodies.

Hypothesis – People living in the contaminated area might be endangered not only by As contaminated dust and water, but also by food products originating from this area. Moreover, there might be certain As accumulating plants and their consumption can represent a serious health risk.

3. Literature review

3.1. Soil Contamination

3.1.1. Sources

The total concentration of RE in soil are the sum of the lithogenic sources and inputs from a wide range of possible anthropogenic sources. These could be summarized in the below equation (Alloway, 2013):

$$M_{total} = (M_{pm} + M_{atm} + M_{sed} + M_f + M_{ac} + M_{tm} + M_{om} + M_{ic}) - (M_{cr} + M_e + M_l + M_v)$$

Where: M = metal(loid), pm = parent material, atm = atmospheric deposition, sed = deposited sediment, f = fertilizers, ac = agricultural chemicals, tm = technogenic materials, om = organic materials, ic = other inorganic contaminants, cr = crop removal, e = soil erosion, l = leaching, and v = volatilization

3.1.1.1. Lithogenic sources

Lithogenic source of RE is the concentrations of elements derived from minerals in the geological parent material on which the soil has developed. It is the primary factor determining the total concentration of RE. Trace elements all together comprise a total of only 1% by mass of the earth's crust. Their individual concentrations do not normally exceed 1,000 mg kg⁻¹, except in ore minerals, which are natural concentrations of minerals containing these trace elements (Alloway, 2013). The exact amounts of trace elements in earth's crust can vary- the principal factors determining these variations are the selective incorporation of particular elements in specific minerals during igneous-rock crystallization, relative rates of weathering, and the modes of formation of sedimentary rocks (Allen, 1995).

Examples:

Contamination of soils due to volcanic activity

In natural ecosystems, soil enrichment with Ni occurs in alluvial soils or in soils subjected to the deposition of volcanic ashes. Copper can be released in the environment (and soil) from natural sources through a variety of processes including volcanic eruption, in fact, volcanoes and wind-blown soil particles have been identified as the major natural sources of Cu and Zn emissions (Alloway, 2013). Adamo et al. (2006) in their research were studying the impact of river overflowing on trace element

contamination of volcanic soils in south Italy, investigating the volcanic soils affected by different numbers of polluted river flooding events with main RE being Cr and Cu. Using micromorphology and Scanning Electron Microscopy/ Wavelength-Dispersive Spectroscopy (SEM/WDS) analyses they found Cr and Cu enriched silt and clay coatings in surface and subsurface soil horizons, which suggested a transfer of metal-rich sediments along the soil pore network with water movement. Bastías et al. (2013) were studying soil contaminated with As due to volcanic and mining activities in Chile. They were using honey as a bioindicator and they found that the samples of honey from Futaleufú showed higher As concentrations after the eruption of the Chaitén Volcano in 2008.

3.1.1.2. Anthropogenic sources

Anthropogenic sources of RE in soils are often referred to as contamination sources. There are many different anthropogenic sources of RE contamination affecting both agricultural and urban soils. These include atmospheric deposition of aerosol particles (<30 µm diameter), raindrops containing RE or gaseous forms, direct applications of agricultural fertilizers, agri-chemicals and various organic materials including sewage sludges, livestock manures, food wastes and composts. Technogenic materials such as ashes, mine wastes and demolition rubble can be contaminants in pedogenic soils or can form the parent materials of new, man-made soils, also called- Anthrosols (Alloway, 2013). Improper waste management can be a source for soil and environmental contamination. For example, Pascucci (2011) found that approximately 157 million tons of municipal solid waste and 2.04 billion tons of industrial solid waste (of which 14.29 million tons of hazardous waste) were produced in 2009 in China. Pascucci (2011) described that these wastes would contaminate green land, drinking water and even air, ultimately threatening human health. However, localized contamination from a predominant single source, such as a metal smelter can have a marked effect on soils, vegetation and possibly also on the health of the local population, especially in countries where there are inadequate emission controls and soil quality standards (Alloway, 2013).

Some RE are chemically very reactive in the environment, which results in their mobility and bioavailability to living organisms (Zovko and Romic, 2011). Most of the materials added to improve cultivated soils (such as: lime, inorganic nitrogenous or potassic

fertilizers, and farmland manure) have low RE contents and when used at normal rates are unlikely to affect RE levels in soils and crops (Allen, 1995).

3.1.2. RE fate in soil

One of the most important soil chemical properties is the ability of the soil to adsorb cations and anions, which is directly linked to the type and amount of secondary minerals including clays, carbonates and hydrous oxides of Fe and Mn, organic matter quality and quantity, the soil pH and redox conditions. This adsorptive capacity of the soil has a profound effect on the bioavailability of RE. Risk elements are adsorbed strongly by the organic and inorganic colloidal constituents of soil. The organic fractions primarily responsible for RE binding are humic and fulvic acids (HA and FA), which may constitute of approximately 75% of the soil organic matter on average, but this varies between soils (Alloway, 2013). Boruvka & Drábek (2004) were studying the RE distribution between fractions of humic substances in heavily polluted soils. They found that, compared to Cd and Zn, Pb showed higher affinity to HA. The amount of metals bound by FA was mainly controlled by the level of pollution. However, determining the proportion of what is termed the 'active organic carbon' (AOC) in soil solid and solution can be challenging. The presence of dissolved organic carbon (DOC) has great implications for transport of RE that strongly bind to HA and FA. In the case of Cu for example, commonly up to 99% of Cu in the soil solution is complexed to FA (Alloway, 2013). Effects of excessive levels of RE on soil properties depend on complex reactions between the trace cations and other components of all soil phases- liquid, solid and gaseous. The mobile fraction of RE behaves like multivalent cations in soil phases and is controlled by dynamic equilibria between solid and liquid phases. The fate of these RE in soils depends upon many soil processes, such as, dissolution, sorption, precipitation, volatilization, absorption and sorption by microbiota (Kabata-Pendias, 2010).

3.1.3. Toxicity

Some of the RE in very small amounts are essential for normal growth and development of living organisms. From a physiological point of view, they are called micronutrients or microelements (e.g., Fe, Mn, Zn, Cu, Mo, Ni, Se), while others are toxic even in small concentrations (e.g., Cr, Cd, As, Hg, Pb). The issue of toxicity is

usually a matter of speciation of discussed elements and their quantity, with the range varying for each element (Zovko and Romic, 2011).

3.2. Arsenic

3.2.1. Arsenic as an element

Arsenic is the twentieth most abundant element in the earth's crust. It is a metalloid with the atomic number 33 and atomic mass of 74.9216 g mol⁻¹. Its outer electron configuration is 4s²4p³, together with N, P, Sb and Bi, As belongs to the fifth main group of the periodic table. As it is with other metalloids, As has semiconductor properties and occurs in nature in various oxidation states including +V, +III, 0, and -III (Alloway, 2013). Because As more easily forms anions, its non-metal properties dominate. When As is in an oxidation state of +V, it acts similar to P, a fact that has many implications for the way in which it reacts in soil, as well as its potential toxicity in plants (Moreno-Jiménez et al., 2012). In the aqueous environment inorganic As appears commonly in the oxidation states +V and +III as arsenous acid (As(III)), arsenic acid (As(V)), and their salts. Inorganic As compounds can be methylated by bacteria, fungi, and yeasts to the organic compounds such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and gaseous derivatives of arsine (Bissen et al., 2003). Under aerobic conditions, the oxidation state of As tends to be +V, and when this occurs at a pH between 2 and 3, arsenic acid (H₃AsO₄) is formed. When the pH rises to values between 3 and 11, this compound disassociates to H₂AsO₄⁻ and HAsO₄²⁻. Under anaerobic conditions, the predominant As species is H₃AsO₃ (Moreno-Jiménez et al., 2012).

3.2.2. Arsenic in soil

Arsenic is a widespread constituent of many types of mineral deposits, particularly those containing sulphides and sulpho-salts. In these and other deposits, As commonly accompanies Cu, Ag, Au, Zn, Cd, Hg, U, Sn, Pb, P, Sb, Bi, S, Se, Te, Mo, W, Fe, Ni, Co, and Pt metals. Under most conditions As is a suitable indicator of deposits of these elements, being particularly useful in geochemical surveys utilizing primary halos in rocks, and secondary halos and trains in soils and glacial materials, stream and lake sediments, natural waters, and vegetation (Boyle and Jonasson, 1973). Arsenic occurs naturally as an impurity in metal ores (e.g. arsenopyrite, copper, gold), in sulfide

compounds (e.g. realgar = AsS , orpiment = As_2S_3), in coal and shales and in rock-phosphate, among others. It has been released into the environment as a result of natural weathering phenomena, but more profoundly as a result of mining and other industrial operations (Naidu et al., 2006). Total As concentrations in the soil solid phase range between 1 and 40 mg kg^{-1} in uncontaminated soils. As it was said the main natural source is weathering from parent materials. Shales, clays and phosphorites (phosphates) commonly contain elevated concentrations of As (shales and clays max. 490 mg kg^{-1} ; phosphates max. 188 mg kg^{-1}) whereas sandstones (max. 120 mg kg^{-1}), limestones (max. 20 mg kg^{-1}) and igneous rocks show typically lower concentrations of As than the world average for soils (Alloway, 2013).

As(III) and As(V) are the major As species found in most soil samples. The distribution of As species in soils depends on the type and amounts of sorbing components of the soil, pH and the redox potential. Under oxic soil conditions ($E_h > 200$ mV, pH 5–8), As is commonly present as As(V). However, As(III) is the predominant form under reducing conditions (Naidu et al., 2006). Arsenic shares chemical similarity with P and therefore acts as an analogue in various biological processes such as uptake in plants and microorganisms, and in terms of its chemical behavior in soils. However, the soil chemistry of As is more diverse than that of P as As exists in more than one oxidation state, and it can form bonds with S and C more readily (Alloway, 2013). An important variable in the study of As in soils is the heterogeneity in which it can appear, such that regions displaying high concentrations may be adjacent to regions that have much lower levels. In such cases, contaminant hot spots must be identified, because they will pose the hardest-to-manage threats (Moreno-Jiménez et al., 2012).

3.2.2.1. Microbial Methylation

Arsenic occurs mainly as the inorganic species arsenite (H_3AsO_3) and arsenate (H_2AsO_4^- , HAsO_4^{2-}) in the natural environment. In the inorganic As species, bacteria can replace the hydroxyl groups by methyl groups. Therefore, methyl arsonate ($\text{CH}_3\text{AsO}_3^{2-}$) and dimethylarsinate ($(\text{CH}_3)_2\text{AsO}_2^-$) are also found in soils. The oxidation of methylated As species to inorganic species can occur under aerobic conditions. Organic As species volatilized to the atmosphere are rapidly oxidized to the inorganic species (Bissen et al., 2003).

3.2.2.2. Arsenic contaminated sites

In terms of anthropogenic sources, annual global production of As is estimated to be between 75 and 100×10^3 t. Natural sources (those in which human intervention does not play an important role) will depend, in many cases, on the geochemistry of each site, principally of the site's lithology and dispersion pathways. The major human activities that produce As are mining, metallurgy, agriculture, forestry, fossil fuel treatment plants, urban waste, and cattle farming. In both mining and metallurgy, As is produced as a by-product of little value, making it an unwanted waste (Moreno-Jiménez et al., 2012). Nriagu et al. (2007) described that As poisoning episodes from exposure to industrial sources have been reported all over the world. For example, in Japan, where cases have been associated with pollution around As mines and pollution of groundwater around As-using industries and industrial waste burial sites. Other examples of contaminated environments with increased risk for As poisoning include agricultural lands treated with arsenical pesticides, urban areas, war zones defoliated or sprayed with As compounds (Nriagu, et al., 2007). In another research, Smith et al. (2002) described that there are more than 10 000 As contaminated sites in Australia. Inorganic As is naturally present at high levels in the groundwater of many countries- Argentina, the United States of America, Bangladesh, Chile, China, Mexico and India. (Murcott 2012). Few of the most As contaminated sites in Czech Republic include Příbram and Kutna Hora, which are historical mining and smelter sites (Száková et al., 2010; Kocourková-Víšková et al., 2015; Tremlová et al., 2017). Locally, mineralized bedrocks (ores) can contain extremely high As concentrations, resulting in geogenic anomalies in soil (Alloway, 2013). Fossil fuel combustion also produces quantities of As that may lead to long-term accumulation from the gases emitted to the surrounding areas. All of these factors release As into the environment and can result in its accumulation in soils (Moreno-Jiménez et al., 2012).

Examples:

Tremlová et al. (2017) were growing six different vegetables (black radish (*Raphanus sativus*), black salsify (*Scorzonera hispanica*), lettuce (*Lactuca sativa*), parsnip (*Pastinaca sativa*), Savoy cabbage (*Brassica oleracea var. sabauda* L.) and Swede turnip (*Brassica napobrassica*)) in As-contaminated soils which originated from mining and smelting sites in the Czech Republic – Kutna Hora and Píbram. They found that Higher proportions of both DMA and methylarsonate (MA) were found in the

aboveground edible parts (leaves) compared to the underground parts (tubers). Ponavic et al. (2016) researched the surface distribution of As in topsoils of the central part of Prague. Contents (medians) of As in their studied samples of Prague topsoils significantly exceed the average concentrations of As in the lithosphere. Their research revealed that a risk higher than 1×10^{-6} was found at all sites (Ponavic et al. 2016). Drahota et al. (2009) researched naturally contaminated soil, sediment and water at the Mokrsko-West gold deposit, Central Bohemia. They studied this site in order to determine the processes that lead to release of As into water and to control its speciation under various redox conditions. They found that some shallow subsurface environments with high organic matter contents were characterized by reducing conditions that indicated a relatively high amount of S^{2-} in the solid phase and a lower dissolved As concentration ($70\text{--}80 \mu\text{g L}^{-1}$) in the pore water. These findings were attributed to the formation of Fe(II) sulphides with the sorbed As (Drahota et al. 2009). In Czech Republic, the areas surrounding Kutná Hora are characterized by former silver mining activity. A high level of RE have been reported in soils close to the historical mines. Králová et al. (2010) in their publication described the Aqua Regia extractable (i.e. “pseudo-total”) element values in soils which exceeded the Czech threshold values (representing 30 mg kg^{-1} As). They also found that plant species growing in these soils represented an ordinary ruderal plant community of 29 species without occurrence of metallophytes. Moreover, the element concentrations in aboveground biomass of plants were low. Transfer factors, given as a ratio of element content in plants and its “pseudo-total” content in soil, varied from 0.0003 to 0.003 for As (Králová et al., 2010). Ash et al. (2016) performed column leaching experiments on soil samples collected from wooded area to the north of Přebíram in the Czech Republic, which were polluted with As and Pb, in order to determine the specificity of Low-molecular-mass organic acids (LMMOA) related release for individual elements, at varying organic acid concentrations. They found that the extent of As and Pb leaching in contaminated surface and subsurface soil depends significantly on the types of carboxylic acid involved, and that the mechanisms of As and Pb release differ between the studied LMMOA. Sysalova et al. (2011) were studying the extraction procedures applied to air pollution with selected RE (As, Cd, Mn, Cr, Mn, Ni, Zn, Pb) in urban Prague areas. They investigated the arsenic species occurrence and stability in presence of air filters (47 mm). They found that As (V) prevailed significantly in all fractions. Száková et al. (2010) were growing beetroot (*Beta vulgaris*, L.) in

contaminated soils from Pribram, Mokrsko and Kutna Hora to investigate the contents of RE, As speciation and possible interactions of elements and betalains. They found that Arsenate was the dominant arsenic compound in the extracts . Although the soils used in the experiment were extremely contaminated, the mobile portions of the RE represented only a small fragment of the total RE content.

3.2.3. Concentrations

Natural levels of As in soil usually range from 1 to 40 mgkg⁻¹, with a mean of 5 mgkg⁻¹ (Chou et al., 2007). Table 1. below shows typical As concentrations in soils in various world regions. In comparison to other RE, As shows a low solubility in well-aerated soils (Moreno-Jiménez et al., 2012).

Table 1. Arsenic concentrations in soils of various world regions (Source- Alloway, 2013 p 274)

Region/ Location	Site/ Soil information	N	Median	Mean	Geometric Mean	Min	Max	Method
World	Uncontaminated soils			7.2		0.1	55	
USA	Topsoils	1.260		7		<0.1	93	Total
Canada	Ap horizons	1.270	6.6					Total
EU	Topsoils	1.590	6			2.5	410	Aqua regia
Czech Republic	Various major soil groups	162			30		3.030	Total

3.2.3.1. Arsenic toxicity

The toxicity of As is determined by its valence state, if it is in an organic or inorganic form, and the physical properties governing its absorption and elimination. As(III) is 60 times more toxic than As(V). Organic As is almost non-toxic, whereas inorganic As is toxic. Ingested organic As has a half-life of about four days. Arsine gas (AsH₃) is the most toxic compound. The fatal dose is 250 mg m⁻³ at an exposure time of 30 minutes. The lethal dose (LD₅₀) for arsenic trioxide is 34.5 mgkg⁻¹, sodium arsenite 4.5 mg kg⁻¹, sodium arsenate 14 to 18 mg kg⁻¹, monomethylarsonic acid 1800 mg kg⁻¹, dimethylarsinic acid 1200 mg kg⁻¹, and trimethylarsine 8000 mg kg⁻¹ (Bissen et al., 2003).

3.2.3.1.1. Effects on human body

Arsenic can cause acute and chronic poisoning. In chronic As ingestion, the absorbed As accumulates in the liver, kidneys, heart and lungs and smaller amounts in the muscles, nervous system, gastrointestinal tract and spleen (Chappell et al., 2001). Long-term exposure through food or air results in hyperkeratosis, hyperpigmentation, cardiovascular diseases, disturbance in the peripheral vascular and nervous systems, eczema, suffering from liver and kidney disorder. Arsenic is deposited in hair, skin, nails, and bones. The normal level of As in hair ranges from 80–250 $\mu\text{g kg}^{-1}$ and 1000 $\mu\text{g kg}^{-1}$ is the indication of toxicity (Arnold et al., 1990).

The source of acute As poisoning is usually pesticides, insecticides or rodenticides. Most cases of acute As poisoning occurs from accidental ingestion. Acute As poisoning causes vomiting, dryness of the mouth and throat, muscle cramps, colicky abdominal pain, tingling of the hands and feet and nervous weakness. If death does not occur within 24h, irreversible organ disorders occur (Bissen et al., 2003).

Arsenic absorbed by the body undergoes hepatic biomethylation to form the metabolites monomethylarsonic acid (MMAA) and DMA for excretion (Chappell et al., 2001). To a certain amount the human body can detoxify the inorganic As compounds As(III) and As(V) by methylation, so that the affinity of arsenic for tissue is reduced. The possibility of methylation of As is limited to an As uptake of 400 to 500 $\mu\text{g d}^{-1}$. The individual sensitivity to As differs. Humans who are not accustomed to the consumption of As die at an As uptake between 0.1 g d^{-1} and 0.3 g d^{-1} . Chronic As consumers can uptake up to 1 g d^{-1} H_3AsO_3 without manifestations of acute poisoning (Bissen et al., 2003). Children, however, are more susceptible to As toxicity. About 90% of the children younger than 11 years living in As-affected villages show elevated levels of As in hair and nails. Infants and children might be at greater risk from As toxicity due to more water consumption on a body-weight basis (Chappell et al., 2001).

Arsenic toxicity causes inactivation of up to 200 enzymes, most notably those involved in cellular energy pathways and DNA synthesis and repair (Chappell et al., 2001). It is genetically harmful because it inhibits the repair of DNA damage and it is also carcinogenic (Bissen et al., 2003).

Arsenic is not an essential trace element for humans, so deficiency does not occur. However, As deficiency manifests in animals as reduced fertility, increased spontaneous abortion rate, low birth-weight in offspring, damage to red blood cells and increased mortality. In plants, root growth is stimulated by small amounts of As in

solution, but evidence of its necessity for plant growth has not been established (Naidu et al., 2006).

3.2.3.2. Methods of determination

The first stage in the determination of RE in soils is the collection of soil samples for analysis. The samples must be representative and collected using a suitable sampling regime i.e. one designed to match the purpose of the study. When samples have arrived at the laboratory, they are spread out, aggregates are broken down and samples are dried and then passed through an aluminum or nylon sieve. Special care is required when handling soils intended for speciation analysis to preserve the intrinsic forms of the analyte elements (Alloway, 2013).

The determination of total As concentrations in biological samples in most cases requires complete destruction of the organic matrix. During this mineralization, all the organic As compounds should be converted into inorganic As and a loss of the analyte should be prevented (Frankenberger, 2001). In samples with high halide concentrations, loss of As can occur. Dry ashing or evaporation of an acid digest to dryness might also result in loss of As, as the oxides may sublime. Arsenic trioxide sublimes at 180°C, whereas arsenic pentoxide (As_2O_5) releases oxygen at temperatures above 300°C and decomposes to arsenic trioxide (Stoeppler, 1992). Dry ashing is usually performed with inorganic oxidants such as magnesium nitrate/magnesium oxide mixtures. For wet ashing, acid mixtures of nitric acid and perchloric acid, or mixtures with nitric acid, perchloric acid, and sulfuric acid are commonly employed (Walter et al., 1997).

There are several analytical determination methods such as, Spectrometric methods (Molybdenum Blue Method, Silver Diethyldithiocarbamate Method); Electrochemical Techniques; Atomic Absorption Spectrometry, Inductively coupled plasma - optical emission spectrometry (ICP OES) and electrochemical methods, such as, colometry (Frankenberger, 2001). The silver diethyldithiocarbamate method is based on the generation of arsine (AsH_3) either with Zn and HCl or NaBH_4 in acidic solutions. However, the silver diethyldithiocarbamate method is mainly used for the determination of As in water samples (Frankenberger, 2001).

For the determination of As compounds, three major steps must be taken into consideration. The As species must be extracted from the sample (unfortunately in situ determination of arsenic compounds is not possible at environmental concentrations).

During the extraction step, the As compounds must not change or decompose chemically. Therefore, the extraction step should be as mild as possible and almost all the As present in a sample must be extracted (Kuehnelt et al., 2001). A combination of various extractants is often necessary to reach all the As. Polar organic solvents or water are commonly used for these purposes. After the analytes (As species) have been brought into the analyte solution, a separation step has to be employed to separate the different compounds. Due to the different chemical properties of the arsenic compounds, they might be anionic, neutral, or cationic; a reliable separation within one single run is not possible. A combination of various separation procedures must be employed (Frankenberger, 2001).

Interesting:

Bastías et al. (2013) were studying the use of honey as a bioindicator of environmental pollution that relate anthropogenic activities and volcanic eruptions to the concentration of As. The purpose of their study was to determine the concentration of total and inorganic As in honey samples collected during the years 2007, 2008, and 2009 in different areas of Chile. They found that As pollution in honey may originate from both natural and anthropogenic sources and that it is appropriate to use honey as a bioindicator of environmental pollution (Bastías et al., 2013).

3.2.3.3. Arsenic Speciation

Speciation of an element is the distribution of an element amongst defined chemical species in a system. Templeton et al. (2000) described that it is often not possible to determine the concentrations of the different chemical species that sum up to the total concentration of an element in a given matrix. Often, chemical species present in a given sample are not stable enough to be determined as such. During the procedure, the partitioning of the element among its species may be changed. For example, this can be caused by a change in pH necessitated by the analytical procedure, or by intrinsic properties of measurement methods that affect the equilibrium between species (Templeton et al., 2000). Also in many cases, the large number of individual species (e.g., in metal–humic acid complexes or metal complexes in biological fluids) will make it impossible to determine the exact speciation. The practice is then to identify various classes of the elemental species (Cornelis et al., 2004).

The speciation of As is an important consideration as its toxicity is mainly species-dependent and not well correlated with the total As concentration, therefore the total As concentration is not considered as an accurate measure of As exposure (Naidu et al., 2006). The different species of As produce diverse toxicological effects in humans, with inorganic forms being more toxic than organic ones. For food control, the objective consists in discerning the toxic inorganic forms from the harmless organo-arsenicals. Although foodstuffs yield total As concentrations below the legal limit, in some cases fish derivatives exceed these values. However, the As present in the latter samples is presumably arsenobetaine and so not toxic. Biomonitoring of occupational exposure to As needs to study different As species (As(III), As(V), MMA, DMA, As–betaine, As–choline, As–sugars) in a wide range of samples (such as: serum, plasma, urine, tissues). It is important to quantify all these species in order to identify the cause of exposure and the metabolism of such species into the body (Cornelis et al., 2004).

3.2.3.3.1. Stability of species

It was already mentioned that the aerobic environment, As is found in the oxidation state of +V. As(V) appears as H_3AsO_4 at pH values below 2, in the pH range from 2 to 11 H_3AsO_4 dissociates to H_2AsO_4^- and HAsO_4^{2-} . With the exception of extreme pH conditions (<4 or >9), or high concentrations of competing ions (e.g. phosphate, silicic acid or silicate) the release of As from its strong bonds with soil particles depends upon redox potential (Eh); the extent of aeration of the soil (Punshon et al., 2017). At low Eh values, As is found in the oxidation state +III as H_3AsO_3 . Up to pH 9 H_3AsO_3 does not dissociate. At higher pH values, it appears as H_2AsO_3^- , HAsO_3^{2-} , and AsO_3^{3-} . At Eh values below –250 mV arsenic compounds such as As_2S_3 can be formed in the presence of sulfur or hydrogen sulfide. These compounds are not soluble under neutral or acidic conditions. Arsine and elemental As are formed under very strong reducing conditions (Bissen et al., 2003). Agronomic cropping systems can be divided with respect to As mobilization on the basis of their redox status. Dominant biogeochemical processes influencing aerobic systems, specifically cereals, upland rice, fruit tree orchards, and community gardens, differ from those that dominate in anaerobic systems, predominantly in flooded rice paddies (Punshon et al., 2017).

3.2.3.3.2. Combinations of techniques

It is crucial to take in account the chemical “form” of an analyte (is oxidation state, atoms and molecules it is bound to, mineral phases in which it is associated). Some approaches for speciation of RE in soils are based around the coupling of chromatographic separation or other separation methods to element-specific detectors such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Alloway, 2013). The identification and quantification of As compounds is easily achieved with element-selective detectors: an electrothermal atomic absorption method (ET-AAS), hydride generation- flame atomic absorption spectrometry (HG-FAAS), an atomic fluorescence (AFS), Inductively coupled plasma optical emission spectroscopy (ICP-OES), ICP-MS and ICP-OEA = ICP AES. However, direct introduction of samples into the flame of the AAS was unsatisfactory because of matrix interference. ICP-MS is being increasingly used as a highly sensitive multi-element analyzer for RE analysis of environmental and biological samples, but it is extremely expensive and therefore inaccessible to many developing countries that have persistent As contamination issues.

Several combinations of derivatization, separation and detection systems have been employed for As speciation. The most common combinations are capillary electrophoresis with ultra-violet detection (CE-UV), HG-AAS, high-performance liquid chromatography coupled to ICP – Mass spectrometry (HPLC-ICP-MS), Ion Chromatography (IC) coupled with ICP-MS and high performance liquid chromatography-HG-AFS spectrometry (HPLC-HG-AFS) (Naidu et al., 2006).

3.2.3.3.3. Arsenic solid phase speciation

Arsenic solid phase speciation is concerned with the identification of the molecular-scale properties of As bearing solid phases and the physicochemical processes and factors that govern their formation. Arsenic in the solid phase is safer than in the aqueous and bioavailable form, meaning that it is not readily available to biological receptors (Smith et al., 1998).

Generally, there are two broad types of methods researchers use to elucidate the type of sorption complex a sorbate forms: indirect methods and direct methods. Ultimately, the goal of solid phase speciation is to yield detailed molecular-scale information about the sorbate/sorbent interactions including the coordination number (CN), next nearest neighbors, bond distances and oxidation state etc. This information can then be used to formulate possible sorption mechanisms. Indirect and direct methods greatly

complement each other to achieve this goal. Indirect methods rely on the quantification of data pertaining to the sorption or desorption of some ion under differing environmental conditions. Some of direct methods of As speciation include synchrotron radiation based x-ray absorption spectroscopy (XAS) including x-ray absorption near edge structure and extended x-ray absorption fine structure spectroscopy (XANES and EXAFS, respectively, commonly referred to as XAFS) (Naidu et al., 2006).

3.3. Arsenic and plants interactions

3.3.1. Arsenic Phytotoxicity

Phytotoxicity is defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances (phytotoxins) or growing conditions (Blok et al., 2008). Elevated soil As concentrations generally decrease the ability of the soil to support plant life, but phytotoxicity of As is often reached prior to accumulation to levels that would be toxic to wildlife or to humans ingesting the plants. However, the inability to grow an economically viable crop on contaminated sites is far from satisfactory (Naidu et al., 2006).

Among the symptoms induced by As exposure in plants are reduced root elongation, loss of root branching, chlorosis in leaves, and shrinking or necrosis in aerial plant parts. Arsenic causes chlorosis in plant leaves because of the induced decrease in chlorophyll levels. There may be other reasons for As-induced chlorosis, such as Fe deficiency, but the primary cause is directly attributed to the inhibition of pigment synthesis. The most notable effect produced by As results from the similarities that exist between arsenate and phosphate. Addition of arsenate causes a decrease in the levels of P in plants, because the entry of both ions is mediated through the same membrane transporter. Impairment of plant development is one of the observed symptoms from As toxicity (Moreno-Jiménez et al., 2012). Arsenate and arsenite both reduce the growth of plant species, such as Yorkshire fog (*Holcus lanatus*), Field Lupine (*Lupinus albus*), and Common wheat (*Triticum aestivum*), when grown under hydroponic conditions. Yoon et al. (2015) in their research found that mung bean (*Vigna radiata*) and cucumber (*Cucumis sativus*), compared to other crops, showed much smaller inhibition of germination by two types of inorganic As (arsenite, and arsenate), while the organic arsenic compound, DMA, had no inhibitory effects on plants. Jiang and Singh (1994) observed marked yield reductions in barley and ryegrass at plant As concentrations as low as 2.5–8.7 mg/kg. The total soil As

concentrations at which phytotoxicity occur are extremely variable across plant species and sites. This results from the fact that the availability of soil As to plants depends on many different factors, including plant species, As species, soil pH, soil Eh and the presence of soil components such as Fe and Al oxides, clay minerals and organic matter. Overall, there appears to be a fairly narrow margin between background soil As concentrations and phytotoxic concentrations (Jiang & Singh 1994). For example, in sandy and loamy soils the geometric mean of reported toxicity thresholds is a total soil As content of only 40 mg kg^{-1} , a value quoted as the upper range of background soil As concentrations. In addition to the phytotoxicity “protection” factor, there is good evidence that in many plants As accumulates in the roots with only limited translocation into shoots and leaves (Naidu et al., 2006). Therefore, it would appear that the “soil–plant barrier” concept proposed by Chaney (1980) provides considerable protection of the food chain in respect to As contamination.

3.3.2. The soil-plant barrier concept

In the “soil-plant barrier concept”, RE are classified in groups for food-chain Risk Assessment. The classification of RE depends on their partitioning behavior in soil, their propensity for root uptake, and their propensity to accumulate in edible portions in plants in relation to critical phytotoxicity concentrations versus critical food concentrations for humans. Using this concept, RE which are highly insoluble, or are retained very strongly by plant roots, are in Group 1. Some examples of such RE are Cr^{3+} , Cr^{6+} , Ti, Sn. Arsenic belongs to Group 2 - this group consists of RE that can be adsorbed by roots but are not readily translocated to edible plant parts (Chaney, 1980). Since 1980, it has become more apparent that As may be a food-chain hazard in paddy rice production systems. This is due to redox-induced mobilization of As. It should be noted that this concept classifies elements on the basis of transfer of contaminants via the soil-root-shoot/edible portion pathway. In urban contaminated soils, areal deposition of contaminants provides a bypass of the “soil-plant barrier”, so that the elements such as As and Pb may pose a risk to human or animal health where there is a continuing source of RE deposition to soil (Swartjes, 2011).

3.3.3. Arsenic accumulation mechanisms in plants

Toxic nonessential elements move into and through a plant along the same pathways as essential and beneficial elements. Thus, the molecular elucidation of plant metal

homeostasis and mineral nutrition has laid the foundation for dissecting nonessential element accumulation (Clemens & Ma, 2016). Arsenic accumulation in plant tissues is influenced by plant-inherent properties and it differs between species, ecotypes and plant parts (Naidu et al., 2006). Plants differ in specific root-response mechanisms to environmental conditions such as nutrient availability, adverse soil chemical conditions, water content, bulk density, and presence of toxic pollutants (Marschner 1996). Plants also can passively and/or actively modify the rhizosphere (the narrow zone at the soil–root interface). This results in altered chemical, biological and physical characteristics of the rhizosphere relative to bulk soil. The spatial extension of the rhizosphere can be greatly enlarged by mutualistic symbiosis between plants and fungi (aka. mycorrhizas). These plant-induced changes in the rhizosphere may influence the bioavailability of As (Naidu et al., 2006). Possibly following active mobilization from the soil or the apoplast, elements are taken up by transporters residing in the plasma membrane of root cells. Translocation to the shoot is dependent on the rate of trapping in different compartments of root cells, the mobility within the root symplast and across barriers such as the endodermis, loading into the xylem, and upward mobility in the xylem. Clemens & Ma (2016) described that element accumulation in leaves requires uptake from the xylem and the apoplast, transport to sites of usage and into storage compartments, and the availability of binding molecules. The distribution between leaves and the loading of seeds is a function of phloem loading and mobility, the rates of various symplast-to-apoplast and apoplast-to-symplast transport processes, the efficiency of xylem-to-phloem transfer and the availability of storage sites. Because As has no biological functions, it enters cells as a “hitchhiker” through transporters for essential or beneficial elements. In a wide range of plants, the most efficiently translocated As species are in fact methylated As species. Much of the As(III) is retained in root cells because of the transport of As(III)-PC (As (III)- phytochelatin) complexes into vacuoles (Clemens & Ma, 2016). To cope with the As stress, plants synthesize metal-binding cysteine-rich, thiol-reactive peptide called phytochelatins (PCs) (Hasanuzzaman 2020). Elemental imaging of rice roots by high-resolution secondary ion mass spectrometry (SIMS) have revealed strong colocalization of As and S in the vacuoles of endodermal and pericycle cells, which is consistent with such transport (Clemens & Ma, 2016).

3.3.3.1. Arsenic Biotransformations in Plants

Advances in speciation techniques such as X-ray absorption spectroscopy (XAS) and HPLC-ICP-MS/ESI-MS (which combines high-performance liquid chromatography with IC-ICP-MS and electrospray ionization mass spectrometry) have enabled researchers to analyze the different chemical forms of As both in the soil and in plant organs. One important finding was that many plant species rapidly reduce As(V) to As(III) inside cells (Figure 1.) Even the observed conversion of As(V) to As(III) in plant growth medium is the result not of microbial activity but of intracellular reduction of As(V) and subsequent efflux of As(III) (Clemens & Ma, 2016).

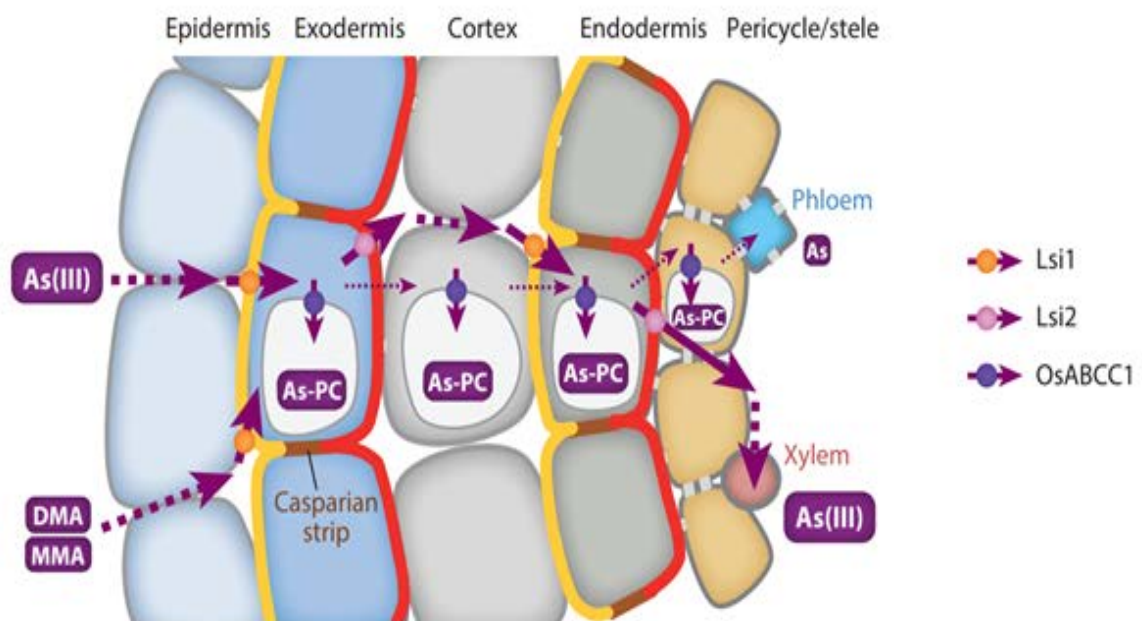


Figure 1. Lateral transport of As in rice roots. As(III) is transported toward the stele by two Si transporters, Lsi1 and Lsi2, localized at the distal (highlighted in yellow) and proximal (highlighted in red) sides of the exodermis and endodermis. OsABCC1 in the tonoplast sequesters As-phytochelatin (As-PC) in the vacuoles and thereby restricts the availability of As(III) for movement across the root. Dashed arrows indicate possible passive diffusion of As; the arrow thickness reflects the relative contribution to overall transport.

Additional abbreviations: DMA, dimethylarsinic acid; MMA, monomethylarsonic acid.

(Source: Clemens & Ma, 2016, p 497)

3.3.3.2. Arsenic and Iron interactions

There is some evidence that Fe and As nutrition are linked. Arsenic and Fe concentrations in shoots of tolerant Common Bent (*Agrostis capillaris*) as well as in

shoots and roots of Sea aster (*Aster tripolium*) were found to be strongly correlated (Porter & Peterson 1975). Sun et al., (2018) were researching how the oxalic acid mobilizes As from Fe(III)-oxide coated sand under hydrodynamic conditions. Because hydrous oxides of Fe are the major sink of As in soil, phyto-available As is related to the Fe hydroxide concentration in soil. Therefore, rhizosphere processes enhancing Fe availability to plants may concomitantly increase As availability in the rhizosphere (Naidu et al., 2006).

3.3.3.3. Arsenate-phosphate antagonism

Phosphates can often contain significant amounts of some RE (Allen, 1995). For example, in their research, Davenport and Peryea (1991) found that the use of high rates of mono-ammonium phosphate (MAP) or monocalcium phosphate (MCP) fertilizers have the potential to alter Pb and As mobility in soils contaminated with lead arsenate pesticide residues.

The P(V) ion plays a prominent role in As(V) interactions in the rhizosphere due to its physicochemical similarities and the well-documented antagonism between these ions in plant uptake (Hurd-Karrer, 1939). The As(V) ion acts chemically as a P(V) analog because both As and P belong to the same chemical group and have comparable dissociation constants for their acids and solubility products for their salts. Accordingly, As(V) is transported across the plasma membrane via the P(V) transport systems having a higher affinity for P(V) than for As(V) (Naidu et al., 2006). It is well known that P(V) additions in solution culture consistently decrease As(V) uptake and mitigate phytotoxicity symptoms (Hurd-Karrer, 1939). An important factor influencing the efficiency of P additions seems to be soil texture. It appears that P fertilization of fine-textured soils generally improves phytotoxicity symptoms even though As concentrations in plants increase, whereas no or negative effects have been observed for coarse-textured soils. Also, the scale of the experiment should be taken in account. Meaning, good mixing of the contaminated soil with the applied P fertilizer can be easily achieved in pot experiments. However, this is difficult to obtain at field scale. For example, P applied in the field as superphosphate in dry form and as phosphoric acid to cultures (such as, Sudan grass (*Sorghum x drummondii*) and corn (*Zea mays*)) have resulted in no phytotoxicity response (Naidu et al., 2006).

Smith et al. (2002) investigated the effects of PO_4^{3-} , Na^+ and Ca^{2+} on the sorption of As(V) and As(III) by an Oxisol, a Vertisol, and two Alfisols. They found that the

presence of P(V) (0.16 mmol L^{-1}) greatly decreased As(V) sorption by soils containing low amounts of Fe oxides ($<100 \text{ mmol kg}^{-1}$), indicating competitive adsorption between P(V) and As(V) for sorption sites. On the contrary, the presence of a similar amount of P(V) had little effect on the amount of As(V) adsorbed by soils with high Fe content ($>800 \text{ mmol kg}^{-1}$). However, As(V) sorption substantially decreased from 0.63 to $0.37 \text{ mmol kg}^{-1}$ as P(V) concentration was increased from 0.16 to 3.2 mmol L^{-1} in selected soils. This suggests increased competition between P(V) and As(V) for soil sorption sites, through either the higher affinity or the effect of mass action of the increasing concentration of P(V) in solution. A similar effect of P(V) on As(III) sorption was observed in the low sorbing Alfisol and high affinity Oxisol. However, the amount of As(III) sorbed by the Oxisol was much greater than the Alfisol for all treatments. The presence of Ca^{2+} increased the amount of As(V) sorbed compared with that of Na^{+} and was manifested through changes in the surface charge characteristics of the soils (Smith et al., 2002). Use of phosphates can be a cause of contamination not only when using them as soil fertilizers- extreme caution should be taken when using phosphates in food production. For example, Naidu et al., (2006) described a powdered milk accident in Japan in 1955. It contained As ranging from $13\,500\text{--}21\,000 \mu\text{g kg}^{-1}$. It was found that contamination of the powdered milk was from sodium phosphate (7.11% As) used in its manufacture.

3.3.3.4. Long-Distance Transport of As

Transfer of RE between organs requires long-distance transport via the xylem and phloem. Knowledge of the actual RE speciation in the xylem and phloem is still limited because of many analytical challenges related to sampling techniques and purity. The transport forms of As in the xylem are generally assumed to be As(III). In As(V) - exposed plants in which As(V) is not fully reduced during root passage, As(V) can additionally be present in the xylem (for example, for wheat and barley). Methylated forms of As can be detected when plants are exposed to them (Clemens & Ma, 2016).

3.3.4. As uptake in different plant species

Plants vary greatly in their sensitivity to enhanced soil As levels and some plants found growing on contaminated soils such as mine sites appear to have evolved tolerance to the high concentration of As in the soil. Such plants have been shown to accumulate As to extreme levels which would be toxic to normal plants. In some cases, plant As

concentrations of up to 8000 mg kg⁻¹ have been recorded in plants growing on contaminated mine wastes (Naidu et al., 2006). Similar to other RE, dicotyledons seem to accumulate generally more As in above-ground parts than monocotyledons (Otte & Ernst, 1994). However, there have been some notable differences found within these groups. For example, beans have been found to accumulate more As in leaves than tomato and radish (Cobb et al., 2000). Studies comparing As accumulation in various tissues of crop plants generally agree that most As is concentrated in below-ground plant parts, whereas least is incorporated into edible tissues such as fruits and seeds (Smith et al., 1998; Adriano, 2001). However, there are some exceptions. Similar or even larger As concentrations in shoots than in roots were found for radish (Cobb et al., 2000; Tlustoš et al., 2002). Potatoes accumulate more As in leaves than in the tuber flesh, whereas the largest concentrations of As are found in the tuber peeling (Jacobs et al., 1970).

Baker (1981) proposed a conceptual model for metal uptake characteristics in plants distinguishing three strategies:

- excluders – maintaining constantly small metal concentrations in shoots over a wide range of soil concentrations;
- indicators – reflecting external metal concentrations in a linear relationship; and
- accumulators – accumulating metals in shoot tissues in excess of root and soil concentrations due to active shoot-root translocation mechanism.

Uptake by different vegetables varies according to RE (and is not always consistent across sites). In Table 2. vegetables are ranked according to their potential to accumulate As. It can be seen, that leafy vegetables tend to be “accumulators”, and fruiting vegetables tend to be “excluders”. This is likely because leaves accumulate ions through transpiration and aerial deposition, and nonessential elements may therefore accumulate in these tissues if the element is transported across the root membrane or deposited on the leaf surface. Fruits are principally formed by movement of nutrients in phloem (the nutrient transport system) into the fruit across a membrane from the xylem (the water transport system) within the plant, and transfer of many elements from xylem to phloem may be controlled, or affected by many counter ions, similar to root uptake processes. For aerial deposition, species morphology (e.g. hairiness of leaves, surface roughness of aerial tissues) may be important in the trapping of atmospheric particulates and aerosols (Swartjes, 2011).

Table 2. Uptake of As by vegetable species. Note these may include both soil and atmospheric pathways. (Sources- Xu and Thornton, (1985); Warren et al., (2003); Woolson, (1973))

Element	As
Species	Lettuce (<i>Lactuca sativa</i>), Radish (<i>Raphanus sativus</i>), Spinach (<i>Spinacia oleracea</i>), Onion (<i>Allium cepa</i> L.), Beetroot (<i>Beta vulgaris</i>), Carrot (<i>Daucus carota</i> subsp. <i>Sativus</i>), Peas (<i>Pisum sativum</i>), Broad beans (<i>Vicia faba</i>) Cauliflower (<i>Brassica oleracea</i>), Potato (<i>Solanum tuberosum</i>), Tomato (<i>Solanum lycopersicum</i>)
↑ Increasing accumulation	

3.3.4.1. Hyperaccumulating plants

Plants capable of accumulating exceptionally large concentrations of RE are called “hyperaccumulators”. However, this trait was discovered relatively recently for As in two fern genera *Pteris* and *Pityrogramma* (Naidu et al., 2006). Ma et al. (2001) were researching Brake fern (*Pteris vittata*) which was found growing on a site in Central Florida contaminated with chromated copper arsenate. They analyzed fronds of plants growing at the site and found that only brake fern contained large amounts of As (As; 3,28–4,98 mgkg⁻¹). Brake fern extracted As efficiently from contaminated sites soils into its fronds: plants growing in the contaminated site contained 1,44–7,53 mgkg⁻¹. Brake ferns from the uncontaminated site contained 11.8–64.0 mgkg⁻¹. These values are much higher than typical values for plants growing in normal soil, which contain less than 3.6 mgkg⁻¹ of As. Arsenic concentration in fern fronds growing in soil spiked with 1,500 mgkg⁻¹. Arsenic increased from 29.4 to 15,86 mgkg⁻¹ in two weeks. As well as removing As from soils containing different concentrations of As, brake fern also removed As from soils containing different As species. Up to 93% of the As was concentrated in the fronds (Ma et al, 2001). These plants (fern genera *Pteris* and *Pityrogramma*) are the first reports of significant As hyperaccumulation by an

unmanipulated plant, and they could have a great potential to remediate As soils cheaply (Naidu et al., 2006).

Figure 2. shows a generalized pattern of As accumulation in plant shoots of terrestrial plants as dependent on substrate concentrations in oxidized soils. The shoot/soil concentration ratio (shoot accumulation coefficient, SAC) for hyperaccumulators is above one, whereas all other plants show SAC values well below this threshold. All hyperaccumulators of As are ferns (*Pteridales* from the genera *Pteris* and *Pityrogramma*), whereas all other plants include wild plants and crop cultivars from various families across the whole plant kingdom (*Pteridophyta*, *Monocotyles*, *Dicotyles*). Soil–plant transfer of As appears to be generally small as the mean SAC of all non-hyperaccumulators in Figure 2. is about 0.025, the largest is 0.28. From this, we can make a conclusion that the majority of non-As-accumulating plants can be termed as excluders.

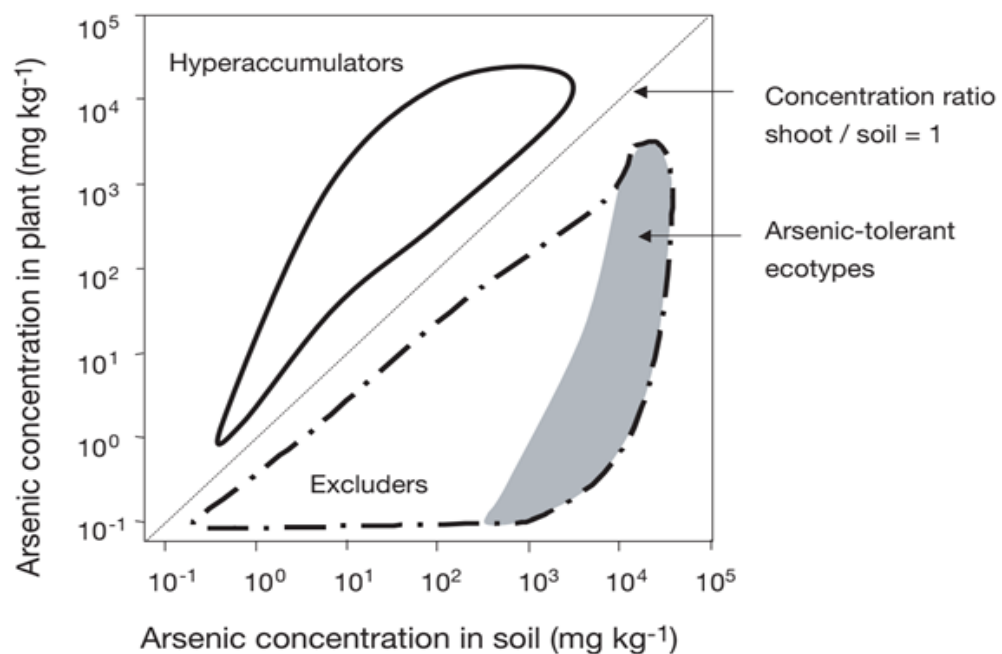


Figure 2. Generalized pattern of As uptake strategies by terrestrial plants (based on unpublished data compilation) (source: Naidu et al., 2006 p 211)

3.3.5. Crops contaminated with Arsenic

Root-to-shoot translocation rates of As are low in most crop species investigated to date. The crucial exception here is rice. A direct comparison with wheat and barley, for instance, showed that translocation in rice is 68–78% and 250–440% higher, respectively, depending on the form of inorganic As applied, and comparisons with

other crop plants, such as tomato, have yielded qualitatively similar results. Thus, higher As mobility is one reason for the massively higher soil-to-shoot As transfer factors in commercially grown rice relative to wheat and barley (Clemens & Ma, 2016). However there have been studies discussing the As contamination for other cereals and fodder plants. For example, Soto et al. (2019) were researching the possible effects of applying the As-resistant and plant growth promoter microorganisms to enhance As tolerance in *Triticum aestivum*. In another example, Dradrach et al. (2020) were researching As accumulation by *Festuca rubra* growing in contaminated sites. From their experiment, they concluded that the differences between the field and greenhouse data indicate that the populations of red fescue, that develop in As rich sites, are specifically As-tolerant.

3.3.5.1. Rice

Rice is both a model species and globally the most relevant source of human As and Cd intake. Significant potential has been shown for breeding low-Cd and low-As varieties in rice (Clemens & Ma, 2016).

Pinson et al. (2015) described more than 1,700 rice accessions that were cultivated over two years in flooded and non-flooded conditions. Consistent with many previous observations, the watering regime strongly influenced As and Cd grain accumulation in opposite ways. On average, flooded conditions led to a 30-fold increase in As accumulation; these increases resulted from the higher bioavailability of As under reducing conditions (as As(III)). The range of concentrations (maximum: minimum ratio) was higher for As than for most other elements (approximately 150 and 125 under non-flooded conditions), and heritability was high (>0.6) for As under both conditions (Pinson et al., 2015). Neumann et al. (2017) in discussed the soil warming influence on As availability in the rice rhizosphere. They found that As concentrations measured in pore water and plant tissue systematically responded to soil temperature, however plant growth, including grain yield and above- and belowground biomass, did not systematically respond. Although in their experiment, grain As concentrations did not change with soil temperature, taking in account the As effect on human health, future studies should explore correlation between climate change and As uptake by rice (Neumann et al., 2017). Hare and Chowdhary (2019) described the implication of As resistant microbes in mineral content and translocation on changes in growth response in rice plants grown in As affected areas. They found that rice supplemented

with As resistant bacteria BBAU/MMM1 (*Bacillus litoralis*) and BBAU/LP3 (*Bacillus infantis*) exhibited enhanced growth characteristics by 32.0% and >23.7% respectively with respect to control (Hare & Chowdhary, 2019). With rice being one of the main foods in many countries (Bastías & Beldarrain, 2016) and one of the most affected crops by As contamination, the above discussed studies show that there are ways for contamination prevention and remediation for rice crops.

3.3.5.2. Crop contamination prevention

To prevent the crop contamination with As, some preparation tasks should be done before sowing. Collecting soil and water samples for analysis and taking in account the possible contamination sources nearby (such as mining sites, contaminated water supplies, power plants) is a must. Punshon et al. (2017) advised that understanding the sources of As to crop plants and influence the dynamics of the agronomic As cycle are key to reducing crop uptake of As now, and preventing exposure in future (Punshon et al., 2017).

Methods for limiting the As uptake by crops have been described in different articles (Clemens & Ma, 2016; Punshon et al., 2017). Those include careful water management to control As bioavailability, amendment and fertilization practices, and mitigation using plant breeding approaches. These agronomic strategies have been proposed and explored to reduce As accumulation in crops. For example, the use of Fe-based amendments increases in the concentration of free Fe oxide in the soil, retarding the release of arsenite from the solid phase into soil solution, whereas Si fertilization to saturate the Si transport pathway which mediates As(III) accumulation (Clemens & Ma, 2016).

Water management techniques should be more broadly discussed when talking about rice. Flooded conditions can mobilize soil-bound As through reductive dissolution of Fe (oxyhydr)oxides, and the reduction of arsenate to the more mobile arsenite. Flooding is traditionally used for weed control, for mobilization of key nutrients such as Fe, P and Zn, and flooding also discourages the buildup of root nematodes over multiple years of rice growth. Flooded conditions can mobilize soil-bound As through reductive dissolution of Fe (oxyhydr)oxides, and the reduction of arsenate to the more mobile arsenite (Dixit & Hering 2003). Although the traditional method for cultivating rice involves flooding leveled, tilled fields before or shortly after planting germinated seedlings, flooded soil is not a biological requirement of rice plants. Water

management strategies that involve periods of oxic soil conditions can decrease As uptake in rice by limiting dissolution of As. However, it should be noted that rice grown in non-flooded or aerobic conditions has a lower yield than intermittently or constantly flooded rice. Intermittent flooding (flooding maintained until full tillering, followed by intermittent irrigation) is a promising management technique to reduce As levels, and can potentially produce higher grain yields than either non-flooded or constantly flooded conditions (Punshon et al., 2017).

However, the development of crops that accumulate high levels of As and yet remain healthy, while preventing arsenic from reaching the edible grain is thought to hold tremendous potential as a strategy for reducing human exposure to dietary arsenic. Over the past years, approaches based on molecular knowledge have rapidly gained momentum as arguably the most promising way to achieve higher food safety. The use of molecular genetics techniques such as alterations in gene expression characteristics, gene editing to alter target specificity, or alternately, using traditional plant breeding techniques are very tangible approaches. These approaches use knowledge of the As uptake and tolerance characteristics of plants to develop varieties with desired characteristics. Comparatively easy to implement is the use of loss-of-function alleles of RE transporters in breeding programs. Such alleles can come from mutagenized populations, preferably in the genetic background of commonly planted elite cultivars. Overall, altering the expression levels of transporters through transgenic approaches can be successful even when the selectivity is not changed (Clemens & Ma, 2016; Punshon et al., 2017).

Possibilities of soil remediation techniques has a great importance. Naidu et al. (2006) described that the abilities of microbes to carry out As conversions involving oxidation, reduction and methylation could be used for remediation of As-contaminated soils and water. Another potential method to treat As-polluted soil may be to use microbes to methylate As and volatilize arsine that could then be trapped. Soil fungal species would be best suited for this since they play a dominant role in As volatilization aerobically in agricultural soils. *Pteris vittate* and *Pityrogramma calomelanos* could be possibly used for phytoremediation of As-contaminated soils (as described in chapter 3.3.4.1.).

4. Conclusion

This study hypothesized that people living in the contaminated area might be endangered not only by As contaminated dust and water, but also by food products originating from this area. Moreover, there might be certain As accumulating plants and their consumption can represent a serious health risk. The hypothesis was confirmed, and it should be added that As is not only a danger for health but can also reduce yields and cause economical damage.

Soil contaminated with As is still a large issue that prevents people living in affected areas to access As free water and food. Economic costs of As toxicity include the expense of medical care, loss of income and reduction of crop productivity and quality. One of the pathways for As entering the food chain is when it occurs in food because it is present in the soil and water and is taken up by crop plants.

As (III) and As(V) are major species found in most soil samples, however As(III) is 60 times more toxic than As(V).

Some plants rapidly reduce As(V) to As(III) inside cells, however the amount of As accumulated and also the plant part in which it is accumulated varies greatly between species. Generally, leafy vegetables (such as lettuce and spinach) tend to be “accumulators” and fruity vegetables (such as tomato) tend to be “excluders”. When looking at cereals translocation in rice is much higher than in other crop plants.

In order to develop good management and remediation strategies for As-contaminated soils, the various reactions of As within the soil and the processes by which it can be transferred to other compartments of the environment need to be understood and predicted.

There are different ways to prevent the As accumulation in crops (such as water regime management, mitigation, amendment and fertilization practices). There are also ways of remediating the As contaminated soil, which include methylation, soil fungal species. Hyperaccumulating ferns *Pteris vittate* and *Pityrogramma calomelanos* could be possibly used for phytoremediation of As-contaminated soils in future.

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