PHYTOAVAILABILITY OF GEOGENIC ARSENIC AND ITS PARTITIONING IN SOIL: A CASE OF STUDY IN A THERMAL AREA OF CENTRAL ITALY

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Abstract

Arsenic (As) is an ubiquitous metalloid that is introduced into the environment from both anthropogenic and geochemical sources. The *As* can be introduced in food chain through plants grown on polluted soil and/or contaminated irrigation water. The element may impair plant growth, moreover its toxicity and cancerogenicity poses a threat for human health. Most plants tolerate soil *As* concentrations up to 50 mg kg^{-1}. However, at higher levels some plants might be negatively affected, while some others develop strategies to adapt to these conditions. It is known that *As* absorption, translocation and accumulation depend on plant species. The *As* tends to concentrate mainly in plant roots and old leaves, with a minor concentration in stems and young leaves, and the lowest concentrations is in fruits. In this study soil *As* mobility, tomato phytoavailability, and *As* plant partitioning were measured in an naturally *As* reach agricultural area (57.49 mg kg^{-1}). The results show that *As* compounds mainly accumulate in the roots (2.85 mg kg^{-1}), whereas only a small portion is translocated to fruits (0.08 mg kg^{-1}) making the risk for human health negligible.

Keywords: *Arsenic, tomato uptake, translocation, partitioning.*

Introduction

Arsenic (*As*) is a metalloid present in the environment both for geogenic or anthropogenic reasons such as mining, industrial and agricultural activities (Meharg et al., 1994). In the last years, this metalloid has obtained a worldwide attention because of its toxicity for both humans (arsenic and its compounds are classified into carcinogenic and listed in group 1 by the International Agency for Research into Cancer) and plants. For plant toxicity, roots are usually the first tissue exposed to *As*, which inhibits their extension and growth. At sufficiently high concentrations, *As* interferes with critical metabolic processes, such as oxidative phosphorylation and ATP synthesis, which can lead to plant death (Finnegan and Chen, 2012). Moreover, *As* translocation to the shoot can severely inhibit plant growth and yield (Garg et al., 2011).

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Several factors, such as concentration, chemical forms (organic or inorganic), oxidation state of *As* (Carbonell-Barrachina et al., 1998) as well as soil microbial community structure and activity (Stazi et al., 2015), play an important role on the behaviour of *As* in the environment and plants uptake therefore its accumulation in products for human consumption (Jia et al., 2014). Moreover, plant uptake and transport systems vary from species to species (Walsh et al., 1975; Marmiroli et al., 2014). In higher plants, arsenate is taken up and translocated via the high-affinity phosphate transporter (Pi) system due to the chemical analogy with Pi (Zhao et al., 2009). One of the mechanisms of tolerance given by the plants, after uptake as arsenate, is its storage in vacuoles after uptake and transformation in arsenite by a process of reduction mediated by glutathione and glutathione S-transferase (Sharma, 2012).
Tomato is one of the main horticultural crop in both Europe and the US, but its fruits can be compromised by *As* contamination. The *As* toxic species can be accumulated in tomato tissues and, consequently, could enter in the human food chain through the ingestion of its edible part.
In this study, a field experiment on tomato crop has been conducted on a naturally polluted agricultural soil in order to measure the mobility of total *As*, its transport in the soil/plant system and the risk for the human health.

Material and methods

The study was carried out in the year 2013 at the experimental farm of University of Tuscia (Viterbo, Italy). The soil was volcanic and classified in the textural class as clay loam. The agricultural field was managed according to the conventional practices. Soil tillage was carried out at 20 cm depth before tomato transplanting that occurred in May, at the same time the soil was fertilized with 80 kg ha^{-1} P_2O_5 (using perphosphate). Soil had a clay-loam texture with sub-acid reaction. The total organic carbon and nitrogen contents were 1.24% and 0.11%, respectively (Table 1).

Table 1
Soil chemical properties

Parameters	
Textural Class	Clay loam
pH $_{(H2O)}$	6.63 ±0.05
pH $_{(KCl)}$	5.56 ±0.02
TOC (%)	1.24 ±0.04
TN (%)	0.11 ±0.01
C/N ratio	10.98±0.29
Available P	14.4±0.43 mg kg^{-1}

Plant and soil sampling

Soil sampling was performed in summertime (August) at the end of the vegetative cycle of the tomato crop. After removing the litter layer six soil samples (0-20 cm depth) were collected from the agricultural field. The soil samples were immediately sieved (<2 mm) and kept at 4 °C prior to analyses. Six plants of the tomato crop were sampled at crop physiological maturity. The parts of each plant, fruits, leaves, stems and roots, were separated in the field and then immediately transferred in laboratory for analyses.

Chemical analysis and sample preparation

Available P concentration in soil was determined by the colorimetric method using by extracting samples with 0.5 M $NaHCO_3$ (Olsen et al., 1954). Soil pH was measured by potentiometry in distilled water and KCl 1:2.5 (w/v). Total Carbon (TOC) and Total Nitrogen (TN) were determined by dry combustion.

The reagents of superpure grade, used for the microwave-assisted digestions, were: hydrochloric acid (36% HCl), nitric acid (69% HNO_3) and hydrogen peroxide (30% H_2O_2) (Merck, Darmstadt, Germany). High-purity water (18 MΩ cm^{-1}) from a Milli-Q water purification system (Millipore, Bedford, USA) was used for the dilution of the standards, for preparing samples throughout the chemical process, and for final rinsing of the acid-cleaned vessels, glasses, and plastic utensils.

Monoelemental, high-purity grade 1 g l^{-1} stock solutions of *As* (As_2O_3 99.995%) was purchased from CaPurAn (CPA chem, Bulgaria). The purity of the plasma torch argon was greater than 99.99%. The external calibration solutions include known concentrations of each target analyte. They were prepared from standard certified elemental solutions (CaPurAn) and Milli-Q water containing 3% HNO_3 in order to obtain a range of the following concentrations: 0.5, 1, 3, 5, 10, 20 , and 40 μg l^{-1} . Yttrium was used as internal standard. The measurement accuracy was assessed using Standard Reference Material 1547 (SRM 1547) from The National Institute of Standards and Technology (Gaithersburg, MD, USA). For soil sample the accuracies of the measurements were assessed using standard reference materials trace metals Loamy Sand 3 (CRM034 –Fluka).

Total arsenic determination in soil and plant

The initial total *As* concentration in the soil was determined by extracting with concentrated HNO_3 and H_2O_2 with a microwave assisted digestion (Mars plus CEM, Italy) operating at an energy output of 1800 W. Briefly 0.5 g of each soil samples were digested placing them in digestion vessels with 10 ml of concentrated HNO_3. Samples and reagents were digested at 165 °C (2 min) – 175 °C (10 min). After the digestion procedure and subsequent cooling, the digested samples were diluted to a final volume of 50 ml with Milli-Q water.

All parts of tomato plants (roots, steam, leafs, berries) were divided into small pieces, washed with water and then dried at 65 °C for 72 h. Approximately 0.250 g of each tomato sample was inserted directly into a 100 ml PFA HP-500 Plus digestion vessels. Two milliliters of 30% (m/m) H_2O_2 , 0.5 ml of 37% HCl and 7.5 ml of HNO_3 69% solution were added to each vessel. The heating program was performed in one step. The temperature was increased linearly from 25 to 180 °C in 37 min and was held at 180 °C for 15 min. After the digestion procedure and

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subsequent cooling, the digested samples were diluted to a final volume of 25 ml with Milli-Q water. Blanks were prepared in each lot of samples. All experiments were performed in triplicate. All digested samples of soil and plant were analyzed at Inductively coupled plasma optical emission spectrometer (ICP OES) with an axially viewed configuration (Optima 8000DV, Perkin Elmer) equipped with an ultrasonic nebulizer was used in the As determination. For detection we have chosen the line with the lowest interferences, and high analytical signal to background ratio, the *As* (I) at 193.69 nm.

Determination of mobility of arsenic in soil and its uptake in plant

Mobility of total *As* in soil was determined by sequential extraction procedure according to Marabottini et al. (2013) with minor modifications. were studied the fractions of soil in which the *As* is poorly linked to the soil components: Water Soluble (WS), Not Specifically Sorbed (NSS) and Specifically Sorbed (SS). These three fractions are considered chemically labile and available to plants uptake.

The water-to-root bioaccumulation factor (BAF) of metalloid describes the ability of plant to accumulate element from water and soil. It was calculated as the ratios of *As* concentrations on root dry weight basis, and the corresponding total concentration of *As* in soil, according to Abreu et al. (2008). The root-to-plant translocation factor (TF) defines the movement and distribution of *As* from root to aerial part of the plant. The TF is operationally calculated as the ratio between the *As* concentration in plant (in shoot or fruits) on a DW basis, and the corresponding total concentration of *As* in roots (%) at the end of the experiment (Liu et al., 2014).

Statistical analysis

Differences in *As* content in the tomato plants and in the soil among various parts of plant (fruits, leaves, stems and roots) were statistically analysed by ANOVA and the means were separated by LSD at $P \leq 0.05$. In order to homogenize the variance, data were transformed as square root (x) and percentages as angular transformation before analysis. Afterwards data were transformed back for tables and figures elaboration.

Results and discussion

Total and bioavailable arsenic and assimilable phosphorous concentration in soil.

In soil were measured 57.49 mg kg^{-1} of *As*. Soil physical and chemical properties are important factors affecting *As* bioavailability. Clay and organic matter are the most responsible for binding the metalloid, while the presence of P influence *As* mobility in soil (Violante et al., 2005). According to the chemical fractionation the labile *As* was obtained by the sum of the *As* in the three fractions water soluble (WS), non-specifically sorbed (NSS), specifically sorbed (SS). These fractions have particular ecological importance, because the metalloid present is considered bioavailable and phytoavailable. The amount of *As* in each fraction is reported in Table 2. The bioavailable *As* was just the 13% of toxic metalloid naturally present in agricultural soil.

Parameters	Clay loam
Total *As*	57.49 ±1.62
Water Soluble *As*	0.37±0.05
Non Specified Sorbed *As*	0.11±0.01
Specifically Sorbed *As*	6.57±0.19

Table 2
The amount of arsenic in various soil fractions

Arsenic uptake and partitioning in plants organs

The higher levels of *As* concentration in the tissues of tomato plants resulted in the root system (4.64 mg *As* kg^{-1} dry matter) and lower concentration values in the other parts of plant following the trend leaves (1.30 mg *As* kg^{-1} dry matter) > stems (0.69 mg *As* kg^{-1} dry matter) > fruits (0.08 mg *As* kg^{-1} dry matter). This was probably due to a sort of strategy adopted by tomato plants to tolerate the *As*, by avoiding and/or limiting *As* transport from roots to other plant parts(Meharg and Macnair, 1992; Burlò et al., 1999).

In this study the BAF, that reflects the accumulation of *As* in in roots from *As* in soil, was 0.65 (about 8% of total *As* in soil).

Results obtained by translocation factor (TF), a representative index to indicate the shift of metalloid from roots to plant shoots or fruits, allow to confirm that *As* concentration in the roots increased more than that in the other part of the plant(Table 3).

shoot/root	leaves/root	fruit/root
0.28	0.15	0.017

Table 3.
The movement of As from root to aerial part of tomato plant expressed as traslocation factor (TF).

A consumption of 1 kg of fresh tomato containing this amount of *As* (0.019 mg kg^{-1} fresh weight) would involve the intake of about 19 µg of *As*, 0.27 µg kg^{-1} body weight (b.w.) of an average adult. However, current international guidelines (European Food Safety Authority, 2010) indicate a tolerable intake of 0.3 to 8 µg kg^{-1} b.w. per day.

Conclusion

This study investigates the uptake, accumulation and translocation of arsenic in tomato plants growth in natural polluted soil with a total *As* of 57.49 mg kg^{-1} and just 12.3% was the bioavailable portion. The results confirmed that *As*-compounds were mainly accumulated in the root, whereas only a little part was translocated to shoot and fruits making the risk to the consumer negligible.

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PHYTODISPONIBILITE DE ARSENIC GEOGENIQUES ET SES PARTITIONNEMENT DANS LE SOL: UN CAS DE L'ÉTUDE DANS UNE STATION THERMALE DE L'ITALIE CENTRALE

RÉSUMÉ
L'arsenic (As) est un métalloïde largement répandu dans l'environnement, il se trouve à la fois des causes naturelles et anthropiques. L'As peut entrer dans la chaîne alimentaire pour l'absorption par les plantes cultivées sur des sols contaminés et/ou irriguées avec l'eau pollué. L'élément peut nuire au développement de la plante, sa toxicité et cancérogénicité constitue un risque pour la santé humaine. Beaucoup de plantes tolèrent une concentration de As dans le sol jusqu'à 50 mg kg^{-1}. De toute façon, à des concentrations plus élevées certaines plantes peuvent être affectées négativement, tandis que d'autres développent des stratégies d'adaptation à ces conditions. Il est bien connu que l'absorption, la translocation et l'accumulation de l'As est espèces-dépendantes. L'As a tendance à se concentrer principalement sur les racines et les feuilles âgées, avec une concentration plus faible dans la tige et les jeunes feuilles, les fruits se trouvent dans des concentrations inférieures. Dans cet étude, on a mesuré de As mobilité dans le sol, la phyto-disponibilité et la distribution des plants de tomates cultivées sur des terres agricoles naturellement pollué par l'As (57.49 mg kg^{-1}). Les résultats montrent que les composés de l'As accumulent principalement dans la racine (2.85 mg kg^{-1}), tandis que seulement une petite partie est transloqué au fruit (0.08 mg kg^{-1}), ce qui réduit le risque pour la santé humaine.

FITODISPONIBILITÀ DELL'ARSENICO GEOGENICO E LA SUA RIPARTIZIONE NEL SUOLO : UN CASO DI STUDIO IN UNA ZONA TERMALE DEL CENTRO ITALIA

RIASSUNTO
L'Arsenico (As) è un metalloide largamente diffuso nell'ambiente per cause sia naturali che antropogeniche. L'As può entrare nella catena alimentare attraverso l'assorbimento da parte delle piante cresciute su suoli inquinati e/o irrigati con acqua contaminata. L'elemento può danneggiare la crescita della pianta, e costituisce un rischio per la salute umana per la sua tossicità e cancerogenicità. Molte piante tollerano una concentrazione di As nel suolo fino a 50 mg kg^{-1}, a maggiori concentrazioni alcune piante possono essere danneggiate, mentre altre sviluppano strategie di adattamento. E' noto che l'assorbimento, la traslocazione e l'accumulo di As dipende dalla specie vegetale. L'As tende a concentrarsi principalmente nelle radici e nelle foglie più vecchie, mentre raggiunge una minor concentrazione nel fusto e nelle foglie giovani, nei frutti in genere si ritrovano le concentrazioni più basse. In questo studio è stata misurata la mobilità dell'As nel suolo, la sua fito-disponibilità e la distribuzione in piante di pomodoro cresciute su un terreno agricolo naturalmente inquinato da As (57.49 mg kg^{-1}). I risultati mostrano che i composti dell'As principalmente si accumulano nella radice (2.85 mg kg^{-1}), mentre solo una piccola parte risulta traslocata nel frutto (0.08 mg kg^{-1}), riducendo il rischio per la salute umana.