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Use of Arsenic Contaminated Irrigation Water for Lettuce Cropping: Effects on Soil, Groundwater, and Vegetal

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Abstract The present study investigated the effects of using arsenic (As) contaminated irrigation water in *Lactuca sativa* L. cropping. Two different arsenic concentrations, i.e., 25 and 85 μg L^{-1} and two different soils, i.e., sandy and clay loam, were taken into account. We determined the arsenic mobility in the different soil fractions, its amount in groundwater, and the phytotoxicity and genotoxicity. Nuclear magnetic resonance (NMR) and inductively coupled plasma (ICP) were used to assess the lettuce metabolic profile changes and the arsenic uptake by the plant, respectively, as a function of the various conditions studied, i.e., As content and type of soil. Data indicated that at both concentrations in sandy soil, arsenic is in part quickly leached and thus present in groundwater and in part absorbed by the vegetable, being therefore readily available for assimilation by consumption. NMR results reported a large modification of the metabolic pattern, which was depending on the pollutant amount. In clay loam soil, the groundwater had a low As content with respect to sandy soil, and NMR and ICP performed on the lettuce did not reveal severe changes related to As, most likely because the metalloid is bound to the colloidal fraction.

Keywords Arsenic · Irrigation water · Nuclear magnetic resonance · Inductively coupled plasma · *Lactuca sativa*

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Introduction

Arsenic (As) is a highly toxic, naturally occurring grayish-white metal-like material. Inorganic forms of arsenic are created when elemental arsenic combines with oxygen, chlorine or sulfur, and it has been recognized as a human poison since ancient times. Because As is highly soluble and mobile in water, groundwater contaminations with arsenic is consequently widespread. Environmental arsenic problems are the result of mobilization under natural geological or soil-formation conditions, but in many cases the higher values indicate contamination from human activities as agricultural and industrial practices. The US Environmental Protection Agency [1], the International Agency for Research on Cancer [2], and the US Department of Health and Human Services [3] have determined that inorganic arsenic is carcinogenic to humans.

Arsenic in soil may leach into the subsurface soil; however, since many arsenic compounds tend to partition to soil or sediment under oxidizing conditions, leaching usually does not transport arsenic in depth [4, 5]. Arsenic is largely immobile in agricultural soils, therefore, it tends to concentrate and remain in the upper layers.

On soil surface and groundwater, As levels are usually low with the values ranging between 1 and 10 μg L^{-1} [5, 6]. Drinking water is recognized as the main source for human inorganic arsenic intake [7]. The World Health Organization [8] and US EPA [9] have fixed the limit for As in drinking water equal to 10 μg L^{-1} in order to avoid long-term effects and chronic diseases.

Arsenic in soil has been extensively investigated. Mäkelä-Kurtto et al. [10] found in Pirkanmaa region (Finland) that in arable soil, only about 1% of total arsenic was in soluble forms, and the arsenic content was higher in the lower mineral layers. Tlustoš et al. [11] observed that the presence of dimethylarsinic acid (DMA) in soil strongly affected the radish growth and the soil properties; the lowest As immobilization was found in Fluvisols while was higher in Luvisols and Chernozems. This was explained on the basis that Fluvisols are characterized by poor sorption capacity and low clay particle content, so that DMA remains available for plant uptake. On the contrary, Chernozems and Luvisols can reduce the phytotoxic levels of DMA via adsorption, volatilization, and/or decomposition, all phenomena occurring efficiently in these types of soils.

In Italy, the largest arsenic concentration is found in the Lazio and Campania regions, predominantly in fluvial and sea sediment. Arsenic contamination in soils, gravitational and clean water, diet and milk in bovine milk chain have already been considered [12, 13]; results showed that the geological presence of As could justify the high concentrations found in drinking water. The use of As-polluted waters for crop irrigation can have dramatic toxic effects also for the human health through contaminated food consumption. Inorganic arsenic is the predominant form in the soil and is toxic for plants; however, the levels of toxicity for the plants are derived from the combination of several factors, namely the environment, soil management, mobility of water, genetic factor regulating availability, uptake, and translocation of this element within the plant.

Also, the primary length reduction, commonly addressed as phytotoxicity, is useful to assess the vegetables' tolerance growth in contaminated soils [14]. The comet assay was introduced in the 1990s to assess the genotoxic effects arising from crop genetic damages due to pollutants [15–18].

Radish internal morphology modifications due to As contamination have been assessed by means of magnetic resonance imaging (MRI) [19, 20], and inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to quantify the As uptake by radish [20]. Recently, the high-resolution magic angle spinning-nuclear magnetic resonance (HRMAS-NMR) tool

has been proposed as a reliable system, based on NMR spectroscopy, for assessing the metabolome of foodstuff. It offers the almost unique opportunity of measuring samples without any chemical and/or physical preparation by producing highly resolved NMR spectra. The full width at half the height of most signals is in the order of about a hertz and is therefore comparable to the one obtained from the liquid sample equivalent. The chemical composition of many foodstuff like cheese [21, 22], meat [23, 24], wheat [25], and bread and flour [26] has been determined by means of ^{1}H-HRMAS-NMR.

Aims of this study were: (1) investigation of the arsenic environmental impact in simulated polluted soil, analyzing the different soil fractions by sequential extractions; (2) measurement of the As levels in gravitational water, the As uptake in vegetal; (3) evaluation of the As genotoxicity (by comet assay) and phytotoxicity (lettuce primary root length reduction); and (4) highlight of metabolic pathways alteration by using the ^{1}H-HRMAS-NMR metabolic profiling approach.

Materials and Methods

Experimental Plots

For our experimental purposes, radish and lettuce are well-suited edible crops for evaluating As germination, uptake, and phytotoxicity as suggested by Wachope [27], but there is little data on the sensitivity of the edible portion of these crops. The present study is included in a biennial project set on radish/lettuce crop sequence that are frequently adopted in farms of the Latium region and are commonly consumed vegetables. The results on radish cultivation have been published [20].

The simulation was performed in 12 lysimetric boxes, six filled with sandy soil and the other six with clay loamy soil, both collected in horticultural farms of Latium (Maccarese and Monterotondo, respectively) and sown with lettuce (*Lactuca sativa* L.). The boxes were divided in three groups, each composed of two sandy soil (S) and two clay loamy soil (CL), and were irrigated with water having different As contents. The latter were 19 μg L^{-1}, 44 μg L^{-1}, and 104 μg L^{-1}, in the forthcoming text indicated as "Control," As 25 and As 85. The arsenic content in Control was the natural abundance in groundwater, the larger concentrations were achieved by using sodium arsenate dibasic heptahydrate [As(V)] ($Na_2HAsO_4{\cdot}7H_2O$; RPE-ACS, Fluka Sigma-Aldrich). The arsenate, also if considered less phytotoxic than arsenite [As(III)], binds more strongly to soil constituent surfaces than arsenite and consequently arsenate is considered to be less mobile than arsenite. Three replicates were performed and the crop was irrigated 12 times so that the total amount of arsenic added for each treatment was 228 μg for Control, 528 μg for As 25, and 1,248 μg for As 85.

Soil Analysis

Soil characterization and elemental analysis are summarized in Table 1 and were determined according to an official method [28]: soil pH was measured with a glass electrode in a 2.5:1 water to soil ratio (*v*/*w*) suspension; particle size distribution with the densimetric method; total organic carbon (TOC) with the Walkley–Black method; organic matter content (OM) was obtained by calculation from the total organic carbon values, i.e., TOC × 1.72; available phosphorus (P_2O_5) with the Olsen method (30-min extraction with 0.5 M sodium hydrogen carbonate [$NaHCO_3$, Merck] pH 8.5, soil to solution ratio 1:20); cation exchange capacity (CEC) was calculated as the sum of exchangeable cations

Table 1 The main characteristics of the soils

Parameter	Measure unit	CL	S	Parameter	Measure unit	CL	S
pH (H_2O)		7.6	8.3	Ca	meq 100 g^{-1}	24.33	3.23
Sand	%	24.4	92.3	K		1.27	0.29
Silt		47.6	3.7	Na		3.21	0.12
Clay		28.0	4.0	Mg		0.7	1.6
TOC		1.04	0.52	Cd	mg kg^{-1}	<0.05	<0.05
OM		1.79	0.9	Cu		1.03	8.01
N	mg kg^{-1}	0.12	0.1	Fe		401.1	56.2
P_2O_5 (Olsen)		25.2	57.6	Ni		0.57	<0.05
K_2O		598.1	138.1	Pb		2.1	1.1
CEC	meq 100 g^{-1}	29.51	5.24	Zn		1.3	2.7

concentration in the soil solution (ammonium acetate extraction) by inductively coupled plasma atomic emission spectrometry (ICP-AES); anion content in soil, water, and plants was evaluated by using the ion chromatography [29].

Soil Extraction and Sequential Extraction Procedure

Arsenic content in soil was determined by means of the total acid digestion (ISO 11466, [30]); 0.5 g of air-dried sample was placed into a glass beaker, and 5 mL of aqua regia (nitric acid to hydrochloric acid, 1:3) was added, covered with a glass lid, and was left for 3 h at 200°C on a sand bath. After the oxidation of organic matter was completed, the sample was cooled, filtered through a 0.45-μm membrane and diluted to 50 mL with distilled water and measured by ICP-AES.

Sequential extraction procedure was performed using a modified version of the BCR method [31], which involves the following steps: (1) extraction with 0.11 M acetic acid solution (Fr1=exchangeable and weak acid soluble fraction); (2) extraction with 0.5 M hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, RPE-ACS Carlo Erba) solution (Fr2= reducible fraction); (3) digestion with 8.8 M H_2O_2 solution and extraction with 1 M ammonium acetate (Fr3=oxidizable fraction); (4) residue from step 3 was digested with *aqua regia* (Fr4=residual fraction). The arsenic concentration was then determined by ICP-AES at the analytical wavelength of 189.042 nm. Three replicates were performed for each sample and blanks were measured in parallel using the extraction reagents.

Phytotoxicity and Genotoxicity Tests

Polluted soil samples (about 500 g from each box) were collected in plastic bags from the upper layer, i.e., about 30 cm in depth and were placed in aluminum basins. Each basin, containing 50 seeds of *Vicia faba*, was irrigated with 120 ml of de-ionized H_2O and incubated at 20±1°C for 5 days to allow the germination. After 5 days of growth, the primary root length of the seedlings was measured, and phytotoxicity was calculated by measuring the reduction of the primary root length of the *V. faba* seedlings grown on polluted soil compared to those grown on unpolluted soil.

The comet test was performed under Alkaline unwinding/Alkaline electrophoresis protocol by Angelis et al. [32] and Menke et al. [33]. Root tips were chopped using a razor

blade; the suspension with released nuclei was filtered through a 20-μm filter to remove most of the tissue debris. Fifty microliters of the filtrate was mixed with agarose and set on a microscopic slide. Nuclei embedded in agarose were lysed for 1 h and a half, and then the slides were placed in the electrophoresis buffer at pH>13 for 40 min before electrophoresis. The protocol was modified in the electrophoresis analysis applying 300 mA, 25 V for 45 min instead of 30 V for 20 min. The prepared slides were stained with 5 μg ml^{-1} of ethidium bromide solution, and comets were viewed by an epifluorescence microscope; image analysis was carried out using an interactive image analyser (IAS 2000, Delta Sistemi, Rome, Italy). The comet length (Comet-L) is chosen as a parameter of DNA damage (micrometer) and is defined by the software IAS 2000 (with a calibrated ocular micrometer disk) as Comet-L (micrometer)=(total length of tail)+(head diameter). An analysis of variance (ANOVA) with the Dunnett post hoc test ($p<0.05$) was performed to determine the significant differences among the samples grown in clay and sandy soils treated with different As concentrations and a negative control (unpolluted soil).

NMR Measurements

About 5 mg of the sample was inserted in an HRMAS-NMR 4 mm teflon rotor together with about 40 mg of heavy water (D_2O) buffer at pH=7.0, 0.01 M (KH_2PO_4/K_2HPO_4) containing 0.5% tetra-silyl-propionic acid (TSP) used as internal standard. Three plants as Control, four for As25, and five for As85 were measured for sandy soil, while for clay loam soil three samples for each treatment were analyzed.

The ^{1}H-NMR spectra were recorded at 25°C with an AVANCE Bruker spectrometer operating at a proton frequency of 400.13 MHz. The sequence used contained the presaturation of the water signal (zgpr, Bruker library) obtained by centering the spectral window at 4.706 ppm and using a 2-s pulse with an attenuation of 64 dB. The spectral window was 11.015 ppm, the numbers of the points of the spectrum were 32 K and 90° proton pulses (4.50 μs and 5.30 dB attenuation) were used. Each spectrum was obtained with 512 scans and the FID, prior to Fourier transformation, was multiplied by an exponential factor (lb) equal to 0.30 Hz. Phase and baseline corrections of the spectra were normality performed by using the software package XWIN NMR 3.5 supplied by Bruker. Integration of the resonances of the spectrum was obtained by using the same software package; relative signals were normalized to the internal standard TSP.

Statistical Analysis

ANOVA was performed in order to test the significance of the observed differences. Software SPSS, version 14.0 [34], was used to determine significant differences ($p<0.05$) between samples cultivated in two types of soil, sandy and clayey, treated with different concentration of As.

Results and Discussion

Arsenic in Soil, Water, and Vegetal

Table 2 reports the As concentration in the four different soil fractions considered (Fr1 to Fr4). Exchangeable and weak acid soluble fraction (Fr1) and reducible fraction (Fr2) increased from Control to As 85 in clay loam soil (CL); conversely in sandy soil (S), the

Table 2 Concentrations of arsenic in the affected soils (mg kg^{-1})

Treatment	Fr1	Fr2	Fr3	Fr4
Control CL	0.85 a	1.07 a	5.87 ab	27.83 b
As 25 CL	1.09 ab	1.05 a	6.68 b	21.93 b
As 85 CL	1.92 bc	1.36 ab	5.88 ab	24.39 b
Control S	1.24 ab	1.40 ab	5.18 a	4.55 a
As 25 S	3.62 d	1.82 b	6.21 ab	4.75 a
As 85 S	2.14 c	1.37 ab	5.71 ab	3.79 a

Numbers in a column followed by different letters indicate significant differences ($p<0.05$). Control= 19 μg As L^{-1}, As 25=44 μg As L^{-1}, As 85=104 μg As L^{-1}

Fr1 exchangeable and weak acid soluble fraction, *Fr2* reducible fraction, *Fr3* oxidizable fraction, *Fr4* residual fraction

same Fr1 and Fr2 fractions increased from Control to As25, while decreased from As25 to As85, probably caused by water percolation that induced a higher accumulation of arsenic in gravitational water and less concentration of the relative soluble fraction easily available for plant in soil. In clay loam soil, the clayey organic colloid binds strongly to the pollutant, mainly the Fr4 fraction (residual) that was less available for plant uptake and leaching; also, the higher level of iron and calcium in clay loam soil (401 mg kg^{-1} Fe, 24.3 mg kg^{-1} Ca, Table 1) influence the solubility and mobility of arsenic.

The level of As in gravitational water from the contaminated boxes was reported in Table 3. As accumulation increase related to the As pollution both in the two types of soil. The As water content rose from 0.012 mg L^{-1} in Control of CL boxes to 0.031 mg L^{-1} in As 85 CL pots. The same trend was observed in percolation water from sandy soil where the element content increased from 0.023 mg L^{-1} in Control of S boxes to 0.040 mg L^{-1} in As 85 S pots. The higher accumulation detected in gravitational water from sandy soil with respect to clay loam could be linked to the different soil structures that cause major percolation of water and a lower concentration of pollutant in soil solution in sandy soils. So, the arsenic percolated through the sandy soil and it was less available for plants.

Beni et al. [12, 13] investigated the risk of high amounts of arsenic in net and bearing stratum water due to the volcanic origin of the soils in the Lazio region. This work underlined the risk of high amounts of Arsenic in net and bearing stratum water in the Lazio region where 75% of the samples were found to be above the legal limit.

Table 3 Arsenic concentrations in gravitational water from polluted soil (mg L^{-1}) and in vegetal growth in affected soil (mg kg^{-1})

Treatment	As in gravitational water	As in vegetal
Control CL	0.012 a	3.0 a
As 25 CL	0.016 a	3.8 b
As 85 CL	0.031 b	11.9 c
Control S	0.023 a	2.5 a
As 25 S	0.019 a	9.3 c
As 85 S	0.040 b	5.0 b

Numbers in a column followed by different letters indicate significant differences ($p<0.05$). Control= 19 μg As L^{-1}, As 25=44 μg As L^{-1}, As 85=104 μg As L^{-1}

With regard to lettuce growth, there was no difference in plant weights between treatments in both types of soil (data not shown). The data in Table 3 showed an As uptake related to As contamination. In fact, in clayey loam soil the lettuce arsenic content increased from 3.0 mg kg^{-1} in the Control boxes to 11.9 mg kg^{-1} in the As 85 pots. In sandy soil, the element content varied from 2.5 mg kg^{-1} in the Control boxes to 9.3 mg kg^{-1} in As 25 and to 5.0 mg kg^{-1} in the As 85 pots; this trend is similar to that observed for the arsenic fraction (Fr1 and Fr2) in sandy soil. In clay loam soil, the As uptake into the lettuce leaves increased with the increasing solution of As concentration, while in sandy soil the As uptake increased only from Control to As 25, and it decreased from As 25 to As 85 because of the major leaching at the higher As concentration.

Marconi et al., in a previous work [20], estimated the As amount in radish by ICP-AES and showed that As content increased accordingly to As soil pollution. The elevated As concentration of sandy soil plots could be due to the greater mobility and availability of the substances brought with the irrigation water, while in the clayey soil, the formation of As complexes with organic matter that makes this element less available for the radish. Furthermore, we underlined that significant effects exist for both treatments and soils while any interaction was observed for the treatment/soil.

Translocation of As from the soil to terrestrial plants made by root uptake or by absorption of airborne arsenic deposited on the leaves and certain species may accumulate high levels [1]. Nevertheless, even when these plants are grown in soil with high As content, the uptake is relatively low [35, 36].

Toxicity in Soil

The results of the comet assay (Table 4) were statistically significant for both types of soil at the two concentrations tested. One of the mechanisms of the DNA damage induced by arsenic could be oxidative stress in *V. faba*. Arsenic induces gene amplification and inhibits some DNA repair mechanisms. Inhibition of enzymes involved in DNA repair by arsenic may be responsible for the DNA damage. Under environmental stresses, plants often produce reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals causing damage to DNA, proteins, and lipids. To minimize the harmful effects of ROS, plants have evolved an effective scavenging system composed of antioxidant molecules and antioxidant enzymes [37] in order to reduce the toxicity of this compound. Further studies and in vitro experiments could be useful to better understand the mechanisms of the toxicity

Table 4 Comet assay performed in sandy soil and clay loamy soil sample, and reduction of primary root length of *V. faba* growth in two types of soil

Treatment	Comet-L (μm)	Primary root length (mm)
Control CL	95.29 a	57.96 a
As 25 CL	245.59 b	42.46 a
As 85 CL	178.41 b	38.08 b
Control S	157.12 a	39.80 a
S As 25	266.57 b	25.20 b
S As 85	202.19 b	26.84 b

Numbers in a column followed by different letters indicate significant differences ($p<0.05$). Control= 19 μg As L^{-1}, As 25=44 μg As L^{-1}, As 85=104 μg As L^{-1}

of the different arsenic chemical forms and how compartmentalization happens in roots besides chemical conditions of the soil that could affect arsenic phytoavailability.

The phytotoxicity test (Table 4) shows a significant result in clay loamy soil at the highest concentration tested (85 μg As L^{-1}) and in sandy soils at both concentrations. The soil texture and phosphorous level could influence arsenic uptake in plants. Increasing As concentration in clay loamy soil, the primary root length decreased, while in sandy soil the different concentrations exert the same results.

Phytoavailability and phytotoxicity of individual arsenic compounds in soils were summarized by Sheppard [38]. In soil-based studies, redox conditions and pH significantly affected the availability and consequent phytotoxicity of inorganic arsenic species. The dominant role of the soil properties and the source of arsenic are evident; inorganic As was fivefold more toxic in sandy and loamy soils than in clay soils.

Metabolic Profile of Lettuce by NMR

Figure 1 shows the ^{1}H-HRMAS-NMR spectrum of a lettuce leaf. In the region between 0 and 2 ppm the signals of fatty acids are present, from 2 to 3 ppm organic acids and amino acids have their resonances, from 3 to 6 ppm most of the peaks belong to carbohydrates, and finally in the lower frequency region from 6 to 8 ppm phenol compound signals can be found. Sobolev et al. [39] reported the detailed assignment of the metabolic profile of lettuce by high-resolution nuclear magnetic resonance spectroscopy. In the present work, we made use of that assignment for assessing the effects of arsenic contamination onto the metabolome as a function of As content and type of soil.

Based on the statistical analysis of the NMR data, we found that the most informative area is the one between 6 and 8 ppm (Fig. 2), containing mainly phenols and aromatic amino acids. Table 5 reports the various areas significant for discrimination according to soil type, and one can see that phenolic compounds are relevant for sandy soil.

For example, in the zone between 7.06 and 6.96 ppm the signals of chlorogenic and chicoric acid compounds are present. Another discriminating variable is the chemical shift at δ=7.64 ppm assigned to an unsaturated proton of the monocaffeoyltartaric acid. The concentrations of these compounds are reported to be high [40], in the order of milligrams

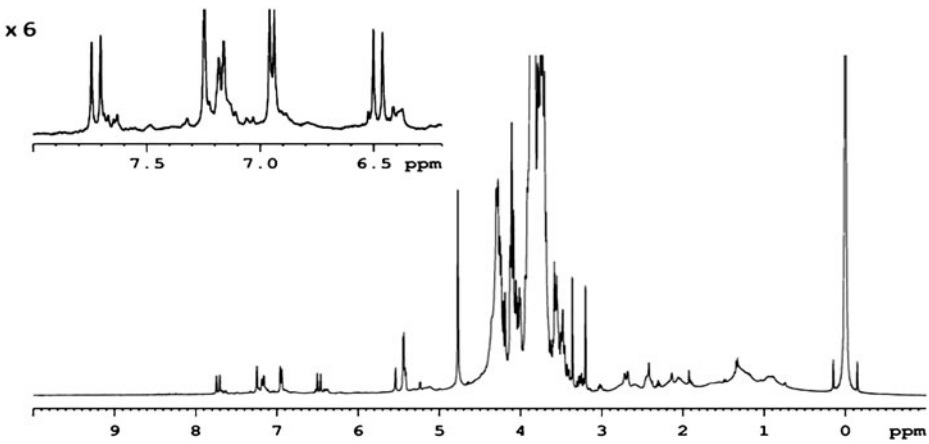

Fig. 1 ^{1}H-HRMAS-NMR spectrum of leaf lettuce in buffered D_2O

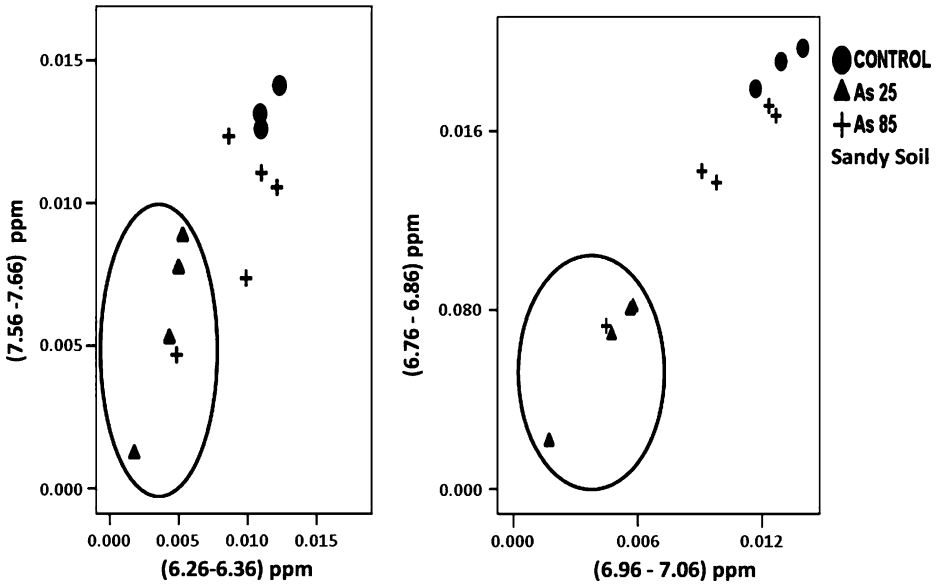

Fig. 2 Discriminant values of any interval area (parts per million) which represent the phenolic compounds in sandy soil

per gram, and were monitored in other studies by analysis for high-performance liquid chromatography.

The decrease of these compounds in the lettuce samples, in particular in those grown on sandy soils polluted at As 25, indicates that phenols can stimulate the enzyme glutathione S-transferases (GSTS) to reduce the toxicity of xenobiotic harmful substances [41]. The GSTS develop these protective actions by catalyzing directly the conjugation of glutathione (GSH), the latter is a three-peptide containing cisteine, glycine and glutamic acid, and exists in both reduced (GSH) and oxidized (GSSG) form. GSH facilitates the redox reactions by

Table 5 Results of ANOVA: significance of the discriminant intervals (parts per million) in As 25 plots

Variable (ppm)	F Sandy soil	Significance (*P*) Sandy soil	F Clay loamy soil	Significance (*P*) Clay loamy soil
7.66–7.56	5.884	0.023	1.136	0.382
7.16–7.06	6.385	0.019	0.959	0.435
7.06–6.96	10.186	0.005	0.784	0.498
6.86–6.76	14.652	0.001	0.780	0.500
6.76–6.66	4.558	0.043	0.828	0.481
6.36–6.26	11.565	0.003	0.783	0.499
5.36–5.26	4.831	0.038	1.042	0.409
4.06–3.96	5.005	0.035	0.462	0.651
3.86–3.76	5.006	0.035	0.680	0.542
3.76–3.66	5.411	0.029	0.995	0.424
3.66–3.56	5.149	0.032	0.913	0.451
3.46–3.56	4.151	0.053	0.328	0.732

reversible oxidation of its thiolic active groups and binds to various electrophilic substances.

Table 6 reports other chemical shifts relevant for discriminating, the most important are found at δ=4.01 ppm and 3.46 ppm. The two resonances belong to amino acids, among which cisteine, glycine and glutamic acid, which participate in glutathione synthesis. This is supported by the reduced amount of these amino acids, which are consumed along with the glutathione activity that goes onward.

The decrease of these compounds, as also phenols, was observed in the lettuce samples polluted at As 25 in sandy soils, while in the samples contaminated at As 85 we found a saturation effect. Probably above the defined level of arsenic concentration, the sites of coordination of complexes that form between the arsenic (III) and the glutathione are saturated [42]. These differences are relative to arsenic concentrations found only in the sandy soils, while in samples from clay loamy soils, there was no significance, neither an effect of the arsenic presence nor consequently a concentration effect.

The data obtained in a previous work [20] showed a morphological structural change that occurred in radish tuber growing in As-contaminated soil by considering T_2-weighted MRI images of the different experimental plots. We found that increasing the As concentration changed the outermost cell layer thickness. This might be a physiological effect of the hypocotyl to the arsenic presence, which tend to create a barrier to avoid As accumulation. This observation is supported by the fact that in sandy soil, As leaching is faster for the low capability of soil particles to keep the As contaminant.

Conclusion

This work underlined the evidence of the use of the same chemical–physical parameter as indicators for investigated As contamination in soil and vegetables. The different amounts of organic matter and clay content in the two types of soil were correlated with the soil exchange capacity that regulates the release of toxins. These differences relative to arsenic concentrations were found only in the sandy soils, while in clay loamy soils, there was no significance, neither an effect of the arsenic presence nor consequently a concentration effect.

NMR data were correlated with those obtained by ICP. In fact, the metabolic profile of lettuce by ^{1}HRMAS-NMR showed a quantitative difference of phenolic compounds in As 25 samples, in the sandy soils where the faster As leaching induce a higher concentration of the relative soluble fraction easily available for plants. While in the As 85 samples, an effect

Table 6 Any ^{1}H chemical shifts of amino acids that are in the discriminant zone between 3.46 and 4.01 ppm

Compound	Assignment	^{1}H (ppm)	Multiplicity
Cisteine (Cys)	αCH	3.98	dd
Glicine (Gly)	αCH	3.55	s
Glutamate (Glu)	αCH	3.76	t
Glutamine (Gln)	αCH	3.78	t
Threonine (Thr)	αCH	3.60	t
Asparagine (Asn)	αCH	4.01	t

dd doublet of doublets, *t* triplet, *s* singolet

of saturation was observed and this is probably due to the fact that the sites of the arsenic (III)-glutathione complexes of coordination are saturated over a defined level of arsenic concentration. We needed to compare the data obtained by different analytical methods in order to have a more comprehensive view of the pollutant action in different environmental compartments.

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