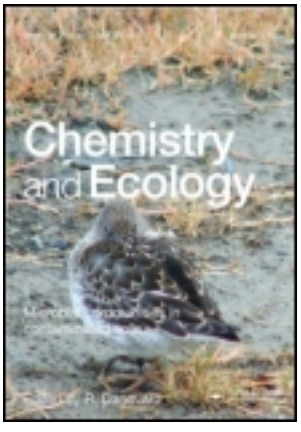

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Effects of arsenic on soil-plant systems

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[a] Department of Production Plants and Anthropic Settlements, ISPESL, Rome, Italy

[b] Agricultural Research Council Research Centre for the Soil Plant System (RPS), Rome, Italy

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Effects of arsenic on soil–plant systems

Elena Sturchio[a]*, Priscilla Boccia[a], Claudia Meconi[a], Miriam Zanellato[a], Simona Marconi[b], Claudio Beni[b], Rita Aromolo[b], Alessandra Ciampa[b], Giampietro Diana[b] and Massimiliano Valentini[b]

[a]*Department of Production Plants and Anthropic Settlements, ISPESL, Rome, Italy;* [b]*Agricultural Research Council Research Centre for the Soil Plant System (RPS), Rome, Italy*

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Arsenic is a highly toxic element and its presence in food composites is a matter of concern for the well-being of both humans and animals. The aim of this study was to evaluate the effects of arsenic on food vegetables and polluted soils. *Vicia faba* seedlings grown on polluted soils were used to evaluate the phytotoxic and genotoxic effects by comet assay. The results of these tests were dependent upon different types of soils. We studied different types of soils and contamination effects on *Raphanus sativus* L. and *Lactuca sativa* L. cropping by using magnetic resonance imaging (MRI) and nuclear magnetic resonance (NMR). For both analytical approaches, we found indicators correlated to As contamination, chemical for NMR, i.e. modification of composition, and morphological for MRI, i.e reorganisation of internal tissues. Samples of vegetables were collected to analyse their micro- and macronutrient contents and level of metals using inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis, which confirms the results obtained by MRI.

Keywords: arsenic; genotoxicity; phytotoxicity; magnetic resonance imaging; nuclear magnetic resonance; ICP-OES

1. Introduction

Arsenic (As) is one of the most toxic elements that can be found in the environment. Despite its toxic effect, inorganic arsenic occurs on earth naturally in small amounts [1,2]. It is widespread in our environment due to natural sources or anthropogenic activities. It occurs in soil and minerals and it may enter air, water and soil through wind-blown dust and water run-off.

These sources are very variable in terms of arsenic risk, however, higher concentrations are found mainly in groundwater, which presents a particular risk because it is often used as a source of drinking water and to irrigate crops for food.

Extrapolating from the main food categories (Concise Food Consumption Database) of the European Food Safety Authority (EFSA), the food sub-classes of cereal grains and cereal-based products, followed by food for special dietary uses, bottled water, coffee and beer, rice grains and rice-based products, fish and vegetables were identified as largely contributing to the inorganic

*Corresponding author. Email: elena.sturchio@ispesl.it

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arsenic (iAs) daily exposure in the general European population [3]. Recently, researchers have emphasised an alarming situation rising from the issue of iAs-contamination in food. They suggest that a diet based on iAs-contaminated paddy rice, which is a primary aliment for half of humanity, can lead to cancer [4].

The main reason for the high iAs accumulation in rice is its method of cultivation; rice is cultivated under anaerobic conditions, in which iAs is highly available for plant uptake. High consumers of rice in Europe, such as certain ethnic groups, are estimated to have a daily dietary iAs exposure of $\sim 1\,\mu g\ kg^{-1}$ b.w. per day [5].

Although iAs-contaminated food is a widespread problem worldwide, no direct evidence linking iAs-contaminated rice consumption to cancer has been provided to date. Moreover, it is recommendable to consider the effective concentration of iAs after the rice is boiled for cooking. Furthermore, different individual genetic polymorphisms might determine different iAs metabolism and biomethylation pathways, leading to different adverse toxicological effects [6].

In Italy, the highest concentration of As was found in Lazio and Campania [7]. The Italian legal limit for total As content in water intended for human consumption has been fixed at $10\,\mu g{\cdot}L^{-1}$ [8].

In the last decade, the most interesting aspect of genotoxicity assessment has been its application to environmental toxicology [9]. One of the key objectives in determining soil health is to acquire indicators that can be used to evaluate the soil's current status; in this regard, the aim of this study was to develop biomarkers for genotoxicity (comet assay) and to evaluate contamination effects on soil functional qualities and plant nutrition in an integrated approach [10].

Root meristems of *Vicia faba* were analysed to study the phytotoxic and genotoxic effects of As. This plant was chosen as test plant because it has large chromosomes and it is particularly suited to the study of soil pollution [11,12].

The analyses were supported by soil–plant chemical–physical characterisation and by spectroscopy analysis.

Absorption spectroscopy studies were useful to determine the total arsenic concentrations in a range of foodstuffs, including vegetables, rice and fish. Magnetic resonance imaging (MRI) is suitable to assess vegetables quality. MRI is primarily used in medical imagining, visualising the structure and function of the body. It also has been used outside the medical field, for example as a non-destructive testing method to characterise the internal structure and quality of several types of products (vegetables and fruits). A previous study by Clark et al. reported data on the application of MRI in studies of fruits and vegetables [13].

A further spectroscopy technique, ^{1}H high-resolution magic-angle spinning (HR-MAS) NMR, was used to observe possible qualitative or quantitative changes in metabolites in samples polluted with different As concentrations. This recently developed technique allows the analysis of materials that swell, become partially soluble, or form solutions in a solvent, even when some solids are still present [14]. The advantages of this technique are that it requires small quantities of sample, between 3 and 5 mg, and no preliminary sample extraction is required, therefore avoiding the problem of sample manipulations which can cause loss or oxidation of several classes of substances.

2. Materials and methods

2.1. *Experimental site*

The simulation was set-up in two sets of lysimetric boxes, using two types of soil, sandy and clay-loamy, having different physical, chemical and functional qualities. The experimental set-up consisted of 12 boxes, sown with lettuce (*Lactuca sativa* L.), a plant species with edible leaves. On a second occasion, the experiment was repeated with radish (*Raphanus sativus* L.) which

represents a useful plant for the evaluation of morphology and structural changes due to As contamination.

In two replicates, the boxes were differently polluted with two concentrations of arsenate, adding 25 μg As $\cdot$ L^{-1} (As 25) and 85 μg As $\cdot$ L^{-1} (As 85), respectively, and one of these was used as the control (C control). These concentrations represent the mean As content observed in irrigation water and the higher values detected in net and bearing stratum water of Lazio, respectively [15]. Furthermore, plants were irrigated using net water with a local As compound equal to 19 μg As $\cdot$ L^{-1}. The final As concentrations were: 19 μg As $\cdot$ L^{-1} for C control, 44 μg As $\cdot$ L^{-1} for As 25 and 104 μg As $\cdot$ L^{-1} for As 85. The irrigation was repeated 12 times. A negative control with deionised H_2O was used in all the phytotoxicity and genotoxicity tests.

2.2. *Phytotoxicity and genotoxicity tests on polluted soils*

2.2.1. *Sample collection*

At the end of the treatments, soil samples ($\sim$500 g from each box) were collected in plastic bags from surface soil (30 cm) and placed in aluminium basins. Each basin, containing 50 seeds of *Vicia faba*, was irrigated with 120 mL of deionised H_2O and incubated at 20 $\pm$ 1 °C for five days to allow germination in order to perform the phytoxicity test and the comet assay.

2.2.2. *Phytotoxicity test*

After five days of growth, seedlings were removed; the primary root length of seedlings was measured to evaluate the potential toxic effects. Phytotoxicity was calculated by measuring the reduction in primary root length of *V. faba* seedlings exposed to polluted soil.

2.2.3. *The comet assay*

The comet test was performed under an alkaline unwinding/alkaline electrophoresis (A/A) protocol as described by Angelis et al. and Menke et al. [16,17]. Briefly, nuclei from chopped *V. faba* root tips were filtered and mixed with agarose and set on a microscopic slide for electrophoresis (300 mA, 25 V for 45 min). Comets were viewed using an epifluorescence microscope; image analysis was carried out using an interactive image analyser (IAS 2000, Delta Sistemi, Rome, Italy). Comet length has been used as a parameter of DNA damage (μm). Each experimental data set was tested by analysis of variance (ANOVA) with Dunnett's test to compare the difference in its mean and standard error at the 0.05 level of statistical significance versus the control group (H_2O deionised). The statistical software package SPSS (Chicago, IL) was used.

2.3. *Soil–plant system analysis*

2.3.1. *Soil characterisation and inductively coupled plasma analysis on plants*

Soil chemical and physical parameters were investigated to evaluate the effects on the soil–plant system due to contamination with arsenic. Soil characterisation and element analysis (Table 1) were carried out using the official soil chemical analysis method [18]. Total soil arsenic was determined by total acid digestion of the soil according to ISO 11466 [19]. Soil samples were analysed using a sequential extraction procedure, a modified version of the BCR (Community Bureau of Reference) sequential extraction procedure [20], on four different soil fractions:

Table 1. Soil characterisation.

Parameter	Unit measure	C	S
pH		7.6	8.3
Sand	%	24.4	92.3
Silt	%	47.6	3.7
Clay	%	28.0	4.0
OM	%	1.8	0.9
N	$mg \cdot kg^{-1}$	0.12	0.10
P Olsen	$mg \cdot kg^{-1}$	25.2	57.6
K_2O	$meq \cdot 100\,g^{-1}$	598.1	138.1
CEC	$meq\ 100\,g^{-1}$	29.51	5.24
Ca	$meq \cdot 100\,g^{-1}$	24.33	3.23
K	$meq \cdot 100\,g^{-1}$	1.27	0.29
Na	$meq \cdot 100\,g^{-1}$	3.21	0.12
Mg	$meq \cdot 100\,g^{-1}$	0.7	1.6
Cd	$mg \cdot kg^{-1}$	<0.05	<0.05
Cu	$mg \cdot kg^{-1}$	1.0	8.0
Fe	$mg \cdot kg^{-1}$	401.1	56.2
Ni	$mg \cdot kg^{-1}$	0.57	<0.05
Pb	$mg \cdot kg^{-1}$	2.1	1.1
Zn	$mg \cdot kg^{-1}$	1.3	2.7

Notes: C, clay-loamy soil; S, sandy soil; OM, organic matter; CEC, cation-exchange capacity.

(1) exchangeable and weak acid soluble fraction, (2) reducible fraction, (3) oxidisable fraction, and (4) residual fraction (Table 2).

Plants harvested two weeks after sowing were collected in plastic bags, transported to the laboratory and prepared for inductively coupled plasma (ICP) analysis. Dried hypocotyls of lettuce and radish were analysed for total element content by ICP spectrometry, following the wet acid digestion method. Nutrient uptake in shoots and hypocotyls, and plant morphology were examined to identify some indicators concerning the soil–plant system.

2.3.2. *Experimental conditions and acquisition parameters MRI–^{1}H HR-MAS NMR*

Fresh radish hypocotyls were analysed using MRI. MRI measurements were performed using a Bruker Avance 300 MHz spectrometer (Bruker Biospin, Milan, Italy) equipped with a cylindrical birdcage single-tuned nucleus (^{1}H) coil probehead with an inner diameter of 60.0 mm. The water signal was monitored and used for the image reconstruction. Gradient–echo (GEFI) and multi-slice–multi-echo (MSME) were performed according to standard procedures. In GEFI, the echo and repetition times were 2.445 and 60.0 ms, respectively, whereas in MSME they were set to 17.5 and 5000.0 ms, respectively.

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

Sample	Exchangeable and weak acid soluble fraction (Fr1)	Reducible fraction (Fr2)	Oxidisable fraction (Fr3)	Residual fraction (Fr4)
Control clay-loamy	0.85a	1.07a	5.87ab	27.83b
As 25 clay-loamy	1.09ab	1.05a	6.68b	21.93b
As 85 clay-loamy	1.92bc	1.36ab	5.88ab	24.39b
Control sandy	1.24ab	1.40ab	5.18a	4.55a
As 25 sandy	3.62d	1.82b	6.21ab	4.75a
As 85 sandy	2.14c	1.37ab	5.71ab	3.79a

Notes: Numbers in a column followed by different letters indicate significant differences ($p < 0.05$). Control, $19\,\mu g\,As \cdot L^{-1}$; As 25, $44\,\mu g\,As \cdot L^{-1}$; As 85, $104\,\mu g\,As \cdot L^{-1}$.

Approximately 5 mg of leaf lettuce sample was inserted in a ^{1}HR-MAS NMR 4 mm Teflon rotor, together with ~40 mg of deuterated buffer at pH 7.0, 0.01 M (KH_2PO_4/K_2HPO_4) containing 0.5% TSP (tetra-silyl-propionic acid) was used as an internal standard.

^{1}H NMR spectra were recorded at 25 °C with AVANCE Bruker spectrometer operating at a proton frequency of 400.13 MHz. The sequece used contained the presaturation of the water signal (zgpr, Bruker library) obtained by centring the spectral window at 4.706 ppm and using a 2-s pulse with an attenuation of 64 dB. The spectral window (SW) was 11.015 ppm, the number of points of the spectrum was 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 30 Hz. Phase and baseline corrections of spectra were normality performed using the software package X-WIN NMR 3.5 supplied by Bruker. Integration of the resonances of the spectrum was obtained by using the same software package: relative signals were normalised to the internal standard TSP.

Statistical analyses of data were performed using SPSS (2006) [21]. ANOVAs were applied to compare arsenic treatments, and differences between means were tested with Duncan's multiple range test.

3. Results

The results of soil characterisation are shown in Tables 1 and 2.

3.1. *Analyses on soil boxes cultivated with lettuce*

3.1.1. *Phytotoxicity and genotoxicity tests on polluted soils*

3.1.1.1. *Phytotoxicity.* As 85 shows a significant statistical values for phytotoxicity in clay-loamy soils and sandy soils at both concentrations tested. At increasing As concentration in clay-loamy soil, the primary root length decreased, whereas in sandy soil the different concentrations have the same results (Figure 1).

3.1.1.2. *Comet assay.* The results of the comet assay are statistically significant for both types of soil at the two concentrations tested (Figure 2).

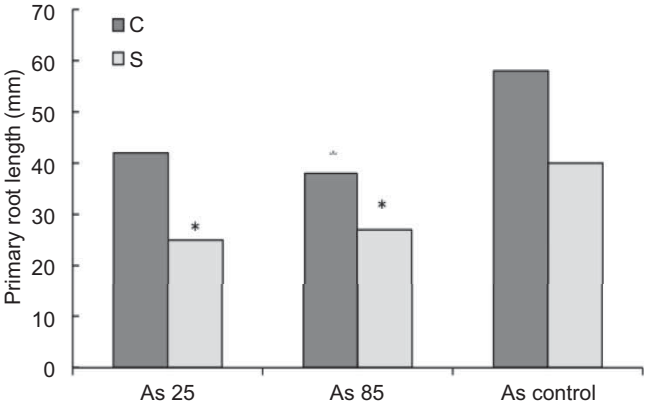

Figure 1. Analyses on soil boxes cultivated with lettuce. Reduction in the length of the primary root of *Vicia faba* grown in two types of soil: clay (C) and sand (S), at two different As concentrations (25 and 85 μg As · L^{-1}) and control samples (19 μg As · L^{-1}). *Significantly different from control at $p < 0.05$.

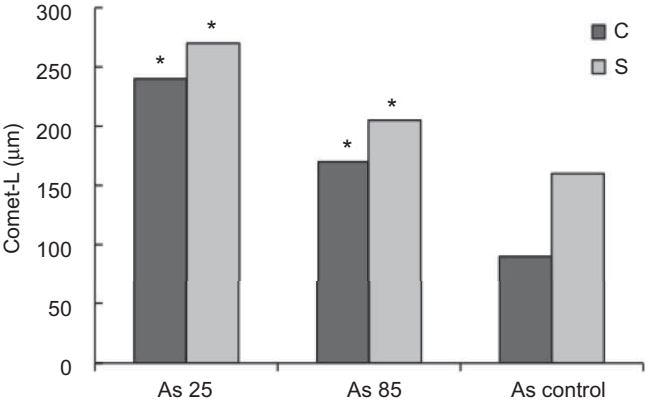

Figure 2. Analyses on soil boxes cultivated with lettuce. Comet assay performed in two types of soil: clay (C) and sand (S), at the two different As concentrations (25 and 85 $\mu g\ As \cdot L^{-1}$) and control samples (19 $\mu g\ As \cdot L^{-1}$). *Significantly different from control at $p < 0.05$.

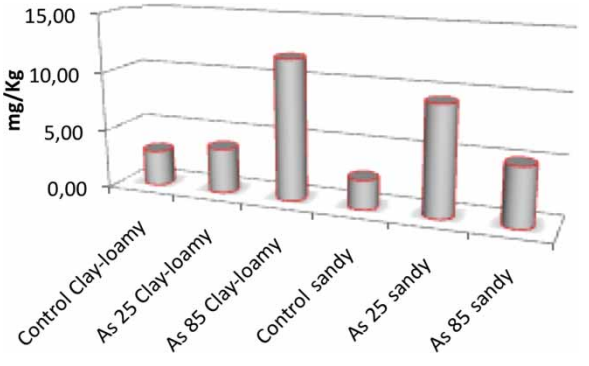

Figure 3. Concentrations of As in lettuce grown in affected soil ($mg \cdot kg^{-1}$). Control, 19 $\mu g\ As \cdot kg^{-1}$; As 25, 44 $\mu g\ As \cdot kg^{-1}$; As 85, 104 $\mu g\ As \cdot kg^{-1}$.

3.1.2. *Analyses on plants*

3.1.2.1. *ICP analysis.* The data in Figure 3 show As uptake in lettuce related to the concentration of As contamination: in clay-loamy soil, the lettuce As content increased from 3.0 $mg \cdot kg^{-1}$ in C boxes to 11.9 $mg \cdot kg^{-1}$ in As 85 pots. In sandy soil, As content varied from 2.5 $mg \cdot kg^{-1}$ in C boxes to 5.0 $mg \cdot kg^{-1}$ in As 85 pots.

3.1.2.2. *Metabolic profile of lettuce by ^1H HR-MAS NMR.* Figure 4 shows the ^1H HR-MAS NMR spectrum of leaf lettuce (0–2 ppm, fatty acids; 2–3 ppm, organic acids and amino acids; 3–6 ppm, carbohydrates; 6–8 ppm, phenols). In the literature there is a detailed study of magnetic resonance spectroscopy on the metabolic profile of different lettuce components, with regards to both polar and nonpolar compounds [22]. Most of the pollutants in clay-loamy soil are bound to oxides of Fe and Al [23,24] and are less available to the water layers, in contrast to the sandy soils in which As is easily available for plants. The spectroscopic results show at an As concentration of 25 $\mu g \cdot L^{-1}$, there is a decrease in the signals in the phenol zone. The decrease in area of these signals is significant ($p < 0.05$) in the ANOVA performed on lettuce samples from both sandy and clay-loamy soil, but is particularly evident for sandy soil (Table 3). The decrease in phenols compounds in the lettuce samples, particularly in those polluted to As 25 $\mu g \cdot L^{-1}$, might be due to

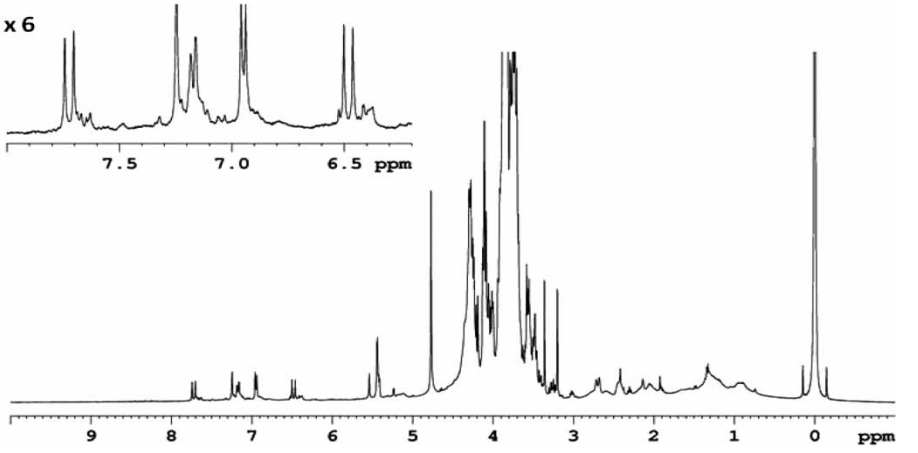

Figure 4. ^{1}H-HRMAS spectrum of leaf lettuce in buffered D_2O. The insert shows the region between 6-8 ppm with a strong vertical expansion (phenol signals). 0–2 ppm, fatty acids; 2–3 ppm, organic acids and amino acids; 3–6 ppm, carbohydrates; 6–8 ppm, phenols.

Table 3. Results of statistical analysis of lettuce NMR, ANOVA of the discriminant intervals (ppm) performed on lettuce samples from both sandy and clay-loamy As-polluted soil (As 25, 44 μg As $\cdot$ kg^{-1}).

	F	Significance (*P*)	*F*	Significance (*P*)
Variable (ppm)	Sandy soil	Sandy soil	Clay-loamy soil	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

glutathione *S*-transferase (GST) stimulation by phenols. The glutathione (GSH) binds to various electrophilic substances, e.g. As(III), and forms complexes via GST that are actively excreted from the cell, with further depletion of GSH if the concentration of the electrophilic substance is higher than the biosynthesis capability [25]. Its principal function is to eliminate toxic xenobiotic substances, such as chemical carcinogenics and pollutant environmental compounds [25].

3.2. *Analyses on soil boxes cultivated with radish*

3.2.1. *Phytotoxicity and genotoxicity tests on polluted soils*

3.2.1.1. *Phytotoxicity.* Both the concentrations tested (25 μg As $\cdot$ L^{-1}, 85 μg As $\cdot$ L^{-1}) show significant statistical values for phytotoxicity only in clay loamy soil (Figure 5).

3.2.1.2. *Comet assay.* The results of the comet assay are statistically significant only in sandy soils at the highest concentration tested (85 μg As $\cdot$ L^{-1}) (Figure 6).

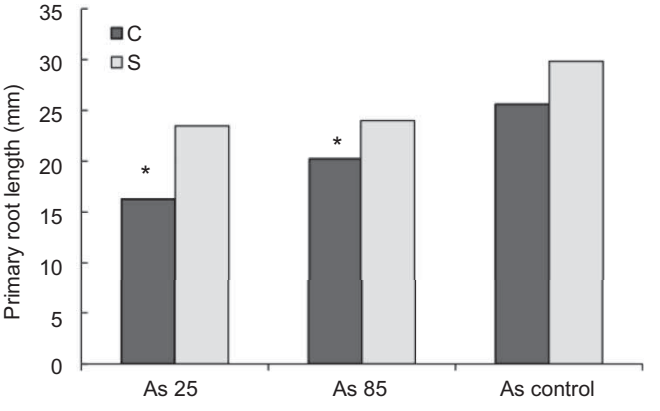

Figure 5. Analyses on soil boxes cultivated with radish. Reduction in the length of the primary root of *V. faba* grown in two types of soil: clay-loamy (C) and sandy (S) at two different As concentrations (25 and 85 μg As $\cdot$ L^{-1}). Control samples (19 μg As $\cdot$ L^{-1}). *Significantly different from control at $p < 0.05$.

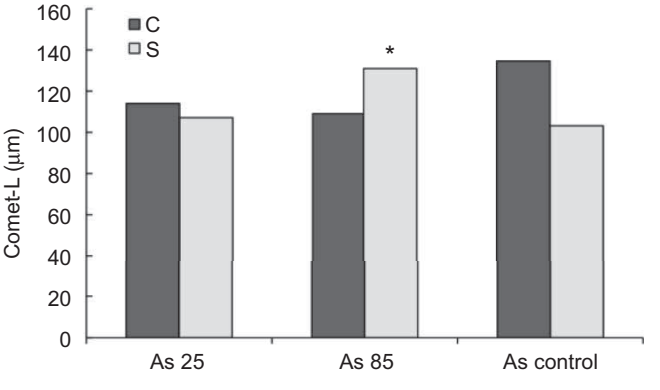

Figure 6. Analyses on soil boxes cultivated with radish. Comet assay performed in clay-loamy soil (C) and sandy soil (S) at two different As concentrations (25 and 85 μg As $\cdot$ L^{-1}). Control samples (19 μg As $\cdot$ L^{-1}). *Significantly different from control at $p < 0.05$.

3.2.2. *Analyses on plants*

3.2.2.1. *ICP analysis.* The data obtained by ICP-OE spectrometer analysis (Table 4) show an increase in As accumulation related to As pollution in clay-loamy soil. The radish As content rose from 0.65 mg $\cdot$ kg^{-1} in C boxes to 1.95 mg $\cdot$ kg^{-1} in As 85 pots. The same trend is observed in sandy soil in which the element content increases from 0.43 mg $\cdot$ kg^{-1} in C boxes to 1.37 mg $\cdot$ kg^{-1} in As 85 pots. The higher accumulation detected in clay-loamy soil compared with sandy soil for

Table 4. As content in radish tuber (mg $\cdot$ kg^{-1}).

Treatments	C Sandy	As 25 Sandy	As 85 Sandy	C Clay-loamy	As 25 Clay-loamy	As 85 Clay-loamy	LSD interaction soil* Treatments ($p < 0.05$)
Weight (g)	27.36a	29.58a	21.75b	17.86a	19.77a	20.88a	3.007
As content (mg $\cdot$ kg^{-1} d.m.)	0.43	0.76	1.37	0.65	1.1	1.95	n.s.

Notes: Numbers in a column followed by different letters indicate significant differences ($p < 0.05$). n.s., non significant. Control, 19 μg As $\cdot$ kg^{-1}; As 25, 44 μg As $\cdot$ kg^{-1}; As 85, 104 μg As $\cdot$ kg^{-1}.

both the crops, lettuce and radish, might be linked to the different soil texture, which causes a major exchange capacity, and to the consequent higher As availability to plants in the clay-loamy soil.

3.2.2.2. *Radish tuber analysis MRI.* By considering T_2-weighted MRI images of the different experimental plots, a progressive collapse of the structure depending on As concentration is observed; black spots appear over the whole hypocotyl. The MRI images differ slightly from what has been reported previously [26], in terms of the regularity of the radial structures and the presence of dark spots. The outermost cell layer changed also; the latter consist of two concentric spherical crowns, the outer characterised by fast-moving water molecules, and the inner containing strongly bound water. It is evident that by increasing the [As] ratio between the diameter of the hypocotyl and the outermost cell layer thickness, i.e. A_1, and the ratio between the outer and the inner spherical crowns thickness, A_2, changes smoothly by 1–5%, for samples grown in sandy soils. For radishes cultivated in clay-loamy soil, variations of 10–20% between A_1 and A_2 are observed. This indicates that the outermost cell layer thickness increases with water As

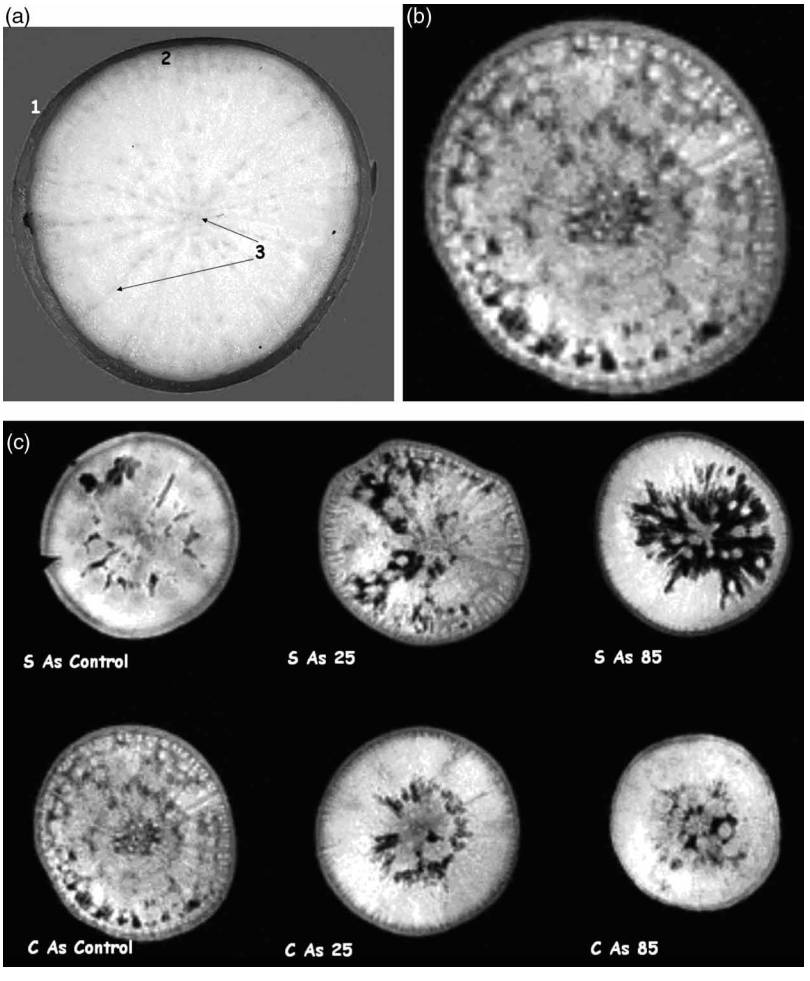

Figure 7. (a) Section of radish tuber (1, outermost cell layer; 2, parenchyma cells; 3, vascular bundles). (b) T_2-weighted MRI images of a radish. (c) T_2-weighted MRI images of radishes treated with different concentrations of As in two types of soil (left to right: control, As 25, and As 85; upper, sandy soil; lower, clay-loamy soil).

concentration, whereas the thickness of outer light region of the outermost cell layer decreases. This might be a physiological response to the presence of As by the hypocotyl, which creates a barrier to avoid As accumulation. This observation is supported by the stable values of A_1 and A_2 in sandy soil, where As leaching is faster because of the low capability of soil particles to contain As (Figure 7a–c).

4. Discussion and conclusions

The results obtained in the radish cultivation study, regarding tuber structural changes by MRI, elemental content and arsenic amount through ICP-OES agree with different chemical–physical soil parameters. In fact, the larger soil-exchange capacity value in clay-loamy soil determines the higher adsorption of As in the plant-system considered, probably because of the longer contact time between the hypocotyl and the soil solution containing As. MRI indicates that the morphology of the outermost cell layer varies with water As concentration, as a consequence of the physiological response of the hypocotyls to the presence of As, particularly in clay-loamy soil. By contrast, in sandy soil, As leaching preserves the radish tuber by morphological modifications, even though radish yield is lower than in clay-loamy soil.

In the lettuce cultivation experiment, the metabolic profile (Figure 4) by ^{1}H HR-MAS NMR shows quantitative differences in phenolic compounds in the As 25 samples (between 6 and 8 ppm), in sandy soils [25]. Probably above of certain arsenic concentration, for example, in the As 85 samples there is a saturation effect in the As(III)–glutathione complexes [27]. These differences in concentrations are found only in sandy soils; clay-loamy soils do not show any effect due to the presence of As or a a concentration effect. Partition of the major elements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, and P) (data not showed) may explain which of them were related to the simulated pollution by As. The elements were mainly associated with the residual fraction (Fr4), with $>90\%$ of the total element content, and the amounts were always higher in clay-loam soils. In oxidisable fractions (Fr3), copper (Cu) ranged from 9 to 12%, and the total amounts were small. Also, the As content in the residual fraction was related ($p < 0.1$) to all elements in the soil. The same pattern has been described in soils contaminated with other pollution sources [28,29]. Andersen *et al.* [30], in arable loamy sand soils in Denmark, found strong correlations between clay content and Cu, Ni and Zn in the residual fraction, as a consequence of their geological origin.

Soil texture and phosphorous level might influence As uptake in plants. The chemical behaviour of As is largely similar to that of phosphorus in soils. In all plant species tested to date, arsenate is taken up via phosphate transport systems. Massive concentrations of arsenic result in phytotoxicity: As participates in many cell reactions (due to its chemical similarity to phosphorus) and replaces phosphorus in the phosphate groups of DNA. Arsenate acts as a phosphate analogue and can disrupt phosphate metabolism, whereas arsenite reacts with sulphydryl groups of enzymes and tissue proteins, leading to inhibition of cellular function and death [31,32].

In soil-based studies, redox conditions and pH significantly affected the availability and consequent phytotoxicity of inorganic arsenical species.

The phytotoxicity test on *V. faba*, performed on clay-loamy soil, shows significative results when the soil was previously cultivated with lettuce and with radish, whereas the same test performed on sandy soils shows significant results only when cultivated with lettuce. These results are probably due to the high As-accumulation capacity of clay-loamy soils compared with sandy soils, in which the As accumulates mainly in the radish tuber. Furthermore, the comet assay on *V. faba*, showing a positive result only at the highest As concentration ($85\,\mu g\,As \cdot L^{-1}$), confirms the hypothesis that the radish tuber has an high accumulation capacity for As, by contrast, the results for soils cultivated with lettuce are significant for all the concentrations tested (25 and $85\,\mu g\,As \cdot L^{-1}$).

As is a weak mutagen and cannot directly induce gene mutations. However, As is a potent comutagen, an agent that will enhance mutagenicity. Inhibition of enzymes involved in DNA repair by As may be responsible for DNA damage. As is also a clastogen that can cause microscopically visible damage or changes to chromosomes (e.g. breaks in chromosomes, change in chromosome number) [31,33]. One of the mechanisms by which DNA damage can be induced by As might be oxidative stress in *V. faba*. Further studies, performing a modified protocol of the comet assay with repair enzymes, are necessary to provide a more comprehensive understanding and confirm that DNA damage is induced by oxidative stress. Under environmental stresses, plants often produce reactive oxygen species, causing damage to DNA, proteins and lipids. To minimise the harmful effects of products, plants have evolved an effective scavenging system composed of antioxidant molecules and antioxidant enzymes [34].

Our results showed that some chemical–physical parameters may be used as indicators of As contamination in soil and vegetables. The different amount of organic matter and clay content in the two types of soil was correlated with the soil-exchange capacity which regulates the release of toxic substances.

In conclusion, major attention should be paid to different aspects, such as different agronomic practices of edible plants and the genetic characteristics (polymorphisms) of As-contaminated organisms. Starting with analysis of the main food categories identified by the EFSA Panel on Contaminants in the Food Chain (e.g. cereal grains, bottled water, coffee, beer, rice grains and rice based products, fish and vegetables), a multidisciplinary study could comprehend the chemical–physical characterisation of the soils and how these may influence As uptake by the plant, the different As accumulation rate among different vegetables, and how cooking may influence the As content of food.

Dietary exposure to iAs should be reduced, and in order to refine risk assessment of iAs there is a need to produce speciation data for different food commodities to support dietary exposure assessment and dose–response data for the possible health effects.

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