ARSENIC CONTAMINATION IN RADISH TUBER INVESTIGATED BY MEANS OF MRI AND ICP OES

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ABSTRACT

*Arsenic (As) is a highly toxic element and its presence in food is a matter of concern for the well-being of both animals and humans. Arsenic-contaminated groundwater is used in agriculture to irrigate crops for food and animal consumption with a potential contamination of the food chain. The purpose of this study was the evaluation of arsenic effect on radish tuber (*Raphanus sativus *L.). Experimental plots with sandy and clay-loamy soil were cultivated with radish and treated with three different concentrations of As water solution: 19, 44 and 104 µg/L. Magnetic resonance imaging was used to visualize the tuber structural changes, and the content of elements and the As amount were evaluated by inductively coupled plasma atomic emission spectroscopy. The data obtained demonstrate that As contamination in radish tuber is underlined with the dual approach.*

PRACTICAL APPLICATIONS

The scope of this research is the evaluation of the effects on the toxicology and the morphology because of the arsenic (As) uptake by edible plants. The focus is on the contamination of the plant, which has implications for food safety and human health. The results obtained by magnetic resonance imaging and inductively coupled plasma atomic emission spectroscopy show that several changes of the internal structure occurred and that some parameters can be used as indicators of the As contamination in food.

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INTRODUCTION

Arsenic (As) is a naturally occurring element widespread in the environment, present in soils and minerals and it may enter air, water and land throughout wind-blown dust and water run-off. The As in the atmosphere comes from various sources: volcanoes release about 3 mg per year and microorganisms release volatile methylarsines to the extent of 20 mg per year. Anthropic activity is responsible for the largest: 80 mg of As per year are released by the use of fossil fuels.

We can found arsenic mostly as three oxidation stases: (1) Arsenides (alloy-like intermetallic compounds); (2) Arsenic (III) or arsenites (organoarsenic compounds); and (3) Arsenic (V) complexed in stable inorganic arsenic oxycompounds (Okada and Yamanaka 1994). The As is chemically similar to phosphorus; it forms colorless and odorless crystalline oxides, As_2O_3 and As_2O_5, which are hygroscopic and readily soluble in water. The As can partially substitute the phosphorus in biochemical reactions, becoming therefore poisonous. It is considered as one of the most toxic elements present in the environment, and despite its effects, inorganic As bonds occur naturally in small amounts. Human beings may be exposed to As through food, water and air, and it may also occur through skin contact with contaminated soil. The World Health Organization (WHO 2001) classified this element as toxic and reported effect by inorganic arsenic onto the human health; i.e., the gastrointestinal and respiratory tracts, skin, liver, cardiovascular, hematopoietic and nervous systems.

The presence of As, even in small amount, in drinking water constitutes a primary risk for food safety and human health. In fact, As contamination of soil and water is a risk for crops, animal and human being, entering in the food chain. Drinking water in the U.S.A. generally contains an average of 2 μg/L of As (USEPA 1982), although 12% of water supplies from surface water sources in the north central region of the U.S.A. and 12% of supplies from groundwater sources in the western region have levels exceeding 20 μg/L (Karagas *et al.* 1998).

The environmental behavior and fate of As and its impact on human health have been largely studied (Kabata-Pendias and Pendias 1992; Okada and Yamanaka 1994; Brooks 1998; Vather and Concha 2001; Styblo *et al.* 2002; Villa-Lojo *et al.* 2002; Mearget and Mazibur 2003). High As concentrations are mainly found in groundwater, which presents a particular risk as it is often used as a source for drinking water and crops irrigation. The use of contaminated groundwater for crops irrigation in Bangladesh and West Bengal (India) areas have been studied in order to assess the entering of As in the human food chain. (Das *et al.* 2003; Al Rmalli *et al.* 2005).

The water use in agriculture, related to the irrigation, is typically of consumption, because water is not returned anymore, but it is fixed by the plants, evaporated and percolated in the subsoil. It is not very demanding in quality, but it needs conspicuous volumes of water in narrow periods. Therefore, quality water is destined to human and animal consumption, while waste water, recovery consortia water and, mainly, groundwater are used for the irrigation. The irrigation is one of the most important uses of the water, especially in Italy, where it allows the development of an agriculture that represents one of the more promising economic activities. In the context of a planning and a rational management of the water resources, the irrigated use must compete with others, above all that drinkable. In respect to the drinking water, the Italian legal limit in force (Legislative Decree 31/2001) and European regulation (Council Directive 80/778/EEC) fixed this limit at 10 μg/L. Furthermore, in Italy a fixed threshold of this toxic element in animal beverage and irrigation waters does not exist. The adoption of disciplinary measures to rule the use of water for irrigation seems to be necessary. Beni *et al.* (2007) reported a study of the As levels in soils, gravitational and clean water, diet and milk in bovine milk chain. The As accumulation in gravitational and clear water and its correlation with bovine milk quality were also investigated (Beni *et al.* 2008). The results showed high concentrations of As in drinking water. Nevertheless, distribution curve of As in soils indicated that the high level of As had geologic origin. The As contents in Italian water was lower than the allowed limit of 10 μg/L decided by the European Union for human consumption; similarly, As concentration in milk was found to be below the common concentration range (20–60 μg/L). This work underlined the risk of high amounts of As in net and bearing stratum water in Lazio region, where 75% of samples were found to be above the legal limit.

Vegetal organisms have different capacity in taking up heavy metals and sequestrating elements. Hyper accumulating plants, which are capable of transferring large amounts of As, have been largely investigated. The concentration of metals in radish leaves and tubers were correlated with total free ionic concentration in rizosphere solution and with their concentrations in soils (Simon *et al.* 2000). Vegetable tolerance growth in contaminated soils can be assesed also by a reduction of the primary length (fitoxicity) (Sturchio *et al.* 2006). Studies with atomic absorption spectroscopy (AAS) were useful to determine the total As concentrations in several foodstuffs, including vegetables, rice and fish (Tam and Lacroix 1982; Arenas *et al.* 1988; Hershey *et al.* 1988; Dabeka *et al.* 1993).

Magnetic resonance imaging (MRI) has been reported as a valuable analytical technique for the quality evaluation of vegetables (Sequi *et al.* 2007; Bellincontro *et al.* 2009; Taglienti *et al.* 2009). MRI is a useful tool to follow noninvasively slight structural modifications occurring in radish as a function of As concentration in irrigation water. The unique proprieties of noninvasiveness and nondestructiveness in producing high-resolution spatial images of any internal section or volume or sample make it an attractive and powerful approach in food science (Clark *et al.* 1997). MRI images provide information about the spin density distribution, mostly water molecules and in same cases sugar, lipid and fat, and about the relationship between spins and cellular tissue. The latter can be obtained from the relaxation times (spin-lattice and spin-spin, T_1 and T_2, respectively), the diffusion coefficients, susceptibility effects and chemical shift differences. MRI is known mainly for its medical and diagnostic application, and because a decade is used for studying foodstuff. The nondestructive and noninvasive siveness features make MRI applying in this research field. Salerno *et al.* (2005) defined the internal morphology and structural changes because of the dehydration of radish tuber by means of MRI. Massantini and Mencarelli (2003) demonstrated the effects of erroneous storage condition of radishes, which causes changes in the morphology and its quality.

The purpose of this study was the evaluation of the effects on radish tuber of different As concentrations by means of MRI spectroscopy and inductively coupled plasma atomic emission spectroscopy (ICP-AES; Thermo Jarrell Ash, IRIS Advantage, Thermo Optek, Milan, Italy), in order to detect the morphological changes in plants and relate it to As contamination.

MATERIALS AND METHODS

Experimental Site

The experimental site was arranged in two sets of lysimetric boxes, filled with two types of soils, one characterized by sandy and the other by clay-loamy particle size distribution, having very different physical, chemical and functional features. The two sets of plot were sown with radish tuber. The irrigation water used had a native As content equal to 19 μg/L and was used to prepare two different solutions of As, adding 25 and 85 μg As/L, respectively. The As, as sodium arsenate dibasic heptahydrate, was obtained from Fluka Sigma-Aldrich (Milan, Italy). Resuming the treatments, with three replicates, became: C = control (19 μg As/L); As 25 (44 μg As/L); As 85 (104 μg As/L). These last two concentrations of As were chosen because they represent the mean content of As observed in irrigation water and the higher values detected in net and bearing stratum water of Lazio region, respectively (Beni *et al.* 2007). Treatments were repeated 12 times during radish cultivation, so that the total amount of As added for each treatment was: 228 μg for C, 528 μg for As 25 and 1,248 μg for As 85.

Soil Sample Collection and Analysis

Soil samples (each about 500 g) were collected from boxes and soil characterization was carried out by the official method (MiPAF 2000) particle

TABLE 1.
SOIL CHEMICAL AND PHYSICAL CHARACTERIZATION

Parameters	CL	S	Parameters	CL	S
pH (H_2O)	7.6	8.3	K (meq/100 g)	1.27	0.29
Sand (%)	24.4	92.3	Na (meq/100 g)	3.21	0.12
Silt (%)	47.6	3.7	mg (mg/kg*)	0.7	1.6
Clay (%)	28.0	4.0	Cd (mg/kg)	<0.05	<0.05
OM (%)	1.79	0.9	Cu (mg/kg)	1.03	8.01
Total N (mg/kg)	0.12	0.1	Fe (mg/kg)	401.1	56.2
Available P (mg/kg)*	25.2	57.6	Ni (mg/kg)	0.57	<0.05
Exchangeable K (mg/kg)†	598.1	138.1	Pb (mg/kg)	2.1	1.1
CEC (meq/100 g)	29.51	5.24	Zn (mg/kg)	1.3	2.7
Ca (meq/100 g)	24.33	3.23			

* As P2O5 (Olsen).

† As K2O.

CL, clay loam soil; S, sandy soil; OM, organic matter; CEC, cation exchange capacity.

size distribution by gravimetric method, pH in H_2O with a potentiometer, total organic carbon (Walkley and Black), total nitrogen (Kjeldahl), available phosphorus (Olsen), cation exchange capacity and exchangeable cations measured in the extracted soil solution (ammonium acetate) by using the AAS.

Chemical and physical parameters reported in Table 1 were used to evaluate the effects on the soil–plant system because of the As contamination. Data show a different pH between clay loam soil and sandy soil; organic matter content and cation exchange capacity were lower in the second one.

Water and Plant Analysis

Gravitational water composition, plant nutrients uptake and plant morphology were estimated in order to determine some soil–plant system contamination indicators. Ten radish tubers (hypocotyls) for each replicates were harvested 40 days after sowing (consumer's maturation time) and then were dried and weighed. Water and dried hypocotyls were analyzed for the total elements content by ICP-AES. The analysis of irrigation water was performed according to EPA standard methods 3120 and 3125 (USEPA 1998) (45-mL water sample, 5-mL nitric acid concentrated, digestion). The extracts of As concentration were detected by means of ICP- AES. The radish tuber As content was determined by using a ICP-AES after wet acid digestion method (0.5-g sample, 3-mL deionized water, 7 mL of 65% HNO_3, 140C). All reagents used were of analytical grade or better. For each group of samples, blanks (deionized water and reagents) were included throughout the entire sample preparation and analytical process.

MRI

Three representative fresh hypocotyls for each treatments were analyzed using MRI through 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. Water signal was monitored and used for the image reconstruction. gradient-echo fast imaging (GEFI) and multi-slice-multi-echo (MSME) experiments, m_gefi_ortho and m_msme_ortho, respectively (Bruker library), were performed according to standard procedures. In GEFI measurements, which generate echoes only by applying gradient pulses, the field of view was 40.0 × 40.0 mm, the matrix size 128 × 128 pixels and spectral width 100.0 kHz. The echo and repetition times were set equal to 2.445 and 60.0 ms, respectively. The number of scans was 1; slice thickness was 2.00 mm; and excitation pulse was a sinc3. The data were processed to obtain images 128 × 128 in size and a field of view of 40.0 × 40.0 mm. The processing mode was FT_MODE, the latter was complex_FFT and spikes elimination was allowed. In MSME experiments, which produce echoes via a spin-echo-based sequence, the field of view was 40.0 × 40.0 mm, the matrix size 128 × 128, spectral width 100.0 kHz, the echo and repetition times were set equal to 17.5 and 5,000.0 ms, respectively, the number of echoes and images was 24, the number of scans and dummy scans was 1, slice thickness was 2.0 mm and the excitation pulse was a sinc3. The data were processed to obtain images 128 × 128 in size, a field view of 40.0 × 40.0 mm, the processing mode FT_MODE, the latter complex_ FFT and with spikes elimination allowed.

Statistical Analysis

Analysis of variance (ANOVA) was performed in order to test the significance of the observed differences. The software SPSS, 13th edition (SPSS Inc., Chicago, IL), was used to determine significant differences ($P < 0.05$) between radishes cultivated in two types of soil, sandy and clayey, treated with different concentration of As, by means of two-way ANOVA.

RESULTS

Plant Measurements

Radish, a dycotiledonae species of Cruciferae family, has discrete patches of xylem and phloem, which occur in a ring and separates the cortex from the parenchyma (Fig. 1). Cambium extends between vascular bundles (3), appearing as a narrow ring producing conducting tissues within the bundles, and the

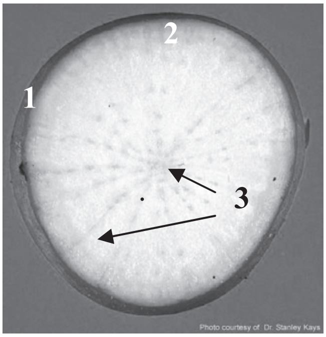

FIG. 1. SECTION OF RADISH TUBER
1, outermost cell layer; 2, cells of parenchyma; 3, vascular bundles.

parenchyma cells between them. The outermost cell layer of leaves, roots and stems constitutes epidermis (1) Parenchyma; and (2) has thin cell walls, with variable physiology and morphology; they are active in photosynthesis, respiration and storage.

The main function of xylem is the upward transport of water and dissolved nutrients. The phloem is the tissue throughout products photosynthesized in leaves and other green parts of the plant are transferred to other parts of the plant. The phloem is also the tissue responsible for the transport of organic materials from storage zones. The relatively short growth period, <2 months, and the complete knowledge of the internal structure makes radish suitable for the evaluation of effects produced on morphology and chemical composition by the use of As-contaminated groundwater.

The results for plants harvested 40 days after sowing are reported in Table 2. In regards to the radishes' fresh weight, in sandy soil plots weights were higher in comparison with those of clayey soil, underlining significant effects for both treatments and soils. In particular, in clay loam soil no difference was found between treatments, while in sandy soil the treatment As 85 gave a significant decrease of the fresh weight, in comparison with the other treatments. The two-way ANOVA showed significance for the treatments/soils interaction.

Data obtained by ICP-AES to estimate the As contamination level of radish are reported in Table 2 and show that As content increased accordingly to As soil pollution. In clay loam soil, the amount raised from 0.65 mg/kg (control) to 1.95 mg/kg (As 85). The same trend was observed in sandy soil where the As content increased from 0.43 mg/kg (control) to 1.37 mg/kg (As 85). The higher radish As concentration of sandy soil plots could be because of the greater mobility and availability of the element brought with the irrigation

TABLE 2.
RADISH TUBER, MEAN FRESH WEIGHT AND TOTAL ARSENIC (As) CONTENT

Soil	Treatments	Weight (g)	As content (mg/kg d.m.)
Sandy	C	27.36a	0.65
	As 25	29.58a	1.10
	As 85	21.75b	1.95
Clay	C	17.86a	0.43
	As 25	19.77a	0.76
	As 85	20.88a	1.37
LSD interaction between soil and treatments ($P < 0.05$).		3.007	n.s.

Means with different letters are significant after calculation of LSD ($P < 0.05$).
C = control (19 µg As/L); As 25 = 44 µg As/L; As 85 = 104 µg As/L.
n.s., test F not significant.

water. Vice versa, in the clayey soil, the formation of As complexes with humic substances and clays makes this element less available for the plant. In regards to As content in radish tuber, significant effects exist for both treatments and soils, while no significance was observed for the treatment/soil interaction. Terrestrial plants may accumulate As by root uptake from the soil or by absorption of airborne As deposited on the leaves, and certain species may accumulate high levels (USEPA 1982). Nevertheless, even when these plants are grown in soil with high As content, the uptake is relatively low (Gebel *et al.* 1998; Pitten *et al.* 1999). Kale, lettuce, carrots and potatoes were grown in experimental plots around a wood preservation factory in Denmark, where waste wood was incinerated to investigate the amount and the pathways for As uptake by plants (Larsen *et al.* 1992). During incineration, the arsenate was partially converted to arsenite and the As obtained from the stack was bound particles primarily. High levels of inorganic As were found in the test plants and in the soil around the factory; statistical analyses showed that the dominating pathway for As transport was by direct atmospheric deposition for kale, while for potatoes and carrots was a combination of soil uptake and atmospheric deposition.

Zavala and Duxbury (2008) estimated normal levels of total As in rice. The authors demonstrate a link between environmental contamination with As and As content in rice grain. Total As concentration in rice varied from 0.005 to 0.710 mg/kg. The mean As concentrations for rice from the U.S.A. and Europe (both 0.198 mg/kg) were statistically similar and significantly higher than rice from Asia (0.07 mg/kg). However, toxic plant level of As derived from a combination of a lot of factors: environment, management and genetic factors that control As availability, uptake and translocation.

The As accumulation by plants depends on As speciation. Uptake of four As species, i.e., arsenite, arsenate, methylarsonic acid (MMA) and dimethylarsinic acid (DMA) by turnips grown in soilless culture conditions showed that the uptake increased with increasing arsenic concentration in the nutrient solution, while the organic arsenicals showed higher upward translocation than the inorganic arsenical (Carbonell-Barrachina *et al*. 2000).

The concentration of As and inorganic phosphate which are present in different vegetables and the relationship among the inorganic arsenic, MMA and DMA with the inorganic phosphate were investigated by the Department of Pharmacology, Bangabnadhu Sheikh Mujib Medical University, Dhaka, Bangladesh (Laizu 2007). Speciation of As (inorganic arsenic, MMA and DMM) and the amount of inorganic phosphate were estimated. The fruiting vegetables contained low level of arsenic, which might have some relationship with higher level of inorganic phosphate. The leafy vegetables contained higher level of inorganic phosphate in contrast to As, though they had no significant relationship between inorganic phosphate and different speciated form of As.

The trend of total amount of As uptake by the turnip plants was MMA < DMA < arsenite < arsenate. In a similar experiment, performed by using tomato plants, the total amount of As uptake (Burlo *et al*. 1999) followed the trend DMA < MMA < arsenate < arsenite, with increasing plant As concentrations according to As concentration in the nutrient solution. The As was accumulated in the root system (85%) with smaller amounts translocating to the fruit (1%). However, plants treated with MMA and DMA had higher As concentrations in the shoots and fruits than those treated with arsenite or arsenate.

Terrestrial plants placed on land bordering As-contaminated waters show relatively low As content, even though the sediments have high As concentrations, above 200 mg/kg (Tamaki and Frankenberger 1992). The As concentrations in vegetables grown in uncontaminated soils and arsenic contaminated soils, as well as other metals and organic contaminants, were generally <12 μg/kg wet weight. A maximum As concentration of 18 μg/kg wet weight was found in unpeeled carrots grown in soil, which contained a mean As concentration of 27 mg/kg, dry weight (Samsoe-Petersen *et al*. 2002). The contaminated water used for irrigation in agriculture can make a problem for people. In fact, the accumulation of As in crop (rice plant) gives a possible effect on nutrient (protein) content of rice grain (Alam *et al*. 2002).

MRI Analysis

Figure 2 shows the MRI image of a radish obtained by using an MSME experiment. The signal intensity is directly proportional to the local transverse

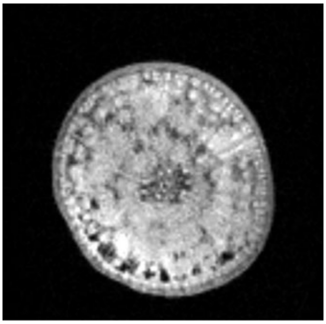

FIG. 2. T_2-WEIGHTED MRI IMAGE OF A RADISH

relaxation time T_2, which in turn depends upon the local mobility of water molecules, the latter being with intra- and intercellular. In this image, brighter zones contain water with high capability of moving freely, while on the contrary darker areas are characterized by slow mobility H_2O.

From Fig. 2, one can clearly see the internal organization of the tissues: the dark external secondary cortex containing low mobility water molecules; the pith, located exactly in the center of the hypocotyl; the collateral sieve-vascular bundle system is partially visible and corresponds to the white radial and discontinuous parenchyma structure. Finally, a region containing water molecules with low-medium mobility and with a radial structure alternates the sieve-vascular system from the pith to the cortex and constitutes a water and nutrient storage area.

MRI image in Fig. 2 slightly differs from what previously reported (Salerno *et al.* 2005), in terms of geometry of the radial structures and presence of dark spots. This is probably because of the different cultivation system, being in open-air the present one and in green house the previous one. The different cultivated variety can also be a factor of influence for such morphological details.

Figure 3 reports T_2-weighted MRI images of radishes irrigated with increasing As concentration: control, 25 μg/L and 85 μg/L from left to right, and cultivated in the two different soils considered, sandy and clay loam, top and bottom, respectively. The morphology of the control samples are very similar and by increasing the As amount in water irrigation a progressive collapse of the structure occurred. The latter is made evident by the black spots over the whole hypocotyl, and by the changing of the thickness and the structural details of the outermost cell layer. The latter consists of two concentric spherical crowns, one external and light in color and the inner being darker. They differ in the wateriness, in terms of water organization within the cellular tissues; the outer has fast moving water molecules, while the other

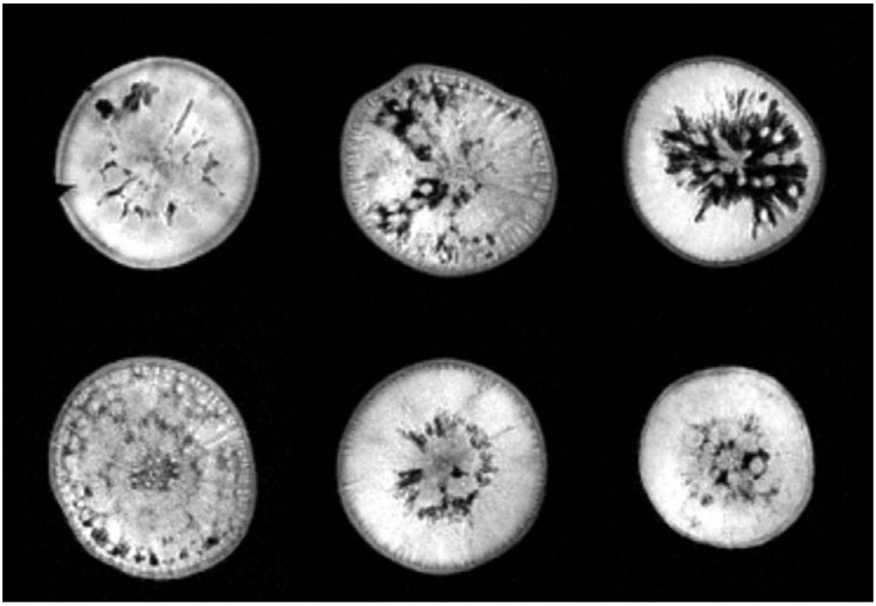

FIG. 3. T_2-WEIGHTED MRI IMAGES OF RADISHES TREATED WITH DIFFERENT CONCENTRATIONS OF ARSENIC IN TWO TYPES OF SOIL
Left to right, control, As 25 and As 85; top, sandy soil; bottom, clay loam soil.

contains water strongly bound. The axial T_2-weighted MRI images have been parameterized by measuring the ratio between the diameter of the hypocotyl and the outermost cell layer thickness, indicated as A_1, and the ratio A_2 between the outer and the inner spherical crown thickness. Table 3 reports the mean values of A_1 and A_2 for the different cases considered, i.e., soil and water As concentration.

Based on these data, one can observe that the As effect is slightly relevant for A_1 an A_2 values, and changes in the range 1–5% occurred, for samples grown in sandy soils. On the contrary, in radishes cultivated in clay loam soil, we observed a progressive increase for A_1 and A_2 values accordingly with water As concentration. This indicates that the outermost cell layer thickness increases with water As concentrationand the outer light region of the outermost cell layer itself decreased the thickness. This might be a physiological response of the hypocotyl to the As presence, which tend to create a barrier to avoid As accumulation. This observation is supported by the fact that in sandy soil, where the washing away is faster for the low capability of soil particles to keep the As contaminant, the A_1 and A_2 values do not vary largely.

TABLE 3.
A_1 AND A_2 VALUES OBTAINED BY T_2-WEIGHTED MRI IMAGES

Soil	Treatments	A_1	A_2
Sandy	C	0.034a	1.32
	As 25	0.032a	1.34
	As 85	0.033a	1.39
Clay	C	0.035a	1.08
	As 25	0.038a	1.14
	As 85	0.048b	1.46
LSD interaction between soil and treatments ($P < 0.05$).		0.008	n.s.

Means with different letters are significant after calculation of LSD ($P < 0.05$).
C = control (19 μg As/L); As 25 = 44 μg As/L; As 85 = 104 μg As/L.
A_1, hypocotyl diameter/outermost cell layer thickness ratio; A_2, outer/inner spherical crowns thickness ratio; n.s., test F not significant.

DISCUSSION

The results showed that some measured chemical–physical parameters can be used as indicators for the evaluation of As contamination soil and radish. The different amount of organic matter and clay content in two type of soil was correlated with the soil exchange capacity that regulates the release of toxic substances. In fact, the larger value of this parameter in clay loam soil determined a higher adsorption of As in the plant system considered, probably because of the longer contact time between hypocotyl and arsenic.

We have also correlated MRI data with those obtained by ICP. MRI indicates that the morphology of the outermost cell layer varies with water As concentration, as a consequence of the physiological response of the hypocotyl to the As presence, particularly in clay loam soil. On the contrary, in sandy soil the faster As leaching preserves the radish tuber by the morphological modification, even if in this type of soil the radish yield is lower than in clay loam ones.

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