Arsenic Species: Effects on and Accumulation by Tomato Plants

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The uptake of arsenic (As) species by *Lycopersicum esculentum*, growing under soilless culture conditions, was studied. A 4 × 3 × 2 factorial experiment was conducted with four As species (arsenite, arsenate, methylarsonate, and dimethylarsinate), three As concentrations (1, 2, and 5 mg L^{-1}) and two tomato cultivars (Marmande and Muchamiel). The phytoavailability and phytotoxicity were primarily determined by the As species. The concentrations of As in plant increased significantly with increasing As concentration in solution. Both MA and DMA showed a higher upward translocation than arsenite and arsenate, and treatments with MA and DMA clearly reduced plant growth and fruit yield. The As concentration in tomatoes treated with arsenite or arsenate were within the range considered normal in food crops; however, the As concentration in tomatoes treated with MA and DMA were close to or even above the maximum limit. When tomato plants are exposed to high concentrations of As in nutrient solutions, they may uptake As to concentrations unacceptable for human food.

Keywords: *Lycopersicum esculentum; arsenic species; food crop contamination; tomatoes*

INTRODUCTION

Arsenic (As) is ubiquitous in nature, with As levels being elevated by mining, industrial, and agricultural activities (Meharg et al., 1994). In the past, indiscriminate application of inorganic arsenicals as pesticides, desiccants, or wood preservatives led to pollution of many agricultural soils and reduction of their productivity (Marin et al., 1993). During the last 30 years, the inorganic arsenical pesticides were replaced by organic herbicides (Woolson, 1983; Marin et al., 1993) that are applied at lower concentrations and have lower toxicity for animals and humans than inorganic arsenicals.

Because the solubility, mobility, bioavailability, and phytotoxicity of As depends on its oxidation state (Masscheleyn et al., 1991), knowledge about the uptake of inorganic and organic As species by plants and about their effects on plant growth and nutrition and fruit yield are essential to understand the behavior of As species in the soil−plant environment. So far, mainly four As species have been determined in the soil−plant system (Sohrin et al., 1997). At the natural pH of soil solutions, arsenate as $HAsO_4^{2-}$ and $H_2AsO_4^-$ is the thermodynamically stable species under aerobic conditions. Arsenate is a chemical analogue of phosphate and may interfere with oxidative phosphorylation (Terwelle and Slater, 1967). Arsenite, present as $As(OH)_3$ in soil solutions, inhibits the activity of enzymes by reacting with thiol groups. Methylarsonic acid [$CH_3AsO(OH)_2$] and dimethylarsinic acid [$(CH_3)_2AsO(OH)$], present as anions in soils, are much less toxic than arsenite or arsenate and may block protein synthesis in plants (Sckerl and Frans, 1969).

Arsenic is not essential for plants and appears not to be involved in specific metabolic reactions when supplied at low concentrations (Marin et al., 1993). At higher concentrations, however, As has been reported to interfere with metabolic processes and to inhibit plant growth, sometimes leading to death (Marin et al., 1993). Because phosphate and arsenate are taken up by the same uptake system, the supply of phosphate to plants may be compromised (Meharg et al., 1994) if the concentration of As in the soil solution is high.

Uptake of As by plants depends on many factors, including plant species (Walsh and Keeney, 1975), As concentration in the soil solution (N. A. S., 1977), soil properties such as pH, clay content and Eh (Marin et al., 1993), and the presence of other ions (Woolson et al., 1973). In a recent paper (Carbonell-Barrachina et al., 1998), the phytoavailability and phytotoxicity of As were reported to be also affected by the chemical species of As; whereas treatments with arsenite and arsenate did not affect plant growth, both methylarsonic acid and dimethylarsinic acid were phytotoxic to *Spartina alterniflora* and *Spartina patens* (wetland plants).

In Spain, soils that had been treated with sodium arsenite and lead arsenate are now frequently used to grow tomatoes and beans (Carbonell-Barrachina et al., 1994; Carbonell-Barrachina et al., 1995). Studies of the effect of organic arsenicals applied to foliage emphasized their herbicidal activity and neglected the possibility of uptake by roots. Dimethylarsinic acid, however, may be found in all soils and may predominate in many of them (Braman, 1975).

Arsenic species may be toxic to tomato plants, may accumulate in this plant, and may enter the human food chain through the fruits. A greenhouse experiment was designed to evaluate the absorption by and phytotoxicity of four As species to two tomato cultivars. The main objective of this study was to establish whether As concentrations in tomato fruits are potentially dangerous to human health.

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MATERIALS AND METHODS

Tomato plants (*Lycopersicum esculentum* Mill) were grown, under greenhouse conditions, in soilless culture in the presence of different As species. Silicean sand served as inert medium. The factorial treatments (4 × 3 × 2, As species × As concentrations × tomato cultivars) were applied in three replicates of a complete randomized design. The treatments consisted of four As species [arsenite, arsenate, methylarsonate (MA), and dimethylarsinate (DMA)] with three As concentrations (1, 2, and 5 mg L^{-1}) and two tomato cultivars (Marmande and Muchamiel). The following As compounds (sodium salts) were added to the nutrient solution for getting the desired As chemical species: $NaAsO_2$ (sodium arsenite), $Na_2HAsO_4 \cdot 7H_2O$ (sodium hydrogen arsenate), $CH_3AsO(ONa)_2 \cdot 6H_2O$ (disodium methylarsonate, DSMA), and $(CH_3)_2AsO(ONa) \cdot 3H_2O$ (sodium dimethylarsinate, SDMA). Controls with no added As were also included.

The concentration of each As species was determined regularly in the respective solution by the hydride generation atomic absorption technique (Masscheleyn et al., 1991).

Seeds were germinated in a commercial preparation of peat moss and vermiculite. Fourteen days after germination, uniform seedlings were selected. Organic residues were washed from the roots with distilled water and the seedlings transferred to hydroponic pots (1 plant pot^{-1}) containing 0.5 L of nutrient solution. The nutrient solution (Feigin et al., 1987) contained (in mg L^{-1}): 126 N; 46.5 P; 136.9 K; 31.6 Mg; 160.5 Ca; 2.0 Fe; 0.8 Mn; 0.3 Mo; 0.5 B; 0.2 Zn; and 0.2 Cu. The nutrient solution was replaced every 4 days.

Arsenic compounds were added after 21 days of acclimatization. Plants were grown for 36 days and then harvested. Roots were washed with tap water, P-free detergent, and rinsed several times with distilled water. Roots, shoots, and fruit were separated, weighed, and then dried at 60−70 °C for 72 h. The dry materials were ground in a stainless steel mill. A 0.5-g dry sample was taken and 5 mL 50% (v/v) HNO_3 and 1 mL of ashing aid suspension containing 20% (w/v) $Mg(NO_3)_2$ and 2% (w/v) MgO were added and the components were mixed well. After evaporation on a sand-bath until total dryness, the residue underwent a first careful ashing process in a muffle furnace: 150 °C for 1 h, 200 °C for 30 min, 250 °C for 1 h, 300 °C for 3 h, 350 °C for 30 min, and 450 °C for 12−14 h. Generally, it was necessary to perform a second shorter ashing process (150 °C for 1 h, 300 °C for 30 min, and 450 °C for 12−14 h), once or twice, until the ash was completely white (Ybáñez et al., 1992). The ash was dissolved in 4 mL of 6 N HCl and filtered through Whatman no. 1 filter paper into a 25-mL volumetric flask. Blanks were prepared by charring 5 mL of 50% (v/v) HNO_3 and 1 mL of ashing aid suspension using the dry ashing process. Arsenic in aliquots of 5 mL of the mineralized sample solution was determined with a Perkin-Elmer Optima, Model 3000, Inductively Coupled Argon Plasma Emission Spectrometer (ICP). The detection limit of this method was 0.05 mg L^{-1}.

Statistical analyses were performed using the PROC ANOVA and PROC GLM procedures available in SAS (SAS, 1987).

RESULTS AND DISCUSSION

The As concentrations used in this study (1, 2, and 5 mg L^{-1}) were selected taking into account that tomato plant is reported as tolerant to As pollution (Wauchope, 1983) and that in a previous study (Carbonell-Barrachina et al., 1997) an As−arsenite concentration of 2 mg L^{-1} was not phytotoxic to tomato plants, cv. Marmande. Arsenic species were found to be stable with respect to oxidation/reduction and methylation/demethylation reactions for a period of 4 days. A phosphorus concentration of 46.5 mg L^{-1} was selected because this concentration is typical of many fertilized soils in which tomatoes are grown (Junta de Extremadura, 1992) and because plants live longer at high P concentrations than at lower levels (Meharg and Macnair, 1991; Carbonell-Barrachina, 1998).

Plant Growth. Muchamiel plants had a higher total dry weight (root + shoot) than Marmande plants (Table 1b). Plants treated with arsenite had the highest total dry weight of all As-treated plants. In Marmande plants, only treatments with arsenite caused a higher dry weight than controls; however, in Muchamiel plants, treatments with both arsenite and arsenate caused a higher dry weight than controls (Table 1a). The influence of As concentration on total dry weight was not significant, demonstrating that the As species is more important than the As concentration in solution in determining the phytotoxic effects of As to tomato plants. Arsenic chemical form in nutrient solution was also the crucial factor determining the phytotoxicity of As to several wetland plants: rice (Marin et al., 1992) and *Spartina patens* and *Spartina alterniflora* (Carbonell-Barrachina et al., 1998).

Arsenic has not been shown to be an essential plant nutrient, although it is essential for animal metabolism (Lepp, 1981). Marin et al. (1992) reported an increase in the growth of rice, growing under hydroponic conditions, after treatments with dimethylarsinic acid at concentrations of 0.05 and 0.2 mg of As L^{-1}. Carbonell-Barrachina (1995) reported an increase in tomato plant growth, at the first stages of development, after an arsenite treatment at a concentration of 2 mg of As L^{-1}. More recently Carbonell-Barrachina et al. (1998) observed that applications of arsenate at concentrations of 0.2 and 0.8 mg L^{-1} (hydroponic culture) significantly increased root, shoot, and total dry matter production of *Spartina alterniflora* and *Spartina patens* compared to control plants.

The reason for the positive growth response of As-treated plants is unclear, but may be linked with phosphorus nutrition. Phosphate and arsenate are taken into plant roots by a common carrier; however, this phosphate/arsenate plasma membrane carrier has a much higher affinity for phosphate than arsenate (Meharg and Macnair, 1990). Besides, phosphate is reported as being a very efficient competitive inhibitor of arsenate uptake (Meharg and Macnair, 1990). Arsenate/phosphate uptake can be suppressed if the plants are P sufficient, which was the case of the present study (Table 2a; Junta de Extremadura, 1992). This suppression is due to a feed back regulation of the arsenate/phosphate transporter (reduced arsenate uptake through the suppression of the high-affinity uptake system; Meharg and Macnair, 1992).

In this particular experiment, root, shoot, and fruit P concentrations were significantly influenced by both As species and As concentration in the nutrient solution. Plants treated with MA and DMA presented significantly higher P levels than plants treated with arsenite and arsenate (Table 2b) and controls (Table 2a). Phosphorus concentrations in tomato plants increased with increasing As levels in the nutrient solution. A similar pattern in P behavior was found by Cox (1995), who studied the effects of different arsenicals on the growth and nutrition of canola (*Brassica napus* L.). Since As can substitute P in plant, but is unable to carry out the role of P in energy transfer, the plant reacts as if there is a P deficiency. Thus, as plant As increases, the plant reacts by increasing P uptake.

The phytotoxic effects of As are indicative of a sudden decrease in water mobility, as suggested by root plas-

Table 1. (A) Effects[a] of and (B) Results of the ANOVA and Duncan Tests for the Effects of Arsenic Concentration and Chemical Form on Dry Weight of Tomato Plants

A

	As con (mg L^{-1})	dry weight (g pot^{-1})			
		root (g pot^{-1})	shoot (g pot^{-1})	total (g pot^{-1})	fruit (g pot^{-1})
		Marmande			
control		4.4 ± 1.5[b]	29.1 ± 1.0	33.6 ± 1.2	9.5 ± 4.8
arsenite	1	4.9 ± 0.6	28.6 ± 1.4	33.5 ± 1.8	9.9 ± 0.2
arsenite	2	5.4 ± 0.4	29.0 ± 2.8	34.4 ± 3.2	7.3 ± 0.6
arsenite	5	5.8 ± 0.4	33.9 ± 2.8	39.8 ± 3.0	10.2 ± 2.4
arsenate	1	4.4 ± 1.1	24.4 ± 4.8	28.8 ± 5.8	5.9 ± 1.2
arsenate	2	4.2 ± 1.5	21.6 ± 6.7	25.8 ± 8.2	7.1 ± 2.5
arsenate	5	4.6 ± 0.8	29.3 ± 4.7	33.8 ± 5.4	6.7 ± 1.0
MA	1	4.0 ± 1.5	19.6 ± 6.6	23.6 ± 8.1	4.7 ± 1.7
MA	2	3.6 ± 1.6	15.3 ± 4.4	18.9 ± 6.0	2.3 ± 2.0
MA	5	3.8 ± 0.5	20.1 ± 2.2	24.0 ± 2.7	3.0 ± 0.3
DMA	1	2.8 ± 0.6	18.2 ± 4.8	21.1 ± 5.5	5.2 ± 1.5
DMA	2	4.1 ± 0.2	22.2 ± 3.0	26.3 ± 3.2	3.5 ± 1.8
DMA	5	3.3 ± 0.6	17.8 ± 3.6	21.1 ± 3.9	1.8 ± 0.9
		Muchamiel			
control		7.7 ± 1.7	36.7 ± 6.8	44.4 ± 7.8	5.1 ± 0.2
arsenite	1	7.5 ± 0.3	42.1 ± 2.7	49.6 ± 2.9	4.7 ± 1.5
arsenite	2	8.6 ± 0.9	41.0 ± 2.6	49.5 ± 2.9	4.8 ± 0.3
arsenite	5	8.3 ± 0.8	40.7 ± 3.4	49.0 ± 4.2	3.6 ± 1.0
arsenate	1	8.4 ± 0.3	41.8 ± 2.6	50.2 ± 2.4	1.5 ± 0.9
arsenate	2	8.1 ± 0.4	36.7 ± 1.7	44.7 ± 2.1	8.5 ± 2.3
arsenate	5	7.2 ± 1.2	35.5 ± 6.2	42.7 ± 7.2	3.7 ± 1.9
MA	1	6.9 ± 0.5	34.7 ± 1.3	41.6 ± 1.8	1.0 ± 1.0
MA	2	7.3 ± 0.7	40.2 ± 5.0	47.5 ± 5.6	–[c]
MA	5	5.4 ± 1.2	25.1 ± 7.7	30.6 ± 8.9	0.9 ± 0.9
DMA	1	5.3 ± 1.5	22.2 ± 7.8	27.5 ± 9.3	0.2 ± 0.2[d]
DMA	2	4.3 ± 0.3	20.5 ± 2.0	24.8 ± 2.2	2.9 ± 2.9
DMA	5	3.2 ± 0.4	10.8 ± 0.2	14.0 ± 0.6	–[c]

B

ANOVA F test

	root		shoot		total		fruit	
source of variation	*F* value		*F* value		*F* value		*F* value	
variety	45.9	***[e]	27.6	***	32.0	***	24.7	***
As sp	12.4	***	18.0	***	17.8	***	15.8	***
As con	0.6	NS	0.6	NS	0.6	NS	0.6	NS
var × As sp	2.4	NS	4.5	**	4.2	NS	1.0	NS
var × As con	1.3	NS	3.4	*	3.1	NS	3.1	NS
As sp × As con	0.5	NS	0.8	NS	0.7	NS	1.9	NS
var × As sp × As con	0.4	NS	0.4	NS	0.4	NS	0.5	NS

Duncan Multiple Range Test

source of variation	root	shoot	total	fruit
tomato variety				
Marmande	4.3 b[f]	23.3 b	27.6 b	5.6 a
Muchamiel	6.7 a	32.6 a	39.3 a	2.7 b
arsenic species				
Arsenite	6.8 a	35.9 a	42.6 a	6.7 a
arsenate	6.1 ab	31.5 a	37.7 a	5.6 a
MA	5.2 b	25.8 b	31.0 b	2.0 b
DMA	3.8 c	18.6 c	22.5 c	2.3 b
arsenic concentration				
1 mg L^{-1}	5.5 a	29.0 a	34.5 a	4.1 a
2 mg L^{-1}	5.7 a	28.3 a	34.0 a	4.6 a
5 mg L^{-1}	5.2 a	26.7 a	31.9 a	3.7 a

[a] Values shown in this table are the mean of three replicates (3 pots with 1 plant pot^{-1}) for each tomato cultivar. [b] Standard error. [c] No fruit production in these treatments. [d] Not enough fruit mass to carry out P and/or As determinations. [e] NS = not significant *F* ratio ($p < 0.05$), *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. [f] Treatment means from the ANOVA test. Values followed by the same letter, within the same source of variation, are not significantly different ($p < 0.05$), Duncan multiple range test.

molysis and discoloration followed by leaf wilting and necrosis of leaf tips and margins (Machlis, 1941; O'Neill, 1995). This limitation in the movement of water into the plant may even result in plant death (Woolson et al., 1971). Plants treated with DMA and MA at a concentration of 5 mg As L^{-1} were stunted with necrosis in leaf tips and margins. Total dry weight of DMA- and MA-treated Marmande plants was 31.5% and 68.9% compared to the controls, respectively.

Root dry weight was different for the two tomato cultivars, with Muchamiel plants having a significantly higher root production than Marmande plants (Table 1a and b). The higher root production of Muchamiel plants would have possibly increased their root-holding

Table 2. (A) Effects[a] of and (B) Results of the ANOVA and Duncan Tests for the Effects of Arsenic Concentration and Chemical Form on Tissue Phosphorus Concentration of Tomato Plants

A

	As con (mg L^{-1})	tissue phosphorus (dw) root (g kg^{-1})	shoot (g kg^{-1})	fruit (g kg^{-1})
		Marmande		
control		2.14 ± 0.06[b]	1.37 ± 0.02	2.47 ± 0.05
arsenite	1	2.43 ± 0.03	1.16 ± 0.03	2.92 ± 0.04
arsenite	2	2.46 ± 0.02	1.62 ± 0.02	3.29 ± 0.14
arsenite	5	2.42 ± 0.04	1.53 ± 0.01	2.79 ± 0.02
arsenate	1	2.46 ± 0.04	1.31 ± 0.05	3.75 ± 0.01
arsenate	2	2.37 ± 0.04	1.76 ± 0.01	3.27 ± 0.01
arsenate	5	2.19 ± 0.02	1.53 ± 0.01	3.23 ± 0.03
MA	1	2.41 ± 0.04	2.83 ± 0.01	3.42 ± 0.01
MA	2	2.74 ± 0.04	2.59 ± 0.05	3.85 ± 0.01
MA	5	2.89 ± 0.03	2.06 ± 0.03	3.73 ± 0.03
DMA	1	2.40 ± 0.03	2.26 ± 0.01	3.33 ± 0.03
DMA	2	2.46 ± 0.02	2.62 ± 0.05	4.06 ± 0.01
DMA	5	3.53 ± 0.04	2.51 ± 0.04	3.83 ± 0.08
		Muchamiel		
control		2.61 ± 0.02	1.78 ± 0.03	3.95 ± 0.06
arsenite	1	2.10 ± 0.03	1.68 ± 0.02	2.86 ± 0.06
arsenite	2	2.07 ± 0.02	1.68 ± 0.01	3.10 ± 0.02
arsenite	5	2.20 ± 0.03	1.98 ± 0.10	3.32 ± 0.03
arsenate	1	2.34 ± 0.07	1.88 ± 0.03	3.70 ± 0.05
arsenate	2	1.91 ± 0.06	1.63 ± 0.03	3.18 ± 0.03
arsenate	5	2.37 ± 0.09	1.95 ± 0.01	3.14 ± 0.03
MA	1	2.44 ± 0.03	2.76 ± 0.02	3.42 ± 0.05
MA	2	2.41 ± 0.03	2.28 ± 0.03	—[c]
MA	5	1.77 ± 0.20	2.32 ± 0.02	3.75 ± 0.02
DMA	1	2.68 ± 0.01	2.26 ± 0.03	—[c]
DMA	2	2.90 ± 0.04	2.27 ± 0.04	3.47 ± 0.03
DMA	5	3.61 ± 0.06	2.71 ± 0.04	—[c]

B

ANOVA *F* Test

source of variation	root *F* value		shoot *F* value		fruit *F* value	
variety	70	***[d]	90	***	13.7	***
As sp	211	***	1111	***	80	***
As con	31	***	5.2	**	13.0	***
var × As sp	32	***	56	***	8.1	***
var × As con	12.1	***	128	***	7.4	**
As sp × As con	53	***	87	***	33	***
var × As sp × As con	20	***	6.5	***	—[e]	—[e]

Duncan Multiple Range Test

source of variation	root	shoot	fruit
tomato variety			
Marmande	2.59 a[f]	1.98 b	3.45 a
Muchamiel	2.40 b	2.13 a	3.33 b
arsenic species			
arsenite	2.28 c	1.61 c	3.05 d
arsenate	2.27 c	1.68 b	3.38 c
MA	2.44 b	2.47 a	3.63 a
DMA	2.99 a	2.44 a	3.52 b
arsenic concentration			
1 mg L^{-1}	2.41 b	2.02 b	3.28 b
2 mg L^{-1}	2.46 b	2.06 a	3.48 a
5 mg L^{-1}	2.62 a	2.07 a	3.42 a

[a] Values shown in this table are the mean of three replicates (3 pots with 1 plant pot^{-1}) for each tomato cultivar. [b] Standard error. [c] No fruit production in these treatments. [d] NS = not significant *F* ratio ($p < 0.05$), *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. [e] The three way-interaction could not be done because some plants did not produced fruit. [f] Treatment means from the ANOVA test. Values followed by the same letter, within the same source of variation, are not significantly different ($p < 0.05$), Duncan multiple range test.

capacity for As, thereby limiting As translocation to the above-ground plant parts. The application of MA and DMA led to a significant decrease in root dry weight compared to controls and to those plants treated with arsenite and arsenate at all As concentrations used in this experiment and for both tomato cultivars. A significant increase in root dry weight was observed when As was applied as arsenite at all the concentrations used.

The As treatments affected shoot dry weight in a similar manner as they did total dry weight. This parallelism between total and shoot dry weights was

Table 3. (A) Effects[a] of (B) Results of the ANOVA and Duncan tests for the effects of Arsenic Concentration and Chemical Form on Tissue Arsenic Concentration of Tomato Plants

A

	As con ($mg\ L^{-1}$)	As concentration (dw) root ($mg\ kg^{-1}$)	shoot ($mg\ kg^{-1}$)	fruit ($mg\ kg^{-1}$)	arsenic concentration ratio (ACR)
		Marmande			
control		5.0 ± 0.2[b]	0.5 ± 0.4	1.1 ± 0.5	0.10 ± 0.03
arsenite	1	131 ± 2	1.1 ± 0.5	2.0 ± 0.1	0.01 ± 0.01
arsenite	2	250 ± 4	3.1 ± 0.7	1.2 ± 0.2	0.01 ± 0.01
arsenite	5	588 ± 1	9.0 ± 0.8	1.6 ± 0.5	0.02 ± 0.01
arsenate	1	118 ± 3	2.7 ± 0.1	1.3 ± 0.1	0.02 ± 0.01
arsenate	2	263 ± 1	5.3 ± 0.7	1.9 ± 0.1	0.02 ± 0.01
arsenate	5	497 ± 9	10.5 ± 1.2	4.8 ± 0.9	0.02 ± 0.01
MA	1	94.6 ± 0.9	3.2 ± 0.6	2.6 ± 0.4	0.03 ± 0.01
MA	2	212 ± 4	6.9 ± 0.6	4.7 ± 0.1	0.03 ± 0.01
MA	5	215 ± 2	40.7 ± 1.0	17.8 ± 0.9	0.19 ± 0.01
DMA	1	107 ± 3	1.8 ± 0.7	3.5 ± 0.2	0.02 ± 0.01
DMA	2	212 ± 1	6.0 ± 0.1	4.8 ± 0.6	0.03 ± 0.01
DMA	5	405 ± 5	10.0 ± 0.7	8.2 ± 0.7	0.03 ± 0.01
		Muchamiel			
control		4.8 ± 0.2	1.1 ± 0.7	1.1 ± 0.5	0.22 ± 0.01
arsenite	1	136 ± 2	0.8 ± 0.3	1.9 ± 0.6	0.01 ± 0.01
arsenite	2	214 ± 1	4.9 ± 0.5	2.2 ± 0.2	0.02 ± 0.01
arsenite	5	462 ± 3	6.8 ± 0.4	2.8 ± 0.4	0.02 ± 0.01
arsenate	1	117 ± 2	1.3 ± 0.4	4.8 ± 1.4	0.01 ± 0.01
arsenate	2	247 ± 4	6.2 ± 0.1	2.3 ± 0.3	0.03 ± 0.01
arsenate	5	518 ± 5	6.9 ± 0.3	4.0 ± 0.8	0.01 ± 0.01
MA	1	103 ± 3	3.2 ± 0.5	8.0 ± 0.5	0.03 ± 0.01
MA	2	167 ± 1	12.2 ± 0.7	–[c]	0.07 ± 0.01
MA	5	170 ± 1	36.3 ± 0.8	26.3 ± 1.2	0.21 ± 0.01
DMA	1	88.0 ± 3.8	2.1 ± 0.4	–[c]	0.02 ± 0.01
DMA	2	164 ± 3	10.8 ± 0.6	5.6 ± 0.4	0.07 ± 0.01
DMA	5	140 ± 3	18.2 ± 0.3	–[c]	0.13 ± 0.01

B

ANOVA *F* test

source of variation	root F value		shoot *F* value		fruit F value		ACR F value	
variety	1261	***[d]	11.1	**	39.8	***	153	***
As species	2860	***	586	***	159	***	736	***
As concentration	13144	***	1421	***	109	***	636	***
var × As species	320	***	28.1	***	15.4	***	77.6	***
var × As concentration	509	***	25.1	***	0.1	NS	50.1	***
As species × As con.	1325	***	333	***	58.9	***	329	***
var × As spe. × As con.	207	***	15.2	***	–[e]	–[e]	28.5	***

Duncan Multiple Range Test

source of variation	root	shoot	fruit	ACR
tomato variety				
Marmande	258 a[f]	8.4 b	4.5 b	0.04 b
Muchamiel	211 b	9.2 a	7.0 a	0.05 a
arsenic species				
arsenite	297 a	4.3 d	2.0 d	0.01 c
arsenate	293 b	5.5 c	3.2 c	0.02 c
MA	160 c	17.1 a	11.8 a	0.10 a
DMA	186 d	8.1 b	6.0 b	0.05 b
arsenic concentration				
1 $mg\ L^{-1}$	112 c	2.0 c	3.6 b	0.02 c
2 $mg\ L^{-1}$	216 b	6.9 b	4.3 b	0.04 b
5 $mg\ L^{-1}$	374 a	17.3 a	9.4 a	0.08 a

[a] Values shown in this table are the mean of three replicates (3 pots with 1 plant pot^{-1}) for each tomato cultivar. [b] Standard error. [c] Not fruit production in these treatments. [d] NS = not significant *F* ratio ($p < 0.05$), *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. [e] The three way-interaction could not be done because some plants did not produced fruit. [f] Treatment means from the ANOVA test. Values followed by the same letter, within the same source of variation, are not significantly different ($p < 0.05$), Duncan multiple range test.

not surprising because the dry weight of shoots constituted the major portion of total dry weight (80−90%). Both MA and DMA were the most phytotoxic As species with respect to shoot dry weight.

Fruit yield was negatively influenced by the presence of all As species, even at the lowest As addition concentration; however, reduction of fruit mass was statistically higher for plants treated with MA and DMA compared to those growing on solutions containing arsenite and arsenate. Arsenic concentration, however, did not affect tomato yield.

Tissue Arsenic Concentration. The total amount of As taken up by tomato plants followed the trend: DMA < MA ≪ arsenate ≅ arsenite, with increasing As

levels in the nutrient solution resulting in a higher As uptake (data not shown; As uptake = As concentration × dry weight). Upon As absorption, tomato plants accumulated As mainly in the root system (85% of total As) and only relatively low quantities were translocated to shoots (14%) and fruit (1%).

When a toxic metal has been absorbed by plants, the most extended mechanism involved in plant tolerance is limiting the upward transport, resulting in accumulation primarily in the root system (Meharg and Macnair, 1990). The strategy developed by tomato plants to tolerate the different species of As was avoidance, limiting As transport to shoots and increasing As accumulation in the root system (Table 3a and b). Avoidance, however, does not explain how tomato root tissues tolerate such extremely high As concentrations (up to 588 mg As kg^{-1}) without exhibiting visual symptoms of toxicity. A possible explanation, not directly deduced from this study, could be that As compartmentalization in tomato roots was so effective that As impact on growth and metabolism was minimal. Arsenic detoxification and compartmentalization in root cells are topics that will need further research to verify their role in plant tolerance to As.

Arsenic uptake by plants is influenced, among many other factors, by the plant species (Walsh and Keeney, 1975). In this particular study, root As concentration was different for each of the tomato cultivars used in this experiment, with Marmande plants having higher As concentrations than Muchamiel plants (Table 3a and b). Arsenic concentrations in roots increased significantly with increasing As levels in the nutrient solution. Arsenic phytoavailability followed the trend: MA < DMA ≪ arsenate ≅ arsenite.

Muchamiel plants accumulated more As in shoots and fruits than Marmande plants (Table 3a and b). The As addition concentration had a significant effect on the shoot and fruit As levels; As concentrations in shoot and fruit tissue increased significantly with increasing As levels in the nutrient solution. Shoot and fruit As concentrations were also influenced by the As species. Treatments with MA and DMA caused higher As concentrations in shoots and fruits than those with arsenite and arsenate, contrarily to what happened in the root system.

Arsenic levels in vegetables, grain, and other food crops at the consumer level are low, even when the crops are grown on contaminated land (O'Neill, 1995). The usual statement (e.g., Lepp, 1981) that toxicity limits the uptake of As to safe levels was not, however, confirmed in our study. Under conditions of exposure to threshold levels in the soil, the statement appears to be true. If, however, crops are grown on contaminated nutrient solutions, they may accumulate residues which are unacceptable. Similar results were obtained after violation of label restrictions on timing of arsenical sprays application during the growing season (Wauchope, 1983).

The statutory limit set for As concentration in fruits, crops and vegetables is 1 mg kg^{-1} on a fresh weight (fw) basis (Mitchell and Barr, 1995); considering an average water content of the tomato fruits in our experiment of 90%, this limit on a dry weight (dw) basis is 10 mg kg^{-1}. In our study, As concentration in tomatoes ranged from 0 to 4.8 mg kg^{-1} (dw) for arsenite and arsenate treatments, and from 0 to 26.3 mg kg^{-1} (dw) for MA and DMA applications, with maximum As levels being found for the highest concentration of MA. These As levels were well within the normal range for As concentrations in food crops in the case of arsenite and arsenate but were close to or even above this maximum As limit in the case of treatments with DMA and especially MA. Therefore, residues from the use of organic-based As pesticides (MA and DMA) are potentially more dangerous to human health than As inorganic sources (arsenite and arsenate) due to their possibility of reaching tomato fruits in higher concentrations.

Organic arsenicals, such as methylarsonic and dimethylarsinic acids, are used as agrochemicals and herbicides, but until the present study these compounds do not seem to have caused any particularly problematic environmental contamination or damage to health (Yamauchi and Fowler, 1994), because methylated As compounds are far less acutely toxic than the inorganic As compounds. However, there are reports on toxicologic problems with organic arsenicals, particularly dimethylarsinic acid, such as damage to DNA (Yamanaka et al., 1991) and mutagenicity (Yamanaka et al., 1989). Therefore, these genetic studies indicate that these major organic As compounds are not innocuous, and that the high levels of As reached in tomato fruit in this experiment could be considered as potentially dangerous to human health. This danger is, however, dependent on As speciation in plant tissues and this is a topic that will need further research to state whether these high levels of total As could be considered as a hazard to animal and human health.

The As concentration ratio (ACR = shoot As concentration/root As concentration) was significantly affected by both As chemical species and As concentration. The data on plant As concentrations and ACR indicated that the As chemical species in the nutrient solution and the As concentration not only determined the phytoavailability of As to tomato plants, but also determined the transport and movement of As within the plant. Considering the data on ACR, both MA and DMA showed a higher translocation from roots to shoots compared to arsenite and arsenate. This higher degree of upward translocation could have contributed to the observed greater toxicity of MA and DMA for tomato plants and to the lower fruit yield. Marin et al. (1992) demonstrated that MA was the most phytotoxic arsenical to rice because this organic arsenical was readily translocated to the shoot and thereby increased its possibility of affecting rice yield.

ABBREVIATIONS USED

MA, methylarsonate; DMA, dimethylarsinate; DSMA, disodium methylarsonate; SDMA, sodium dimethylarsinate.

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