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Arsenic Toxicity and Accumulation in Turnip As Affected by Arsenic Chemical Speciation

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Arsenic (As) uptake by turnip, growing under soilless culture conditions, was studied. A 4 × 3 factorial experiment was conducted with four As species [arsenite, arsenate, methylarsonic acid (MMAA), dimethylarsinic acid (DMAA)] and three As concentrations (1.0, 2.0, and 5.0 mg L^{-1}). Arsenic phytoavailability and phytotoxicity were primarily determined by As speciation. Organic arsenicals, especially MMAA, were clearly phytotoxic to this turnip cultivar. Plant As concentrations significantly increased with increasing As application rates. Both organic arsenicals showed a higher upward translocation than their inorganic counterparts, contributing to the greater phytotoxicity and lower dry matter productions of these organic treatments. Both inner root and outer root skin As concentrations were above the maximum limit set for As content in food crops (1.0 mg kg^{-1}). If turnip plants are exposed to a large pulse of As, as growth on contaminated nutrient solutions, they will accumulate residues at levels that are unacceptable for animal and human consumption.

Keywords: *Arsenic absorption; arsenic adsorption; arsenic speciation; Brassica napus; food contamination; food crops*

INTRODUCTION

Arsenic (As) is ubiquitous in our environment and has both natural and anthropogenic sources. The most important natural sources of As are low-temperature volatilization and volcanic action, whereas the largest anthropogenic sources are the exploitation and smelting of metalliferous ores, the burning of fossil fuels, and agricultural activities (Mitchell and Barr, 1995). The toxicity of As to biological systems has made it a useful constituent of insecticides, herbicides, fungicides, desiccants, and wood preservatives (Johnson and Hitbold, 1969; Marin, 1995). However, indiscriminate use of these As substances has led to elevated concentrations of plant-available As in many soils, which may reduce soil productivity (Liebig, 1966; Marin, 1995) and be toxic to plants (Deuel and Swoboda, 1972; Marin, 1995).

Typical uncontaminated agricultural soils contain 1−20 mg of As kg^{-1} of soil (Wauchope, 1983), but contaminated soils associated with mineralized zones may contain levels as high as 2600 mg of As kg^{-1} of soil (Meharg et al., 1994). Soluble As concentrations vary as low as 0.007 mg kg^{-1} in uncontaminated upland soils to 13 mg kg^{-1} in highly contaminated mining areas. Soluble As levels will also significantly increase under low redox potential conditions (Carbonell-Barrachina et al., 1999).

Recognition that different As compounds vary in their solubilities, mobilities, bioavailabilities, and phytotoxicities has directed attention toward the specific compounds or chemical forms of the element present in the environment. Thus, studies concerning the uptake of different As species, both inorganic and organic, by plants and their effects on plant growth, fruit yield, and nutrition are essential to understanding As behavior in the soil−plant environment. So far, mainly four As species have been determined in the water/soil/plant system (Sohrin et al., 1997). Arsenate is the thermodynamically stable form under aerobic conditions, of which one or two protons are dissociated at natural soil solution pH values [$H_2AsO_4^-$ and $HAsO_4^{2-}$]. Arsenate is a chemical analogue of phosphate and may interfere with oxidative phosphorylation (Terwelle and Slater, 1967). Arsenite is a neutral species at natural pH values [$As(OH)_3$] and inhibits the activity of enzymes by binding to thiol groups. Methylarsonic acid [$CH_3AsO(OH)_2$; MMAA] and dimethylarsinic acid [$(CH_3)_2AsO(OH)$; DMAA] also form anions in soils but are much less toxic than inorganic species (Sohrin et al., 1997) and may block protein synthesis (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 (Liebig, 1966; Lepp, 1981; 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). As phosphate and arsenate are taken up by the same uptake system, this may cause a number of problems in terms of phosphate nutrition for plant species. First, arsenate will compete with phosphate for uptake and, second, the suppressed phosphate/arsenate uptake system will also reduce phosphate accumulation (Meharg et al., 1994).

Organic herbicides have resulted in less attention by researchers than their inorganic counterparts due to their lower rate of application and toxicity (Woolson, 1983). The studies on As organic species effects on plants have emphasized its herbicidal activity when

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applied to the foliage, neglecting the possibility of root absorption. Braman (1975), however, pointed out that DMAA may be a ubiquitous As compound found in all soils and may predominate in many.

The bioavailability, uptake, and phytotoxicity of As is controlled by a large number of factors including the source and concentration of the element (NAS, 1977), As chemical form (Carbonell-Barrachina et al., 1998), soil pH, and redox and drainage conditions (Marin et al., 1993) as well as the type and amount of organic matter present (Mitchell and Barr, 1995). Uptake is also dependent on seasonal effects, plant species (Walsh and Keeney, 1975), and a number of physical and chemical factors operating at the soil−root and root−shoot interfaces (Mitchell and Barr, 1995).

In Spain, an indiscriminate application of inorganic arsenicals as pesticides led to a buildup of As residues in many agricultural soils and reduction of their productivity; these polluted soils are now frequently used for vegetable growing, including turnips, tomatoes, beans, radishes, etc. Besides, at the present moment, there is a great concern about As pollution in Spain due to a recent environmental accident in a pyrite mine located in the city of Aznalcóllar, Sevilla (southern Spain). In this accident ~4.5 hm^3 of pyritic sludges, containing among other pollutants ~5000 mg of As kg^{-1}, was spilled into the Agrio and Guadamar Rivers and the surrounding agricultural areas. Some of the pollutants, including As, reached Doñana National Park, the largest wetland area in Europe and affected soils, plants, and even animals.

Arsenic species from these polluted soils may accumulate in any of the agricultural plants being grown in them and enter the human food chain through their edible parts. We designed a greenhouse experiment that allowed the study of As absorption and phytotoxicity to turnip plants in relation to its chemical form. The main objective of this study was to determine whether As could accumulate in the edible part of turnip plants in concentrations potentially dangerous for human health. The distribution of the adsorbed/absorbed As among outer root skin, inner root, and shoots is also reported here.

MATERIALS AND METHODS

Turnip plants (*Brassica napus* L.), cv. Virtudes-Martillo, were grown in soilless culture containing different chemical forms and concentrations of As. The experiment was carried out under greenhouse conditions, using siliceous sand as inert medium for cultivation. The factorial treatments (4 × 3, As species × As concentrations) were applied in three replicates of a complete randomized design. The treatments consisted of four chemical forms of As (arsenite, arsenate, MMAA, and DMAA) with three As concentrations (1.0, 2.0, and 5.0 mg L^{-1}). The sodium salts used were $NaAsO_2$ (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 amount and form of As in solution were analyzed regularly using a hydride generation atomic absorption technique (Masscheleyn et al., 1991) to verify that the chemical form of the added As did not change over time. Arsenic species were found to be stable with respect to oxidation/reduction and methylation/demethylation reactions for a period of 4 days. Thus, the nutrient solution was replaced every 4 days to maintain the desired treatments.

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 deionized water, and seedlings were weighed and transferred to hydroponic pots containing 0.5 L of nutrient solution. A single pot, representing a specific As form−As rate treatment, contained one seedling. The basal nutrient solution (Feigin et al., 1987) contained (in mg L^{-1}) the following: 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.

Arsenic treatments started after 14 days of acclimatization; seedlings were weighed, and no As was found when plant tissues were analyzed. Plants were grown for 60 days and then harvested (plants were 88 days old after germination). Plants were washed with tap water and P-free detergent and rinsed several times with distilled−deionized water. Roots and shoots were separated, and fresh and dry (60−70 °C for 72 h) matter productions were determined. Roots were divided in two parts: outer root skin and inner root, to distinguish between the adsorption and absorption processes. Dried samples were ground in a stainless steel mill to obtain a homogeneous sample. Plant tissue samples were dry-ashed by applying the methodology of dry mineralization developed by Ybáñez et al. (1992). Digested samples were filtered and diluted with distilled−deionized water to 25 mL. Arsenic in the extracts was determined by hydride generation atomic absorption spectrometry (HGAAS) with a Perkin-Elmer (PE) model 2100 spectrometer with a PE MHS-10 hydride generator. Blanks were analyzed to assess possible As contamination.

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

RESULTS AND DISCUSSION

Arsenic concentrations used in this experiment (1, 2, and 5 mg L^{-1}) are typical of highly contaminated agricultural soils (Lepp, 1981; Wauchope, 1983; Meharg et al., 1994) and were selected to give a range of concentrations up to the expected toxic levels (5 mg of As L^{-1} from previous experiments using other agricultural and wetland plants; Carbonell-Barrachina et al., 1995, 1997, 1998; Carbonell et al., 1998). Toxicity symptoms and even crop yields may be correlated with soluble As concentrations, with toxic levels in the range of 2−50 mg kg^{-1} for many crops (Wauchope, 1983).

The normal range for soluble phosphorus (P) is 8−20 mg kg^{-1} (Junta de Extremadura, 1992). The P level used in this study (46.5 mg L^{-1}) is above this normal range, but it was selected because (1) the levels of As used were also higher than usual, and to maintain a ratio P/As similar to that of agricultural soils a high P concentration was also necessary, and (2) it would allow plants to live for a longer period of time than lower P concentrations (Meharg and Macnair, 1991).

Plant Growth. Turnip plant growth (as represented by roots and shoots dry matter production) was significantly affected by As treatments. Our results demonstrated that the As chemical form was more important than the As level in solution in determining the phytotoxic effect of As on this turnip cultivar (Table 1). Arsenic form has been reported as the crucial factor determining As phytotoxicity in several wetland plants: rice (*Oryza sativa* L.) (Marin et al., 1992) and *Spartina patens* and *Spartina alterniflora* (Carbonell-Barrachina et al., 1998).

Arsenic chemical form in solution influenced root and shoot dry weights (Table 1). Plants treated with arsenate had the highest biomass production; treatments with arsenite and DMAA caused shoot and root dry weights similar to those of controls, and plants treated with MMAA had significantly lower dry weights than control plants. The influence of As concentration on total

Table 1. Effects of and Results of the ANOVA and Duncan Tests for the Effects of Arsenic Concentration and Chemical Form on Dry Weight of Turnip Plants

	As rate (mg L^{-1})	dry matter production (g pot^{-1})			
		root skin	inner root	roots	shoots
control		1.55[a] ± 0.07[b]	5.80 ± 0.60	7.34 ± 0.67	7.02 ± 0.59
As(III)	1.0	0.74 ± 0.15	4.69 ± 1.48	5.43 ± 1.63	6.88 ± 1.14
As(III)	2.0	1.25 ± 0.39	8.11 ± 3.03	9.36 ± 3.43	10.04 ± 2.35
As(III)	5.0	2.59 ± 0.01	6.55 ± 2.63	9.14 ± 2.63	6.94 ± 2.67
As(V)	1.0	2.12 ± 0.01	5.05 ± 1.13	7.17 ± 1.13	6.33 ± 0.60
As(V)	2.0	1.71 ± 0.16	11.60 ± 0.33	13.30 ± 0.17	11.24 ± 1.75
As(V)	5.0	1.39 ± 0.36	10.31 ± 3.82	11.70 ± 4.16	11.03 ± 1.52
MMAA	1.0	0.43 ± 0.08	1.86 ± 0.69	2.29 ± 0.75	3.40 ± 1.29
MMAA	2.0	1.53 ± 0.01	2.22 ± 0.36	3.75 ± 0.36	2.04 ± 0.19
MMAA	5.0	1.55 ± 0.01	2.33 ± 1.25	3.88 ± 1.25	2.77 ± 0.37
DMAA	1.0	2.60 ± 0.01	7.34 ± 3.31	9.94 ± 3.31	8.94 ± 2.18
DMAA	2.0	1.64 ± 0.01	3.81 ± 1.65	5.45 ± 1.65	6.44 ± 1.86
DMAA	5.0	0.99 ± 0.06	5.70 ± 0.48	6.69 ± 0.54	8.21 ± 1.08

ANOVA *F* Test

source of variation	root skin	inner root	roots	shoots
As form	***[c]	**	**	***
As rate	NS	NS	NS	NS
As form × As rate	***	NS	NS	NS

Duncan Multiple-Range Test

source of variation	root skin	inner root	roots	shoots
arsenic form				
As(III)	1.53 a[d]	6.45 a	7.98 a	7.95 a
As(V)	1.74 a	8.99 a	10.72 a	9.53 a
MMAA	1.17 b	2.14 b	3.30 b	2.74 b
DMAA	1.74 a	5.62 a	7.36 a	7.86 a
arsenic rate				
1.0 mg L^{-1}	1.47 a	4.74 a	6.21 a	6.39 a
2.0 mg L^{-1}	1.53 a	6.43 a	7.97 a	7.44 a
5.0 mg L^{-1}	1.63 a	6.22 a	7.85 a	7.24 a

[a] Values shown in this table are the mean value of three replicates (three pots with one plant per pot). [b] Standard error. [c] NS = not significant *F* ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [d] 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.

root and shoot dry productions was not significant, implying that As chemical form is more important than As concentration in determining As phytotoxicity to this plant species.

Arsenic has not been shown to be an essential plant nutrient, although it is essential for animal metabolism (Lepp, 1981). Stimulation of growth by As additions (mainly as arsenate) has been, however, reported to increase growth of maize (Woolson et al., 1971a), peas, wheat, and potatoes (Jacobs et al., 1970), and rye, soybean, and cotton (Cooper et al., 1932). It is possible that arsenate additions may displace phosphate from the soil in certain situations with a resultant increase in plant P availability (Jacobs et al., 1970), thereby affecting growth. However, this speculation cannot be responsible for the increased turnip plant dry matter production observed in our experiments because soil-free systems were used.

Marin et al. (1992) also reported an increase in the growth of rice in hydroponic studies containing DMAA at rates of 0.05 and 0.2 mg of As L^{-1}. Carbonell-Barrachina et al. (1995) reported an increase in tomato plant growth after an arsenite treatment at a concentration of 2.0 mg of As L^{-1}. More recently, Carbonell-Barrachina et al. (1998) observed that applications of arsenate at rates of 0.2 and 0.8 mg L^{-1} (hydroponic culture) significantly increased root, shoot, and total dry matter production in *Sp. alterniflora* and *Sp. patens* compared to control plants.

The reason for the observed positive growth response is unclear but may be linked with P nutrition. Phosphate and arsenate are taken into plant roots by a common carrier; however, both high- and low-affinity phosphate/arsenate plasma membrane carriers have a much higher affinity for phosphate than arsenate (Meharg and Macnair, 1990), and phosphate is reported to be a very efficient competitive inhibitor of arsenate uptake (Meharg and Macnair, 1990). Arsenate/phosphate uptake can be suppressed in plant roots if the plants are P sufficient, which was the case in the present study (Table 2; Reuter and Robinson, 1986). This suppression is due to a feedback regulation of the arsenate/phosphate transporter; arsenate uptake is reduced through the suppression of the high-affinity uptake system (Meharg and Macnair, 1992). Conversely, Cox (1995), who studied the effect of different arsenicals on the nutrition of the canola plant, postulated that because 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. There was a significant interaction between turnip plant growth and plant P, showing that the growth of this cultivar of turnip was dependent on plant P status. In Figure 1, turnip shoot dry weight has been depicted versus P concentration in shoots as an example of the "negative" relationship between P and plant growth,

Table 2. Effects of and Results of the ANOVA and Duncan Tests for the Effects of Arsenic Concentration and Chemical Form on Phosphorus Concentration of Turnip Plants

	As rate (mg L^{-1})	phosphorus (g kg^{-1})		
		root skin	inner root	shoots
control		3.46[a] ± 0.02[b]	8.39 ± 0.29	4.35 ± 0.06
As(III)	1.0	2.89 ± 0.03	6.01 ± 0.30	3.73 ± 0.03
As(III)	2.0	2.36 ± 0.03	5.56 ± 0.33	2.77 ± 0.02
As(III)	5.0	2.59 ± 0.04	4.94 ± 0.02	3.27 ± 0.04
As(V)	1.0	3.36 ± 0.03	5.00 ± 0.35	3.94 ± 0.04
As(V)	2.0	2.99 ± 0.02	4.81 ± 0.04	3.71 ± 0.04
As(V)	5.0	2.92 ± 0.03	4.73 ± 0.10	3.40 ± 0.14
MMAA	1.0	5.35 ± 0.09	7.38 ± 0.14	7.21 ± 0.20
MMAA	2.0	10.72 ± 0.08	8.20 ± 0.36	9.87 ± 0.12
MMAA	5.0	6.87 ± 0.09	8.20 ± 0.19	7.77 ± 0.07
DMAA	1.0	2.55 ± 0.03	7.47 ± 0.31	3.57 ± 0.05
DMAA	2.0	3.12 ± 0.01	5.57 ± 0.12	4.19 ± 0.04
DMAA	5.0	3.77 ± 0.09	7.96 ± 0.29	3.93 ± 0.04

ANOVA *F* Test

source of variation	root skin	inner root	shoots
As form	***[c]	***	***
As rate	***	**	***
As form × As rate	***	**	***

Duncan Multiple-Range Test

source of variation	root skin	inner root	shoots
arsenic form			
As(III)	2.62 c[d]	5.56 c	3.26 d
As(V)	3.09 b	4.87 d	3.69 c
MMAA	7.65 a	7.51 a	8.29 a
DMAA	3.13 b	6.92 b	3.90 b
arsenic rate			
1.0 mg L^{-1}	3.53 c	6.48 a	4.62 b
2.0 mg L^{-1}	4.80 a	5.77 b	5.14 a
5.0 mg L^{-1}	4.04 b	6.39 a	4.59 b

[a] Values shown in this table are the mean value of three replicates (three pots with one plant per pot). [b] Standard error. [c] NS = not significant *F* ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [d] 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.

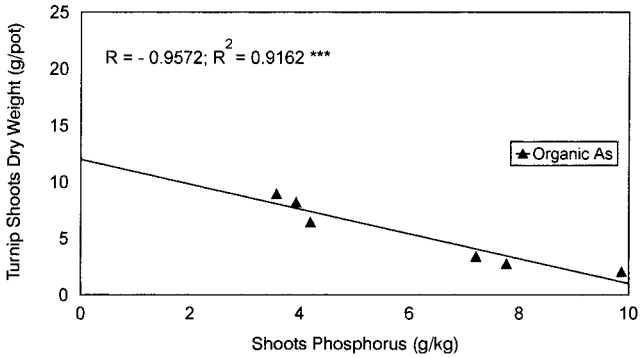

Figure 1. Turnip shoot dry biomass production as a function of shoot phosphorus concentration (***, significant at $p < 0.001$).

implying that Cox's hypothesis was valid for this plant species, especially for the organic arsenicals.

In this particular experiment, root and shoot P concentrations were significantly influenced by both As chemical form and As concentration in the nutrient solution (Table 2). Plants treated with MMAA presented significantly higher P levels than plants treated with inorganic arsenicals and controls except in the inner root, where control plants had the highest P level. Although P concentrations in turnip plants were dependent on the As level in solution, there was not a clear relationship between plant P and solution As.

The phytotoxic effects of As are indicative of a sudden decrease in water mobility, as suggested by root plasmolysis and discoloration followed by leaf wilting and necrosis of leaf tips and margins (Machlis, 1941). This limitation in the movement of water into the plant may result in plant death (Woolson et al., 1971b). MMAA was clearly phytotoxic to turnip plants (Table 1) and significantly decreased plant growth at all of the As rates of application used in this study. All MMAA-treated plants were stunted, with necrosis in leaf tips and margins; their dry matter productions of roots and shoots were only 45.0 and 39.0%, respectively, compared to the controls. Some of the plants growing in the nutrient solution containing MMAA seemed to have limited water; they took up small volumes of nutrient solution compared to control plants.

Tissue Arsenic Concentration. The total amount of As taken up by turnip plants (roots + shoots) followed the trend MMAA < DMAA < arsenite < arsenate, with increasing As levels in the nutrient solution resulting in a higher As uptake for all As species and concentrations (data not shown; As uptake = As concentration × dry weight). The highest residues of As are found in plant roots (e.g., sugar beet and radish), with intermediate values in the vegetative top growth (e.g., spinach and grasses) and edible seeds and fruits containing the lowest levels of As (Lepp, 1981). Upon As absorption, turnip plants accumulated As mainly in the root system (75% of total As), and only relatively low quantities (25%) were translocated to shoots.

Berry (1986) suggested three strategies of plant tolerance to metals: avoidance (limited uptake by roots or limited transport to shoots), detoxification (subcellular compartmentalization of metal or by binding to cell walls), and biochemical tolerance (specialized metabolic pathways and enzymatic adaptations). From the data in Table 3, it seems that the strategy developed by turnip plants to tolerate the different chemical forms of As was avoidance, limiting As transport to shoots and increasing As accumulation in the root system. This, however, does not explain how turnip root tissue tolerates such extremely high As concentrations (up to 115.5 mg of As kg^{-1}) without exhibiting visual symptoms of toxicity. Arsenic detoxification and compartmentalization in root cells may be involved in this tolerance process and are topics that will need further research to verify their roles in plant tolerance to As toxicity.

There is another factor that may have contributed to the low toxicity of As to the turnip root system. All of the arsenicals are strongly adsorbed to root surfaces from solution. This adsorption is apparently limited only by availability. For this reason, observed As concentrations in roots are very high in hydroponic experiments (Wauchope, 1983), including ours, and are higher than in other plant parts in most soil-grown plants. Our results clearly demonstrated that there is a significant difference between As concentrations caused by adsorption (outer root skin) and those caused by absorption (inner root), with the highest levels being due to adsorption onto the root surfaces. The significance of this pathway in relation to the total As uptake by humans has yet to be identified and may be worthy of further research.

Arsenic concentrations in roots significantly increased with increasing As levels in the nutrient solution. The

Table 3. Effects of and Results of the ANOVA and Duncan Tests for the Effects of Arsenic Concentration and Chemical Form on Arsenic Concentration of Turnip Plants

		arsenic (mg kg^{-1})				
	As rate (mg L^{-1})	root skin	inner root	shoots	RAI[a]	ACR[a]
control		6.4[b] ± 0.1[c]	2.9 ± 0.1	0.9 ± 0.1		0.32 ± 0.01
As(III)	1.0	85.2 ± 3.1	11.7 ± 0.2	3.5 ± 0.2	11.8 ± 0.2	0.30 ± 0.03
As(III)	2.0	98.8 ± 4.9	20.5 ± 0.3	6.5 ± 0.3	10.3 ± 0.2	0.32 ± 0.01
As(III)	5.0	116 ± 1	42.0 ± 3.3	6.9 ± 0.3	8.7 ± 0.8	0.16 ± 0.01
As(V)	1.0	83.6 ± 5.8	10.1 ± 0.6	4.1 ± 0.5	10.5 ± 0.7	0.40 ± 0.01
As(V)	2.0	107 ± 2	24.4 ± 1.6	10.6 ± 0.3	12.6 ± 1.0	0.43 ± 0.03
As(V)	5.0	108 ± 3	40.5 ± 1.1	14.3 ± 0.2	8.1 ± 0.1	0.35 ± 0.01
MMAA	1.0	39.5 ± 2.0	8.3 ± 0.2	8.6 ± 0.4	8.3 ± 0.2	1.04 ± 0.05
MMAA	2.0	59.5 ± 2.2	10.1 ± 0.2	10.0 ± 0.3	5.1 ± 0.1	0.98 ± 0.02
MMAA	5.0	69.6 ± 1.4	32.2 ± 0.6	18.3 ± 0.2	6.5 ± 0.2	0.56 ± 0.01
DMAA	1.0	24.6 ± 1.4	9.7 ± 1.0	10.4 ± 0.5	9.2 ± 1.0	1.15 ± 0.08
DMAA	2.0	66.7 ± 2.0	10.9 ± 0.7	13.9 ± 0.3	5.6 ± 0.3	1.25 ± 0.11
DMAA	5.0	69.0 ± 1.7	21.7 ± 0.6	17.0 ± 0.4	4.3 ± 0.1	0.80 ± 0.04

ANOVA *F* Test

source of variation	root skin	inner root	shoots	RAI	ACR
As form	***[d]	***	***	***	***
As rate	***	***	***	***	***
As form × As rate	***	***	***	***	***

Duncan Multiple-Range Test

source of variation	root skin	inner root	shoots	RAI	ACR
arsenic form					
As(III)	99.8 a[e]	24.7 a	5.6 d	10.3 a	0.26 d
As(V)	99.3 a	25.0 a	9.7 c	10.4 a	0.39 c
MMAA	56.2 b	16.9 b	12.3 b	6.6 b	0.86 b
DMAA	53.4 b	14.1 c	13.8 a	6.4 b	1.06 a
arsenic rate					
1.0 mg L^{-1}	58.2 c	10.0 c	6.7 c	9.9 a	0.72 a
2.0 mg L^{-1}	82.9 b	16.5 b	10.2 b	8.4 b	0.74 a
5.0 mg L^{-1}	90.5 a	34.1 a	14.1 a	6.9 c	0.47 b

[a] RAI, root absorption index; ACR, arsenic concentration ratio. [b] Values shown in this table are the mean value of three replicates (three pots with one plant per pot). [c] Standard error. [d] NS, not significant *F* ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [e] 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.

data on the root absorption index (RAI = root As concentration/nutrient solution As level) seemed to indicate that the chemical form of As present in the nutrient solution mainly determined the phytoavailability of As to turnip plants, although the As concentration also played an important role. Arsenic phytoavailability followed the trend MMAA ≅ DMAA < arsenate ≅ arsenite. The higher the As level in the nutrient solution, the higher the As concentrations in roots and shoots.

The As addition rate had a significant effect on shoot As level; As concentration in shoot tissue increased significantly with increasing As levels in the nutrient solution. Tissue As levels found in shoots were lower than those found in the root system. Shoot As concentrations were also influenced by the As chemical form. Contrarily to the As accumulation pattern in root as affected by the As chemical form, both organic arsenicals caused higher As accumulations in shoots compared to the inorganic arsenicals.

The tissue As concentration ratio (ACR = shoot As concentration/inner root As concentration) was significantly affected by both As chemical form and application rate; these two variables not only determined the phytoavailability of As to turnip plants but also controlled the transport and movement of As in the plant. A higher proportion of absorbed organic arsenicals, especially MMAA, was translocated to shoots, and that which was translocated was more toxic than similar DMAA levels in the plant tops. This study, therefore, has also shown that MMAA and DMAA, usually thought of as contact herbicides (Wauchope, 1983), are quite active via root entry and xylem transport.

It is rare that As accumulations in plants reach levels that are harmful to animals and man because growth is reduced before the content will reach toxic levels (Lepp, 1981). For instance, Mitchell and Barr (1995) reported that the concentration of As in barley, grown on a contaminated area with total As content in soil ranging from 20 to 335 mg kg^{-1}, tended to increase as the soil content increased, but the As concentration in the grain peaked at 0.41 mg kg^{-1} (less than the 1 mg kg^{-1} limit recommended for foodstuffs), indicating that little of the soil's As was translocated to the edible parts of barley even when present in the roots or shoots. This usual statement was not confirmed in our study. Under conditions of exposure to threshold levels in the soil, the statement appears to be true; after treatments equivalent to between 5 and 85 years of application, As was below detection limits in the edible portion of peas and sweetcorn (Jacobs et al., 1970). If, however, crops are exposed to a large pulse of As, as growth on contaminated nutrient solutions, they may accumulate residue levels that are unacceptable for human consumption. These high As concentrations found in plant tissues of soilless culture studies are likely due to the fact that

soluble As concentrations in the nutrient solutions are not high enough to cause plant death but are high enough to make plants absorb As continuously, leading to very high levels of pollutant.

The statutory limit set for As content in fruit, crops, and vegetables is 1.0 mg kg^{-1} (fresh weight) (Mitchell and Barr, 1995); considering an average water content of the edible part of the turnip in our experiment of 90%, this limit on a dry weight (dw) basis is 10 mg kg^{-1}. In our study, As concentrations in the skin of turnip roots were always above this maximum limit and ranged from 24.6 to 116 mg kg^{-1} (dw) for As-treated plants. These high As concentrations are, without a doubt, a result of all the arsenicals being firmly adhered to the root surfaces from solution (Wauchope, 1983). Arsenic concentrations in the inner roots were lower than those of the root skins, ranging from 8.3 to 42.0 mg kg^{-1} (dw), but were still above this maximum limit for almost all of the treatments. Therefore, and taking into account a possible incorporation of As in the food chain, soil residues from the use of As-based pesticides and herbicides are potentially dangerous for human health due to the feasibility of high levels of As accumulating in the edible part of turnips and even more if these turnips are consumed with peelings because of the As adsorption to the root surface.

The organic arsenicals are still registered for agricultural use as herbicides (Gao and Burau, 1997), and to date they do not seem to have caused any environmental problem or damage to health, because methylated As compounds are far less acutely toxic than the inorganic As compounds. The high levels of As reached in turnips in this experiment could be considered as potentially dangerous to human health even if As was still present in turnips as the applied organic species, because there are genetic studies that demonstrate that organic arsenicals may cause toxicological problems, such as damage to DNA (Yamanaka et al., 1991), and mutagenicity (Yamanaka et al., 1989). The severity of these problems, however, depends on As speciation, and this is a topic that will need further research to state whether the high levels of total As should be considered as a real hazard to animal and human health.

ABBREVIATIONS USED

MMAA, methylarsonic acid; DMAA, dimethylarsinic acid; DSMA, disodium methylarsonate; SDMA, sodium dimethylarsinate.

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