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EFFECT OF ARSENITE ON THE CONCENTRATIONS OF MICRONUTRIENTS IN TOMATO PLANTS GROWN IN HYDROPONIC CULTURE

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ABSTRACT: As a part of the study of environmental contamination by arsenic (As), we have undertaken the analysis of the effects caused by arsenic on the processes of absorption and accumulation of the micronutrient elements, boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in tomato (*Lycopersicum esculentum* Mill, cultivar Marmande). Therefore, an experiment has been carried out in a hydroponic culture in which different levels of As were used (added in the form of sodium arsenite) in concentrations of 2, 5, and 10 mg/L together with the corresponding control plants. Contamination by As causes changes to take place in both the absorption and transport of all the micronutrients. A reduction in the absorption of B, Cu, Mn, and Zn is observed while there is an increase in the absorption of Fe. In all the cases, there is a reduction in the transport of these elements towards the aerial part of the plant, no doubt as a result of the structural damage caused to the plant.

INTRODUCTION

Arsenic in the form of sodium arsenite is used as pesticide in vineyards as a treatment for *Stereum hirsutum*, *Phomopsis viticola*, and *Sparganothis pillenana*, which, unfortunately, are very common in our province (Alicante) and in certain other regions of Spain. It is carefully applied (as a spray), a winter treatment to the

stocks while they are dormant (26). As with any pesticide, careless application may result in drift to non-target crops (40).

On soils where sodium arsenite has been widely used may have high concentrations of As. These soils are now frequently used for tomato growing.

The phytotoxicity of As depends mainly upon which chemical form is present, with the arsenite being more toxic than the arsenate whereas both of these are more toxic than organic As compounds (32).

All the anions (borate, arsenite, arsenate, etc.) are strongly adsorbed onto the root membrane, they are then transported metabolically, selectively transferred to the symplast, and finally transported to the aerial part of the plant (40). Particularly, all the arsenicals (arsenate and arsenite) are strongly adsorbed to root surface from solution. For this reason, arsenic concentrations observed in roots are very high in hydroponic experiments (3,5,40).

Arsenate is a well-known decoupler of phosphorylation in mitocondria and arsenate can inhibit leaf uptake of other chemicals. Where non-lethal amounts are available translocation may result in comparable concentrations in foliage and in roots (40).

The transport of arsenite from roots is bounded by its high toxicity for radicular membranes (32), because it reacts with sulfhydryl groups of proteins (36) yielding disruption of the root functions (27), even cellular death. As a result of this, the largest quantities of As residues, when we have worked with tomato plants, are found in roots, medium amounts of residues are found in the high vegetative parts (leaves and stems) and the smallest quantities are to be found in the seeds and fruit (3,5,39).

Since As is chemically similar to phosphorus (P), it is likely to take part in many cellular reactions. Specific organic As compounds, such as arsenobetaine, arsenocholine, or arsenolipids, have been found in some organisms and it has been demonstrated that As replaces the P in the phosphate groups of DNA (16). However, although it has recently been proved to be essential to animal metabolism, the incorporation of As into the metabolic processes of higher plants has received very little attention.

In this paper, we attempt to evaluate the effect of As in the form of arsenite (using non-lethal amounts) on the nutrient uptake and accumulation in tomato plants of the Marmande cultivar grown in a hydroponic culture.

MATERIALS AND METHODS

The experiment was performed in hydroponic culture in optimized nutritional conditions, made up by 48 tomato (*Lycopersicum esculentum* Mill, cultivar Marmande) plants, two plants in 24 pots, were used so that there were four treatments with 12 plants in each: 0, 2, 5, and 10 mg/L of As in solution as sodium arsenite.

First, the seedbed was prepared and when the plants had reached an adequate height (15-20 cm), they were transplanted to hydroponic pots. After a week, the As treatment started.

Sampling was made in three developmental stages in the vegetative and reproductive cycle of plants—flower bud formation, fruit set, and fruits ripening. For each level four plants have been chosen, two complete flowerpots, representative of medium state of each one of treatments.

Each specimen was divided into roots, stems plus branches, leaves, and fruit, each fraction was individually treated. The procedure to which each one of the samples has been subjected, was formed by the following phases: washing with distilled water (20), drying at 50-60°C (15), pulverization (25), and mineralization (3,5,30,35).

Arsenic was determined by atomic absorption spectrophotometry with hydride generation (3,5). The spectrophotometer used was a Perkin Elmer (PE) Model 2100 with a hydride generator (PE) MHS-10.

Copper, Fe, Mn, and Zn were determined by atomic absorption spectrophotometry (41) and B was determined by the Wolf method (9,13,42).

RESULTS AND DISCUSSION

Boron

Boron is the only non-metal among the micronutrients and so because of its non-metallic character, it is similar to As. Boron is probably taken up by plants as the undisocciated boric acid (23,37), although the process is not well understood. If borate is adsorbed, its uptake is a metabolically mediatised process. At the fruit ripening stage, B uptake is significantly reduced at the level of 10 mg As/L (Table 1).

At the first two samplings, the levels of B found in the stems are constant. In stems at fruit ripening, the level of 10 mg As/L is completely different to the others

TABLE 1.- Concentration of B (mg kg^{-1} of fresh material). Each value is the mean for four plants (four repetitions for sample).

ROOTS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	2.9	3.0 a	6.7 a
2 mg L^{-1}	3.1	3.8 ab	6.5 a
5 mg L^{-1}	3.2	3.4 a	8.0
10 mg L^{-1}	2.9	4.0 b	5.2
	N.S.	**	***
STEMS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	2.7 a	2.9 a	8.5 a
2 mg L^{-1}	2.3	2.8 a	9.4 a
5 mg L^{-1}	2.6 a	2.6	9.5 a
10 mg L^{-1}	2.8 a	3.1 a	3.7
	**	***	***
LEAVES			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	8.1 a	12.9 a	20.7 a
2 mg L^{-1}	8.4 a	12.8 a	19.8 a
5 mg L^{-1}	9.7	12.5 a	20.5 a
10 mg L^{-1}	12.1	19.2	23.1
	***	***	***
FRUIT			
TREATMENT	2nd Sample	3rd Sample	
Control	0.8 a	1.0	
2 mg L^{-1}	1.2 a	1.0	
5 mg L^{-1}	0.9 a	1.0	
10 mg L^{-1}	1.1 a	0.9	
	***	N.S.	

NS, *, ** and *** stand for not significant or significant at P = 0.05, 0.01 and 0.001 respectively (ANOVA Test). In the same column, mean value with the same letter are significantly equal at P= 0.05 (Duncan's Test).

growth periods (B transport is significantly reduced). According to the findings of Michael et al. (24) in tobacco, the rate of transpiration has a decisive influence on the upward transport of B in the plant, which suggests that if the transpiration decreases, (the plants treated with the higher levels of As took up a very small amount of the nutritive solution, therefore the rate of transpiration was low), B concentrations in stems decrease too.

In the leaves, it has been observed that at all the series of samples, a situation of synergy exists, that is, the level of B increases as the As content increases (opposed results were obtained by Blatt (1) in 1.990 using cauliflowers (*Brassica oleracea*). By studying each series of samples individually, we can see that with the first sampling, the level of B increases on passing from one treatment to the other, although the first two treatments are statistically interrelated. In the last two series of samples (fruit set and fruits ripening), the results obtained for the first three concentrations of As are statistically equal and the 10 mg/L level of As shows significant differences compared with the controls. In general, it can be said that the effect of As on the foliar concentration of B (sinergy) is only significant at the level of 10 mg As/L.

Copper

There is ample evidence to indicate the existence of a P-Cu interaction regarding the availability and uptake of Cu by the roots (2,19). Therefore, owing to the well-known similarity between P and As and the fact that these elements can be interchanged as far as their metabolic functions or roles in plants are concerned (16,40), it is possible to explain the Cu/As antagonism that is clearly observed in the root levels of Cu found in our experiment (Table 2).

The development throughout the cultivation cycle in leaves, shows a continuous decrease. This phenomenon is logical if we consider that Cu is related to the growth of plants which is maximum at the early development stages. In leaves, in the series of samples corresponding to the fruits ripening, the Cu/As antagonism is observed to disappear. This is probably owing to the plant's small demand for this element at these stages.

Cooper together with Zn and B are micronutrients which are essential to the balance of nutrients in the plant that regulate such an essential process as transpiration (4,31). For this reason, the withering of a plant is closely related to the content of these elements (Cu, Zn, and B). Plants that receive a balanced

TABLE 2.- Concentration of Cu (mg kg^{-1} of fresh material). Each value is the mean for four plants (four repetitions for sample).

ROOTS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	2.9 a	3.4 a	6.3
2 mg L^{-1}	3.0 a	3.4 a	4.4 a
5 mg L^{-1}	2.4 b	3.0 b	4.2 a
10 mg L^{-1}	2.4 b	2.9 b	3.8 a
	**	**	***
STEMS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	1.3	1.3	3.5 a
2 mg L^{-1}	1.3 a	1.3	3.4 a
5 mg L^{-1}	1.2 a	1.1	2.9 a
10 mg L^{-1}	1.4 a	1.3	1.2
	***	N.S.	***
LEAVES			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	3.0 a	2.2 a	1.6
2 mg L^{-1}	2.8 a	2.3 a	1.6
5 mg L^{-1}	2.2 b	1.7 b	1.9
10 mg L^{-1}	2.0 b	1.8 b	1.8
	***	***	N.S.
FRUIT			
TREATMENT	2nd Sample	3rd Sample	
Control	0.8 a	1.0 a	
2 mg L^{-1}	0.7 a	1.0 a	
5 mg L^{-1}	0.6 a	0.8 a	
10 mg L^{-1}	0.7 a	0.4	
	***	***	

NS, *, ** and *** stand for not significant or significant at P = 0.05, 0.01 and 0.001 respectively (ANOVA Test). In the same column, mean value with the same letter are significantly equal at P= 0.05 (Duncan's Test).

supply are less likely to suffer from water stress (4,31). Vegetables with low levels or a deficiency in Zn and Cu (such is the case with the treatments high in As used in our study) tend to wither (in fact, this is what happened to the tomato plants treated with high levels of As). The function of Cu in this respect is thought to be related to the activity of enzymes, such as phenolase and laccase [in Cu deficient tissues phenolase activity is lowered and an accumulation of phenols occurs (31)]. In plants that are Cu deficient, the decrease in lignification can cause the xylem vessels to collapse and consequently the transport of water is blocked (this is confirmed by the fact that the plants treated with the higher levels of As take up a very small amount of the nutritive solution). Moreover, if this phenomenon is related to a decrease in the levels of Zn and Ca, it will lead to a greater fragility of the plants (this phenomenon was observed in the tomato plants which were treated with high levels of contaminant).

For fruits at fruit ripening, the Cu content decreases as the As level in the culture solution increases. There is a significant reduction at the level of 10 mg As/L.

Iron

Its absorption is greatly influenced by other cations and it has been observed that a competitive situation exists: Fe/Cu, Fe/Mn, and Fe/Zn. Consequently several researchers have pointed out the existence of an antagonistic effect (7,8,17,23,34). At the stage corresponding to the flower bud formation, it is observed that as the As level in the nutritive solution increases, the root Fe content also increases (Table 3). This situation is compensated by a decrease in other cations, such as Mn^{2+}, Cu^{2+}, and Zn^{2+} (to keep charge equilibrium or electroneutrality). In the two remaining series of samples (fruit set and fruits ripening), the situation described above undergoes changes at 10 mg As/L, owing to a combination of various factors—a decrease in plant growth when a high level of contaminant is present (less demand for nutrients, more toxicity to the root membranes)—a restriction on the transport of Fe due to the interaction with Zn and Ca, and the continuance of the antagonistic effect with Zn, Mn, and Cu. As a result of these circumstances, the maximum levels of Fe are found with the 5 mg As/L level, while the results found using the 10 mg As/L level stand out from the rest, its Fe concentrations are the smallest (due to the greater importance of the first two factors).

TABLE 3.- Concentration of Fe (mg kg^{-1} of fresh material). Each value is the mean for four plants (four repetitions for sample).

ROOTS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	90.4 a	90.1	108.0 a
2 mg L^{-1}	97.5 a	99.6 a	108.3 a
5 mg L^{-1}	156.3	106.6	127.6
10 mg L^{-1}	173.5	99.9 a	71.9
	***	***	***
STEMS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	8.5	6.5 a	18.7 a
2 mg L^{-1}	5.7 a	6.7 a	18.6 a
5 mg L^{-1}	6.6 ab	5.7	17.9 a
10 mg L^{-1}	7.4 b	6.5 a	7.1
	***	**	***
LEAVES			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	22.2 a	18.5 a	16.9 a
2 mg L^{-1}	22.6 ab	23.9	18.0 a
5 mg L^{-1}	23.1 b	15.3	24.2 b
10 mg L^{-1}	28.7	18.6 a	22.2 b
	***	***	***
FRUIT			
TREATMENT	2nd Sample	3rd Sample	
Control	4.8	3.8	
2 mg L^{-1}	4.8	5.0	
5 mg L^{-1}	4.0	4.8	
10 mg L^{-1}	4.5	3.8	
	N.S.	N.S.	

NS, *, ** and *** stand for not significant or significant at P = 0.05, 0.01 and 0.001 respectively (ANOVA Test). In the same column, mean value with the same letter are significantly equal at P= 0.05 (Duncan's Test).

In stems as fruits are ripening, the level or content of Fe present corresponds to the reduced need of Fe in the plants treated with the highest level of As, 10 mg As/L (reduced growth).

At flower bud formation and fruit ripening and as the level of As in the solution increases, an increase in the foliar level of Fe is observed. At fruit set, the situation is not clear since other factors intervene, such as a decrease in the Fe demand and a concentration effect due to a reduction in the foliar mass and the progressive accumulation of Fe by the plants that have been subjected to high levels of contaminant.

Manganese

The uptake or absorption of Mn (21) is metabolically regulated in a similar way to that of other divalent cations, such as Ca^{2+} and Mg^{2+}. With regard to its chemical behaviour (10,34), Mn has the properties of cations, such as Mg^{2+} and Ca^{2+} as well as the properties of heavy metals, such as Fe^{2+} and Zn^{2+}. For this reason, it would not be surprising for these species of ions to have an influence on the uptake and translocation of Mn. However, the reduction in the root levels of Mn (Table 4) does not appear to be due to the absorption of increasing concentrations of the cations mentioned above.

In the roots, the levels of Mn are found to be significantly reduced as the quantity of As present is increased. The three series of samples show a similar situation, since the results obtained with the control plants are statistically different from those obtained with the other treatments. This indicates that the effects of As are similar in all the treatments with As.

The development of the Mn levels throughout the cultivation cycle is characterized by an increasing accumulation in the whole plant and in all treatments, although it is true that the variations found in the reference plants and the specimens subjected to treatments with lower As levels were shown to be greater than those found in the other plants.

In stems and leaves, Mn concentrations are found to be significantly reduced as the level of As in the culture solution is increased (there is an antagonistic effect between Mn and As).

The presence of Mn^{2+} is necessary in various biochemical reactions (phosphoglucomutase, enolase, phosphokinase, phosphotransferase, ect.). However, it does not form a part of the prosthetic groups of the enzymes involved but acts as

TABLE 4.- Concentration of Mn (mg kg^{-1} of fresh material). Each value is the mean for four plants (four repetitions for sample).

ROOTS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	7.9	8.1	14.3
2 mg L^{-1}	4.7 ab	5.6 a	6.3 a
5 mg L^{-1}	3.8 a	5.3 a	7.7 a
10 mg L^{-1}	5.8 b	5.6 a	4.6
	***	***	***
STEMS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	2.4	4.6 a	23.1 a
2 mg L^{-1}	1.7 a	4.7 a	17.2 a
5 mg L^{-1}	1.8 a	4.1 a	17.5 a
10 mg L^{-1}	1.4	3.4	6.1
	***	*	***
LEAVES			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	6.6	15.5	30.9 a
2 mg L^{-1}	5.3 a	13.4	29.2 a
5 mg L^{-1}	4.5 a	11.5	29.3 a
10 mg L^{-1}	3.3	5.8	15.7
	***	***	***
FRUIT			
TREATMENT	2nd Sample	3rd Sample	
Control	0.7	0.9 a	
2 mg L^{-1}	0.8	0.9 a	
5 mg L^{-1}	0.7	0.9 a	
10 mg L^{-1}	0.7	0.6	
	N.S.	*	

NS, *, ** and *** stand for not significant or significant at P = 0.05, 0.01 and 0.001 respectively (ANOVA Test). In the same column, mean value with the same letter are significantly equal at P= 0.05 (Duncan's Test).

an inorganic ion (6,14). Therefore, if these reactions are reduced or limited by the different toxic effects of As, Mn may not be required in such high quantities and the plant may develop several ion selection mechanisms which will reduce the global Mn content.

In our experiment, the high levels of As produced visible symptoms of toxicity first in the older leaves and the yield suffered drastic reductions (up to approximately 80 %). However, a deficiency in Mn shows initially in the young leaves and the reduction in their production is almost total (11). Therefore, we cannot associate the toxicity of As with a simple deficiency of Mn.

Zinc

One of the most widely studied interactions of Zn is the deficiency of this element induced by P (22,29). The existence of this antagonism together with the evidence that As is chemically similar to P which it can substitute in many metabolic processes (16,40), could explain the fact that high quantities of As cause a deficiency in Zn. This phenomenon is clearly apparent from the data shown in Table 5, where it can be seen that as the level of As in solution increases the Zn content in all the plant decreases to a significant extent in the majority of cases.

The Zn content in the roots decreased in absorption as the As content in the culture medium increased. Because Zn is not very mobile, the highest concentrations of Zn are found in roots and stems (19). This restriction on the absorption of Zn caused by the contaminant also results in a reduction in its level in the rest of the plant.

As Wauchope (40) pointed out in 1983, arsenite is so toxic that it simply destroys all the tissues with which it comes into contact, probably by reacting with protein sulfhydryl groups (thiol), thus causing degradation of the membranes, disrutption of the root functions and even death to the cells and rapid necrosis if contact has been taken place through the leaves. One of the numerous functions of Zn in plants is to control the mechanism of the transformation cysteine-cystine (6,23) (cysteine <--> cystine), that is, the equilibrium between the thiol and the disulphide form. This is the regulating process of the redox phenomenon in cells which becomes inactive through lack of Zn. Therefore, if in the presence of As with its negative effect on the thiol groups, there is a deficieny of Zn which results in an inadequate or faculty regulatuon of the thiol-sulphide equilibrium, the result

TABLE 5.- Concentration of Zn (mg kg^{-1} of fresh material). Each value is the mean for four plants (four repetitions for sample).

ROOTS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	5.5	5.1	6.3
2 mg L^{-1}	2.9 a	3.1 a	3.0
5 mg L^{-1}	2.2 a	2.3 a	2.3 a
10 mg L^{-1}	2.3 a	2.2 a	1.8 a
	***	***	***
STEMS			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	3.9	3.8	13.9
2 mg L^{-1}	1.5 a	2.1	7.6
5 mg L^{-1}	1.0 a	0.9 a	2.6
10 mg L^{-1}	1.1 a	0.9 a	0.7
	***	***	***
LEAVES			
TREATMENT	1st Sample	2nd Sample	3rd Sample
Control	3.4	3.2 a	2.9 a
2 mg L^{-1}	2.9 a	2.8 a	2.8 a
5 mg L^{-1}	2.8 a	2.1 b	2.5 b
10 mg L^{-1}	2.9 a	2.3 b	2.5 b
	***	***	***
FRUIT			
TREATMENT	2nd Sample	3rd Sample	
Control	1.6 a	2.3 a	
2 mg L^{-1}	1.7 a	2.7 a	
5 mg L^{-1}	1.4 a	1.7	
10 mg L^{-1}	1.1	1.1	
	***	***	

NS, *, ** and *** stand for not significant or significant at P = 0.05, 0.01 and 0.001 respectively (ANOVA Test). In the same column, mean value with the same letter are significantly equal at P= 0.05 (Duncan's Test).

will be an extreme situation for this type of protein (the ones containing thiol groups).

Zinc carries out many different functions in vegetables, mainly due to its participation in the formation, constitution, and function with various enzyme systems (33,38). One such function, namely its intervention in the synthesis of tryptophan, precursor of the auxins (a natural phytohormone which regulates the growth of plants), has been demonstrated (12,18). A Zn deficiency results is a low auxin content in the growth meristems with malformation in the leaves which is seen in their lack of growth and reduced size. Also, a Zn deficiency results in histologic and morphologic changes that take place in the leaves resulting in effects which are both visible and very characteristic. Yellow spots are observed among the main veins which retain their green colour. The leaves are thick and form tiny rosettes. All these symptoms coincide with those shown by the plants observed in this study.

In the fruit, as occurs in stems and leaves, the micronutrient content decreases as the level of As in the culture solution increases, so that this fact can explain the reduced growth of the plants corresponding to the treatments with a greater As content as well as some of the symptoms, such as the curling of leaves and a marked foliar necrosis shown by various specimens.

CONCLUSIONS

At fruit ripening, there is a decrease in the absorption and transport of B, undoubtedly as a result of the disorders caused by As in the root at the level of 10 mg As/L. In the leaves, it has been observed that a situation of synergy exists at all the series of samples, that is, the level of B increases as the As content increases.

An antagonistic As/Cu effect was detected which is related to the similarity between P and As and a possible interchange of their metabolic functions.

An increase in the level of As results in an increase in the absorption of Fe which is related to the antagonism Fe/Cu, Fe/Mn, and Fe/Zn.

The toxic effects exhibited by the plant may result in the elaboration of different ion selection mechanisms which drastically reduced the global Mn content. The presence of As has a negative effect on the thiol groups, and when there is an add deficiency of Zn as there is an inadequate or faulty regulation of the

thiol-sulphide equilibrium, the result will be an extreme situation for this type of protein (containing thiol groups). The reduction in the Zn concentration (Zn is related to the synthesis of auxins) would explain, to some extent, the reduced growth of the plants with a high As content.

The level of As results in the collapse of the xylem vessels which affects the transport of water and this is evident from the withered state of the plant.

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