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Physiological and Mineralogical Properties of Arsenic-Induced Chlorosis in Barley Seedlings Grown Hydroponically

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ABSTRACT

The experiment was carried out to investigate the effects of arsenic (As) on the physiological and mineralogical properties of barley (*Hordeum vulgare* L. cv. 'Minorimugi'). The plants were grown in nutrient solution treated with 0, 6.7, 33.5, and 67 μM As (0, 0.5, 2.5, and 5 ppm As, respectively) in the phytotron. Dry matter yield of shoots and roots decreased significantly with the As treatments, indicating that barley plants are As-sensitive and As-toxicity depends on the As concentration in the rooting medium. Necrosis in older leaves and chlorosis symptoms (whitish color) in the fully developed young leaves were observed at the 33.5 and 67 μM As treatments. Arsenic concentration, accumulation, and translocation increased with the increase of As concentration in the rooting medium. Arsenic was mostly concentrated in roots and a little amount was moved to shoots, indicating that As was not easily translocated to shoots of barley seedlings. Concentrations and accumulations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), and copper (Cu) decreased significantly in shoots for 33.5 and 67 μM As treatments as compared to the 0 μM As treatment. Concentrations of P, K, Ca, Mg, Mn, and Cu decreased in roots, but Zn concentration increased in roots at 67 μM As treatment. Accumulations of P, K, Ca, Mg, Mn, Zn, and Cu in roots also decreased significantly at 67 μM As treatment. Accumulation of P and the cations showed negative relationship with As. Concentration of Fe decreased in shoots at 33.5 and 67 μM As treatments where chlorosis was induced in the young leaf but increased in roots at 33.5 and 67 μM As treatments. It was suggested that As

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might induce iron (Fe)-chlorosis in the plants. Among the micronutrients, Fe translocation was more affected than others by As. Phytosiderophore (PS) accumulation in roots, which is a symptom of Fe-deficiency in grasses, did not change significantly between 0 and 33.5 μM As treatments; indicating that As-induced chlorosis did not enhance PS accumulation in roots and decreased due to As-toxicity at 67 μM As treatment.

Keywords: accumulation, arsenic, barley, chlorophyll index, chlorosis symptom, concentration, phytosiderophore, translocation

INTRODUCTION

Arsenic (As), the enigmatic metalloid, is known as "bish" among the Bengali speaking people both in Bangladesh and West Bangle, India. It is present everywhere in the earth including soil, water, and air. At present, As pollution in soil is ubiquitous in Bangladesh. It is well known that As problem has become a matter of great concern in Bangladesh since late 1993. Arsenic is known to us as a phytotoxic agent (Stiles, 1958; Steevens et al., 1972) although some plants like one of the brake fern (*Pteris vittata* L.), that naturally grown on a site in Central Florida contaminated with chromated copper arsenate, can accumulate huge amounts (3280-4980 ppm) of As in fronds (Ma et al., 2001). Arsenic phytotoxicity largely depends on physicochemical and chemical properties of soils, like soil pH, concentration of soil phosphate (P), iron (Fe), aluminum (Al), the amount of organic matter, and the sensitivity of the crops. For sensitive species, 5 mg kg^{-1} soil extractable As is toxic, while 50 mg As kg^{-1} soil could reduce growth 50% of a less sensitive species (Lepp, 1981). Long-term impacts of As on agricultural yield and its phytotoxicity in soil and/or water are a major concern (Ali et al., 2003). Contamination of this toxic agent in groundwater has been reported from Bangladesh (Dhar et al., 1997; Nickson et al., 1998; Chowdhury et al., 1999), West Bangle, India (Mandal et al., 1996), China (Huang et al., 1992), and Taiwan (Smith et al., 1992). About 90% of Bangladeshi people use groundwater as drinking water (WHO, 2001). In some areas of Bangladesh, groundwater contains up to 2 mg As L^{-1} (Tondel et al., 1999). Inorganic As is the predominant form of As in soil (Johnson and Hiltbold, 1969) and in groundwater (Samanta et al., 1999). In aerobic and anaerobic soil conditions, arsenate and arsenite are the predominant species, respectively (Masscheleyn et al., 1991; Onken and Hossner, 1995). Arsenic uptake by several species of plants (Asher and Reay, 1979; Meharg et al., 1994) was investigated, but it is limited to food crops (Huq et al., 2001).

Graminaceous plants differ from other plants by their inability to utilize Fe, supplied as stable Fe^{3+}-chelates [e.g., ethylenediamine-di (o-hydroxyphenylacetic acid) (EDDHA)]. When graminaceous and dicotyledons plants are grown in calcareous soils containing low amounts of available Fe, the former type are often more effective than the latter in resisting Fe-deficiency

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chlorosis (Buchanan et al., 2000), suggesting that the graminaceous plants acquire Fe from the soil by different mechanism. Root washings of barley (*Hordeum vulgare* L.) roots contain an organic compound that can solubilize Fe^{3+} in the rhizosphere. In response to Fe-deficiency, wheat, oat, and rice roots exhibit a stimulated release of Fe^{3+}-chelating compounds (Phytosiderophores, PS). This response of graminaceous plants is specific to Fe-deficiency and the roots of dicotyledons and nongraminaceous plants do not release Fe-chelating organic compounds under Fe-deficiency (Marschner, 1995; Buchanan et al., 2000). The compounds are called as mugineic acid family and are effective Fe^{3+}-chelators (Kawai et al., 1988). They have been named as PS that are produced in roots and secreted into the rhizosphere of graminaceous plants, solubilize Fe^{3+} in soil and carry it into plant roots under Fe-deficient condition (Marschner, 1995; Buchanan et al., 2000). The physiological characteristics of PS have been investigated (Takagi, 1976; Takagi et al., 1984; Römheld and Marschner, 1990). It is known that, when manganese (Mn), zinc (Zn), and copper (Cu) are present in the medium at high concentrations, the metals may induce Fe-deficiency in plants (Mengel and Kirkby, 1987). However, little work has been done on As-induced chlorosis in graminaceous plants. To the best of our knowledge, there is no information related to As-induced chlorosis and PS formation in barley. The effects of As on physiological and mineralogical properties of barley grown in nutrient solution were investigated.

MATERIALS AND METHODS

Seed Germination and Plant Culture

Barley seeds (*Hordeum vulgare* L. cv. 'Minorimugi') were surface-sterilized with 2% (w/v) chlorinated lime [$Ca(OCl)_2$] for 45 minutes, rinsed with tap water continuously for 1 h, and soaked between moistened towels covered with wrapping paper at 25°C for 24 h. Germinated seeds were placed on a plastic net of the seed box containing 2 mM calcium chloride ($CaCl_2$) solution in the phytotron (8 a.m.–10 p.m., 14 h photoperiod, 280 μmol m^{-2} s^{-1} light intensity; and 17/10°C day/night temperatures). After seven days, the solution in the seed box was replaced by 1/5-strength modified Hoagland-Arnon solution (Hewitt and Smith, 1975) containing 4.0 μM Fe-ethylenediaminetetraacetic acid (EDTA). The full-strength modified Hoagland-Arnon solution contained 6.0 mM potassium nitrate (KNO_3); 4.0 mM calcium nitrate [$Ca(NO_3)_2$]; 1.0 mM ammonium phosphate ($NH_4H_2PO_4$); 2.0 mM magnesium sulfate ($MgSO_4$); 3 μM boric acid (H_3BO_3); 0.5 μM manganese sulfate ($MnSO_4$); 0.2 μM copper sulfate ($CuSO_4$); 0.4 μM zinc sulfate ($ZnSO_4$); 0.05 μM molybdic acid (H_2MoO_4); and 20.0 μM Fe-EDTA (Takagi, 1993). The plants were allowed to grow until the length of the second leaf was about 20% of that of the first leaf. The seedlings were then transplanted in bunches (3 plants were wrapped with sponge rubber)

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and 16 bunches were placed in one pot (10 liters) filled with ½-strength modified Hoagland-Arnon solution containing 10 μM Fe-EDTA. The plants were grown up to 21 DAT (days after treatment) and harvested when nutrient deficiencies symptom or inhibitory effects were the most apparent. The concentrations of As in the medium were 0, 6.7, 33.5, and 67 μM from sodium arsenite ($NaAsO_2$) (Kanto Chemical Company, Tokyo, Japan). The pH (6.5) of the solutions was monitored daily with digital pH meter and adjusted with 1 M hydrochloric acid (HCl) and/or 1 M sodium hydroxide (NaOH). The nutrient solutions were renewed every week, aerated throughout the experiment, and the solution level was maintained by adding deionized water.

SPAD Value

The chlorophyll content of fully developed (3rd leaf) new leaves on 21 DAT was measured using a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo, Japan), because the plants did not fully develop 4th leaf at the 67 μM As concentrations.

Analysis of Elements in Plant Samples

The plant samples were collected and washed with deionized water three times. Shoots and roots were separated and dried at 55°C for 48 h. For mineral nutrition, oven-dried samples were digested with a nitric acid-perchloric acid mixture (Piper, 1942) and analyzed. Arsenic was measured by Hydride Generation Technique (Hitachi HFS-3) and P was determined colorimetrically using a UV-visible Spectrophotometer (model UV mini 1240, Shimadzu Company, Kyoto, Japan) at 420 nm wavelengths after developing the yellow color with vanadomolybdate as described by Barton (1948) and Jackson (1958). Amount of potassium (K), calcium (Ca), magnesium (Mg), Fe, manganese (Mn), zinc (Zn), and copper (Cu) were determined by atomic absorption spectroscopy (AAS).

Measurement of PS Accumulated in Roots

Three bunches of plants from each treatment were collected in the morning (8:30 a.m.) to assay for PS accumulation in roots. The roots were carefully washed with deionized water and kept in the refrigerator (−20°C). The plants were lyophilized, separated into shoots and roots, and the weight was measured. Lyophilized roots were homogenized with a mortar and pestle in 10 mL ethanol (80%, v/v) making volume 50 mL. The root extract was filtered and concentrated by evaporation at 55°C under vacuum. The compounds extracted from roots were dissolved in MQ water (18.2 MΩ cm^{-1}), purified by Milli-RO 60 (Millipore Corporation, USA), introduced to an Amberlite IR-120B cation exchange

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resin column and the resin was washed with deionized water. The cationic fraction absorbed to the resign was eluted with 100 mL 1 M ammonium hydroxide (NH_4OH), concentrated under vacuum and assayed for PS (Kawai et al., 1993). Total PS content in roots was measured by the Fe solubilizing assay of Takagi (1976).

Collection and Measurement of PS Released by the Roots

The roots of a bunch of plants were soaked in beakers containing 500 mL deionized water for 3 h starting from 8 a.m. on 21 DAT. The plants were transferred to the respective pots after collection of root washings. Approximately 10–15 mg thymol (Kanto Chemical Company, Tokyo, Japan) was added to each beaker for preventing microbial degradation of PS. The root washings were introduced to an Amberlite IR-120B cation exchange resin similarly to the procedure for the determination of PS accumulation and the released amount of PS was measured.

Experimental Design

The experiment was a completely randomized block design with 3 replications. Data were analyzed by analysis of variance (SAS, 1988).

Calculation for the Parameters

Concentration = mg or μg of element g^{-1} dw (dry weight); accumulation in shoot = mg or μg of element $plant^{-1}$ shoot; accumulation in root = mg or μg of element $plant^{-1}$ root; and translocation % = nutrient accumulation in shoot $\div$ total accumulation (shoot + root) $\times$ 100.

Reagents

All chemicals used were of analytical reagent grade. All solutions were prepared previously with MQ water. Stock solution of As was prepared by dissolving $NaAsO_2$ in MQ water and was kept at room temperature in acid washed reagent bottle.

RESULTS AND DISCUSSION

Visible Symptoms

The plants grown under 33.5 and 67 μM As produced severe leaf tip necrosis (the older leaves were turned yellow, fell off, and finally died). Arsenic-toxicity

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in plants is characterized by leaf wilting followed by necrosis of leaf tips and margins (Machlis, 1941). The youngest leaves failed to unfold, leaf size (both length and width) were reduced drastically. Interveinal chlorosis at 6.7 μM As and whitish chlorosis at 33.5 and 67 μM As treatments were observed in the fully developed young leaf. Shaibur et al. (2006) reported that As-toxicity produced whitish chlorosis symptom in the young leaf of hydroponic rice seedlings after 14 d of As treatments at 13.4 and 26.8 μM levels. Wilting of younger leaves was a typical symptom of As-toxicity. Besides wilting, premature senescence of leaves occurred. With the severe toxic treatment (67 μM As), curling of the younger leaves followed by shriveling occurred. The most common symptom, however, was growth reduction. Root length was reduced significantly at 67 μM As treatment. Arsenic-toxicity resulted in poor root growth (root length) and roots were discolored (reddish) and felt 'slippery' to the touch, perhaps due to deterioration of the middle lamella by As. It is known that radicular membrane shows severe symptoms of As-toxicity (Sachs and Michael, 1971) because it reacts with sulfhydryl groups of proteins (Speer, 1973), leading to root function disruption (Orwick et al., 1976) and finally cellular death. Root shortness due to As-toxicity is referred to as "little root."

Dry Matter Yield

Inorganic As had negative effect on shoot and root growth. The highest shoot dry matter yield was recorded at 0 μM As and the lowest was at 67 μM As treatment (Figure 1). Arsenic concentration in nutrient solution was accountable for 19, 69, and 72% shoot dry matter reduction at the treatments of 6.7, 33.5, and 67 μM As, respectively, but the results for roots were 8, 33, and 60%, respectively. These results indicated that barley shoots were more sensitive to As than roots and As-toxicity depends on the concentration of As in the rooting medium. The

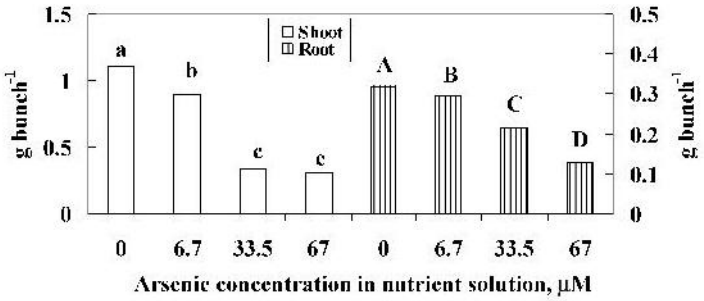

Figure 1. Dry matter yield of shoots and roots of barley seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

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reductions of total dry matter yield were 16, 61, and 69% for 6.7, 33.5, and 67 μM As, respectively. Results indicated that barley plants were As-sensitive and even 6.7 μM As (0.5 ppm) could be the toxic level in the hydroponic culture. Abedin et al. (2002) found considerable reduction in straw and root biomass due to 4 and 8 mg As L^{-1} in a pot experiment in the greenhouse. Growth of bean (*Phaseolus vulgaris* L.) as represented by roots, stem plus branches, leaves, and fruit dry weights, was significantly affected by $NaAsO_2$ (Carbonell-Barrachina et al., 1997a). Biomass of shoots and roots was reduced in rice at 0.8 and 1.6 mg As (dimethylarsinic acid = DMAA) L^{-1} (Marin et al., 1993), but Onken and Hossner (1995) reported a contrasting result in rice. Marin et al. (1992) found that the root biomass decreased significantly at 0.2 and 0.8 mg $MMAAL^{-1}$ (monomethylarsonicacid) in 'Lemont' and 'Mercury' cultivars of rice. However, in the same experiment they also found that a very low concentration of As (0.05 mg DMAA L^{-1}) could increase root growth of the cultivar.

SPAD Value

Chlorophyll content (fully developed 3rd leaf, average of 5 points of a same leaf) decreased significantly at 33.5 and 67 μM As treatments as compared to 0 and 6.7 μM As treatments. The lowest value was recorded at 67 μM As treatment (Figure 2). Chlorosis in the fully developed youngest leaves at 33.5 and 67 μM As levels suggested that As hindered chlorophyll formation. It has already been reported that As could reduced SPAD value significantly in the young leaf hydroponic rice seedlings at 6.7, 13.4, and 26.8 μM levels (Shaibur et al., 2006).

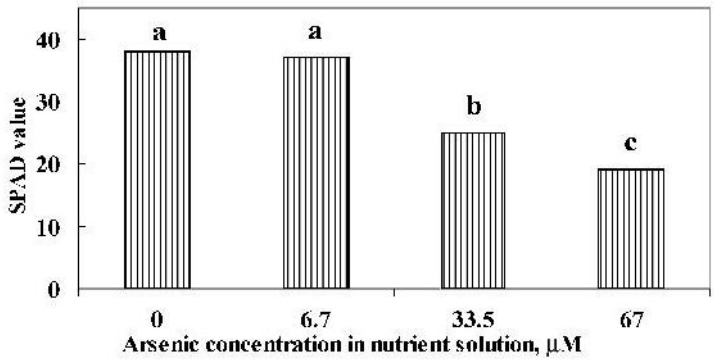

Figure 2. SPAD value measuring as chlorophyll content in fully developed young leaves (3rd) of barley seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

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Arsenic Concentration, Accumulation, and Translocation

Concentrations and accumulations of As were increased significantly both in shoot and root by As treatments as compared to the control (Figures 3I and 3II), indicating that the bioavailability of As depends on the concentration of As in the rooting medium and the higher the concentration the higher the bioavailability. It was found that the As concentrations in roots were 253, 64, and 21 times greater than the shoots of 6.7, 33.5, and 67 μM As treated plants, respectively, but the values of accumulation were 87, 50, and 10 times greater in the same treatments. There was a trend that As concentration in roots was higher than that of shoots in barley. In this experiment, As translocation increased with the increase of As concentration in the nutrient solution. At 6.7 μM As treatment, only 1.10% of absorbed As was translocated from roots to shoots, indicating As could not be translocated easily and mostly be concentrated in roots. At 33.5 and 67 μM As treatments, the values were 2.6 and 9.4%, respectively. Shaibur et al. (2006) reported that As concentration increased in shoot and root of rice seedlings with the increasing As in the rooting medium and roots contained almost 8–16 fold higher As concentration than the shoots. Abedin et al. (2002) found, As concentration in all plant parts of rice plants increased with the

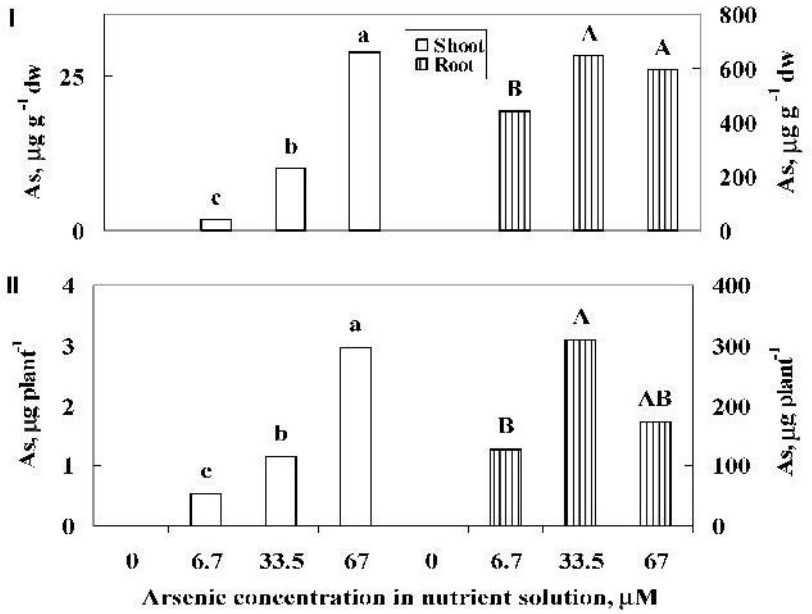

Figure 3. Effect of As on As concentration (I) and accumulation (II) in shoots and roots of barley seedlings grown in nutrient solution. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

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increase of arsenate concentration of the irrigation water. Concentrations of As in roots were greater than the other parts (Abedin et al., 2002) but Ma et al. (2001) found the As concentration in frond of break fern (*Pteris vittata* L.) was almost 24 times greater than the roots. This may be explained by the fact that fern is an As-hyperaccumulator but rice and barley are As sensitive plants.

Phosphorus Concentration, Accumulation, and Translocation

In shoots, P concentration increased significantly at 6.7 μM As treatment but decreased at 33.5 and 67 μM As treatments as compared to 0 μM As treatment (Figure 4I). Our result is in agreement with the result of Wallace et al. (1980). Reduction of P concentrations may be due to the fact that the arsenate is taken up by the phosphate transport system (Asher and Reay, 1979; Meharg and Macnair, 1990) and decreasing P concentration and accumulation. Since As has chemical similarity to P, it is likely to take part in many cellular reactions (Lepp, 1981). Arsenic can substitute for P in plant and the plant might respond as if they were suffered from P-deficiency. Principle functions of P in plants are related to energy transfer and protein metabolism (Mengel and Kirkby, 1987). It is known that As inhibits respiration by blocking the electron transport chain of mitochondria or uncoupling of oxidative phosphorylation (Siegel and Sisler, 1977). Arsenic may hinder these metabolic activities and ultimately decrease the growth. Carbonell-Barrachina et al. (1997b) found that As increased P concentration in stem and leaves of bean plant at harvesting stage which may be due to pH of the solution and varietal differences. Our data showed that 33.5 and 67 μM As treatments decreased P concentration in roots and the lowest value was obtained at 67 μM As treatment (Figure 4I). The P accumulation both in shoots and roots decreased significantly at 33.5 and 67 μM As treatments and the lowest was at 67 μM As treatment (Table 1). Translocation was also negatively affected by As, probably due to competition between the elements (Table 2).

Potassium Concentration, Accumulation, and Translocation

In shoots, K concentration decreased at 33.5 and 67 μM As treatments. In roots, the significant reduction was also obtained at 33.5 and 67 μM As treatments (Figure 4II), which is in agreement with the result of Wallace et al. (1980), who showed that depression of K concentration was taken place in roots of bush bean plants due to As in the nutrient solution. Antagonism between K^+ and Ca^{2+} is well known (Mengel and Kirkby, 1987). But in this experiment we found that both K and Ca concentration decreased in presence of As. Accumulation decreased significantly both in shoots and roots at 33.5 and 67 μM As treatments and it was more severe for the highest As concentration in the solution (Table 1). In shoots, the highest (50.66 mg plant^{-1}) and the lowest (6.53 mg plant^{-1})

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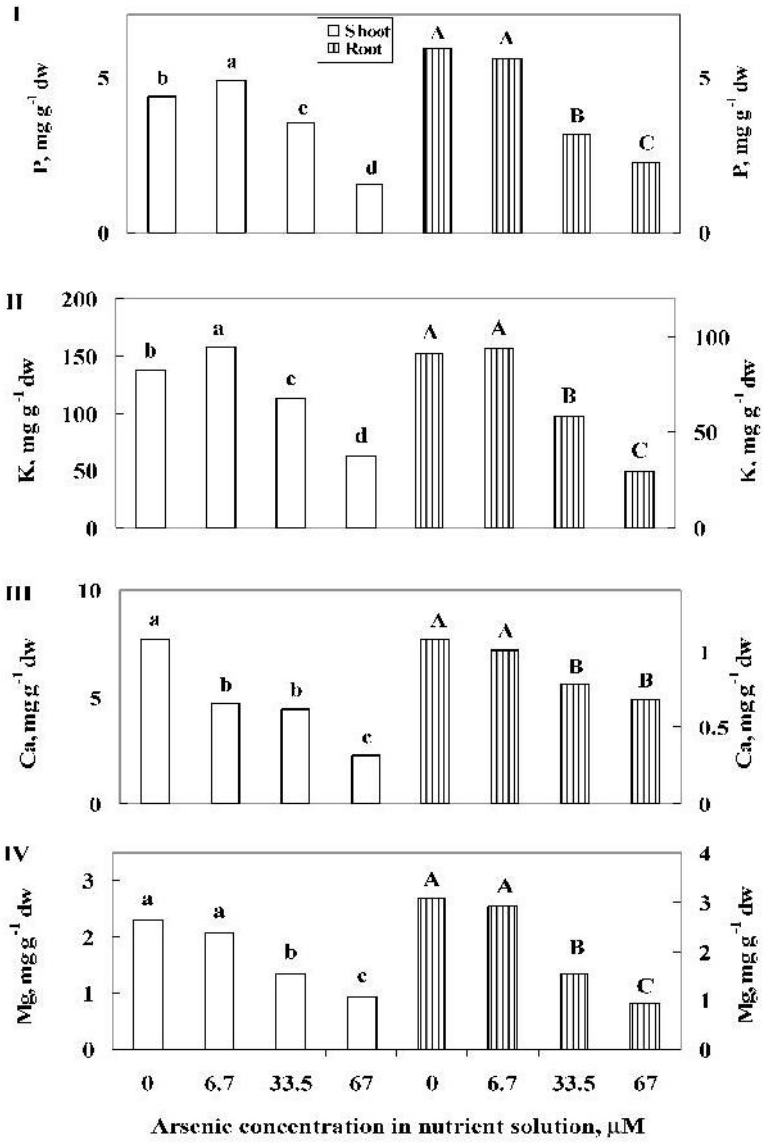

Figure 4. Effect of As on P (I), K (II), Ca (III), and Mg (IV) concentrations in shoots and roots of barley seedlings. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

accumulation was obtained at 0 and 67 μM As treatments, respectively. Similar trends were also found in roots at the same treatments and the values were 9.72 mg plant^{-1} and 1.26 mg plant^{-1}, respectively. Potassium translocation was not markedly affected by the As (Table 2).

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Table 1
Accumulation of nutrients in shoots and roots of barley seedlings grown in nutrient solution with different levels of As

	mg plant^{-1}				μg plant^{-1}			
Treatment	P	K	Ca	Mg	Fe	Mn	Zn	Cu
				Accumulation in shoots				
0 μM	1.60 a	50.66 a	2.84 a	0.85 a	23.52 a	5.46 a	6.17 a	1.50 a
6.7 μM	1.47 a	47.07 a	1.39 b	0.62 b	18.01 b	4.95 a	5.73 b	1.52 a
33.5 μM	0.40 b	12.84 b	0.50 c	0.15 c	4.75 c	1.41 b	1.62 c	0.32 b
67 μM	0.16 c	6.35 c	0.23 d	0.09 d	3.13 d	0.49 c	1.36 c	0.17 c
				Accumulation in roots				
0 μM	0.62 A	9.72 A	0.11 A	0.33 A	19.01 B	1.16 A	1.50 A	0.76 A
6.7 μM	0.57 A	9.25 A	0.10 A	0.29 B	19.76 B	1.03 B	1.50 A	0.71 B
33.5 μM	0.25 B	4.16 B	0.06 B	0.11 C	27.39 A	0.98 B	1.34 A	0.57 C
67 μM	0.12 C	1.26 C	0.03 C	0.04 D	17.51 B	0.36 C	0.85 B	0.20 D

[a]Means followed by different letters in each column are significantly different ($p = 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

Calcium Concentration, Accumulation, and Translocation

Calcium concentration decreased significantly with the increase of As concentration and the lowest value was obtained at 67 μM As treatment (Figure 4III). Decrease in the plant transpiration (Carbonell-Barrachina et al., 1997b) might cause a reduction in the upward transport of Ca, and, therefore, Ca concentration might be decreased in the shoots. Wallace et al. (1980) also showed that depression of Ca concentration in leaves and roots of bush bean plants was due to As-toxicity. Mono and disodium methanoarsonate (MSMA and DSMA) could increase Ca concentration linearly in shoots of canola plants (Cox, 1995).

Table 2
Translocation (%) of elements from roots to shoots in barley seedlings grown in nutrient solution with different levels of As

Treatment	As	P	K	Ca	Mg	Mn	Zn	Cu
0 μM	0	72 a	84 a	96 a	72 a	83 a	80 a	66 a
6.7 μM	1.11 c	72 a	84 a	93 b	68 a	83 a	80 a	68 a
33.5 μM	2.61 b	62 b	76 b	90 c	58 b	59 b	55 b	36 c
67 μM	9.41 a	59 b	83 a	89 c	71 a	57 b	62 b	47 b

[a]Means followed by different letters in each column are significantly different ($p = 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

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In roots, the lowest Ca concentration was observed at 67 μM As treatment. Accumulation of Ca was also negatively influenced by As and the highest (2.84 mg plant^{-1} shoot) and the lowest values (0.23 mg plant^{-1} shoot) were at 0 and 67 μM As treatments, respectively (Table 1). Accumulation of Ca reduced almost 12 times in shoots and 3.67 times in roots by 67 μM As treatment. Translocation of Ca was not much affected by As and the values were 96, 93, 90, and 89% for 0, 6.7, 33.5, and 67 μM As treatments, respectively (Table 2). In this experiment, the concentration and accumulation of Ca decreased at the highest As concentration, indicating that As hindered absorption of Ca.

Magnesium Concentration, Accumulation, and Translocation

The effect of As on shoot Mg concentration was as profound as that of P, K, and Ca. Concentration of Mg decreased significantly at 33.5 and 67 μM As treatments (Figure 4IV). Carbonell-Barrachina et al. (1997b) showed the opposite result in stems at harvesting stage when sodium arsenite was applied at 5 mg As L^{-1} (67 μM As) to bean plants. In roots, the concentration of Mg decreased significantly at 33.5 and 67 μM As treatments and the lowest value was obtained at 67 μM As treatment (Figure 4IV). Accumulation was also depressed significantly both in shoots and roots by As and the lowest value was obtained at the highest As treatment (Table 1). For translocation, the lowest value was recorded at 33.5 μM As treatment; but, when the concentration of As was the highest, translocation was remained similar to the control plants (Table 2).

Iron Concentration, Accumulation, and Translocation

Iron concentration in shoots of 6.7 μM As treatment was almost similar to the control plants but decreased significantly at 33.5 and 67 μM As treatments (Figure 5I). Our result was not in agreement with the result of Wallace et al. (1980) who found that Fe concentration increased in shoots of bush bean plants at pH 4.5 due to the increase of As concentration (10^{-4} M) in the nutrient solution. Carbonell-Barrachina et al. (1998) found that As treatments seemed to statistically increase Fe concentration and decrease Fe uptake in leaves of bean. Marin et al. (1993) found almost no change of Fe concentration in shoots when DMAA was used in nutrient solution for rice. These dissimilarities were most probably due to concentration effect, plant species, and the methodology of those experiments. It was determined that Fe concentration in barley roots increased at 33.5 and 67 μM As treatments. This may be due to concentration effect because dry matter yield decreased with the As treatments (Figure 1). Wallace et al. (1980) and Carbonell-Barrachina et al. (1994) also found the similar results in bush bean and tomato (*Lycopersicum esculentum* Mill) plants, respectively. Accumulation of Fe in shoots decreased significantly due to the

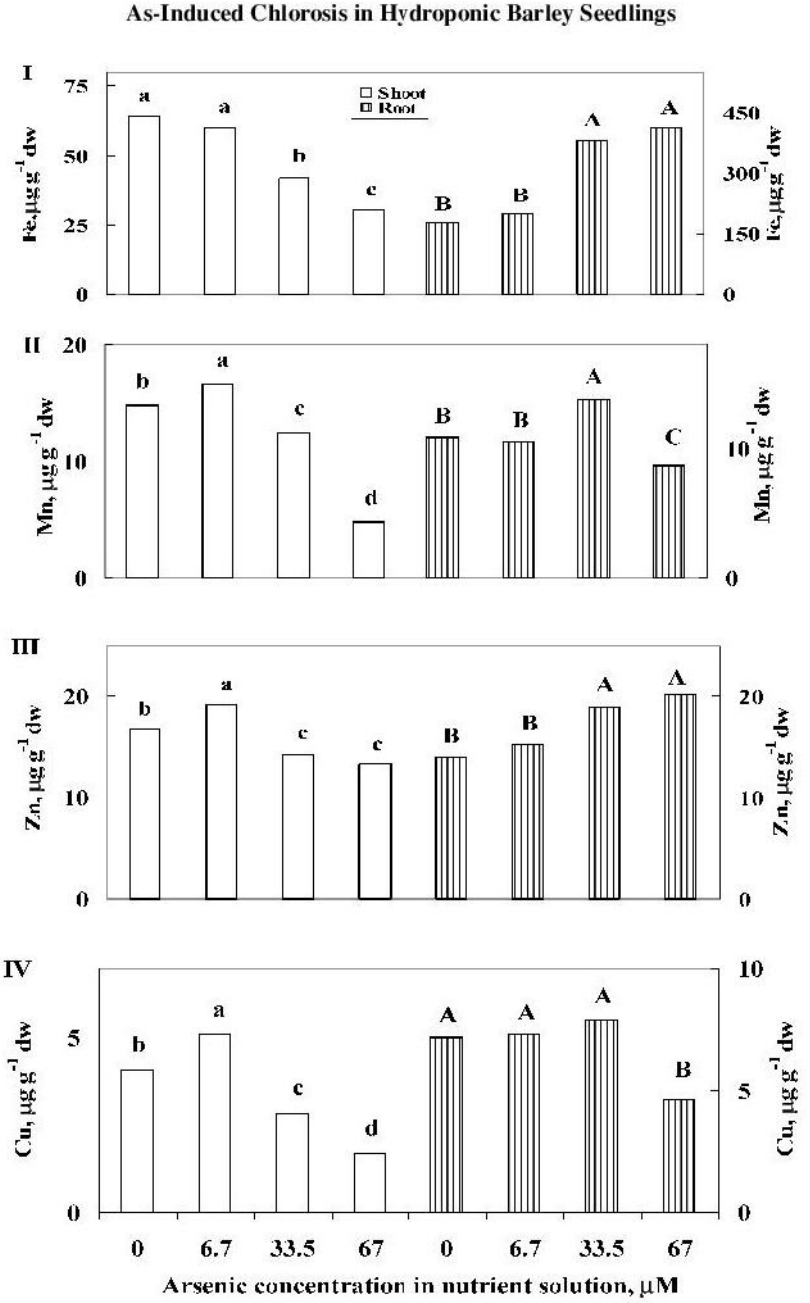

Figure 5. Effects of As on Fe (I), Mn (II), Zn (III), and Cu (IV) concentrations in shoots and roots of barley seedlings. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

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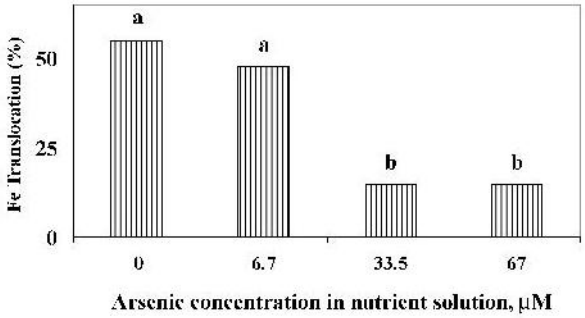

Figure 6. Translocation of Fe from roots to shoots in barley seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

increase of As concentration in the nutrient solution (Table 1), but accumulation of Fe did not decrease in roots. This indicated that As hindered Fe translocation to the shoots and accordingly translocation was decreased at 33.5 and 67 μM As treatments (Figure 6). This result was in agreement with the result of Carbonell-Barrachina et al. (1994) who found that the translocation of Fe, Cu, Mn, and Zn from root to the aerial parts decreased in tomato plant.

Iron and Mg deficiencies are somewhat similar in visual symptoms, because, both are characterized by a failure in chlorophyll production. However, Fe-deficiency, unlike Mg-deficiency always begins in the younger leaves (Mengel and Kirkby, 2001). The younger leaves of barley in our experimental showed chlorosis at 33.5 and 67 μM As treatments. It is known that the older leaves were first affected in Mg-deficiency (Maynard, 1979). In this respect, this syndrome observed in our experiment differed from that of Mg-deficiency. In our experiment, interveinal chlorosis was observed in the plant of 6.7 μM As treatment and whitish chlorosis was in the plants of 33.5 and 67 μM As treatments, indicating the chlorosis was probably due to Fe-deficiency induced by As. In leaf, 30-50 μg Fe g^{-1} dw is considered as the Fe-deficient level (Bergmann, 1988). In the treatment of 33.5 and 67 μM As, the plants contained 42 and 30 μg Fe g^{-1} dw indicating Fe-deficiency in the plants.

Manganese Concentration, Accumulation, and Translocation

Manganese concentration in shoots increased significantly for the 6.7 μM As but decreased at 33.5 and 67 μM As treatments (Figure 5II). In roots, Mn concentration increased at 33.5 μM As but decreased abruptly at 67 μM As treatment. Significant reduction of Mn concentration in shoots and roots was primarily due to toxic effect of As. Our result was in agreement with the result of Wallace et al. (1980) and Carbonell-Barrachina et al. (1998) who found that

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As at high concentration depressed Mn concentrations in leaves and roots of bean plants. Accumulation of Mn decreased according to the increase of As in the nutrient solution (Table 1). It was found that translocation of Mn from roots to shoots decreased at 33.5 and 67 μM As treatments (Table 2).

Zinc Concentration, Accumulation, and Translocation

Concentrations of Zn increased significantly in shoots at 6.7 μM As treatment and decreased at 33.5 and 67 μM As treatments as compared to other treatments (Figure 5III), indicating that As has the similar effect on Zn and Fe at 33.5 and 67 μM As treatments. For most plant species, Zn concentration in leaves below 10 to 15 μg g^{-1} dw are indicative of Zn-deficiency and concentrations in the range of 20 to 100 μg g^{-1} dw are considered to be sufficient level (Boehle and Lindsay, 1969). The data were within the deficient level which was most probably due to As-toxicity. It is well known that P could induce Zn-deficiency (Marschner and Schropp, 1977). Because of the similarity of As and P, As may substitute for P in many metabolic processes (Lepp, 1981; Wauchope, 1983). The characteristics of As may explain the fact that high concentration of As resulted in low Zn concentration in shoots as compared to the control plants. In roots, the concentration increased significantly at 33.5 and 67 μM As treatments (Figure 5III), this may be concentration effect because accumulation of Zn decreased by As treatments (Table 1). Zinc accumulation decreased significantly in shoots with the increase of As in the nutrient solution and the lowest values in shoots and roots were obtained at 67 μM As treatment (Table 1). Translocation was also negatively influenced by As (Table 2). It was suggested that As could reduce not only Zn concentration but also Zn accumulation and translocation from roots to shoots in barley.

Copper Concentration, Accumulation, and Translocation

The concentrations of Cu in shoots decreased significantly at 33.5 and 67 μM As treatments. In roots, the concentration was almost similar at 0, 6.7, and 33.5 μM As treatments, but decreased at 67 μM As in the nutrient solution (Figure 5IV). Accumulation of Cu in shoots was negatively influenced by the As (Table 1). Similar tendency was obtained in roots and the lowest accumulation was at 67 μM As treatment (Table 1). Translocation was also significantly decreased at 33.5 and 67 μM As treatments (Table 2).

Considerable reduction of Cu concentrations both in shoots and roots were obtained at 67 μM As treatment. This may be due to the fact that As hindered the absorption of Cu by roots and subsequent translocation from roots to shoots. Our result was not in agreement with the result of Wallace et al. (1980) who found that, due to increase of As concentration in the nutrient solution, Cu concentration increased in all plant parts of bush bean, though they noted that

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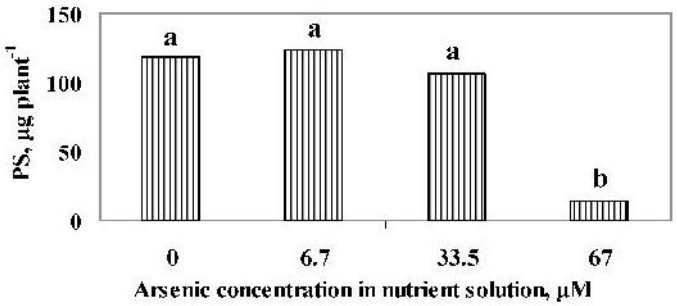

Figure 7. The phytosiderophores (PS) accumulation in roots of barley seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

this might be due to concentration effect. The other probable causes may be the difference of the plant species and the pH of the culture solution. Carbonell-Barrachina et al. (1998) found that Cu concentrations in roots and leaves of bean increased significantly in As-stressed plants as compared to the control plants. They found that Cu uptake was significantly decreased, but at the same time an increase in their concentrations was observed, suggesting concentration effect.

Accumulation and Release of Phytosiderophores

Total amount of PS accumulated in roots before the PS release time decreased due to As-toxicity at 67 μM treatment as compared to other treatments (Figure 7). The present study showed that the plants of 0, 6.7, and 33.5 μM As treatments accumulated PS in roots. The plants of the 33.5 μM As treatment showed chlorosis symptoms (white in color in the young leaves) and the chlorophyll content was lower than the plants of 0 and 6.7 μM As treatments. However, the PS accumulation did not change as compared to 0 and 6.7 μM As treatments. The Fe concentration in leaves (30-50 μg g^{-1} dw) was within the range of critical deficiency level of Fe (Bergmann, 1988) (Figure 5I). It is inferred that this Fe-deficiency symptom might be induced by As, but PS formation was not enhanced in roots probably due to As-toxicity. Phytosiderophores production at 67 μM As treatment might be reduced due to As-toxicity. We did not find any release of PS on 21 DAT in this experiment. This may be logical because the experiment was conducted in +Fe condition. It is known that PS release of the plant grown under +Fe condition is very small or none (Yoshida et al., 2004).

CONCLUSION

Results showed that As-induced necrosis in the older leaf and whitish chlorosis symptom in the younger leaf at 33.5 and 67 μM As treatments, suggesting that

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As might induce Fe-chlorosis in barley. Iron concentrations of shoots of 33.5 and 67 μM As treatments were within the range of the critical deficiency level. Arsenic concentration increased in shoots and roots with the increase of As concentration in the nutrient solution and As tended to accumulate in roots of barley. Arsenic reduced the concentration of P, K, Ca, Mg, Fe, Mn, Zn, and Cu in shoots at 33.5 and 67 μM As treatments. In general, accumulations in shoots and translocations of the elements reduced by the As treatments. It is known that heavy metals described above can induce Fe-deficiency. In our experiment, chlorotic symptom was observed despite low concentrations of Mn, Zn, and Cu at 33.5 and 67 μM As treatments. Therefore, the chlorotic symptom may not be 'heavy metal-induced Fe-deficiency.' The accumulation of PS in the roots of As-induced Fe-deficient barley was not enhanced, rather decreased significantly at 67 μM As treatment as compared to the other treatments.

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