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Critical Toxicity Level of Arsenic and Elemental Composition of Arsenic-Induced Chlorosis in Hydroponic Sorghum

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Abstract Physiological and mineralogical responses of sorghum (*Sorghum bicolor* L. cv. Fast sorghum) in hydroponic culture at elevated concentrations of arsenic (As) were evaluated. Seedlings were grown in the presence of 0, 6.7, 33.5 and 67 μM As levels (0, 0.5, 2.5 and 5 mg As l^{-1}) up to 14 days after treatments (DAT). Shoot and root dry matter yield were repressed by higher As levels. At low As level (6.7 μM) shoot dry matter yield was enhanced by 2.3% but at 33.5 and 67 μM As levels, the yield decreased by 52 and 79%, respectively. The root growth was similarly enhanced (8%) at the lower As level while the growth decrement at the higher As levels were 33 and 68%, respectively for the two treatments. Considering 10% dry weight (DW) reduction, the critical toxicity level of As was calculated to be 11.7 μg g^{-1} DW for shoot and 367 μg g^{-1} DW for root, indicating that shoot was more sensitive to As-toxicity than root. A whitish chlorotic symptom was observed in the fully developed young leaves at the 67 μM As level. The lowest chlorophyll content was also observed at this As level. Arsenic concentration increased both in shoot and in root with increase in solution As concentration. The concentrations of As and Fe were about 16, 28 and 17 times; and 2, 25 and 144 times higher in root than shoot at 6.7, 33.5 and 67 μM As levels, respectively. The concentrations of K, Fe and Cu were significantly lower while Ca, Mg and Mn concentrations were higher in the shoot at the 67 μM As level compared to the control plants. On the other hand, Fe, Mn, Zn and Cu concentrations were higher in root at the 67 μM As level. In the shoot, accumulation and translocation of metal micronutrients, particularly that of Fe, decreased significantly because of the presence of As. The present observations suggested that As might induce a toxic effect on sorghum by hampering the translocation of the metal micronutrients. It is suggested that "As-induced Fe-

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deficiency" caused chlorotic symptoms in the hydroponically grown sorghum.

Keywords Arsenic · Chlorosis · Hydroponic · Iron · Sorghum · Translocation

1 Introduction

The toxic metalloid arsenic (As) is a class Va semimetal that occurs in the environment including plants in both organic (monomethylarsonic acid, MMAA and dimethyl arsenic acid, DMAA) and inorganic (arsenate [As^{5+}] and arsenite [As^{3+}]) forms (Artus 2006). It has been listed as the number-one hazardous substance by the US Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov/cxcx3.html). Arsenic has achieved great notoriety because of the toxic effect of some of its compounds (Kesselring 1948; O'Neill 1995). Its position is 20th in terms of abundance in the earth's crust, 14th in the seawater and 12th in the human body (Woolson 1975; Mandal and Suzuki 2002). Arsenic contamination of drinking water is considered as a global problem (Ahmed et al. 2006) and the As calamity in Bangladesh can be described as the largest known mass poisoning in the history (Rabbani et al. 2002).

In terms of extent of severity and the risk to human exposure, Bangladesh is the most As impacted area (Smith et al. 2000). In some places the concentration of As in groundwater may be up to 2 mg l^{-1} (Tondel et al. 1999). Contamination by As is a problem not only in groundwater but also in soil. The background concentration of As in Bangladesh soil is generally below 10 mg kg^{-1} but it may reach 80 mg l^{-1} in places where contaminated water is used for irrigation and it has been estimated that there is a build up of As in soil to the tune of 5 kg ha^{-1} $year^{-1}$ (Huq et al. 2003).

It has been shown by many authors that when rice is grown with As contaminated water or on As contaminated soils most of the As is accumulated in the roots and straws with a smaller fraction transferred to the grains (Abedin et al. 2002; Imamul Huq et al. 2007). Edible parts of leafy vegetables have been reported to concentrate higher amounts of As compared to the fruit vegetables (brinjal, tomato and chilly; Farid et al. 2003). Imamul Huq et al. (2006) reported that many vegetables, particularly leafy vegetables, Japanese Mustard spinach (*Brassica rapa* L. var. pervirdis; Shaibur et al. 2007) concentrates high contents of As in leaf tissues without showing any visible toxicity symptom. Necrosis in the old leaves and chlorosis in the fully developed young leaves in hydroponic rice (Shaibur et al. 2006) and barley (*Hordeum vulgare* L. cv. Minorimugi) (Shaibur et al. 2008) are the symptoms of As toxicity. Sorghum is a plant that is often irrigated and thus could be exposed to As by As impacted groundwater. Sorghum is an arable crop grown under aerobic soil conditions where arsenate will be the predominant form of As. On the other hand, As in groundwater is about 50% in the arsenite form (Samanta et al. 1999). So far, no or very little information is available on the response of sorghum to As in its growth medium. As such, the present experiment was conducted to evaluate the response of sorghum in hydroponic culture to elevated As concentrations applied as arsenite.

2 Materials and Methods

2.1 Plant Culture

Seeds of sorghum (*Sorghum bicolor* L. cv. Fast sorghum; Sakata Seed Corporation, Yokohama, Japan) were surface sterilized with 2% (*w*/*v*) chlorinated lime [$Ca(OCl)_2$] for 30 min to eliminate pest contamination, rinsed with tap water continuously for 1 h. The sterilized seeds were allowed to germinate in the greenhouse on steel tray filled up with perlite. The seeds germinated after 4 days. Modified Hoagland–Arnon nutrient solution was supplied at 1/5-strength concentration to the germinated seedlings. Seedlings were grown for up to 14 days and at the third leaf stage the seedlings were transplanted to 1/2-strength nutrient solution with As treatments. The full-strength modified Hoagland–Arnon solution contained 6.0 mM KNO_3; 4.0 mM $Ca(NO_3)_2$; 1.0 mM $NH_4H_2PO_4$; 2.0 mM $MgSO_4$; 20.0 μM Fe^{3+}–EDTA (Fe^{3+}–ethylene diamine tetraacetic acid, sodium salt, trihydrate); 3 μM H_3BO_3; 0.5 μM $MnSO_4$; 0.2 μM $CuSO_4$; 0.4 μM $ZnSO_4$ and 0.05 μM H_2MoO_4 (Takagi 1993).

Seedlings were transplanted to plastic buckets (capacity 10 l) containing 1/2-strength modified solution on the 14th day of germination. Each bucket contained eight seedlings (plants were wrapped with sponge rubber) containing 10 μM Fe^{3+}–EDTA. On the fourth day after treatments (DAT), an additional

5 ml of 20 mM Fe^{3+}–EDTA was added to the media. However, on the seventh DAT the concentration of Fe was 20 μM with all the treatments when the solution was renewed. The plants were allowed to grow for 14 DAT with As treatments at the rates of 0, 6.7, 33.5 and 67 μM applied as sodium meta-arsenite ($NaAsO_2$; Kanto Chemical Company, Tokyo, Japan). The pH (6.5) of the solutions monitored daily with digital pH meter (Horiba Korea, Seoul, Korea) was adjusted as and when required with 1 M HCl or 1 M NaOH. The solution was renewed after 7 days and was aerated throughout the experiment. All solutions were prepared in deionized water. The day-night temperature of the greenhouse was 30 and 10°C, respectively. The experiment was arranged in randomized blocks with three replications.

2.2 Chlorophyll Index (SPAD-value)

The chlorophyll index based on SPAD-value of fully developed (fifth leaves) flag leaves at 14 DAT was measured using a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo, Japan). The fifth leaves were taken because these leaves were the fully developed youngest leaves of the chlorotic plants. In each leaf, the average of the SPAD-values was calculated. Average of the data of three plants was calculated.

2.3 Analysis of Plant Samples

The collected seedlings were washed with deionized water three times. Shoot and root were separated and dried at 55°C for 48 h. The ground oven dried samples were digested with a nitric acid-perchloric acid mixture (Piper 1942). Amount of K, Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectroscopy (Loeppert and Inskeep 2001). Phosphorus was determined colorimetrically using a UV-visible spectrophotometer (model UV mini 1240, Shimadzu Corporation, Kyoto, Japan) by the method of Barton (1948) and Jackson (1958). Arsenic in the samples was determined by hydride generation AAS (Hitachi HFS-3).

2.4 Calculation for the Parameters

Concentration is defined as the amount of element per gram of samples in dry weight (DW) basis (mg or μg of the element per gram DW), while uptake/accumulation refers to the total amount of element per plant shoot or per plant root (mg or μg of element per plant).

2.5 Statistical Analysis

Data were subjected to analysis of variance. Differences between means were evaluated using a Ryan–Einot–Gabriel–Welsch multiple range test ($p \leq 0.05$; SAS 1988) using computer origin 5 at Iwate University, Morioka, Japan.

3 Results and Discussion

3.1 Visible As-Toxicity Symptoms in Sorghum

Arsenic-toxicity impaired shoot height and root length. The growth at the 67 μM As level was the least. The terminology ‘little shoot and little root” are being used for shoot and root shortness, respectively, caused by As-toxicity. Similar visible symptoms were observed in hydroponic rice grown at 13.4 and 26.8 μM As levels (Shaibur et al. 2006). No distinct differences of visible symptoms between the shoots grown at 0 and 6.7 μM As levels were observed.

Seedlings at 67 μM As level were stunted with necrosis in the tips of old leaves and whitish chlorotic symptom in the fully developed young leaves. These were the most conspicuous visible symptoms in As-stressed sorghum seedlings. Chlorotic symptom was not as pronounced either for the 6.7 or 33.5 μM As levels. The leaves curled in sunlight at the beginning of 33.5 and 67 μM As treatments.

Root length was not affected at the 0, 6.7 and 33.5 μM As exposures but root length was limited at the 67 μM As level and the roots felt “slippery” to the touch. At the 33.5 and 67 μM As exposures root growth was retarded, roots became shorter and thinner and particularly the lateral root formation was depressed. The color of the root at the 6.7 μM As level was slightly reddish as compared to control plants. The intensity of the reddish color however, increased with increasing As levels in the growth medium.

At night and at morning, the seedlings in the 0 and 6.7 μM As exposures showed dew like water drops in the leaf tip, which, however, was absent for the

67 μM As treated plants. This symptom indicated that As-toxicity might limit the upward water movement in the plant which in turn, might decrease the upward movement of nutrient elements. It has been reported that As-toxicity produced water deficit symptom of rice in the day time (Shaibur et al. 2006) and earlier root coloring to yellowish brown or brown (Yamane 1989).

3.2 Dry Matter Yield

Both shoot and root growths were reduced when the roots were exposed to As-toxicity (Fig. 1). The severity increased with the increase of As concentration in the medium. Abedin et al. (2002) observed significant reduction in straw and root biomass of rice with the increasing arsenate concentration in irrigation water. Yamane (1989) reported that As-toxicity suppressed the development of rice root. In this experiment, As was responsible for 52 and 79% growth reduction in shoot at the 33.5 and 67 μM As levels, respectively; while the values were 33 and 68% for root, respectively, indicating that the shoot was more sensitive to As-toxicity than the root of sorghum. Reduction of shoot growth by As-toxicity was also reported by Tsutsumi (1980). Carbonell-Barrachina et al. (1998) reported that the growth of root, stem, leaf and fruit of greenhouse hydroponic bean plant decreased at 26.8 μM As (2 ppm) and 67 μM As exposures (5 ppm As). However, Onken and Hossner (1995) reported that growth of rice increased on Beaumont soil (fine, montmorillonite, thermic Entic Pelludert) treated with 5 mg As kg^{-1} as sodium arsenite or sodium arsenate. Polynomial two order growth curves of sorghum to As-toxicity are presented in Fig. 2a and b. The critical toxicity levels of As in shoot and in root of hydroponic sorghum seedlings were calculated from the growth curve considering 10% DW reduction (Ohki 1984). The highest value was 1337.5 mg in shoot for 6.7 μM As treated plants and we considered that the dry weight was constant between 0 and 6.7 μM As treatments and the plant growth was not decreased at 6.7 μM As treatment. Therefore, the control data were not included for the calculation of polynomial two order growth curves (Fig. 2a and b). The calculated critical toxicity level of As in shoot was 11.7 μg As g^{-1} DW but the value in the root was 367 μg As g^{-1} DW. The critical toxicity level of As in sorghum may has not been determined yet.

Previously, we reported the data of As-stressed rice (Shaibur et al. 2006) and barley (Shaibur et al. 2008), but the critical toxicity levels of As of those plants have not been reported. Based on the reported data, the calculated critical toxic levels of As in rice were 21.0 μg As g^{-1} DW in shoot and 325 μg As g^{-1} DW in root. In barley, the calculated critical levels were 1.20 μg As g^{-1} DW in shoot and 75.3 μg As g^{-1} DW in root that could reduce 10% DW. These calculated values may aid in the determination of the critical toxicity levels of other elements.

3.3 Chlorophyll Index (SPAD-value)

No change of SPAD-value for the fifth leaves was found for plants grown at 0, 6.7 and 33.5 μM As exposures, but the value decreased sharply for the 67 μM As exposure (Fig. 3). This result demonstrated that As exhibits a concentration dependent inhibitory effect on chlorophyll synthesis. It is well established

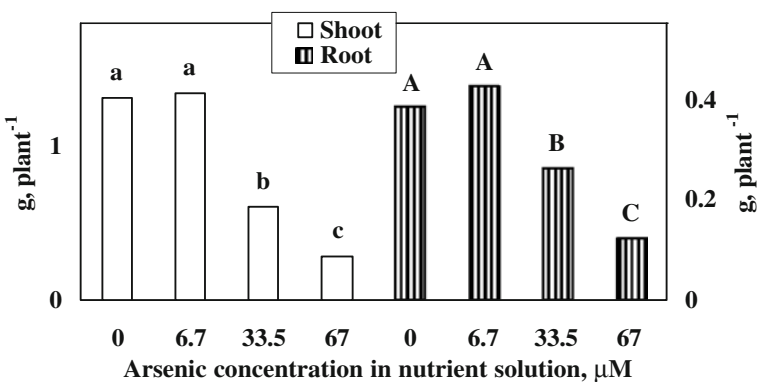

Fig. 1 Dry matter yield of sorghum seedlings grown with different levels of As. *Bars* with different letters are significantly different ($p<0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test

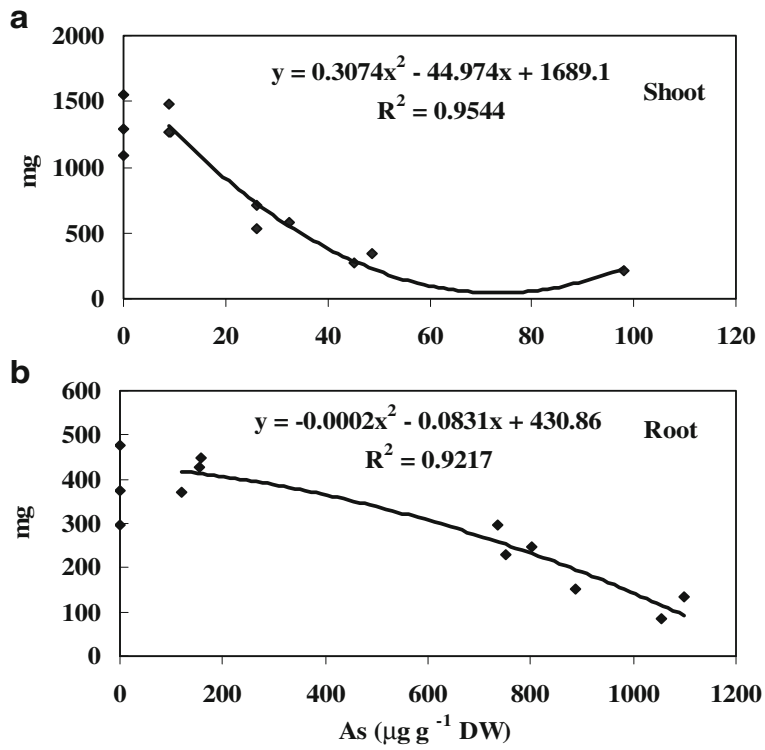

Fig. 2 Two order polynomial growth curve (**a**) shoot and (**b**) root of sorghum seedlings with different As concentrations in plant tissues

that Fe plays a vital role for the formation of chlorophyll in plant leaves (Marschner 1998). In this experiment, Fe concentration decreased in shoot with increasing As concentrations in the medium (Fig. 4a). It is reported that the plants show Fe-chlorosis when the leaves contain 30–50 µg Fe g^{-1} DW (Bergmann 1988). In the present experiment, the Fe concentrations in the shoot of 0, 6.7 and 33.5 µM As exposed plants were over those values (Fig. 4a). However, the seedlings at 67 µM As level contained Fe concentration almost similar to the critical deficiency level, resulting in whitish chlorotic symptoms in the young leaves. The Fe concentration in shoot of 67 µM As treated plants includes stem, old and young leaves but the whitish chlorotic symptom was observed only in the young leaves. It is thus suggested that the As-induced chlorosis in young leaves was Fe-chlorosis in sorghum (Mengel and Kirkby 2001). Similar observations have been made with hydroponic rice (Shaibur et al. 2006) and barley too (Shaibur et al. 2008).

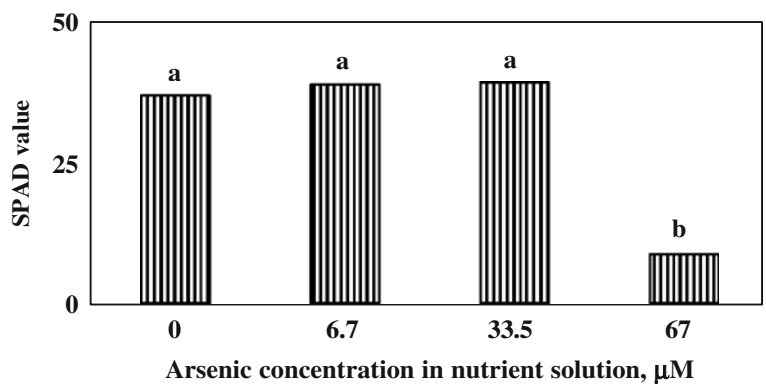

Fig. 3 SPAD-value in fully developed young leaves (fifth) of sorghum seedlings with different levels of As. Bars with different letters are significantly different ($p<0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test

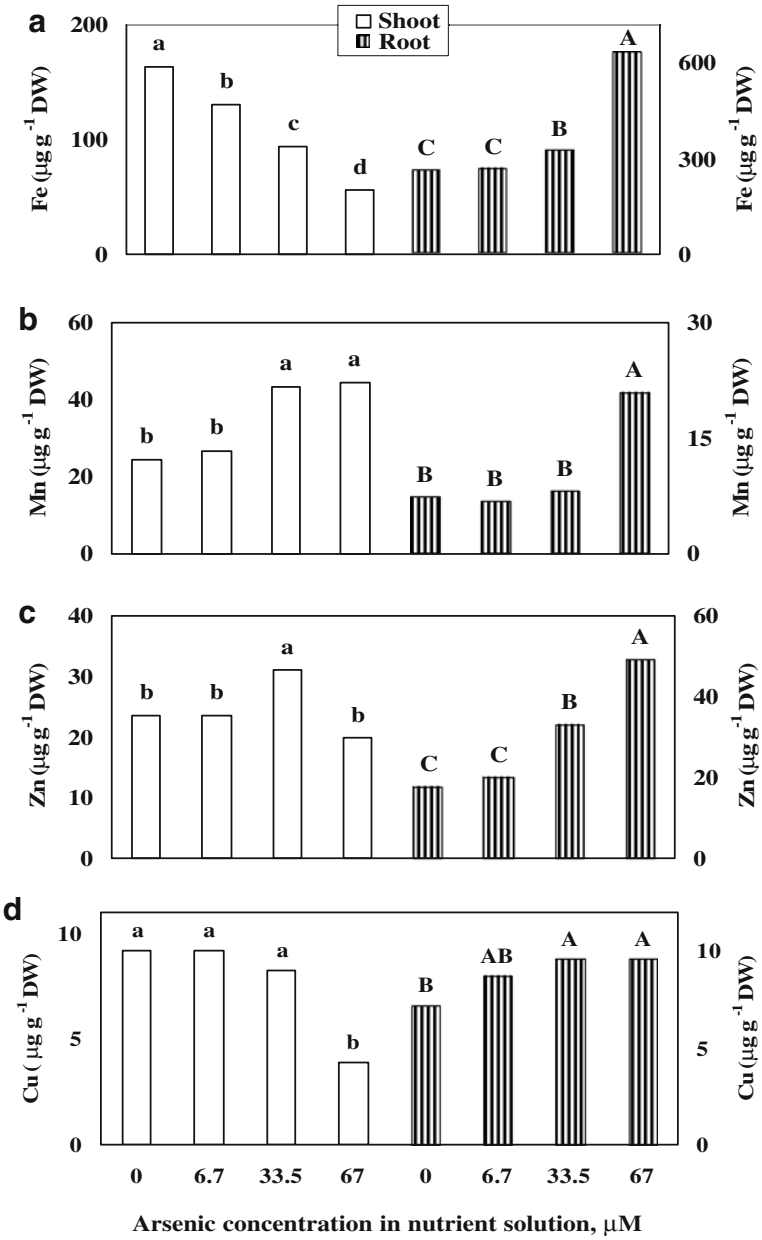

Fig. 4 Effect of As on the concentration of (**a**) Fe, (**b**) Mn, (**c**) Zn and (**d**) Cu in shoots and roots of sorghum seedlings. Bars with different letters are significantly different ($p<0.05$) according to a Ryan-Einot–Gabriel-Welsch multiple range test

3.4 Effect of As on As Concentration, Accumulation and Translocation

Arsenic concentration increased both in shoot and in root with increasing As concentration in the medium (Fig. 5a). Arsenic concentration followed the trend of root > shoot. The concentration of As in the root was almost 16, 28 and 17 times higher than that of shoot for the 6.7, 33.5 and 67 μM As treatments, respectively. This phenomenon is similar to rice (Shaibur et al. 2006; Imamul Huq et al. 2007) and barley (Shaibur et al. 2008). Phytoremediating plants however show the reverse phenomena (Ma et al. 2001). Arsenic concentration and uptake in both shoot and root was found to have promptly increased in rice when As was fed in the form of DMAA (organic As) (Marin et al. 1993). Arsenic is not easily translocated when As is absorbed in chemical forms other than DMAA (Liebig 1966; Frans et al. 1988; Marin et al. 1992). As a corollary to this, it could be assumed that in plants like

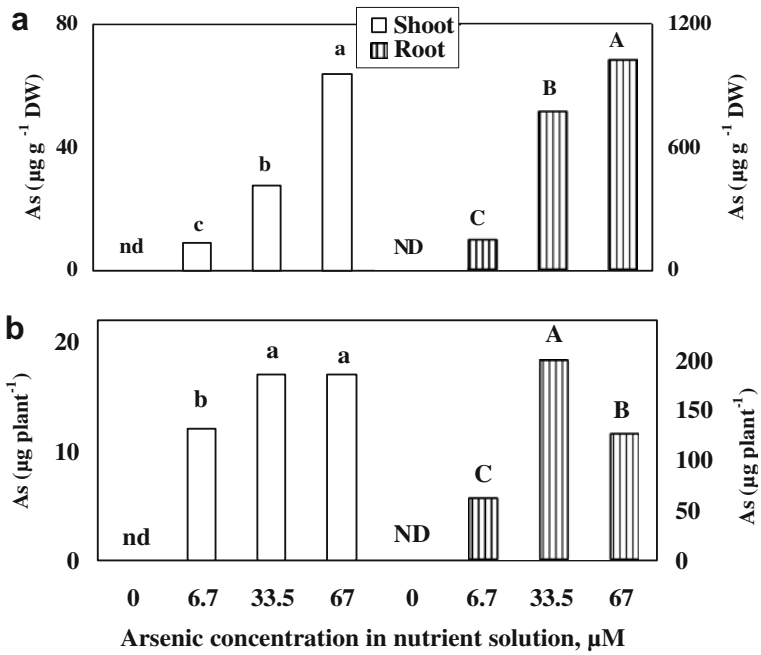

Fig. 5 Effect of As on the (**a**) concentration and (**b**) accumulation of As in shoots and roots of sorghum seedlings. Bars with different letters are significantly different ($p<0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test. *nd* not detected

sorghum, rice or barley, the transformation of inorganic As to organic forms is rather slow compared to what happens in the identified phytoremediators.

In the present case inorganic $NaAsO_2$ was applied and the concentration in root was much higher than that in shoot. Arsenic anions (arsenite and arsenate) may rapidly adsorb to the root surface and lead to intense high As concentration in the hydroponic culture (Wauchope 1983). Another reason to increase As concentration in root could be the Fe-plaque of root. Iron plaque is the coating of Fe-hydroxides/oxides, commonly formed on the root of rice (*Oryza sativa* L.) by the oxidation of root Fe by released oxygen and oxidants into the rhizosphere (Armstrong 1967; Chen et al. 1980). Arsenic may be interfering in the root cells by two mechanisms: Firstly, part of arsenite may be oxidized to arsenate in the root rhizosphere and co-precipitate with Fe^{3+} (Otte et al. 1991). Siderophores by microbes or phytosiderophores exuded by root may complex with Fe^{3+} at the root-plaque interface and mobilize Fe^{3+}-bound arsenate, taken up through phosphate co-transporters (Liu et al. 2005). This may stimulate uptake of Fe and arsenate on the root surface and may increase As and Fe concentration in arsenite treated plants. In the present experiment, the media was aerated, therefore, some arsenite might have been converted to arsenate and might have increased the concentration and accelerated accumulation processes. Secondly, arsenite may be accumulated on the Fe-plaque in the form of $H_3AsO_3^0$ and then be transported into the root via aquaporins (Liu et al. 2005).

Arsenic accumulation was higher in root and lower in shoot (Fig. 5b), this may be due to the fact that Fe-plaque can act as a barrier to the uptake of toxic metals (Batty et al. 2000; Chen et al. 2005) on the roots. Accumulation was lower in the 67 µM As treated plants as compared to the 33.5 µM As treated ones, which may be due to the fact that root growth was the least in the 67 µM As treated plants (Fig. 1). Accumulation in roots was 5, 13 and 7 times higher than that of shoot (Fig. 5b) for the 6.7, 33.5 and 67 µM As treatments, respectively.

The translocation of As was 17, 8 and 13% in plants exposed to 6.7, 33.5 and 67 µM As, respectively (Fig. 6). It has already been reported that As (arsenite) translocation from root to shoot is limited by its high toxicity to root membranes (Sachs and Michael 1971). Liu et al. (2005) concluded that the main barrier to uptake and translocation of As, fed in the form of arsenite, might be the root tissue rather than Fe-plaque. Our finding was not in agreement with the findings of Carbonell-Barrachina et al. (1997) who found that about half of the absorbed

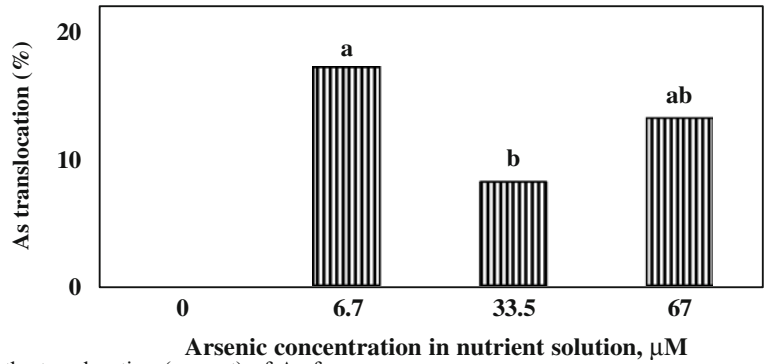

Fig. 6 Effect of As on the translocation (percent) of As from roots to shoots of sorghum seedlings. *Bars with different letters* are significantly different ($p<0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test. Translocation refers to the ratio of accumulation of element in shoot to the total accumulation (shoot + root). The translocation is expressed in percent

As was translocated to the aerial parts of bean plants when $NaAsO_2$ was applied to the medium. The sensitivity of plants to As-toxicity may be reduced by the ability of the plants to reject As (i.e. not adsorb it) or to avoid translocation of As to the sensitive parts.

3.5 Effect of As on Macroelements Concentration, Accumulation and Translocation

Phosphorus concentration increased significantly in shoot for the 6.7 and 33.5 μM As treatments as compared to the control plants (Fig. 7a). In the case of 33.5 μM As treatment, the phenomenon could be a concentration effect because the accumulation decreased (Table 1). It is known that P competes with As in the absorption by root. Generally P concentrations in common plants are 2–4 mg g^{-1} DW (Liao et al. 2004) or 3–4 mg g^{-1} DW (Mengel and Kirkby 2001) during the vegetative growth stage. The P concentrations in the present experimental plants were a little higher (Fig. 7a). Arsenic-toxicity did not reduce P translocation (%), suggesting that absorbed As may not compete with the P anions on the loading site of xylem tube in sorghum seedlings. The polynomial two order relationship between P and As concentration in plant tissues was significantly related with $R^2=0.9134$ for shoot and $R^2=0.5833$ for root (Fig. 8a and b), although the effect of As on P concentration in plant did not appear to be much affected.

The concentration of K decreased both in shoot and root at the 67 μM As level (Fig. 7b). Similar results for rice have also been reported (Shaibur et al. 2006). The reduction of K concentration was not a dilution effect as the growth was not enhanced or rather decreased due to As-toxicity (Fig. 1). The K concentration of shoot for the control plant was almost 61.0 mg g^{-1} DW. It has been reported that K concentration in shoots of hydroponic rice was 44.1 mg g^{-1} DW (Shaibur et al. 2006) and 60.2 mg g^{-1} DW in hydroponic barley (Alam et al. 2001) for control plants. Accumulation of K also decreased with the increasing As concentration (Table 1). Arsenic did not affect the translocation of K much (Table 2). It appeared that there was little or no antagonistic relationship between As and K.

Calcium concentration in shoot increased with increasing As concentration in the medium and the highest value was found for the 67 μM As treatment (Fig. 7c). This is a concentration effect of As-toxicity on sorghum seedlings, because the accumulation decreased (Table 1). Calcium concentration in root was not much affected by the As. This finding is not in agreement with our previous findings with hydroponic rice (Shaibur et al. 2006), but in agreement with the findings of Yamane (1989) for rice.

Magnesium concentrations were similar both in shoot and in root at the 0 and 6.7 μM As levels. It, however, increased in the shoot and decreased in the root at the 33.5 and 67 μM As levels (Fig. 7d). The increase of Mg concentration was most probably due to concentration effect as the growth was repressed by the higher As concentrations in the medium. Previously, it was observed that Mg concentration decreased significantly in shoot and root of hydroponic rice seedlings at the 26.8 μM As level (Shaibur et al. 2006). Yamane (1989) also reported a similar result in rice. In this experiment, Mg accumulation

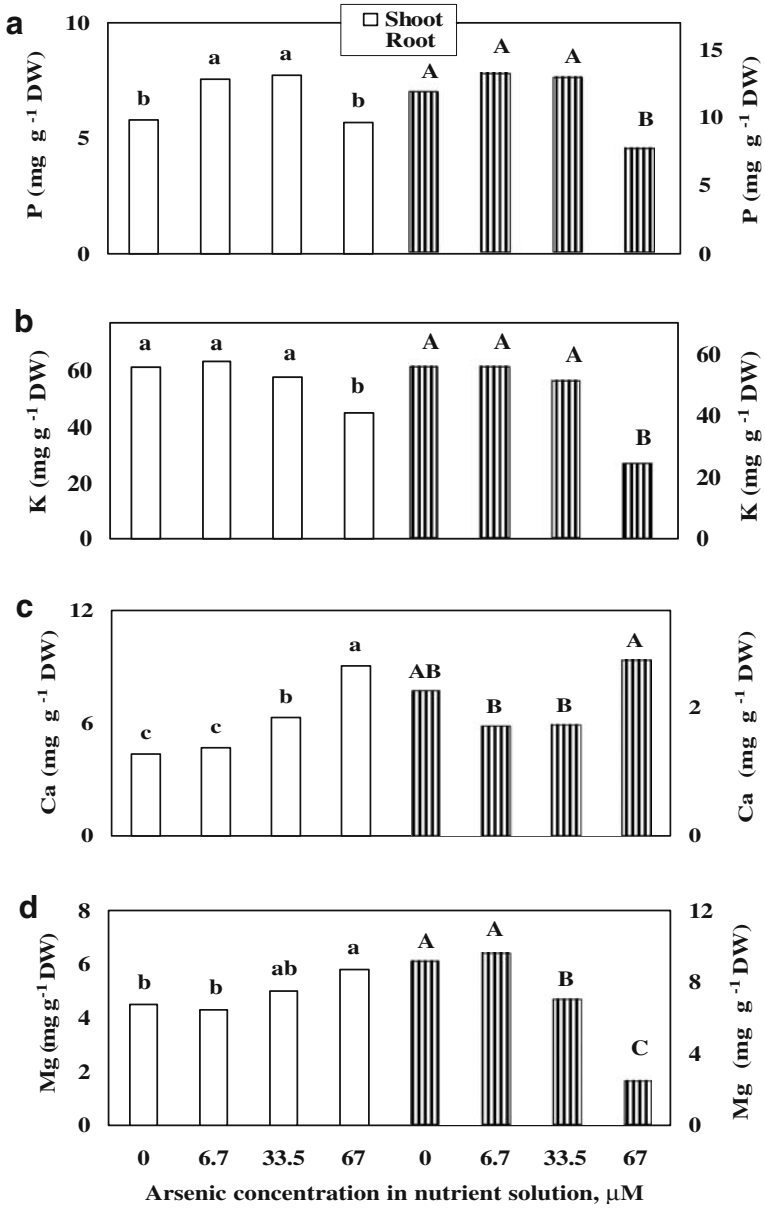

Fig. 7 Effect of As on the concentration of (**a**) P, (**b**) K, (**c**) Ca and (**d**) Mg in shoots and roots of sorghum seedlings. *Bars with different letters* are significantly different ($p<0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test

was reduced in shoot and root by the As-toxicity at the 33.5 and 67 μM As levels (Table 1). Arsenic-toxicity enhanced Mg-translocation largely in the plants treated with 67 μM As (Table 2). On the other hand, Mg translocation was significantly reduced by As-toxicity in hydroponic rice (Shaibur et al. 2006). In leaf tissues, the threshold value for the occurrence of Mg-deficiency symptoms is in the region of about 2 mg g^{-1} DW (Mengel and Kirkby 2001). The test plants contained 4.5, 4.3, 5.0 and 5.8 mg Mg g^{-1} DW in the plants at 0, 6.7, 33.5 and 67 μM As levels, respectively, indicating the fact that Mg was not in the critical deficiency level. This further substantiates the earlier statement that the chlorosis was induced due to Fe-deficiency.

3.6 Effect of As on Microelements Concentration, Accumulation and Translocation

Iron concentration decreased in shoot and increased in root with increasing As concentration in the medium (Fig. 4a). In shoots, the decrease in Fe concentration

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

Treatment (μM As)	Milligrams per plant				Micrograms per plant			
	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Accumulation in shoot								
0	7.58 a	79.7 a	5.72 a	5.86 a	215 a	32.5 a	30.5 a	12.1 a
6.7	10.1 a	84.9 a	6.28 a	5.74 a	175 b	36.0 a	31.6 a	12.3 a
33.5	4.66 b	34.7 b	3.82 b	3.01 b	57.1 c	26.2 b	18.5 b	5.01 b
67	1.49 c	12.6 c	2.52 c	1.62 c	15.4 d	12.5 c	5.61 c	1.07 c
Accumulation in root								
0	4.42 A	21.2 A	0.825 A	3.49 A	97.2 A	2.67 A	6.71 B	2.67 B
6.7	5.44 A	23.3 A	0.695 B	3.56 A	109 A	2.74 A	8.33 A	3.60 A
33.5	3.28 B	13.2 B	0.438 C	1.76 B	82.3 B	2.04 B	8.43 A	2.47 B
67	0.97 C	3.01C	0.325 D	0.30 C	77.4 B	2.59 A	5.86 B	1.13 C

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

was in the order of 20, 43 and 66% for the 6.7, 33.5 and 67 μM As treatments, respectively. The increase of Fe contents in the roots was in the order of 2, 25 and 144% for the same treatments, respectively. Seedlings contained 56 μg Fe g^{-1} DW in shoot when treated with 67 μM As which was a little higher than the critical deficient levels of Fe in leaves (30–50 μg g^{-1} DW; Bergmann 1988). However, it was also reported that the Fe concentration below 65 μg g^{-1} DW in shoot is inadequate for chlorophyll synthesis and subsequent plant growth (Tang et al. 1990). The experimental plants at 67 μM As level showed whitish chlorotic symptom in the fully developed young leaves. It needs to be mentioned here that the values for Fe include the

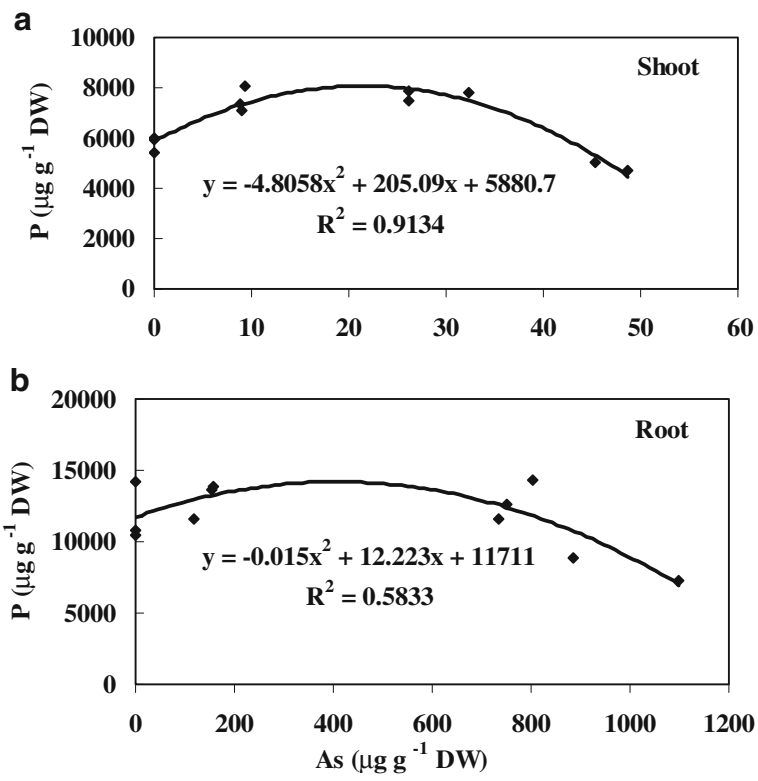

Fig. 8 Polynomial two order correlation between As and P (**a**) in shoot and (**b**) in root varies with the As treatments in sorghum seedlings

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

Treatment (μM As)	P	K	Ca	Mg	Fe	Mn	Zn	Cu
0	63 a	79 a	87 a	63 b	69 a	92 a	82 a	82 a
6.7	65 a	78 a	90 a	62 b	62 b	93 a	79 a	77 a
33.5	59 a	72 b	90 a	63 b	41 c	93 a	69 b	67 b
67	62 a	81 a	89 a	85 a	17 d	83 b	49 c	48 c

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

total iron concentration; the active Fe (Fe $^{2+}$) however has not been estimated. To correlate the degree of chlorosis with As induced Fe-deficiency, the estimation of the active Fe in tissue is a criterion which demands further study.

Iron-chlorosis is somewhat similar to Mg-chlorosis, but Mg-chlorosis is first found in the old leaf (Maynard 1979) and Fe-chlorosis is found in the young leaf (Mengel and Kirkby 2001). Yamane (1989) reported that Fe contents increased in the As treated rice. In the present experiment, the accumulation of Fe in shoot was much more depressed by As than that occurred in root (Table 1). Among the microelements, Fe translocation was the most affected (Table 2), suggesting further that the whitish chlorotic symptom induced by As was most probably due to Fe-translocation problem. It was also suggested that As may induce Fe-chlorosis in hydroponic rice (Shaibur et al. 2006) and barley (Shaibur et al. 2008). It has been found in the present case that Fe and As concentration in root was higher (Figs. 4a and 5a) than in the shoots. Our observations suggest that As might have induced Fe-deficiency in sorghum at the 67 μM As level. The highest Fe and As concentrations were also found in

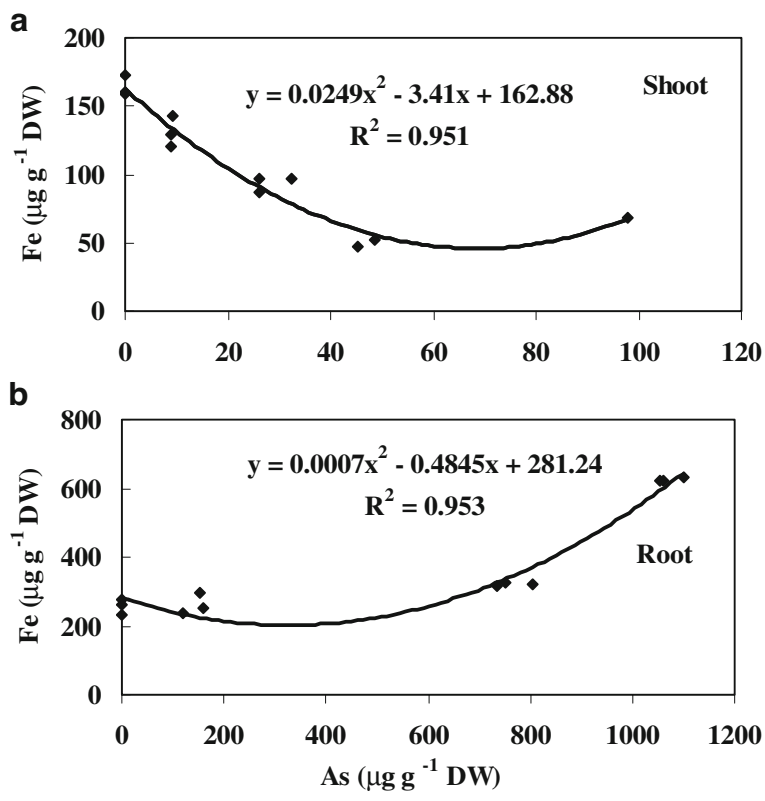

Fig. 9 Polynomial two order correlation between As and Fe (**a**) in shoot and (**b**) in root varies with As treatments in sorghum seedlings

the root of *Aster tripolium* in As polluted salt marshes soil (Otte et al. 1991). Yamane (1989) reported that 90% of the total As was accumulated in the root and more than half of it co-precipitate with Fe at the exterior of the epithelial cells of the root.

The two order polynomial relationship between Fe and As concentration was correlated with values for R^2 for shoot and root at 0.951 and 0.953, respectively (Fig. 9a and b). The relationships were exactly opposite for the two plant parts. The relationships further suggested that As might play a role in the uptake and translocation of Fe in sorghum at elevated As concentration. Similar relationships have also been reported by Porter and Peterson (1977) and De Koe et al. (1988).

Manganese concentration increased in shoot with increasing As concentration in the medium (Fig. 4b). The highest concentration of Mn was also found in the roots at the 67 μM As level (Fig. 4b). This increase of Mn concentration was most probably due to concentration effect as the growth decreased for the higher As concentrations. The critical deficiency level of Mn in plants are similar for most plant species and is in the range of 10 to 20 μg Mn g^{-1} DW of mature leaves (Marschner 1998; Mengel and Kirkby 2001). Edwards and Asher (1982) cited the critical toxic levels as 200 μg Mn g^{-1} DW in maize and 5,300 μg Mn g^{-1} DW in sunflower. Based on the criteria, it seemed that As did not induce Mn-toxicity in the present case and Mn was probably not involved for the induction of Fe chlorosis. Accumulation of Mn (Table 1) in shoot and translocation (Table 2) from root to shoot decreased at the highest level of As treatment.

Arsenic did not affect Zn concentration in shoots markedly but increased it in roots (Fig. 4c). The Zn concentration was around 20–30 μg g^{-1} DW in shoot; and 20–50 μg g^{-1} DW in root. The shoots of this experimental plants contained normal concentration of Zn usually found in plant tissues (raging from 19.8 to 30.6 μg Zn g^{-1} DW). The concentration of Zn in shoots (almost 20 μg Zn g^{-1} DW) for the 67 μM As treated plants was greater than the critical level for Zn-deficiency and was within the normal levels. For most plant species, Zn concentrations in leaves below 10 to 15 μg Zn g^{-1} DW are indicative of Zn-deficiency and concentrations in the range of 20–100 μg Zn g^{-1} DW are sufficient (Boehle and Lindsay 1969). Generally, concentrations in the range around 150 to 200 μg Zn g^{-1} DW of plant tissues are considered as toxic (Sauerbeck 1982). Our experimental result suggested that the chlorosis in the fully developed young leaves was not due to Zn-toxicity either. Translocation (%) of Zn from root to shoot decreased significantly and the lowest translocation was found at the 67 μM As level (Table 2), indicating that As suppressed Zn translocation.

Copper concentration showed a significant decrease in the shoot of the 67 μM As treated plants. On the other hand, there has been a gradual increase of the element in the roots with increasing As treatments (Fig. 4d). Accumulation was also negatively affected in shoot though it was not affected much in root (Table 1). Translocation (%) was affected negatively and the lowest translocation (%) was found at the 67 μM As level (Table 2). The critical deficiency level of Cu in vegetative plant parts is generally in the range of 1–5 μg Cu g^{-1} DW depending on the plant species, plant organ, developmental stage and nitrogen supply (Thiel and Finck 1973; Robson and Reuter 1981). For most crop species, the critical toxicity level of Cu in the leaves is 20–30 μg Cu g^{-1} DW (von Hodenberg and Finck 1975; Robson and Reuter 1981). Copper concentration in shoot was within the critical deficient level at the 67 μM As level (Fig. 4d). In this experiment, the Cu concentration in shoot was normal or within the critical deficient level, suggesting that the chlorosis symptom induced at 67 μM As level might not be due to Cu-toxicity.

It is apparent from the present study that in hydroponic culture, As at elevated concentration induces Fe-deficiency chlorosis. Arsenic at elevated concentration in the growth medium also destabilized the normal mineral nutrition in plants tissues.

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References

Abedin, M. J., Cotter-Howells, J., & Meharg, A. A. (2002). Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. *Plant and Soil*, *240*, 311–319.

Ahmed, K. M., Imamul Huq, S. M., & Naidu, R. (2006). Extent and severity of arsenic poisoning in Bangladesh. In R.

Naidu, E. Smith, G. Owens, P. Bhattacharya, & P. Nadebaum (Eds.) *Managing arsenic in the environment from soil to human health* (pp. 525–540). Collingwood Victoria: CSIRO Publishing.

Alam, S., Kamei, S., & Kawai, S. (2001). Amelioration of manganese toxicity in barley with iron. *Journal of Plant Nutrition*, *24*, 1421–1433.

Armstrong, W. (1967). The oxidising activity of roots in water-logged soils. *Physiologia Plantarum*, *20*, 920–926.

Artus, N. N. (2006). Arsenic and cadmium phytoextraction potential of Crambe compared with Indian mustard. *Journal of Plant Nutrition*, *29*, 667–679.

Barton, C. J. (1948). Photometric analysis of phosphate rock. *Analytical Chemistry*, *20*, 1068–1073.

Batty, L. C., Baker, A. J. M., Wheeler, B. D., & Curtis, C. D. (2000). The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis* (Cav.) Trin ex Steudel. *Annals of Botany*, *86*, 647–653.

Bergmann, W. (1988). *Ernährungsstörungen bei Kulturpflanzen. Entstehung, visuelle und analytische Diagnose*. Jena: Gustav Fischer.

Boehle, J., & Lindsay, W. L. (1969). Micronutrients. The fertilizer shoe-nails. Pt. 6. In the limelight–zinc. *Fertilizer Solutions*, *13*, 6–12.

Carbonell-Barrachina, A. A., Burló, F., & Mataix, J. (1998). Response of bean micronutrient nutrition to arsenic and salinity. *Journal of Plant Nutrition*, *21*, 1287–1299.

Carbonell-Barrachina, A. A., Burló-Carbonell, F., & Mataix-Beneyto, J. (1997). Effect of sodium arsenite and sodium chloride on bean plant nutrition (Macronutrients). *Journal of Plant Nutrition*, *20*, 1617–1633.

Chen, C. C., Dixon, J. B., & Turner, F. T. (1980). Iron coatings on rice roots: Morphology and models of development. *Soil Science Society of America Journal*, *44*, 1113–1119.

Chen, Z., Zhu, Y. G., Liu, W. J., & Meharg, A. A. (2005). Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa* L.) roots. *New Phytologist*, *165*, 91–97.

De Koe, T., Rozema, J., Broekman, R. A., Otte, M. L., & Ernst, W. H. O. (1988). Heavy metals and arsenic in water sediment and plants near the Jales gold and silver mine in North Portugal. In A. A. Orio (Ed.) *Environmental contamination, 3rd international conference, Venice, September 1988* (pp. 152–154). Edinburgh: CEP Consultants.

Edwards, D. G., & Asher, C. J. (1982). Tolerance of crop and pasture species to manganese toxicity. In A. Scaife (Ed.) *Proceedings of the 9th International Plant Nutrition Colloquium, Warwick, England* (pp. 145–150). Farnham Royal, Bucks, UK: Commonwealth Agricultural Bureau.

Farid, A. T. M., Roy, K. C., Hossain, K. M., & Sen, R. (2003). A study of arsenic contaminated irrigation water and its carried over effect on vegetable. In M. F. Ahmed, M. A. Ali, Z. Adeel (Eds.), *Fate of Arsenic in the Environment* (pp. 113–121). Bangladesh University of Engineering and Technology, Dhaka, Bangladesh/The United Nations University, Tokyo, Japan.

Frans, R., Horton, D., & Burdette, L. (1988). Influence of MSMA on Straighthead, Arsenic Uptake and Growth Response in Rice. *Arkansas Agric. Exp. Stat.* Report Series 302. Fayetteville, AR.

von Hodenberg, A., & Finck, A. (1975). Ermittlung von Toxizitäts-Grenzwerten für Zinc, Kupfer und Blei in Hafer und Rotklee {De}. *Z. Pflanzenernähr. Bodenk.*, *138*, 489–503.

Huq, I. S. M., Alam, S., & Kawai, S. (2003). Arsenic in Bangladesh environment and its impact on food chain through crop transfer. Abstract no. 22–45. Abstracts of the Annual Meeting, Japanese Society of Soil Science and Plant Nutrition. 20–22 August 2003, (Vol 49, pp. 177). Tokyo, Japan.

Imamul Huq, S. M., Binte Abdullah, M., & Joardar, J. C. (2007). Bioremediation of arsenic toxicity by algae in rice culture. *Land Contamination & Reclamation*, *15*(3), 327–333.

Imamul Huq, S. M., Correll, R., & Naidu, R. (2006). Arsenic accumulation in food sources in Bangladesh. In R. Naidu, E. Smith, G. Owens, P. Bhattacharya, & P. Nadebaum (Eds.) *Managing Arsenic in the Environment from Soil to Human Health* (pp. 283–293). Collingwood Victoria: CSIRO Publishing.

Jackson, M. L. (1958). Total elemental phosphorus. In M. L. Jackson (Ed.) *Soil chemical analysis* (pp. 175–176). Englewood Cliffs: Prentice-Hall.

Kesselring, J. (1948). *Arsenic and old lace*. London, UK: Warner Chappell Plays.

Liao, X. Y., Chen, T. B., Lei, M., Huang, Z. C., Xiao, X. Y., & An, Z. Z. (2004). Root distributions and elemental accumulations of Chinese brake (*Pteris vittata* L.) from As-contaminated soils. *Plant and Soil*, *261*, 109–116.

Liebig Jr., G. F. (1966). Arsenic. In H. D. Chapman (Ed.) *Diagnostic criteria for plants and soils* (pp. 13–23). Riverside, CA: University of California, Division of Agricultural Sciences.

Liu, W. J., Zhu, Y. G., & Smith, F. A. (2005). Effects of iron and manganese plaques on arsenic uptake by rice seedlings (*Oryza sativa* L.) grown in solution culture supplied with arsenate and arsenite. *Plant and Soil*, *277*, 127–138.

Loeppert, R. H., & Inskeep, W. P. (2001). Iron. In D. L. Sparks (Ed.) *Methods of soil analysis, Part 3—Chemical methods. SSSA Book Series 5* (pp. 639–664). Madison: Soil Science Society of America and American Society of Agronomy.

Ma, L. Q., Komar, K. M., Tu, C., Zhang, W., Cai, Y., & Kennelley, E. D. (2001). A fern that hyperaccumulates arsenic. *Nature*, *409*, 579.

Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: A review. *Talanta*, *58*, 201–235.

Marin, A. R., Masscheleyn, P. H., & Patrick Jr., W. H. (1992). The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant and Soil*, *139*, 175–183.

Marin, A. R., Pezeshki, S. R., Masscheleyn, P. H., & Choi, H. S. (1993). Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants. *Journal of Plant Nutrition*, *16*, 865–880.

Marschner, H. (1998). *Mineral Nutrition of Higher Plants*. London: Academic.

Maynard, D. N. (1979). Nutritional disorders of vegetable crops. A review. *Journal of Plant Nutrition*, *1*, 1–23.

Mengel, K., & Kirkby, E. A. (2001). Principles of plant nutrition (5th Ed.). Dordrecht: Kluwer.

Ohki, K. (1984). Zinc nutrition related to critical deficiency and toxicity levels for sorghum. *Agronomy Journal*, *76*, 253–256.

O'Neill, P. (1995). Arsenic. In B. J. Alloway (Ed.) *Heavy metals in soils* (pp. 106–121, 2nd Ed.). London, UK: Blackie.

Onken, B. M., & Hossner, L. R. (1995). Plant uptake and determination of arsenic species in soil solution under flooded conditions. *Journal of Environmental Quality*, *24*, 373–381.

Otte, M. L., Dekkers, M. J., Rozema, J., & Broekman, R. A. (1991). Uptake of arsenic by *Aster tripolium* in relation to rhizosphere oxidation. *Canadian Journal of Botany*, *69*, 2670–2677.

Piper, C. S. (1942). *Soil and plant analysis*. Adelaide: Hassell.

Porter, E. K., & Peterson, P. J. (1977). Arsenic tolerance in grasses growing on mine waste. *Environmental Pollution*, *14*, 255–264.

Rabbani, G. H., Chowdhury, A. K., Shaha, S. K., & Nasir, M. (2002). Mass arsenic poisoning of ground water in Bangladesh. Global Health Council Annual Conference, Abstract in Proceedings, Washington, DC, May 28–June 1.

Robson, A. D., & Reuter, D. J. (1981). Diagnosis of copper deficiency and toxicity. In J. F. Loneragan, A. D. Robson, & R. D. Graham (Eds.) *Copper in soils and plants* (pp. 287–312). London: Academic.

Sachs, R. M., & Michael, J. L. (1971). Comparative phytotoxicity among four arsenical herbicides. *Weed Science*, *19*, 558–564.

Samanta, G., Chowdhury, T. R., Mandal, B. K., et al. (1999). Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India and Bangladesh. *Microchemical Journal*, *62*, 174–191.

SAS. (1988). SAS/STAT user's guide, no. 1, ANOVA, version 6, (4th Ed). Statistical Analysis System Institute, Cary.

Sauerbeck, D. G. (1982). Which heavy metal concentrations in plants should not be exceeded in order to avoid detrimental effects on their growth. *Landwirtschaftliche Forschung Sonderh*, *39*, 108–129.

Shaibur, M. R., Huq, S. M. I., & Kawai, S. (2007). Effect of arsenic on hydroponic Japanese mustard spinach. Abstracts of the Eighth Conference of the East and Southeast Asian Federation of Soil Science. 22–23 October 2007, (pp. 100). Tsukuba International Congress Center, Tsukuba, Ibaraki, Japan.

Shaibur, M. R., Kitajima, N., Sugawara, R., Kondo, T., Huq, S. M. I., & Kawai, S. (2006). Physiological and mineralogical properties of arsenic-induced chlorosis in rice seedlings grown hydroponically. *Soil Science and Plant Nutrition*, *52*, 691–700.

Shaibur, M. R., Kitajima, N., Sugawara, R., Kondo, T., Huq, S. M. I., & Kawai, S. (2008). Physiological and mineralogical properties of arsenic-induced chlorosis in barley seedlings grown hydroponically. *Journal of Plant Nutrition*, DOI 10.1080/01904160701854074.

Smith, A. H., Lingas, E. O., & Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bulletin of the World Health Organization*, *78*, 1093–1103.

Takagi, S. (1993). Production of phytosiderophores. In L. L. Barton, & B. C. Hemming (Eds.) *Iron chelation in plants and soil microorganisms* (pp. 111–131). New York: Academic.

Tang, C., Robson, A. D., & Dilworth, M. J. (1990). The role of iron in nodulation and nitrogen fixation in *Lupinus angustifolius* L. *New Phytologist*, *114*, 173–182.

Thiel, H., & Finck, A. (1973). Ermittlung von Grenzwerten optimaler Kupfer-Versorgung für Hafer und Sommergerste. *Zeitschrift fuÉr PflanzenernaÉhrung und Bodenkunde*, *134*, 107–125.

Tondel, M., Rahman, M., Magnuson, A., Chowdhury, I. A., Faruquee, M. H., & Ahmad, S. A. (1999). The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. *Environmental Health Perspectives*, *107*, 727–729.

Tsutsumi, M. (1980). Intensification of arsenic toxicity to paddy rice by hydrogen sulphide and ferrous iron. I. Induction of bronzing and iron accumulation in rice by arsenic. *Soil Science Plant Nutrition*, *26*, 561–569.

Wauchope, R. D. (1983). Uptake, translocation and phytotoxicity of arsenic in plants. In W. H. Lederer, & R. J. Fensterheim (Eds.) *Arsenic: Industrial, biomedical, environmental perspectives, arsenic symposium* (pp. 348–374). Gaithersburg, MD: Van Nostrand Reinhold Company.

Woolson, E. A. (1975). Arsenical Pesticides. ACS Symp. Ser. 7. In E. A. Woolson (Ed.). Washington, DC: American Chemical Society.

Yamane, T. (1989). The mechanisms and countermeasures of arsenic toxicity to rice plant. *Bulletin of the Shimane Agricultural Experimental Station*, *24*, 1–95 (In Japanese with English summary).