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ARSENIC DISTRIBUTION IN DIFFERENT PARTS OF BARANUNIYA (*Portulaca oleracea* L.) TREATED WITH ELEVATED CONCENTRATIONS OF ARSENIC

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ABSTRACT

Shaibur MR, Islam T, Adjadeh TA, Huq SMI, Kawai S (2012) Arsenic distribution in different parts of baranuniya (*Portulaca oleracea* L.) treated with elevated concentrations of arsenic. *Int. J. Sustain. Crop Prod.* 7(3), 36-46.

This experiment was conducted to study the distribution of arsenic (As) in different parts of Baranuniya (Summer purslane; *Portulaca oleracea* L.) treated with elevated concentrations of As. The seedlings were treated with 0, 10, 25 and 50 µM As from sodium-meta arsenite ($NaAsO_2$). Dry weight (DW), visible growth, shoot height, leaf number and the width of the leaf blade were limited by the higher concentrations of As on 14 days after treatments (DAT). Arsenic treated plants did not show chlorosis or necrosis in the leaves but contained higher concentrations of As in the leaves and stems. Our result indicates that As can be accumulated in the food stuff and present at a threat to crop quality as well as human and animal health. The result also indicates that Baranuniya is As-sensitive plant and could not be used as a phytoremediator of As. Considering 10% DW reduction, the calculated critical toxicity levels (CTL) of As were 0.145 µg g^{-1} DW in leaf, 0.225 µg g^{-1} DW in stem and 33.57 µg g^{-1} DW in root, respectively.

Key words: *arsenic, dry and fresh weight, leafy vegetables, visible symptom*

INTRODUCTION

Arsenic (As) is a toxic metalloid. It is considered as the king of poison and is the most important pollutant in nature. In the past, As was used as a suicidal and homicidal agent. Many historians believe that the French Emperor Napoleon Bonaparte was poisoned to death like “Vawal Raja” of Bangladesh with As (*Senko Bish* in Bengali; Murshed 2005). Arsenic has great affinity to form or to occur in many minerals. More than 200 minerals of As have been identified and approximately 60% of which are arsenates. The most common As mineral is arsenopyrite (iron arsenic sulfide; FeAsS). The oxidation states of As are -3, 0, +3 and +5; of which As^0 and As^{3+} are the characteristics of reducing environments. The most mobile forms of As are AsO_2^-, AsO_4^{3-}, $HAsO_4^{2-}$ and $H_2AsO_3^-$ (Kabata-Pendias 2001). Under most environmental conditions As^{5+} is present as the $H_2AsO_4^-$ species, while As^{3+} as the $H_3AsO_3^0$ species is only dominant in low pH and low Eh environments (Kabata-Pendias 2001; Crecelius *et al.* 1986).

Bangladesh (Abedin and Meharg, 2002) and Japan’s (Kitagishi and Yamane, 1981) paddy soils are contaminated with As. Pollution in Japanese soil with As was reported extensively (Kitagishi and Yamane, 1981). Japanese paddy soils have high sorption capacity for As and accumulated considerable amounts by receiving As-contaminated irrigation water (Kitagishi and Yamane, 1981). Paddy rice is especially susceptible to As-toxicity as compared to upland crop plants (Tsutsumi 1981). Japanese paddy fields of Miyagi, Shimane, Oita and Miyazaki Prefectures were polluted with As from nearby mines (Tsutsumi 1981). Among them, Tsuwano town of Shimane Prefecture is well known for scientific studies of the As contaminated soil. Sasagadani mine was mostly responsible for As pollution of Shimane Prefecture. The river originated near the mine and polluted the nearby paddy fields by As contaminated irrigation water. Yamane (1979) reported that vast amounts of waste materials containing high content of As had been deposited without covering and treatment, have been transported by the Tsuwano, the Takano and other rivers into paddy fields. The percolated As from wastes and mine water to the river water could be presumed to be source of As pollution in those areas.

Not only in Japan, As contamination is the global problem (Ahmed *et al.* 2006) and the calamity in Bangladesh is described as the largest As poisoning (Rabbani *et al.* 2002). The determination of As in the groundwater was first made in West-Bengal in 1983 and in Bangladesh in 1987 (Khandker *et al.* 2006). Arsenic contamination was first detected in Bangladesh by the Department of Public Health Engineering (DPHE) in 1993 (Ravenscroft *et al.* 2005; DCH 2006), but the fact remained behind the screen till 1996 (DCH 2006) and the credits go to the Chemistry Division of the Atomic Energy Centre, Dhaka for the first detection of As (AECD; Ali 1995). The first group arsenicosis patient was identified in Chomogram village, Chapai Nowabgonj District, Bangladesh in 1994 (Khandker *et al.* 2006). However, the actual time of initiation of As contamination in groundwater and arsenicosis were not clearly identified. Before reporting the As contamination in the groundwater in Bangladesh, some people died by some unknown reasons, where As poisoning might be the main factor. The scale of As digester in Bangladesh is greater than any other As disaster seen before. In terms of the numbers of people exposed, it is the most serious groundwater As problem in the world (Acharyya *et al.* 2000). It is beyond the accidents at Bhopal in 1984 and Chernobyl in 1986 (Murshed 2005).

Arsenic is known to us as the phytotoxic agent and is expected to negative effect on plant growth (Shaibur *et al.* 2008; Shaibur *et al.* 2009a; Shaibur and Kawai, 2011a, b; Shaibur and Kawai, 2012; Shaibur *et al.* 2012). The phytotoxicity of As depends on some properties, e.g. Phosphorus (P; Kabata-Pendias 2001) and iron (Fe; Shaibur *et al.* 2009b) concentrations in the growth media. It was reported that rice grown on apple orchard soil containing 77 mg As kg^{-1} produced no yield in the first year, however, the toxicity was partly reduced after 3 years of cultivation without any special treatment (Kitagishi and Yamane, 1981). Plants growing on As contaminated soil may accumulate As which could be one of the major cause of reduction of As level in soils. Naturally, many weed crops are growing in rice field in Bangladesh and Japan. Baranuniya or Baralaniya (Bengali) or Summer purslane (English) or Suberihiyu (Japanese) is one of the weed leafy vegetable growing mostly in the local areas of Bangladesh and Japan in summer. Sometimes it is called as pigweed, little hogweed, postelijn, pourpier, portulat, garden purslane or fatweed as its leaves and stems are very fleshy and succulent (TROPILAB^{R}INC, 2006; available at: http://www.tropilab.com/purslanetincture.html). Purslane derives from the Latin “portulacca” and the old French “pourcelaine”. Many reports have already been published regarding As-toxicity and As-distribution in plant parts but there is little information about As distribution in leafy weed vegetables Baranuniya. Therefore, the present research was conducted to study the distribution of As in Baranuniya treated with elevated concentrations of As. In this study, we focused on the visible symptom and As-distributions in roots, stems and leaves to understand if the plant is As-sensitive or As-accumulator.

MATERIALS AND METHODS

Seedling collection and acclimation at the greenhouse environment

Baranuniya (Summer purslane; *Portulaca oleracea* L.) were collected from the Botanical garden of Iwate University, Morioka, Japan on 16 August, 2006 and transferred in 10-L opaque plastic PVC bucket containing 9 L of half-strength Hoagland-Arnon nutrient solution (Hoagland and Arnon, 1950) in the greenhouse for 8 days and allowed to acclimate to the environmental condition of the greenhouse. During the acclimation period, seedlings got sufficient strength for starting As treatments. The composition of full-strength solution has been given in Table 1. The pH of the nutrient solution was not adjusted during the acclimation stage. Four more or less uniform seedlings were taken in one bucket, among them 3 similar seedlings were chosen considering 3 replications. We did not have any chance to understand the real age of the seedlings, because the seedlings were randomly collected from the garden. Arsenic was added as sodium meta-arsenite ($NaAsO_2$; Kanto Chemical Company, Tokyo, Japan). Arsenite was used in aerated condition because some arsenite may be converted into arsenate with aeration. The agricultural soils may contain both arsenite and arsenate together. The underground water contained almost 50% arsenite and 50% arsenate (Samanta *et al.* 1999). Nutrient solution was renewed in every 7 days after treatments (DAT) and was aerated continuously during the experiment. The pH (5.5) was adjusted daily with a digital pH meter (Horiba Korea, Seoul, Korea) and with 1 M HCl and/or 1 M NaOH at around 4 p.m. during the experiment (August-September, 2006). Seedlings were treated with 0, 10, 25 and 50 µM As. The duration of the As treatments was 14 days.

Table 1. Compositions of the full-strength modified nutrient solution used in this study (Hoagland and Arnon, 1950)

Salt	Strength	Salt	Strength
KNO_3	6.0 mM	$CuSO_4$	0.2 µM
$Ca(NO_3)_2$	4.0 mM	$ZnSO_4$	0.40 µM
$NH_4H_2PO_4$	1.0 mM	H_3BO_3	3.0 µM
$MgSO_4$	2.0 mM	H_2MoO_4	0.05 µM
$MnSO_4$	0.50 µM	Fe^{3+}-EDTA	20.0 µM

EDTA = monosodium Ethylene Diamine Tetraacetic acid, $C_{10}H_{12}N_2O_8NaFe.3H_2O$

Determination of critical toxicity level (CTL)

A two-order polynomial growth curve between DW and As concentration was set up and the following equation 1 was used to calculate the CTL of As in this experiment (Shaibur and Kawai, 2011a, b).

$$ax^2 - bx + c = 0 \text{---------------} (1)$$

$$\therefore\ x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Number (1) equation has an intercept on the Y axis. Here “c” (constant; value of y) is the intercept in every equation in this report. The reduction of 10% DW was calculated from the intercept point.

Shoot height and root length

Shoot height was determined from the culm base of the standard plant to the tip of the longest leaf and root length was calculated from the junction of root-shoot to the tip of the longest root.

Determination of water loss

On 3 DAT, we added appropriate quantity of deionized water to maintain the water levels in the bucket. For every case, we recorded the added amount of water and made the sum and finally summarized the result of water loss.

Reagents

All chemicals used were of analytical reagent grade. All solutions were prepared previously in MQ water (18.2 MΩ cm^{-1}), purified by Milli-RO 60 (Millipore Corporation, USA) and stored in the laboratory in natural condition. Stock solution of As was prepared by dissolving $NaAsO_2$ in MQ water.

Digestion and determination of arsenic

Seedlings were harvested, washed with tap water first followed by deionized water three times. Shoots and roots were separated with stainless still scissors; leaves were separated from the stems by clean hand, put them in clean paper packet and dried at 55ºC for 48 hours (Shaibur and Kawai, 2011a, b; Shaibur and Kawai, 2012). The oven dried samples were weighted and digested with nitric acid-perchloric acid mixture (5:1 = V/V). Arsenic was measured by Hydride Generation Atomic Absorption Spectrophotometric (HGAAS) technique using the instrument Hitachi HFS-3. The volume of the digested solution was made at 50 mL with MQ water. The samples were further diluted up to 100-2000 times. Therefore, the interference of nitrate on As determination might be minimized. Reduced nitrogen oxides (resulting from HNO_3 digestion) and nitrite could suppress instrumental response for As (Huang and Fujii, 2001).

Calculation for the parameters

Concentration in µg of element g^{-1} DW or FW (fresh weight); accumulation in shoot in µg of element plant^{-1} shoot; accumulation in root in µg of element plant^{-1} root; and translocation % in nutrient accumulation in shoot/total accumulation (shoot + root) × 100 were calculated. Recovery of As is the total amount of As accumulated in root, stem and leaf and finally the value was expressed in %.

Statistical analysis

The experiment was arranged in randomized blocks with three replications. Data on leaf, stem and root DW or FW, shoot height, root length, leaf number, width of leaf blade and As were subjected to ANOVA. Differences between means were evaluated by the Ryan-Einot-Gabriel-Welsch multiple range test (P = 0.05) using computer origin 5 at Iwate University.

Fig. 1. Figure showing the visible symptoms of As-stressed Baranuniya in the different concentrations of As (µM). Arsenic did not show any visible toxicity symptom. This picture was taken in a cloudy day after washing with de-ionized water

RESULTS

Visible symptom

Seedlings fall down on the lid in the day time at 25 and 50 µM As treatments on 2 DAT. Water deficit symptom was also observed under sunlight. Within few days, almost all of the old leaves of nodes died and fallen down on the lid at 50 µM As treatment. The most common symptom was visible growth reduction without showing necrosis or chlorosis in the leaves (Fig. 1). The visible number of leaves (Fig. 2a) limited with increasing As concentrations in the solution. Shoot height and the number of branches were also limited by As-toxicity. Root was brown in color and discoloration was not much pronounced in the As-treated plants. Root was slippery to the touch and branching of root was limited by As-toxicity. Visible width, length and size of leaf blade were limited with the As-treatments.

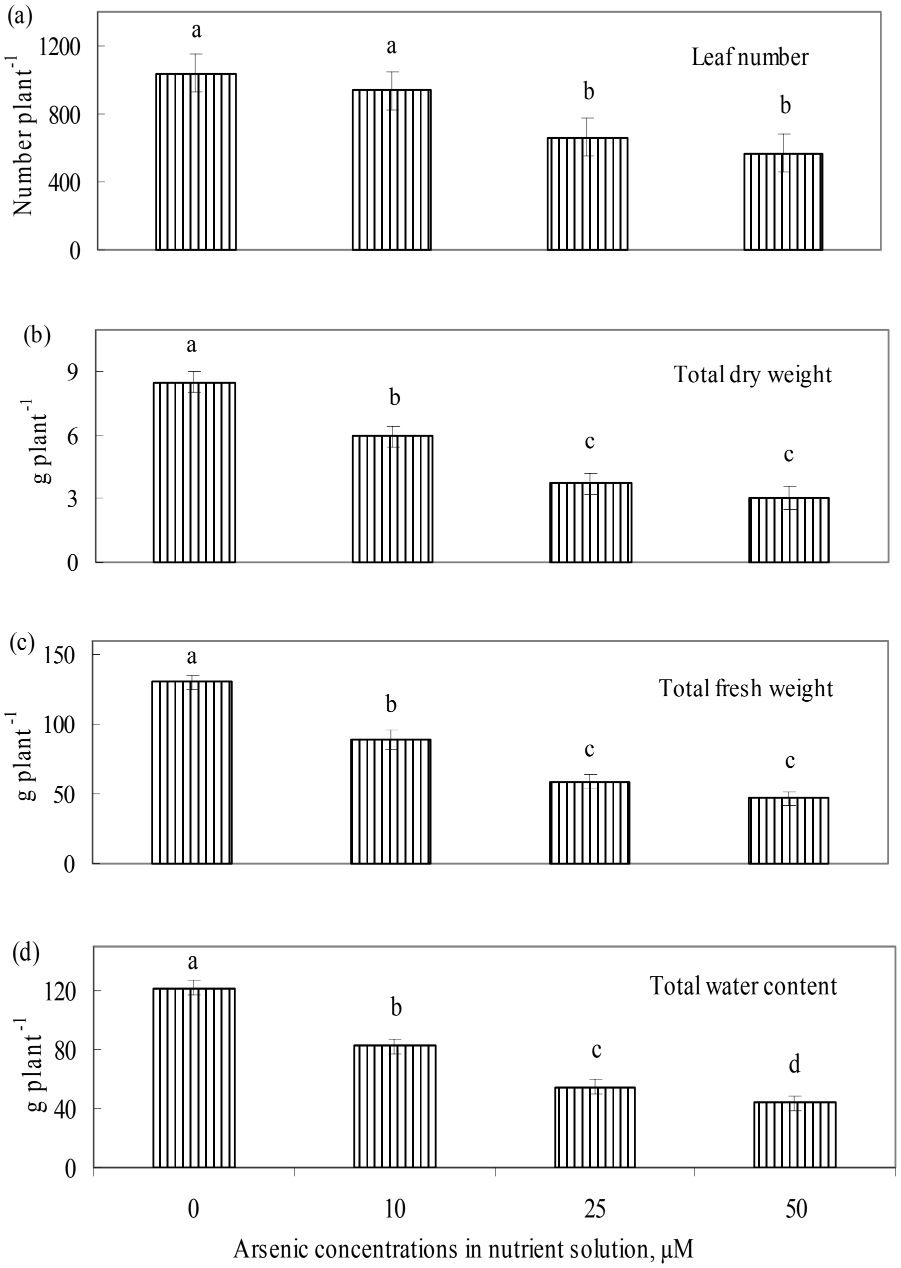

Fig. 2. (a) Leaf number; (b) Total dry weight; (c) Total fresh weight; and (d) Total water content of Baranuniya seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test

Dry and fresh weight, tiller number, branch number and leaf number

Total DW and FW limited with increasing As concentrations in the solution (Figs. 2b, c). Dry weight and FW of root, shoot and leaf were limited by the higher As concentrations used in this experiment (Figs. 3a, b). Baranuniya did not produce any new tiller in control plants. Several new roots came out from the main roots and the formation of new roots limited with increasing As concentrations in the nutrient solution. The highest numbers of new roots were found in the control and the lowest was found in the 50 µM As treatment. Leaf number limited with increasing As concentrations in the nutrient solution (Fig. 2a).

Shoot height and root length

Shoot height limited significantly with increasing As concentrations in the nutrient solution, however, root length was not much affected by the applied As (Fig. 4). The highest shoot height was obtained in the control and the lowest value was obtained in the 50 µM As treatment, respectively. The root length was almost similar in the 0, 10 and 25 µM As treatments, but limited in the 50 µM As treatment as compared to others (Fig. 4).

Water content in plant parts and water loss through evapotranspiration

Total water content in plant tissues limited with increasing As concentrations in the nutrient solution (Fig. 2d). Similarly, the water content in plant parts and water loss through evapotranspiration were also limited with increasing As concentrations (Figs. 5a, b). The experimental plants contained 95, 94, 94 and 94% water in leaf; 93, 93, 93 and 94% in stem; and 88, 90, 85 and 82% in root, respectively. In the first week, the control plants lost 3 L of water and the lost values were 2, 1 and 1 L for 10, 25 and 50 µM As treatments, respectively. Similar trends were also obtained in the 2nd week of As exposure and the values were 3.5, 3, 2 and 2 L for 0, 10, 25 and 50 µM As treatments, indicating that the control plants lost additional 0.5 L of water in 2nd week as compared to the control plants of 1st week.

Arsenic concentration, accumulation, translocation and critical toxicity levels of As

Arsenic concentrations seem to be decreased in leaves, stems and roots both in DW and FW basis with increasing As concentrations in solution (Table 2). The highest concentration was recorded at 10 µM As treatment. Similarly, accumulations of As were also decreased, however, the translocation was not affected much by the applied As treatments (Table 2). It was observed that As decreased DW of leaf, stem and root (Fig. 3a). The CTL of As was calculated considering 10% DW reduction of plant tissue by As. Two order polynomial relationship between As concentration and DW of leaves, stems and roots were significantly correlated (Figs. 6a, b, c). The calculated CTL of As were 0.145 µg g^{-1} DW in leaf, 0.225 µg g^{-1} DW in stem and 33.57 µg g^{-1} DW in root, respectively.

DISCUSSION

Visible symptoms

Seedlings showed water deficit symptom under sun light on 2 DAT, suggesting that water movement from root to the shoots was limited by As-toxicity, which was supported by the water loss data (Fig. 5b). Leaves of control plants were fresh and oily to look at, however, this symptom was absent in As-treated plants. Moreover, the seedling faded to look at and was soft to the touch in As-treated plants in day time. Arsenic-toxicity was responsible for thinner coloring of leaves (fade) and logging. These were the immediate response of As-toxicity in the plants. Arsenic-treated rice seedlings showed thinner coloring of leaves and curled leaves under sunlight (Shaibur and Kawai, 2011a, b). After 4 DAT, immature senescence of old leaves occurred (Kabata-Pendias 2001) and the severity increased with increasing As concentrations in the medium. This might be due to the lack of nitrogen and lack of root-born cytokinins (Marschner 1998). The plants of 10 µM As treatment was shorter than the control plants, but turgidity was almost similar to the control, suggesting that plant might try to increase adaptability with time to the toxic environment. The symptoms of As-toxicity are variously described as leaf wilting, violate coloration (increased anthocyanin), root discoloration and cell plasmolysis, however, the most common symptom was growth reduction (Kabata-Pendias 2001). The plant did not show any visible As-toxicity symptoms in the leaves after washing with deionized water, rather the leaves looked like fresh, indicating that As hindered the water movement from roots to the shoots when the plants were grown in As-containing medium. Our result indicating that the turgidity decreased with increasing As concentrations caused by water deficit. The visible leaf size and shape were smaller in the As-treated plants. We did not see any necrosis and chlorosis in the leaves. Whitish chlorotic symptom in the young leaves and necrotic symptom in the old leaves were the symptoms of As-toxicity in hydroponic rice at 13.4 and 26.8 µM As treatments (Shaibur *et al.* 2006; Shaibur and Kawai, 2011a, b). Therefore, we are suggesting that necrosis and chlorosis may not always be the symptoms of As-toxicity in the As-sensitive plants. The vertical stems of weeds must mechanically sustain their own weight against the influence of gravity (Niklas 1993). They also must be sufficiently stiff and strong to resist bending and avoid breaking when subjected to large, externally applied mechanical forces (Niklas 1993). But As-treated plants were not sufficient strong, suggesting that As might loss the mechanical support of the stem of Baranuniya.

Root was brown and was not affected much by the As-treatments (Fig. 1). This was most probably due to the fact that the root of Baranuniya was naturally brown and the change of root color by As-toxicity was not pronounced. It was reported that As-toxicity was responsible for earlier root coloring to reddish in the 6.7, 13.4 and 26.8 µM As treatments in hydroponic rice (Shaibur and Kawai 2011a, b) and yellowish brown or brown (Yamane 1989). Root discoloration and cell plasmolysis are the symptoms of As-toxicity.

Table 2. Arsenic concentration (μg As g^{-1} DW or FW) and accumulation (μg As $plant^{-1}$) in leaf, stem, root and translocation (%) from root to shoots of Baranuniya grown in nutrient solution with different levels of As

Treatment	-------μg As g^{-1} DW-------			-------μg As g^{-1} FW-------			------μg As $plant^{-1}$-----			Translocation
(μM)	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	(%)
0	0	0	0	0	0	0	0	0	0	0
10	3.42a	4.61a	397a	0.192a	0.339a	39.3a	7.40a	13.4a	202.7a	10.04a
25	2.73b	3.44b	370ab	0.154b	0.153c	57.3b	4.12b	10.2b	117.1b	7.08b
50	2.68b	4.07ab	354b	0.153b	0.263b	62.9c	2.47c	9.74b	86.3c	10.56a

DW = dry weight and FW = fresh weight. Means followed by different letters in each column are significantly different (p= 0.05) according to a Ryan-Einot-Gabriel-Welsch multiple range test

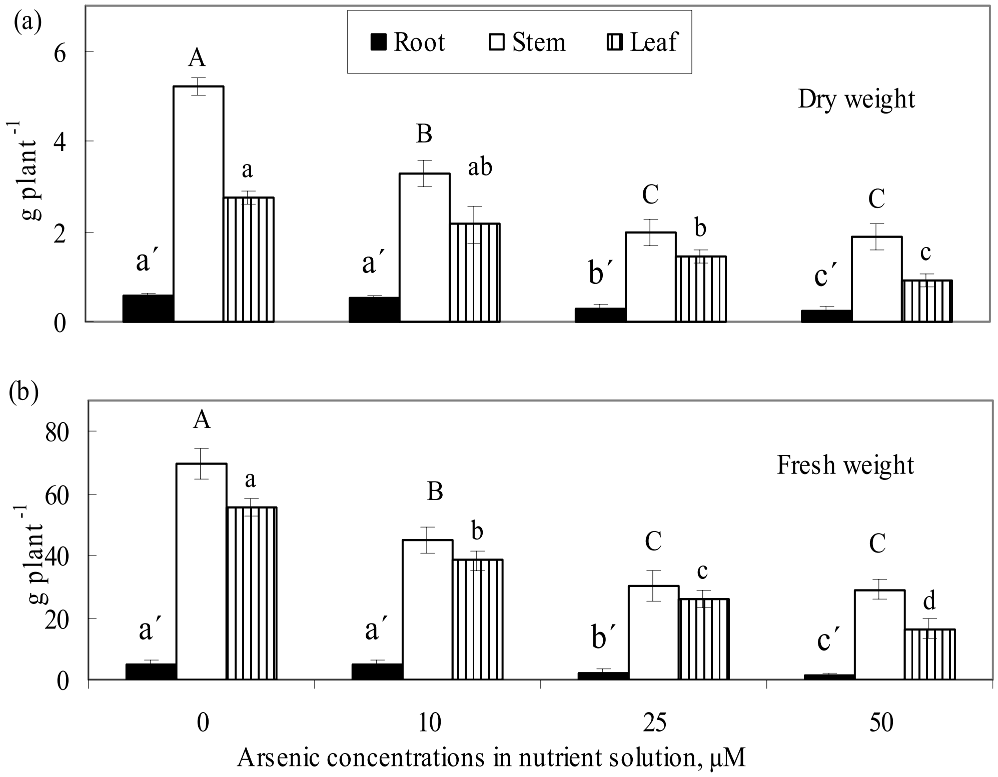

Fig. 3. (a) Dry weight and (b) Fresh weight of leaf, stem and root of Baranuniya seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test

Dry and fresh weight

Reduction of stem DW might be associated with the reduction of height and branching of the stem with As-toxicity. The other probable cause may be that As limited water movement and ultimately reduced the uptake of nutrient elements. Reduction of FW was most probably due to the reduction of water content in the plant parts (Fig. 5a). The result of the present study suggested that the threshold value of Baranuniya might be between 0-10 μM As in hydroponic culture considering 10% DW reduction (Ohki 1984). Reduction of root growth might be associated with the reduction of protein activity, because arsenite inactivates the sulfhydryl groups of root proteins (Speer 1973) causing disruption of the root functions (Isensee *et al.* 1971; Orwick *et al.* 1976) and even cellular death. Arsenic breaks the root structure and reduces the water translocation from root to the shoots and might be responsible for the lower uptake or absorption of nutrients. There are some reports of the stimulating effects of As on the activity of soil microorganisms (Kabata-Pendias 2001). However, it is well-known that As is a metabolic inhibitor; therefore, DW reduction of plants under a high level of bioavailable As may be possible (Kabata-Pendias 2001).

Shoot height and root length

Arsenic-toxicity limited shoot height significantly and the highest value was recorded in the control plant (Fig. 4). In a greenhouse pot experiment, the reduction of shoot height and root length was taken place by arsenite and arsenate (Abedin and Meharg, 2002). Similarly, As-toxicity limited rice plant height (Shaibur and Kawai, 2011a, b). However, no reduction of plant height was recorded up to 125 mg As kg^{-1} dry soil in rice, but 63% reduction was taken place at 312.5 mg As kg^{-1} (Tsutsumi 1980). In the present experiment, the result was not so clear in root, indicating that As-toxicity on root length was not much pronounced like shoot.

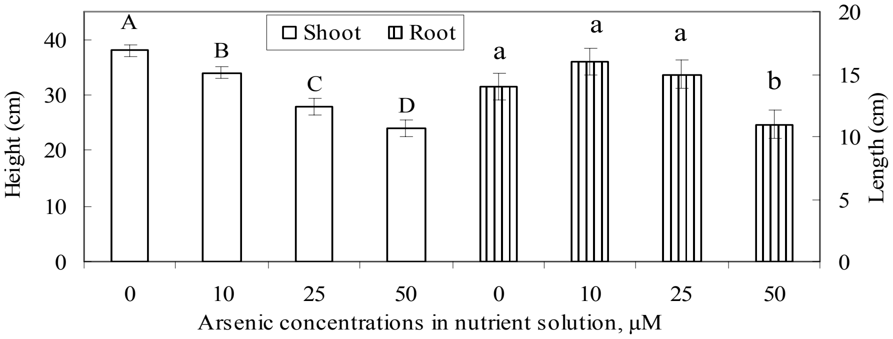

Fig. 4. Shoot height and root length of Baranuniya seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test

Water content and water loss

Arsenic toxicity limited water content in plant parts (Fig. 5a). Water loss data (Fig. 5b) indicated that As limited water movement from root to the shoots. The reduction of water movement may be related to the reduction of nutrient uptake in As-stressed plants. Reduction of water loss might be involved with the reduction of DW with As-toxicity. This may be due to the fact that As may be responsible for inactivation the water transport site or break the structure of root membrane (Orwick *et al.* 1976). In case of second week, the water loss was higher in As-treated plants, suggesting that As-toxicity was higher in early stage of plants and with time plants may try to overcome As-toxicity. When we considered total amount of water loss, it was found that water loss through evapotranspiration decreased with increasing As concentrations in the nutrient solution (Fig. 5b).

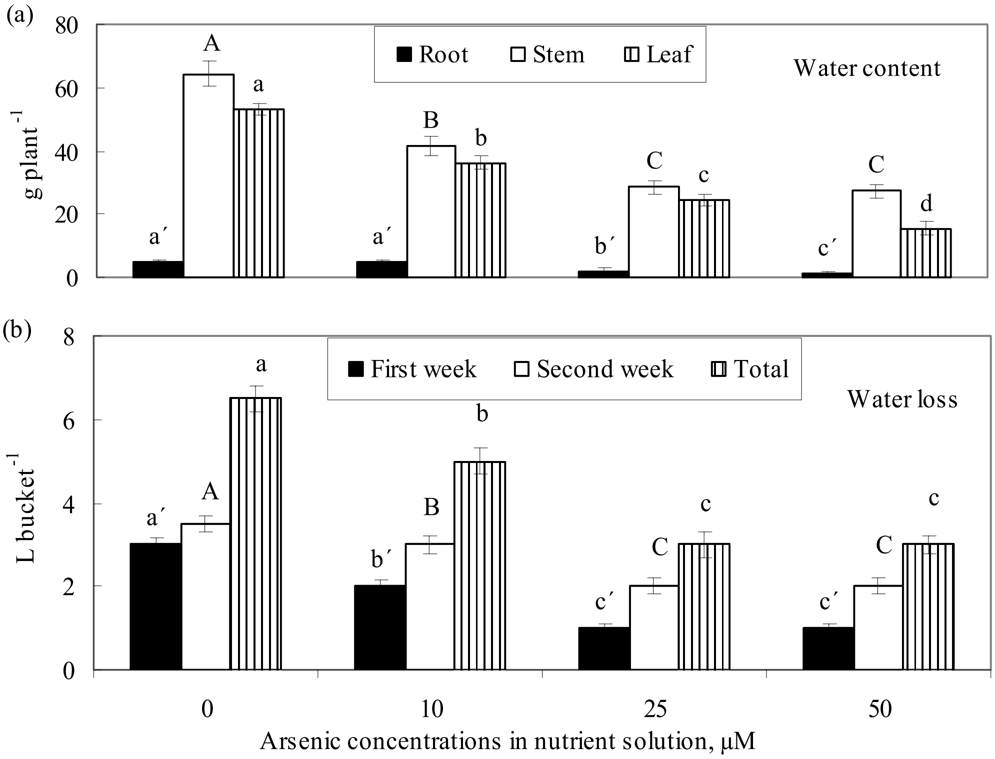

Fig. 5. (a) Water content and (b) Water loss of roots, stems and leaves of Baranuniya seedlings with different levels of As. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan- Einot -Gabriel-Welsch multiple range test

Arsenic concentration, accumulation and translocation

Reduction of As concentration in plant parts was taken place with increasing As concentrations in the medium. This may be due to, the growth was higher in plants treated with lower concentration of As as compared to the higher As concentrations. Our result indicated that Baranuniya could not be used in As polluted areas for cleaning up the soil. We found, As concentration decreased in leaf with increasing As concentrations in the nutrient solution (Table 2), this might be due to the reduction of water movement from root to the shoots (Fig. 5b) and reduction of leaf, stem and root DW and FW (Fig. 3a) by As. The other probable cause might be that Baranuniya is As-sensitive plant and just avoids its toxicity by avoiding the absorption of As. Generally, plants try to limit As-toxicity by rejecting (i.e. not adsorbing it) or not translocate it to sensitive parts. Avoidance of As absorption by some plants may be due to selective anion membrane transport that allows the passage of nitrate and phosphate but not As (III) or As (V) (Harper and Haswell, 1988). High concentration of As was found in leaf and stem without having necrosis and chlorosis, indicating that Baranuniya may contains As at toxic level without showing any visible toxicity symptom.

Arsenic concentrations were 3.42, 2.73 and 2.68 μg g^{-1} DW in leaves (Table 2), but the older leaves died and fallen down on lid. We did not measure As concentrations of the died leaves. The plant is supposed to die before their leaf concentration reaches 10 μg As g^{-1} DW (Wallace *et al.* 1980) though some plants could accumulate higher concentration of As. The concentrations of As were 17.8, 17.7 and 17.5 times higher in DW as compared to FW in leaf. Similarly, the values were 13.6, 22.5 and 15.5 times higher in stem; and 10.1, 6.46 and 5.63 times higher in roots in the 10, 25 and 50 μM As treatments, respectively. In every case, the concentrations values were around 18 times higher; therefore, the recommended value of As concentration in FW basis should be around 18 times lower than that of the DW recommended value set by WHO. A similar suggestion was made recently (Shaibur and Kawai, 2009; Shaibur *et al.* 2009a). Though, the recommended value largely depends on the water content in the plants part. Generally, the leafy vegetables contained >90% water in the aerial parts (Shaibur and Kawai, 2009).

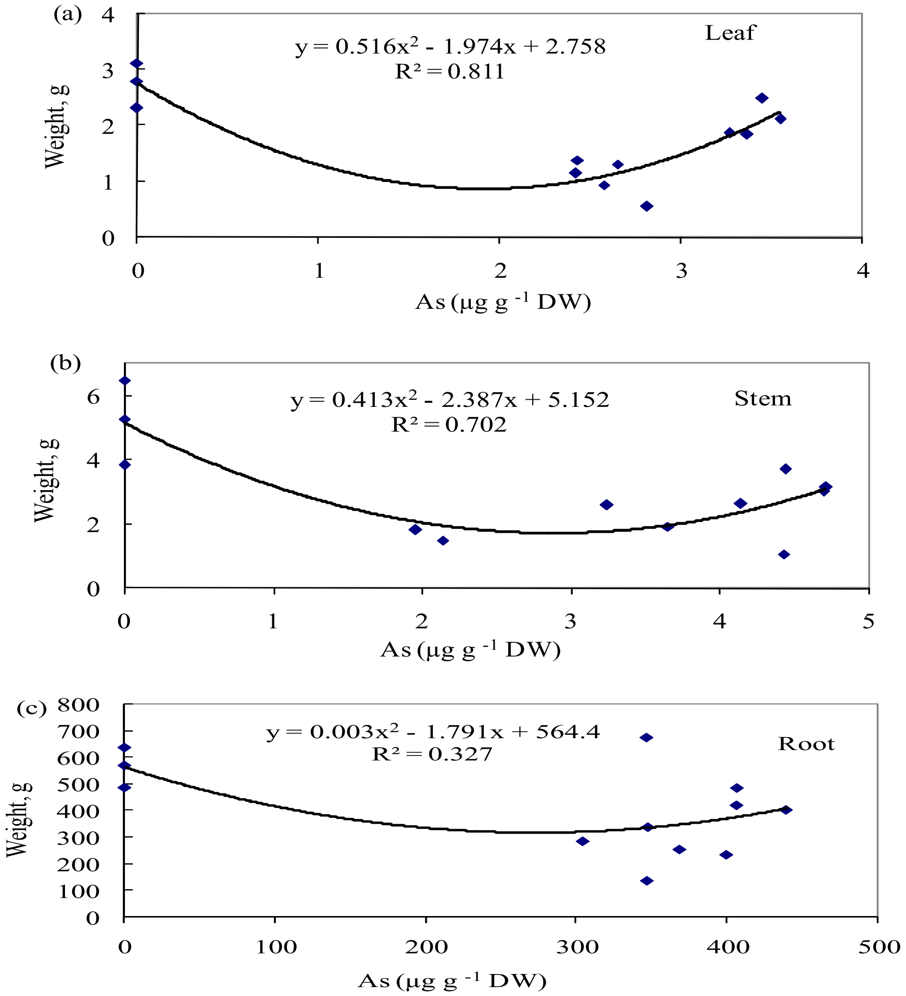

Fig. 6. Typical two order polynomial growth curve of (a) Leaf, (b) Stem and (c) Root of Baranuniya seedlings containing different concentration of As in plant tissues

Critical toxicity level of As

The CTL of As in the leaves was calculated as 3.68 or 0.145 µg As g^{-1} DW considering 10% DW reduction (Ohki 1984.). The 3.68 µg As g^{-1} DW is not acceptable as 10% growth reduced in 0.145 µg As g^{-1} DW (Fig. 6a). Therefore, the CTL of As in Baranuniya leaves was 0.145 µg As g^{-1} DW.

Our experimental plant contained 4.61, 3.44, 4.07 µg As g^{-1} DW in stem in the 10, 25 and 50 µM As treatments, respectively (Table 2) and DW decreased in the 10 µM As treatment (Fig. 3a). Baranuniya is not As-hyperaccumulator rather As-sensitive plant, because growth decreased much and plant showed water deficit symptom in the day time. The CTL of As in stems was calculated as 5.56 or 0.225 µg As g^{-1} DW. In this experiment, the 5.56 µg As g^{-1} DW was not acceptable as 10% growth was reduced in the 0.225 µg As g^{-1} DW (Fig. 6b). Therefore, the CTL of As in Baranuniya stems was 0.225 µg As g^{-1} DW.

Arsenic concentration decreased in root with increasing As concentrations in the nutrient solution and As was mostly concentrated in root (Table 2). Root contained 397, 370 and 354 µg As g^{-1} DW in the 10, 25 and 50 µM As treatments, respectively. Reduction of As concentration in root might be due to the fact that As might reduce new root formation and might decrease the root surface area. The CTL of As in root was calculated as 509 or 33.6 µg As g^{-1} DW. In this experiment, 509 µg As g^{-1} DW is not acceptable as 10% growth was reduced in the 33.6 µg As g^{-1} DW (Fig. 6c). Therefore, the CTL of As in Baranuniya root was 33.6 µg As g^{-1} DW.

Justification for hyperaccumulator or not

Arsenic concentrations in plants may vary with the location and pollution sources. Sometimes plants may concentrate extremely high contents of As above 6000 µg g^{-1} DW and above 8000 µg g^{-1} AW (ash weight; Kabata-Pendias 2001). Some sea plants could concentrate a greater proportion of As from water (Kabata-Pendias 2001). Arsenic levels exceeding 1000 mg kg^{-1} in fronds of plants grown on soil containing 100 mg As kg^{-1} are remarkable and would be considered as hyperaccumulator (Meharg 2003). However, in spite of containing up to 3470 mg As kg^{-1} in aboveground biomass (Porter and Peterson, 1977) from a soil containing 26500 mg As kg^{-1}, bent grass (*Agrostis capillaris* L.) are not classified as hyperaccumulator (Tu and Ma, 2002). Values of bioconcentration factor (BF) may be better to characterize As hyperaccumulation than As concentration in tissue, as the plant tissue concentration does not account for the As concentration in the soil (Tu *et al.* 2002). The BF in frond is defined as the ratio of As concentrations in fronds of Chinese brake fern to that of water soluble As in soil where the plant was grown (Tu *et al.* 2002). In all confirmed hyperaccumulators, the shoot to root ratios of contaminant concentration is >1, whereas the ratios are invariable <1 in non-accumulators (Raskin and Ensley, 2002). The ratio of As concentration in leaf or stem and root is <1, therefore, Baranuniya is not As-hyperaccumulator rather it is As-sensitive plant.

Translocation (%) could also be used to identify As-hyperaccumulator. When the translocation is more than 50% then the plants could be called as the accumulator. Baranuniya translocated only 7-11% of absorbed As to the aerial parts (Table 2), therefore, is not a hyperaccumulator. Sometimes, transfer factor (TF) is used for determination of accumulator. The TF is defined as the ratio of As concentration in shoot to that in root, is a good index of translocation in a plant (Tu *et al.* 2002). It is actually the ratio of As concentration in shoot to that of root. Sometimes, TF is also called as translocation factor (Tu *et al.* 2002).

CONCLUSION

Baranuniya or Summer purslane or Suberihiyu is an As-sensitive plant. Necrosis and chlorosis may not always be the symptoms to show As-toxicity in the leafy vegetables. Without showing visible toxicity symptoms, Baranuniya concentrated higher concentrations of As in the leaves and stems. Considering the bioaccumulation factor and translocation (%), it is concluded that Baranuniya is not As-hyperaccumulator rather it is very sensitive to As. Arsenic was mostly concentrated in root, moderate concentration was in the stem and the lowest concentration was in the leaf, indicating that As can enter into edible tissues through absorption by root and subsequently translocation to the shoots. The contamination of Baranuniya with As and possible adverse effect on animal health by feeding the contaminated plants need to be considered, because Baranuniya (3.42 µg As g^{-1} DW in leaf and 4.61 µg As g^{-1} DW in stem) contains higher concentrations of As in the aerial parts without showing visible toxicity symptoms.

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