Effect of Arsenic on Nutritional Composition of Japanese Mustard Spinach: An Ill Effect of Arsenic on Nutritional Quality of a Green Leafy Vegetable

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Abstract: We tried to observe the effect of arsenic (As) on the concentration, accumulation and translocation of nutrient elements in a dicots plant Japanese Mustard spinach (JM-spinach; *Brassica rapa* L. var. *pervirdis*) grown hydroponically. The treatments of As were 0, 6.7, 33.5 and 67 µM from $NaAsO_2$ (equivalent to 0, 0.5, 2.5 and 5 µg mL^{-1} As, respectively) for 14 days. Fresh weight (FW) enhanced by 13.1% in shoot and 96.5% in root in the 6.7 µM As level, however, limited by the 33.5 and 67 µM As levels both in shoots and in roots. Our result indicated that little concentration of As had stimulating effect on JM-spinach FW, however, toxicity increased with increasing As in the medium. In shoot, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn) concentrations were limited by the 67 µM As treatment. Similar to concentrations, accumulations of these elements together with copper (Cu) were also limited in shoots by the higher As concentrations. No doubt, As limited the nutritional quality of JM-spinach by hampering the concentrations and accumulations of nutrient elements in plant tissues. [Nature and Science 2010;8(8):186-194]. (ISSN: 1545-0740).

Key words: accumulations; arsenic; JM-spinach; concentrations; stimulation

1. Introduction

The toxic metalloid arsenic (As) is widely distributed in nature, e.g.- in soil, water, air, plant, animal and human body. On the basis of abundance, As is ranked 20th in the earth crust, 14th in the seawater and 12th in the human body (Mandal and Suzuki, 2002). Generally, the background of As levels in top soils is low (Kabata-Pendias, 2001). Arsenic concentration in natural agricultural soil is called as background concentration. Groundwater of Bangladesh contains high concentrations of As but its sources have not been clearly identified yet. Some researchers have reported that groundwater contamination with As is attributed to the anthropogenic sources (Anawar et al., 2002). Where As contaminated irrigation waters are used widely for agriculture may have high concentrations of As. The higher As in the rooting medium reduced the plant growth (Shaibur et al., 2006; Shaibur et al., 2008ab). Severe toxic effect of As changes the concentration, accumulation and translocation of nutrient elements in plants (Shaibur et al., 2008cd; Shaibur et al., 2009abc). Concentration or content, uptake or accumulation or absorption or total content, translocation or transfer factors (TF) are widely used terminologies in the field of plant nutrition. Concentration refers to a unit amount of the specific element in a unit amount of samples, whereas accumulation or uptake or total amount or total content or absorption refers to the total amount of an element in shoot or root of a plant (Shaibur et al., 2007)). The unit of concentration is mg or µg of an element g^{-1} dry weight (DW) or fresh weight (FW) of shoot or root and accumulation is mg or µg of the element $plant^{-1}$ shoot or $plant^{-1}$ root (Shaibur et al., 2007). The terminology content is being used instead of concentration especially in the case of soil, though content is used for concentration in plant also (Imamul Huq et al., 2005). Translocation is mostly related to the accumulation or uptake in plant science (Shaibur et al., 2006).

Underground drinking water of Bangladesh contains a hazardous level of As has led to concern regarding the potential effects on the health of people in the As affected areas (Ahmad et al., 1999). Of late, it has been reported that more than 35 million people in Bangladesh are at risk of As poisoning (Rabbani et al., 2002) and 21 million of those are exposed to As concentration above Bangladesh standard, 0.05 µg mL^{-1} (BRAC, 2000). The ground water is used for agricultural purposes. An increase of As concentrations in cultivated medium lead to an increase in As levels in the edible parts of vegetables (Farid et al., 2003; Imamul Huq et al., 2005; Shamsuddoha et al., 2005; Shaibur et al., 2009a). Plants do not concentrate As to be toxic to human if it is grown in non-contaminated agricultural soils, but the concentration in plant tissues could significantly be elevated in vegetables if they are grown on As-contaminated conditions (Farid et al., 2003; Shamsuddoha et al., 2005; Imamul Huq et al., 2006; Shaibur et al., 2009a). Generally, As enters into the plants and affects the food safety. Bangladesh is mainly a country of agriculture and uses groundwater for agricultural purposes. Vegetables are important for human beings as vegetables contain many vitamins and nutrient elements. Usually, vegetables contain 80-90% water (Shaibur and Kawai, 2009). All of these could be affected by the higher concentration of As in the growth medium.

Spinach is cultivated in all over the world including Bangladesh, because it is one of the main vegetables. It is a popular vegetable due to its high nutritional value and good taste. It contains high concentration of carotene and vitamin "C" (Rashid, 1999). In Bangladesh, spinach is cultivated using underground water but the underground water is highly contaminated with As. The nutritional quality of spinach could be affected if it is grown in As contaminated condition. There are hardly information about the nutritional quality of spinach affected by As. Therefore, the present research was conducted hydroponically to observe how the concentration, accumulation and translocation of the nutrient elements in JM-spinach are being affected by the higher As concentrations.

2. Materials and methods

Cultivation of JM-spinach: Perlite was used to germinate the seed of JM-spinach (*Brassica rapa* L. var. *pervirdis*; Kaneko Seed Company Limited, Maebashi, Gunma, Japan). Seed box (plastic) containing seed and sufficient moisture was put in the glasshouse of Iwate University, Japan at around 22ºC in day and 15ºC at night, respectively. After 12 days of sowing, modified Hoagland-Arnon (Hoagland and Arnon, 1938) nutrient solution of 1/5-strength was used in every 3 days. Uniform seedlings at 5th leaf stage (after 34 days of sowing) were hand transplanted onto a 10 L plastic pot containing 9 L ½-strength nutrient solution in the greenhouse for 7 days for adjustment to the greenhouse environment and for getting sufficient strength to resist As-toxicity. Each pot contained 8 bunches (2 plants/bunch). Daily temperature fluctuation was from 8 to 20ºC in the greenhouse. We used aeration in our experimental pot. The pH was maintained at 5.5 in every 24 h at around 4 pm. Solution was renewed every week (January & March- 2006). Plants were grown up to 14 days after treatments (DAT). Arsenic treatments were 0, 6.7, 33.5 and 67 µM from $NaAsO_2$. Evaporated water was replenished with deionized water. 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 $MgSO_4$; 3.0 µM H_3BO_3; 0.5 µM $MnSO_4$; 0.2 µM $CuSO_4$; 0.4 µM $ZnSO_4$, 0.05 µM H_2MoO_4 and 20.0 µM EDTA-Fe^{3+}.

Sample preparation: At 14 DAT, seedlings were harvested and washed with deionized water properly. Water remaining on the surface of the plants was blotted with tissue paper and some primary readings were taken (shoot height, root length, tiller number etc.). Seedlings were separated with the stainless steel scissor into shoots and roots. Fresh weights of shoots and roots were taken and dried at 60 ± 2˚C for 48 h in an air-circulated oven (Isuzu Seisakusho Company, Tokyo, Japan). For mineral nutrition, oven dried samples were digested with a nitric acid-perchloric acid mixture (5:1 V/V) and analyzed. Amount of K, Ca, Mg, Fe, Mn, Zn and Cu were determined with atomic absorption spectroscopy (170-30 Hitachi, Tokyo, Japan). Phosphorus was determined colorimetrically using a UV-visible Spectrophotometer (model UV mini 1240, Shimadzu Corporation, Kyoto, Japan) at 420 nm wavelengths after developing the yellow color with vanadomolybdate (Imamul Huq and Alam, 2005).

Definition of the used terminologies: Concentration = mg or µg of element g^{-1} DW; accumulation in shoot = mg or µg of element $plant^{-1}$ shoot; accumulation in root = mg or µg of element $plant^{-1}$ root; accumulation was calculated by multiplying the concentration value with the DW of shoots or roots; and translocation (%) = nutrient accumulation in shoot/ total accumulation (shoot + root) × 100 (Shaibur et al., 2009abc).

Statistical analysis: The experiment was conducted in a completely randomized blocks with 3 replications. Data were presented as the means of 3 replicates (seedlings). Results were analyzed by ANOVA and with Duncan Multiple Comparison using the SAS 6.12 software package (SAS, 1988) using computer origin 5 at Iwate University, Japan. All statistically significant differences were tested at the $P \leq 0.05$ level.

Chemicals: Analytical reagent grade of chemicals were used. These were purchased from Cica Kanto Chemical Company, Tokyo, Japan. Solutions were prepared previously with MQ water [(18.2 MΩ cm^{-1}), purified by Milli-RO 60 (Millipore Corporation, USA)]. Arsenic stock solution was prepared by dissolving $NaAsO_2$ in MQ water and was kept at room temperature (around 25°C) in acid washed reagent bottle.

3. Results and discussion

Fresh weight: Fresh weight enhanced by 13.1% in shoot and limited by 50.9 and 86.6% for the same in 6.7, 33.5 and 67 µM As levels, respectively (Fig. 1 and Fig. 2a). Similarly, in root, FW enhanced by 96.5 % and limited by 4.10 and 74.4 % in the 6.7, 33.5 and 67 due to the limitation of plant growth with As-toxicity (Fig. 2a). The greatest amount of water was lost by the 6.7 µM As treated plants (Fig. 3). The highest amount of water might be lost through leaf surface. In 6.7 µM As level, the plant growth was the highest, resulting in the highest loss. However, the growth was the lowest in the 67 µM As level, resulting in the lowest loss of water (Fig. 3). This result indicated that the highest As concentration in the nutrient solution limited the availability of water to the roots. It is generally considered that As-stress inhibits plant growth by making water deficiency and hampering the nutrient balance like concentrations, accumulations and translocations (Shaibur et al., 2006).

Water content and water loss: Water content in the plants was measured by minusing the DW from that of FW. Seedlings contained 92.7, 92.2, 92.5 and 84.7% of water in the aerial parts in the 0, 6.7, 33.5 and 67 µM As levels, respectively. The WC content in leaf tissues limited with increasing As concentration in the medium (Fig. 2b). The limitation of WC in leaf tissues might be due to the limitation of plant growth with As-toxicity (Fig. 2a).

The greatest amount of water was lost by the 6.7 µM As treated plants (Fig. 3). The highest amount of water might be lost through leaf surface. In 6.7 µM As level, the plant growth was the highest, resulting in the highest loss. However, the growth was the lowest in the 67 µM As level, resulting in the lowest loss of water (Fig. 3). This result indicated that the highest As concentration in the nutrient solution limited the availability of water to the roots. It is generally considered that As-stress inhibits plant growth by making water deficiency and hampering the nutrient balance like concentrations, accumulations and translocations (Shaibur et al., 2006).

Width of leaf blade and leaf length: Width of leaf blade was measured in the middle position of the fully expanded 5th leaf. At 6.7 µM As level, the width seems to be enhanced as compared to control, however, were limited by 33.5 and 67 µM As levels (Fig. 4a). This result may suggest that little concentration of As may enhance the activity of elongating hormone. Similar results were also obtained for leaf length (Fig. 4a).

Leaf number and leaf area: Leaf numbers were similar in the 0, 6.7 and 33.5 µM As levels, though they seemed to be enhanced in the 6.7 µM As level as compared to others (Fig. 4b). Leaf number enhanced by 14.4% in the 6.7 µM As level but limited by 6.25 and 34.4% in the 33.5 and 67 µM As levels, respectively. Leaf area was also the highest in the 6.7 µM As level but limited by the higher As levels (Fig. 4b). Leaf area enhanced by 10.2% in 6.7 µM As level, but limited by 49.6 and 75.8% in the 33.5 and 67 µM As levels, respectively. Leaf area was measured by multiplying the length of leaf blade of the 5th leaf to the width of the middle position of the same leaf. The JM-spinach seedlings were planted at 5th leaf stage. On 14 DAT, only two new leaves came out at 67 µM As level though they were not fully developed.

Tiller number: We did not see any new tiller even in control treatment after introducing the plants with As-toxicity. Therefore, it was not wise to make a comment regarding this. It could only be said that the duration of the experiment might not be sufficient for the formation of new tiller of JM-spinach or JM-spinach could be the single tiller plant.

Root volume: Root volume increased in 6.7 µM As level as compared to others (data not shown), however, decreased with increasing As concentrations. Our result showed that little concentration of As in the growth medium showed slight stimulating effect on root growth. We believe that the data of root volume in As-treated JM-spinach seedlings has not been determined yet.

Arsenic: Arsenic concentration (Shaibur and Kawai, 2009) and accumulation (data were not shown) enhanced with increasing As concentration in the nutrient solution. This was believed to be due to high available As in the nutrient solution. Our result showed that the phytoavailability or bioavailability of As was depended on the As concentrations in the medium. The enhancement of As concentrations (Shaibur and Kawai, 2009) was not proportional with the As concentrations in the medium. Arsenic concentration was 13.1, 13.0 and 6.57 times higher in DW as compared to FW at 6.7, 33.5 and 67 µM As levels, respectively (Shaibur and Kawai, 2009).

Phosphorus: Phosphorus concentrations were higher or within the normal concentration in shoots though it was limited by the 67 µM As level as compared to other treatments (Tables 1 & 2). Similar trends were also found in roots (Tables 1 & 2). Phosphorus accumulation limited both in shoot and in root in the 33.5 and 67 µM As levels as compared to others (Table 3). Limitation of P accumulation was expected as the FW of the shoots were limited by those As levels (Fig. 2a). Laizu (2007) reported that the leafy vegetable spinach contained 0.34 ± 0.11 mg P g^{-1} freeze dry

Figure 1. Photograph of JM-spinach (Japanese Mustard spinach) grown in different levels of As. This picture was taken after 14 days of As treatments.

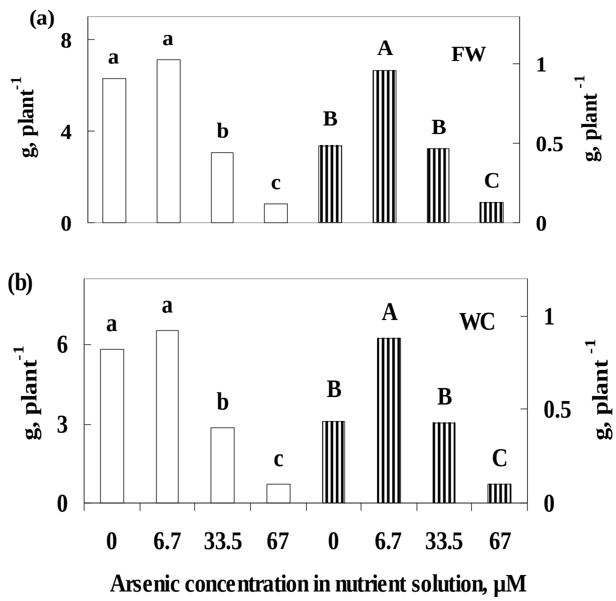

Figure 2. (a) Fresh weight, FW and (b) Water content, WC in shoots and roots of As-stressed JM-spinach (Japanese Mustard spinach) seedlings grown in nutrient solution. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test.

weight, kalmi contained 1.70 ± 1.41 mg P g^{-1} freeze dry weights, pui shak contained 0.45 ± 0.19 mg P g^{-1} freeze dry weight, data shak contained 1.54 ± 0.50 mg P g^{-1} freeze DW and lal shak contained 2.74 ± 0.92 mg P g^{-1} freeze dry weights. In our experiment, P translocation was not much affected by the applied As treatments (Table 4).

Potassium: Arsenic-toxicity limited K concentrations both in shoot and root in the 67 µM level (Table 1), indicating that As hampering K nutritional status in JM-spinach. Limitation of K concentrations might also be due to Na concentrations, because As was used as $NaAsO_2$. In this experiment, K concentration in the shoot of control seedlings was normal but in the highest As treatment it was in deficient level (Tables 1 & 2). Potassium concentrations as high as 100 to 140 mg g^{-1} DW can occur in hydroponically grown plants (Alam et al., 2003). In the present experiment, the highest As concentration limited not only K concentrations (Table 1) but also accumulations (Table 3). However, K translocation was not much affected by the applied As levels (Table 4).

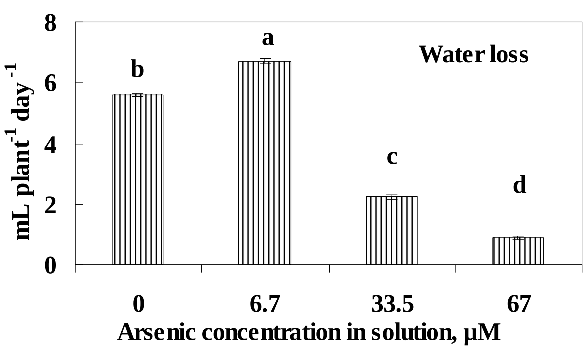

Figure 3. Water loss of As-stressed JM-spinach (Japanese Mustard spinach) grown in nutrient solution with different levels of As. This water loss included the loss of water through evaporation and evapotranspiration. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test.

Table 1. Concentration of Nutrients in Shoots and Roots of Leafy JM-spinach Grown Hydroponically with Different Levels of As

	------------------mg g^{-1} DW---------------				---------------------µg g^{-1} DW-----------------			
Treatment (µM As)	**P**	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**
	Concentrations in shoots							
0	7.73 a	102 a	24.7 a	5.92 a	61.5 b	26.6 b	41.6 b	4.87 a
6.7	6.48 a	94.9 a	22.5 a	5.12 a	80.1 a	23.6 b	43.2 b	4.60 a
33.5	7.95 a	93.1 a	22.2 a	6.61 a	82.7 a	33.1 a	61.7 a	5.17 a
67	3.23 b	38.0 b	10.1 b	3.93 b	46.0 c	14.7 c	30.8 c	4.80 a
	Concentrations in roots							
0	6.42 a	33.5 a	3.35 a	2.29 b	423 c	16.7 a	28.3 b	35.4 a
6.7	5.45 a	29.9 ab	3.25 a	3.49 a	528 b	10.6 b	28.5 b	38.7 a
33.5	6.32 a	34.8 a	3.62 a	2.63 b	383 c	19.4 a	48.9 a	38.0 a
67	2.76 b	26.5 b	2.71 b	3.70 a	727 a	5.54 c	18.2 c	39.3 a

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

Table 2. Concentration Range of Nutrient Elements in Common Leaf Tissues, this Values were Collected from the Literature

Range	------------------ (mg g^{-1} DW)-----------------				-------------------- (µg g^{-1} DW)------------------			
	P	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**
CDL	[1]1-2	[3]23	[5]1.5-2.1	[1]2	[6]30-50	[1]10-20	[1]0-15	[2]1-5
NL	[2]3-5	[4]100-140	[2]1-50	[2]1.5-3.5	[1]50-100	[3]30-300	[7]20-50	[1]<10
CTL	[2]>10	----------	---------	----------	[2]>500	[1]200-5300	[1]150-200	[2]>20-30

CDL (critical deficient levels); NL (normal levels); CTL (critical toxic levels); DW (dry weight). [3]Nutrient concentrations associated with critical and adequate nutritional status in leaf blade of hydroponic sweet potato (O'Sullivan et al., 1993).

References: [1]Mingle and Kirkby, 2001; [2]Marschner, 1998; [3]O'Sullivan et al., 1993; [4]Alam et al., 2003; [5]Dell and Robinson, 1993; [6]Bergmann, 1988; [7]Pedler et al., 2004.

Calcium: Calcium concentrations were similar in shoots in the 0, 6.7 and 33.5 µM As levels but was limited by the 67 µM As level (Table 1). Similar results were also found in roots for the same treatments (Table 1). Our experimental plants contained normal concentrations of Ca in shoots in spite of limiting by the highest As concentration (Tables 1 & 2). We found an intimate relationship between P and Ca concentrations in shoots and roots of JM-spinach, because P and Ca concentrations were limited only by the highest As concentration in the medium. Accumulations of Ca limited both in shoots and roots in the 33.5 and 67 µM As levels (Table 3). Limitation of Ca accumulations might be related to the limitation of P accumulations, because plant roots absorb Ca together with negative charged phosphate groups of membrane lipids (Caldwell and Haug , 1982).

Magnesium: Concentration of Mg limited in shoot in the 67 µM As level (Table 1), indicating that As-toxicity limited the nutritional value of Mg in JM-spinach. The experimental plants contained higher concentrations of Mg in the leaf tissues than the normal concentrations (Tables 1 & 2). In most instances, elevated Mg contents improve the nutritional quality of plants (Marschner, 1998). It means, limitation of Mg concentrations in shoots also limited the nutritional quality of JM-Spinach in the 67 µM As level.

Table 3. Accumulations of Nutrients in Shoots and Roots of JM-spinach Grown Hydroponically with Different Levels of As

Treatment (μM As)	----------mg plant^{-1}----------				----------μg plant^{-1}----------			
	P	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**
	Accumulation in shoot							
0	3.55 a	47.0 a	11.3 a	2.72 a	28.2 b	12.2 a	19.1 b	2.23 a
6.7	3.53 a	51.8 a	12.4 a	2.80 a	43.5 a	12.8 a	23.7 a	2.51 a
33.5	1.85 b	22.0 b	5.23 b	1.57 b	19.7 c	7.97 b	14.7 c	1.25 b
67	0.41 c	4.85 c	1.26 c	0.48 c	5.73 d	1.86 c	3.99 d	0.57 c
	Accumulation in root							
0	0.346 a	1.79 b	0.180 b	0.123 b	22.7 b	0.911 a	1.51 b	1.91 b
6.7	0.410 a	2.26 a	0.245 a	0.258 a	39.6 a	0.781 a	2.15 a	2.91 a
33.5	0.253 b	1.41 b	0.146 c	0.107 c	15.5 c	0.833 a	1.96 ab	1.52 b
67	0.056 c	0.54 c	0.055 d	0.073 d	14.8 c	0.104 b	0.36 c	0.79 c

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

Table 4. Translocations (%) of Elements from Roots to Shoots in JM-spinach Grown Hydroponically with Different Levels of As

Treatment	**P**	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**
0	91.1 a	96.3 a	98.4 a	95.7 a	55.4 a	93.1 a	92.7 a	54.0 a
6.7	89.8 a	95.8 a	98.0 a	91.5 a	52.3 a	94.2 a	91.6 a	46.2 b
33.5	87.9 a	94.0 a	97.3 a	93.7 a	56.2 a	90.9 a	88.2 a	44.4 b
67	87.6 a	89.6 a	95.7 a	86.6 a	27.7 b	94.1 a	90.9 a	42.2 b

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

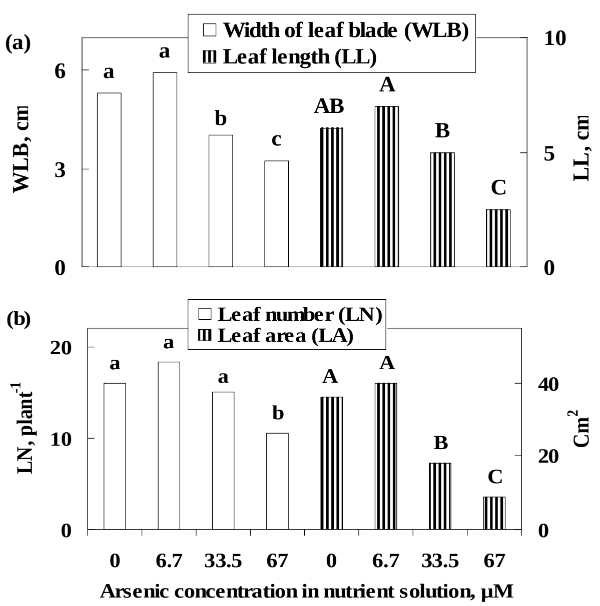

Figure 4. (a) Width of leaf blade (WLB) and Leaf length (LL); and (b) Leaf number (LN) and Leaf area (LA) of As-stressed JM-spinach (Japanese Mustard spinach) seedlings grown in nutrient solution. Bars with different letters are significantly different (p <0.05) according to a Ryan-Einot-Gabriel-Welsch multiple range test.

In leaf tissues, the threshold value for the occurrence of Mg-deficiency symptoms is in the region of about 2 mg Mg g^{-1} DW although this is dependent on a number of factors including plant species (Mengel and Kirkby, 2001).

Iron: Iron concentration enhanced in shoots in the 6.7 and 33.5 μM As levels as compared to control (Table 1). However, Fe concentration was limited by the 67 μM As level as compared to other As levels (Table 1). Iron concentration in shoots was normal in 0, 6.7 and 67 μM As levels, however, was in the CDL in 67 μM As level (Tables 1 & 2). Our result indicated that As limited the nutritional quality of Fe in the 67 μM As level in the nutrient solution. Iron accumulation in shoot was limited in the 33.5 and 67 μM As levels as compared to control (Table 3).

Accumulations of Fe in root enhanced at 6.7 μM As level as compared to others (Table 3). Our result showed that root Fe concentration of JM-spinach was very high relatively to that of aerial parts (Table 1). Iron concentration in root was almost 70% and in shoot was almost 30%. The great part of total root Fe is formed of extraplasmic iron, which constitutes about 75% (Zribi and Gharsalli, 2002). Iron translocation from root to shoots was not much affected by the 6.7 and 33.5 μM

As levels as compared to control, however, was limited by the 67 µM As level (Table 4).

Manganese: The lowest concentration of Mn in shoots was recorded in the 67 µM As level. Shoot of 67 µM As treated plants contained Mn concentration in the CDL (Tables 1 & 2). The CDL levels of Mn in plants may vary between 10-20 µg kg^{-1} DW in fully expanded leaves, regardless of plant species or cultivar or prevailing environmental conditions. Below the CDL, dry matter production and net photosynthesis decreased rapidly (Marschner, 1998). Manganese is mostly involved with the activity of some enzymes. The most well known and best documented example of a Mn-containing enzyme is the 33 kDa polypeptide (protein) of the water-splitting system associated with photosystem-II (PS-II system; Marschner, 1998). In photosynthesizing cells, this role in PS-II is the most sensitive function of Mn to be impaired by Mn-deficiency. This phenomenon might be involved especially in the highest As concentration in this experiment, as the Mn concentration reduced much in that particular treatment.

Manganese concentration decreased much in the roots of 67 µM As level as compared to others (Table 1). Limitation of Mn concentration might be responsible for the limitation of root biomass production. In Mn-deficient plants, shortage of carbohydrates may take place resulting in the inhibition of root growth (Marschner, 1998). The rate of root elongation seems to be highly affected by Mn-deficiency (Abbott, 1967). Sometimes, in Mn-deficient plants the formation of lateral roots could be ceased completely (Abbott, 1967), therefore, limitation of Mn concentration might be responsible for the limitation of lower root biomass and lower lateral root in the highest As treatment in this experiment. Arsenic-toxicity did not affect Mn translocations (Table 4).

Zinc: Zinc concentration was limited in shoots by the 67 µM As level as compared to others (Table 1). Similar results were also obtained in the root for the same treatment (Table 1). The most characteristics visible symptoms of Zn-deficiency in dicotyledons are stunted growth due to shortening of internodes (rosetting) and drastic decrease in leaf size (Marschner, 1998). Under Zn-deficiency, shoot growth is usually more inhibited than the root growth (Zhang et al., 1991). In spite of limiting Zn concentration in 67 µM As level, the leaves of our test plants contain normal concentrations of Zn ranging from 30.8 to 61.7 µg Zn g^{-1} DW (Tables 1 & 2).

Accumulations of Zn limited in shoot at 33.5 and 67 µM As levels as compared to others (Table 3). Effect of As-toxicity has striking similarities to our previous findings of hydroponic rice (Shaibur et al., 2006). We did not see any change of Zn translocations with As-toxicity (Table 4).

Copper: Arsenic-toxicity did not affect Cu concentrations much in shoots and roots of JM-spinach (Table 1). Our test plants contained deficient concentrations of Cu in shoot tissues (Tables 1 & 2) and these lower concentrations of Cu might be responsible for the limitation of growth at the higher As levels. Spinach is basically much more sensitive than pea, rye and rape to Cu-deficiency (Marschner, 1998). In this experiment, accumulations were limited both in shoots and roots in the 33.5 and 67 µM As treatments (Table 3). Translocations of Cu were limited by the applied As in the nutrient solution (Table 4).

4. Conclusions

In general, As-toxicity limited concentrations and accumulations of nutrient elements at 67 µM level. No doubt As-toxicity limited the nutritional quality of JM-spinach by hampering the nutrient balance in the higher As concentration in the medium, though other biochemical reasons might also be involved which demands further study. Little concentration of As (6.7 µM) did not limit elemental concentrations and accumulations, rather fresh weight increased. Little concentration of As in the growth medium might not be harmful for JM-spinach nutritional quality, however, this supposition needs to be verified with experiment in practical agricultural field. The findings of the current result may be informative for the people of Bangladesh where As-contaminated water is being used for vegetables production.

Acknowledgement:

Dr. MR Shaibur is grateful to the Japanese Government for providing him the Monbukagakusho Scholarship which enables him to do this research in Japan.

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Date of submission:18/06/2010