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Effect of phosphorus on the concentrations of arsenic, iron and some other elements in barley grown hydroponically

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Abstract

Hydroponic barley (*Hordeum vulgare* L. cv. minorimugi) was treated with 10 µM arsenic (As) together with 500, 250, 50 and 0 µM phosphorus (P) for 14 days to observe the response of the seedlings. The plants were also treated with 0 µM As + 0 µM P. Iron (Fe)-plaque was visible in the roots under As-treated and P-depleted conditions. The intensity of reddish coloration in the roots decreased with increasing P in the media. However, reddish coloration was not found in the absence of both As and P. The results show that both As and P played a vital role in the formation of Fe-plaque. Additionally, Fe-plaque formation was dependent on the concentration of P in the media. Iron concentration in the roots of the As treated plants without P was higher than that observed in the roots treated with 0 µM As + 0 µM P which suggests that As may have been adsorbed with Fe on the surface of roots, forming Fe-plaque. Arsenic concentration in the shoots and the roots was low under 500 and 250 µM P conditions while high under 50 and 0 µM P conditions. It was found that a higher concentration of P (50 µM; 5 times of As level) was required in the medium to reduce As concentration in the plant tissues.

Keywords: As-Fe complex, barley roots, phosphate, reddish color Fe-plaque, translocation

1. Introduction

Arsenic (As) is an important environmental and health concern due to its chronic and epidemic toxicity. It is widely distributed in nature. Small amounts of As may hamper the growth and nutritional quality of plants. Plants respond to As-toxicity by showing some physiological changes in their shoots and roots. One of the symptoms of As - toxicity in roots is the formation of iron (Fe)-plaque in barley (Shaibur *et al.*, 2008, 2009) and rice (Shaibur and Kawai, 2011a, b). Formation of a reddish color on the root surface of aquatic plants under As-toxicity is due to Fe-plaque. Aquatic plants, such as rice, form Fe-plaque as coating of Fe hydroxides/oxides on their roots. Iron-plaque results from the oxidation of roots by release of oxygen or oxidants into the root rhizosphere (Chen *et al.*, 2005; Liu *et al.*, 2005) and has been reported to consist of a mixture of amorphous and crystalline Fe (Bacha and Hossner, 1977; Chen *et al.*, 1980).

The presence of phosphate can give rise to decrease or increase in the uptake of As by plants depending on the species of As or plants and on the composition of the rooting medium (Tsutsumi, 1980). Arsenic uptake has been shown to increase after application of P in soil (Small and McCants, 1962; Rahman and Parkpian, 2004). It has also been reported that application of P fertilizer to soil increased (Peryea, 1998) or decreased (Hanada *et al*., 1975) the phytoavailability or bioavailability of As. Phosphorus and As are in the same group on the periodic table and have similar atomic configuration. Phosphate and arsenate are analogues of each other and therefore compete for the same sorption sites in root apoplast and for the same uptake system in the root plasmalemma (Asher and Reay, 1979 ; Rahman *et al*., 2008).

In most of the cases, As in the nutrient solution decreased the concentration of nutrients elements like potasium (K), calcium (Ca), magnesium (Mg), Fe, manganase (Mn), zinc (Zn), and cooper (Cu) in the aerial parts of rice seedlings (Shaibur *et al.*, 2011a, b; Shaibur *et al*., 2012). Some reports have shown that Fe plaque can act as a barrier to the uptake of heavy metals such as Cu, cadmium (Cd), Mn and nickel (Ni)(Greipsson 1995; Wang and Peverly 1999; Batty *et al*., 2000). Some reports have shown contrary results indicating that Fe plaque was found to increase the uptake of toxic and nutrient elements (Zhang *et al*., 1998; Ye *et al.*, 2001). It therefore appears that the overall effect of Fe plaque on the uptake of toxic and nutrient elements may be related to the amounts of Fe plaque (Zhang *et al.*, 1998).

Arsenic (III) is a powerful inhibitor of the sulfhydryl groups found in some enzymes and tissue proteins. They attack plant cell membranes causing an inhibition of cellular function and death (Sizova *et al.*, 2002). Many studies have been done by adding P in the presence of arsenite or arsenate to soil or under hydroponic conditions. However, physiological data related to the response of plants at high to low P conditions in hydroponic culture are hardly available. Physiological response of plants to As - toxicity may vary with decreasing P levels. The present experiment was, therefore, conducted with different P levels with the aim to determine the effective P concentration required to reduce As absorption in barley. Thus, physiological and nutritional responses of barley to As -toxicity under varied P levels were examined focusing on the concentrations of As, P and Fe in plant tissues. We chose barley because it is widely distributed around the world.

2. Materials and Methods

2.1. Seedling preparation and plant culture

The seeds (*Hordeum vulgare* L. cv. Minorimugi) were grown with methods described by Shaibur *et al*. (2008). The plants were grown up to 14 days after treatments (DAT) and harvested when nutrient deficiency symptoms or inhibitory effects were very apparent. Arsenic was used in the form of sodium meta-arsenite ($NaAsO_2$; Kanto Chemical Company, Tokyo, Japan) and P was added as ammonium phosphate ($NH_4H_2PO_4$; Kanto Chemical Company, Tokyo, Japan). The solutions of the growth media were renewed every week, aerated throughout the experiment (September-October 2006) and their levels were maintained by adding deionized water. Arsenic concentration in the medium (i.e. 10 μM) was used on the basis of the results of preliminary experiments. Arsenic concentration of 10 μM did not produce any acute toxicity symptom. We used $NaAsO_2$ in the medium under aerated conditions so as to enhance the conversion of some arsenite to arsenate. Together with As, the following concentrations of P were added 500, 250, 50 and 0 μM. There was also a control where the plants were treated with 0 μM As + 0 μM P.

2.2. Chlorophyll index

The chlorophyll index of the third leaf was measured after harvesting using a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo, Japan). The third leaf was the fully developed youngest leaf which emerged after the initiation of the As treatments. Chlorophyll index was measured at five different points on each leaf and then values for three leaves were averaged.

2.3. Chemicals used

All the chemicals used were of analytical reagent grade. The solutions were prepared with MQ water (18.2 MΩ cm-1, purified by Milli-RO 60, Millipore Corporation, USA). Stock solution of As was prepared by dissolving $NaAsO_2$ in MQ water.

2.4. Experiment setup, sample collection and data analysis

Our pots had 16 bunches (3 plants/bunches) which were separated into 4 groups. Thus, each group consisted of 4 bunches. Three bunches were taken from each group and this was done in triplicates. The seedlings were collected and washed with deionized water on 14 DAT. Shoots and roots were separated and dried at 60 ± 5˚C for 48 hour in an oven (Isuzu Seisakusho Company, Tokyo, Japan). Oven-dried samples were digested with a nitric acid-perchloric acid mixture and analyzed for elements. The amounts of K, Ca, Mg, Fe, Mn, and Zn and Cu were determined by the methods previously described by Shaibur and Kawai (2011a, b). Arsenic was measured using hydride generation atomic absorption-flame emission spectrophotometer (AA-6200; Shimadzu Corporation, Kyoto, Japan). The concentrations of As detected in all samples were higher than 0.01 µM which was the detection limit of the instrument. All the data were subjected to analysis of variance with the computer system "sas" at Iwate University. Differences between means were evaluated by using the Ryan-Einot-Gabriel-Welsch multiple range test ($p<0.05$). We duplicated the reagent blank to ensure accuracy in the analysis.

2.5. Explanation of used terminologies

Concentration was expressed in mg or µg of element g^{-1} dry weight (DW) while accumulation in shoot or root as mg or µg of element $shoot^{-1}$ or $root^{-1}$. Translocation of nutrient was determined as accumulation in shoot over total accumulation plant (i.e. in shoot + root) in percentage.

3. Results

3.1. Visible symptoms

Shoots subjected to 10 µM As + 500 µM P, 10 µM As + 250 µM P and 0 µM As + 0 µM P treatments were almost similar in color (Figure 1). However, interveinal chlorosis was observed in the young leaves of 10 µM As + 50 µM P and 10 µM As + 0 µM P treatments (data not shown). A severe growth reduction (i.e. wilted leaves and reduced turgidity) and necrosis in older leaves were observed in the 10 µM As + 0 µM P treatment (Figure 1).

Intensity of reddish coloration in roots increased with decreasing P concentrations in the growth medium which also contained As (Figure 1). Conspicuous reddish coloration (Fe-plaque) appeared in plants at 10 µM As + 0 µM P treatment (Figure 1). Iron-plaque, however, was not observed at 0 µM As + 0 µM P treatment. Roots were very long and thin at 0 µM As + 0 µM P treatment but not highly branched (Figure 1).

3.2. Chlorophyll index

In the presence of As, chlorophyll index decreased with decreasing P concentrations in the medium (Table 1). The lowest chlorophyll index was found in the 10 µM As + 0 µM P treatment. Chlorophyll index was relatively higher in the 0 µM As + 0 µM P treatment compared with that of 10 µM As + 0 µM P (Table 1).

3.3. Leaf number, leaf blade width, DW, shoot height and root length

Leaf number and leaf blade width decreased with decreasing P in the medium (Table 1). Arsenic treated plants showed decreased shoot and root DW with decreasing P in the medium. The lowest DW was

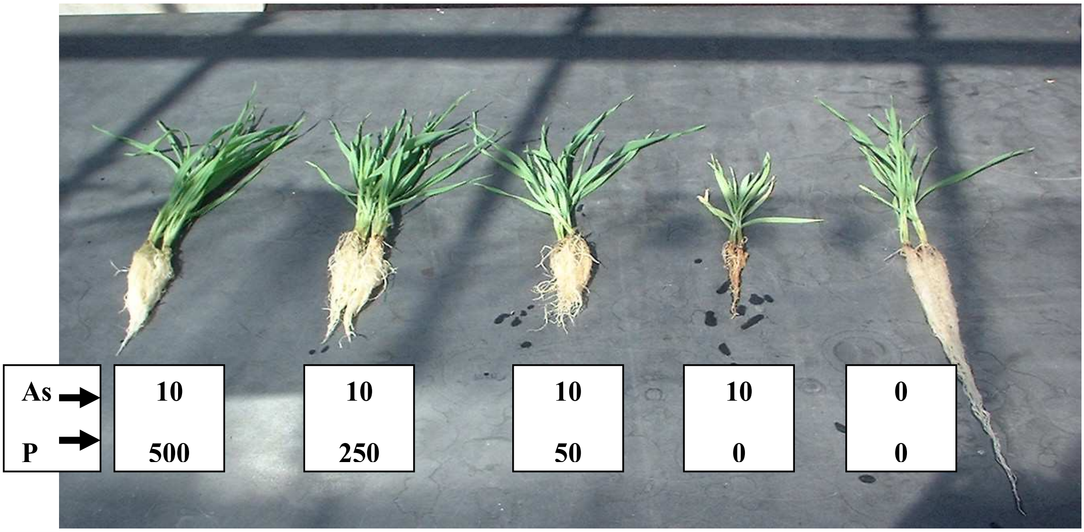

Figure 1. Photograph of barley seedlings as affected by different P (µM) levels in nutrient solution. This picture was taken after 14 days of As exposure. Figure showing that reddish color intensity increased with decreasing P and conspicuous reddish color Fe-plaque was formed in absence of P but in presence of As.

Table 1. Agronomic parameters of barley seedlings grown in different levels of P and As.

Treatments (µM)		Dry weight (mg plant^{-1})		Height (cm)	Length (cm)	Leaf width (mm)	Leaf no. (plant^{-1})	Chlorophyll index
As	P	Shoot	Root	Shoot	Root			
10	500	156.8a	61.5a	37.7a	13.0b	9.33a	4.78a	38.6a
10	250	170.5a	63.3a	35.2a	15.0b	9.00a	4.44a	38.4a
10	50	128.5b	57.5a	38.3a	14.3b	8.67a	4.00ab	33.3b
10	0	67.0d	20.3b	24.7b	10.0c	5.00b	3.00b	14.2c
0	0	106.1c	62.5a	36.7a	35.3a	9.00a	3.56b	33.6b

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

observed at the 10 μM As + 0 μM P treatment (Table1). Shoot height did not seem to have been affected much except at the10 μM As + 0 μM P treatment. Root length was lowest at the10 μM As + 0 μM P treatment (Figure 1).

3.4. Macro and micronutrients

Phosphorus concentration increased in both shoots and roots with increasing P concentration in the medium. The lowest P concentrations was found in the shoots and roots at 0 μM As + 0 μM P treatment (Table 2).

A similar trend was found in the case of P accumulation (Table 3). In the case of K and Mg, the lowest concentrations (Table 2) and the lowest a accumulations (Table 3) were found at the 10 μM As + 0 μM P treatment. Translocation of K and Mg was the highest at the 10 μM As + 0 μM P treatment (Table 4).

Table 2. Concentrations of elements (mg/μg g^{-1} DW) in shoots and roots of barley seedlings grown in different levels of P and As.

Treatments (μM)		P	K	Ca	Mg	Fe	Mn	Zn	Cu
		----------------mg g^{-1} DW				----------------μg g^{-1} DW			
As	P	Concentrations in shoots							
10	500	4.86a	82.0a	5.69ab	1.12b	82.4a	18.5b	16.7a	7.78b
10	250	4.44a	82.7a	5.69ab	1.17b	79.3a	22.6b	14.8a	7.78b
10	50	2.86b	73.6a	4.70b	1.06b	59.4b	22.9b	7.21b	9.96a
10	0	0.79c	54.5b	5.87ab	0.83c	26.3c	6.58c	5.79c	5.91c
0	0	0.46d	80.0a	6.58a	1.53a	63.8b	29.6a	7.72b	9.03a
		Concentrations in roots							
10	500	5.64a	75.1a	1.41a	1.75b	129.1b	15.7b	22.0b	23.6b
10	250	4.91a	72.2a	1.63a	1.66b	119.0b	19.7b	19.2c	32.2a
10	50	1.67b	46.0b	1.51a	1.33c	113.8b	30.3a	17.5c	16.2c
10	0	1.80b	20.5c	1.29a	0.36d	262.6a	3.26c	23.8b	28.1ab
0	0	0.47c	53.8b	1.49a	2.58a	24.3c	34.5a	40.1a	25.4b

Means followed by the different letters in each column are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. Concentration refers to the amount of element g^{-1} of samples in dry weight (DW; mg/μg of element g^{-1} DW).

Iron concentrations in the shoots increased with increasing P level in the medium (Table 2). Iron concentration in the roots, on the other hand, was not very much affected by the level of P (Table 2). However, interestingly, Fe concentration increased abruptly in the roots at 10 μM As + 0 μM P treatment (Table 2), suggesting that Fe might have been adsorbed together with As onto the root surface in the absence of P. Iron translocation decreased in the 10 μM As + 0 μM P treated plants compared with the other treatments (Table 4). The amount of Fe translocated at 10 μM As + 0 μM P treatment was 24.3% whereas at the 0 μM As + 0 μM P treatment it was 81.4% (Table 4).

In the presence of P, Mn concentration in the shoots was not very much affected by As. However, the concentration of Mn in the shoots decreased under depleted P conditions, a result which was similar to what happened in the case of Fe, Zn and Cu concentrations (Table 2). Similar to Fe and Zn, translocation of Mn decreased with decreasing P concentrations in the medium (Table 4).

3.5. Arsenic

Arsenic concentrations were higher in the shoots and roots of the 10 μM As + (50 or 0 μM P) treatments but lower in the 10 μM As + (250 or 500 μM P) treated plants (Figure 2A). In the shoots, the highest As concentration was found at 10 μM As + 0 μM P. Similar to concentration, As accumulation was also higher under the 10 μM As + 50 and 10 μM As + 0 μM P conditions (Figures 2A, 2B). Arsenic translocation was not very much affected by the P levels in the medium although it was lower at 10 μM As + 50 μM P where reddish coloration was observed on the roots (Figures 1, 3).

4. Discussion

The plants showed chlorosis in the young leaves at the 10 μM As + 0 μM P treatment which may have been due to low Fe concentration in the shoots (Marschner, 1998). The low Fe concentration in the shoots was most probably due to toxic effect of As. The critical deficient level (CDL) of Fe (66-72 μg Fe g^{-1} DW) was almost similar for C3 and C4 plant species (Marschner, 1998). At this critical concentration, plants show whitish chlorosis in the leaves. In the present experiment, the old leaves and chlorotic young leaves were digested together and the plants contained 59.4 and 26.3 μg Fe g^{-1} DW in shoots (Table 2) at 10 μM As + 50 μM P and 10 μM As + 0 μM P treatments, respectively. Therefore, the lower concentration of Fe might be mostly responsible for the occurrence of chlorosis in the young leaves. Arsenic damages the chloroplast membrane (chloroplasts are the targets of the toxic As) and disorganizes the membrane structure (Miteva and Merakchiyska 2002; Stoeva *et al.*, 2005), resulting in lower chlorophyll in low P containing solution. Arsenic decreases chlorophyll and carotenoid contents (Miteva and Merakchiyska 2002; Stoeva *et al.*, 2005). Almost all the elements decreased in the shoots at the 10 μM As + 0 μM P treatment (Table 2), a condition which may have also been responsible for the occurrence of chlorosis in the young leaves (Marschner, 1998). Meharg (2004) suggested that rice exhibited Fe-deficiency due to the formation of Fe-plaque on its roots in the presence of As. It is reported that Fe-plaque may act as a “buffer” for antimony Sb (V) and Sb (III) in the rhizosphere (Huang *et al.*, 2012). Most aquatic plant species, including rice can form Fe-plaque on their roots by oxidizing Fe (II) to Fe (III) (Greipsson, 1995; Wang *et al.*, 1999). We observed conspicuous reddish coloration in the roots at the 10 μM As + 0 μM P treatment (Figure 1). Reddish plaque formation in or on rice root may be governed by As concentration (Shaibur and Kawai 2011a, b), P nutritional status (Liu *et al.*, 2004) and Sb status (Huang *et al.*, 2012) in the growth medium. Rice treated with 6.7 μM As (arsenate) showed reddish coloration in the roots after 24 hours of being grown in the medium without P (Liu *et al.*, 2004). Our result also showed that in the presence of As, Fe-plaque was formed under depleted P conditions and the intensityof the plaque decreased with increasing P in the medium (Figure 1).

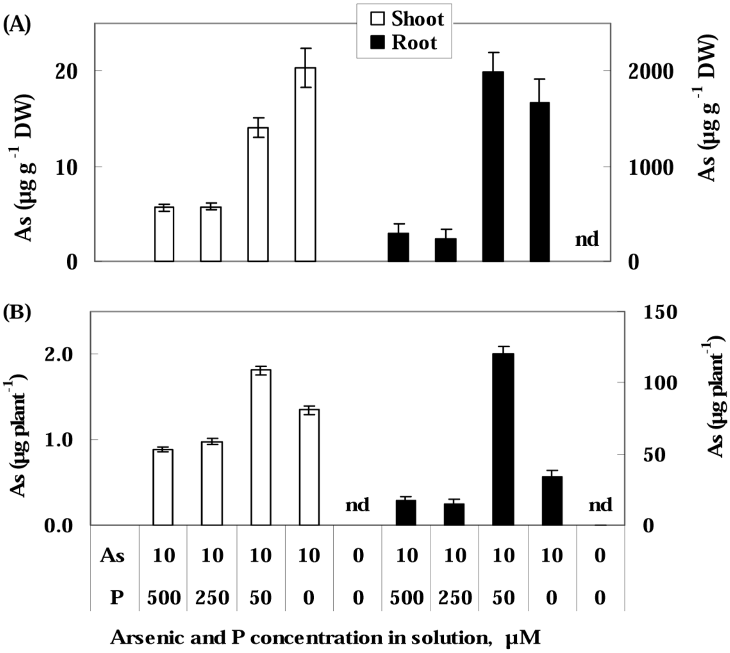

Figure 2. Effect of P on the (A) concentration and (B) accumulation of As in shoots and roots. nd = not detected, DW=dry weiht.

In the roots, Fe concentration was 24.3 µg g^{-1} DW at the 0 µM As + 0 µM P treatment (Table 2). Iron concentration was, however, 262.6 µg g^{-1} DW at the 10 µM As + 0 µM P treatment. This sharp increase might have been caused by the addition of As to the medium. It seems that a strong Fe-As complex may have been formed in the absence of P in the medium and adsorbed onto the cell wall or the membrane of the roots. This phenomenon may have resulted in the high concentration of Fe and As in or at the surface of the roots (Table 2; Figure 2A). It was reported that As and P could strongly be adsorbed on amorphous Fe oxide (Jacobs *et al*., 1970), though antagonistic relationship occurs between them (Asher and Reay, 1979).

Iron-plaque formation may increase Fe concentration in roots. It is formed in or at the root surface of wetland plants through the oxidation of root Fe (Batty and Younger, 2003). Oxygen or an oxidant is released by the roots of aquatic plants (Armstrong, 1967). Parts of the arsenite applied may have converted to arsenate as a result of the oxidation in the root rhizosphere. It is known that arsenate has high affinity for Fe-plaque as it co-precipitates with Fe^{3+} on the plaque (Otte *et al*., 1991; Meharg, 2004).

Arsenite itself could be concentrated on the Fe-plaque of roots in the form of $H_3AsO_3^0$ and then transported into root cells via aquaporins (Meharg and Jardine, 2003). These phenomena may have been involved in the increase of Fe and As in the roots of the plants.

Arsenic concentrations were significantly higher in shoots and roots of the plants grown in low P or P-depleted media than in those of the plants grown in

Table 3. Accumulations of elements (mg/µg plant-1) in shoots and roots of barley seedlings grown in different levels of P and As.

Treatments (µM)		P	K	Ca	Mg	Fe	Mn	Zn	Cu
		----------------mg plant^{-1} ----------------				-----------------µg plant^{-1}------------------			
As	P	Accumulation in shoots							
10	500	0.778a	12.8a	0.895a	0.177ab	12.9a	2.93b	2.64a	1.21a
10	250	0.754a	14.1a	0.970a	0.199a	13.5a	3.85a	2.51a	1.33a
10	50	0.372b	9.44b	0.604b	0.137c	7.81b	2.99b	0.91b	1.28a
10	0	0.053c	3.63c	0.379c	0.056d	1.71c	0.43c	0.38c	0.40c
0	0	0.049c	8.47b	0.689b	0.161b	6.82b	3.15ab	0.81b	0.96b
		Accumulation in roots							
10	500	0.349a	4.60a	0.087a	0.107b	8.00a	0.95d	1.38b	1.40b
10	250	0.312a	4.57a	0.103a	0.106b	7.54a	1.25c	1.21b	2.04a
10	50	0.096b	2.65c	0.087a	0.077c	6.59ab	1.77b	1.00c	0.94c
10	0	0.036c	0.41d	0.026c	0.007d	5.33b	0.06d	0.48d	0.57d
0	0	0.029d	3.36b	0.094b	0.161a	1.52c	2.16a	2.50a	1.59b

Means followed by the different letters in each column are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. Accumulation/uptake refers to the total amount of element plant^{-1} shoot or plant^{-1} root (mg/µg of element plant^{-1}).

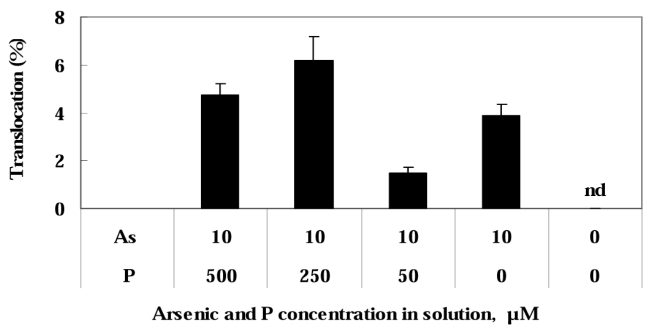

Figure 3. Effect of P on the translocation of As from roots to the shoots. Translocation refers to the ratio of accumulation of element in shoot to the total accumulation (shoot + root). The translocation was expressed in %. nd = not detected.

in high P-supplied media (Figure 2A). It is reported that As concentrations were significantly higher in the roots of P-depleted medium compared with those in P-containing medium in rice treated with arsenate (Liu *et al.*, 2004). Our results show that the accumulations of As and Fe in the roots were positively correlated but, negatively in the shoots. This relationship of the two elements might have been responsible fo r the formation of Fe-arsenate or Fe-plaque inside or outside of roots. A similar positive correlation between arsenate and Fe concentrations was reported to have occurred in the roots of duckweed (Rahman *et al.*, 2008). The formation of Fe-plaque in or at root surface of plants has not been well investigated considering the relationship among concentrations and translocations of As, P and Fe.

Dry weight and chlorophyll index decreased in the 10 µM As + 0 µM P treatment compared with P containing treatments (Table 1). This was most probably due to the decline in the concentrations and accumulations of P, K, Mg, Fe, Mn, Zn and Cu in the shoots (Tables 2, 3) coupled with the increase in As concentration in both the shoots and roots (Figure 2A). Growth reduction in the 10 µM As + 0 µM P treatment was also probably due to the presence of As and the absence of P in the medium. Furthermore, growth reduction in As treated and low P or P-depleted media was most probably due to the reduction of enzyme activity (Jain and Gadre, 2004; Mishra and Dubey, 2006) and/ or due to As-induced oxidative stress (Singh *et al.*, 2007). Arsenic induces toxicity by attaching itself to the sulfhydryl group of root protein resulting in the disruption of root functions (Speer, 1973) and even cellular death (Sizova *et al.*, 2002).

Phosphorus concentration and accumulation in plant tissues decreased with decreasing P in the media (Tables 2, 3). On the contrary, As concentration increased in both the shoots and roots. Competition could occur between phosphate and arsenate during uptake. Arsenate uptake was negatively correlated with phosphate uptake in duckweed (Rahman *et al.*, 2008). Mkandawire and Dudel, (2005) suggested that arsenate uptake in *Lemna gibba* L. might occur through the phosphate uptake system. Phosphorus deficiency in shoots and roots of barley may result in an increase in the release of oxygen from the roots which may have stimulating effect on Fe-plaque formation. Kirk and Du (1997) reported that P deficiency may result in the release of oxygen from rice roots.

Our result shows that As-toxicity was higher in low P or P-depleted conditions. At the same As concentration, the plants grown in media with low P were severely affected. Thus, it could be concluded that application of P might be effective in reducing As-toxicity. Pigna *et al*. (2009) reported that application of inorganic P fertilizer prevented As-toxicity in wheat grown in a pot experiment. Therefore, under low P or P-depleted conditions, high amounts of As might be absorbed resulting in the higher concentration and accumulation in both shoots and roots. Furthermore, our results show that more than 50 µM P was required to reduce As absorption. It is known that P is immobilized depending on the characteristics of soils. Phosphate concentration in soil solution may not always exceed 50 µM even after the application of P fertilizer. The mechanism for the effect of P on As absorption by plants in soil conditions is not well understood (Hanada *et al.*, 1975; Peryea, 1998; Pigna *et al.*, 2009) and therefore needs to be further investigated. More studies focused on P and As concentrations in soils need to be conducted in order to determine the amount of P needed to minimize As-toxicity in the field.

Table 4. Translocations of elements (%) in shoots and roots of barley seedlings grown in different levels of P and As.

Treatments (µM)		P	K	Ca	Mg	Fe	Mn	Zn	Cu
As	P	Translocation (%)							
10	500	68.8ab	73.6b	91.2a	62.3b	62.1b	75.2b	66.2a	46.3b
10	250	70.6ab	75.5b	90.4a	65.3b	64.2b	75.5b	67.4a	39.4bc
10	50	79.2a	78.1b	87.4a	64.0b	53.4c	63.3c	47.7b	57.8a
10	0	59.0b	89.8a	93.4a	88.1a	24.3d	86.9a	44.3b	40.9bc
0	0	62.1b	71.5b	88.0a	50.0c	81.4a	59.2	24.4c	37.5c

Means followed by the different letters in each column are significantly different (p= 0.05) according to 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 was expressed in %.

5. Conclusions

The formation of reddish color Fe-plaque was related to the P concentration in the growth medium containing As. The most intense reddish color was found in the 10 µM As + 0 µM P treatment. Arsenic and Fe were mostly concentrated in the plant roots grown in P-depleted medium. Arsenic-toxicity was directly correlated with As accumulation, which increased with decreasing P concentration in the growth medium. The severest As-toxicity was observed when there was no P in the growth medium. The P concentration needed to reduce As absorption was found to be > 50 µM which was 5 times higher than the concentration of As. Under natural field conditions, P concentration in soil solution may not be higher than 50 µM even after the application of P fertilizer. Further study is, therefore, necessary to elucidate the amount of P required in the form of fertilizers to minimize As-toxicity in plants.

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