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## Effect of Phosphorus or Silicon on the Arsenic Content of Japanese Mustard Spinach (*Brassica rapa* var. *perviridis*) Grown on Arsenic Contaminated Soil

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### Authors' contributions

This work was a part of the PhD thesis of the lead author JCJ. The second author SK was the principal supervisor. Both the authors designed the study, managed the experiment and analyzed the samples. The author JCJ conducted statistical analysis, wrote the protocol and the first draft of the manuscript. Both the authors read and approved the final manuscript.

Original Research Article

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### ABSTRACT

Two experiments were conducted about the effect of the application of phosphorus (P) or silicon (Si) on the arsenic (As) contents of Japanese mustard spinach (JMS) (*Brassica rapa* var. *perviridis*) grown on As contaminated soil (30.6 mg As kg<sup>-1</sup>). The P was applied with four different P fertilizers derived from both organic or inorganic sources viz. chicken manure (CM), bone powder (BP), fused magnesium phosphate (FMP), and super phosphate (SP). The P was applied at two rates (50 and 100 kg citrate soluble P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). The As contents (mg kg<sup>-1</sup> dry weight [DW] and/or µg plant<sup>-1</sup>) in the edible part of JMS reduced significantly as compared to the As contents in control plant with the application of P, irrespective of the form of fertilizer and to the application rate of P. There was no significant difference among the P fertilizers as well as the rate of P application in reducing As contents in the plant. The growth of the plant and P concentration in the plant part increased significantly with the application of P. In the Si application experiment, Si was applied from three different sources viz. potassium silicate (PS), magnesium trisilicate pentahydrate (MTP) and Iherzolite (LE). Silicon was applied at the rate of 1,050

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kg SiO<sub>2</sub> ha<sup>-1</sup>. The As contents in the edible part of JMS increased, but not significantly, as compared to the As contents in control plant with the application of Si irrespective of the source of Si. There was no significant difference in the As contents in the edible part of the plant among the Si sources. The magnesium content in the plant part was increased significantly with the application of MTP and LE. The growth of the plant and the other mineral concentrations in the plant part was statistically similar as compared to the control plant. The results of two experiments indicated that the application of P could reduce the As contents in the edible part of JMS whereas Si had no effect to reduce As content in JMS under the employed condition.

*Keywords: Arsenic; soil; contamination; organic and inorganic phosphorus fertilizer; silicon fertilizer; Japanese mustard spinach.*

## 1. INTRODUCTION

Arsenic (As) is a toxic and carcinogenic element that occurs naturally in the environment through geological activities. Arsenic is naturally present in the soil all over the world with a variation depending on the origin of the soil [1] and the background concentrations are around 5 mg kg<sup>-1</sup> [2]. Soil contamination with As occurs through both natural and anthropogenic activities such as mining and smelting operations [3], fossil fuel combustion [4, 5] and As-based agrochemicals [6], fertilizers and disposal of municipal and industrial wastes [7]. Irrigation in agricultural land with As contaminated groundwater or wastewater, particularly in Bangladesh, India and south-east Asia, leads the accumulation of As in both soils and plants, posing risks to soil ecosystems and human health [8–13].

Phosphorus (P) is an essential nutrient element to plant while As is an unessential element with toxicity to plant. Arsenic exists in the environment in both inorganic and organic forms, and both arsenite and arsenate are often found in both anaerobic and aerobic soil environments. Both As and P belong to the V family on the periodic table of chemical elements. Due to their similarities in electron configurations and in chemical properties, they behave similarly in many ways in the soil-plant system. Under oxic/aerobic soil conditions, As is predominantly present as arsenate [14] which is chemically an analogue of phosphate. In soil, due to this similarity, dissolved phosphate and arsenate competes with each other for the same soil sorption binding sites and this competition results in a reduction in their sorption by soil and an increase in solution concentrations [15–17]. They also compete for the same sorption sites in the root apoplast and for the same uptake carriers in the root plasmalemma [18–23]. Phosphate can decrease or increase the uptake of As by plants, depending on the speciation of As, the species of plant and the plant growth medium [24]. Arsenic uptake of plant was usually suppressed by P uptake when P was added [21, 22, 25–29]. The physiological behaviors of As and P in plant system are very different. Therefore, the phosphate concentration of the soil solution must be considered as a prime factor determining uptake and toxicity of arsenate in plants. Hurd-Karrer [30] demonstrated that As toxicity to plants can be prevented or at least reduced by phosphate addition. Since then many researchers have investigated the interactions between phosphate and arsenate uptake by plants [21, 26–28, 31–33]. Plant responses to arsenate and phosphate depend on growth conditions. Hurd-Karrer [30] found that in nutrient solution a P:As ratio [mg kg<sup>-1</sup>] of at least 4:1 was needed to protect wheat roots against As toxicity. It was reported that phosphate addition improved plant growth in nutrient solutions containing sufficient arsenate to be toxic at low P concentrations [34] and also decreased As concentrations in plants [35].

In soil systems, results were ambiguous; deleterious, negligible or even beneficial effects of P addition on As uptake and plant growth have been reported [30, 36, 37].

Silicon is not recognized as an essential element for plant growth but the beneficial effects of Si on the growth, development, yield, and disease resistance have been observed in a wide variety of plant species [38]. Silicon fertilizers are routinely applied to several crops including rice and sugarcane to enhance high and sustainable crop yields. The beneficial effect of Si is more evident under stress conditions. This is because Si is able to protect plants from multiple abiotic and biotic stresses [39]. Numerous studies have shown that Si is effective in controlling diseases caused by both fungi and bacteria in different plant species [40, 41]. Silicon also enhances plant resistance to insect pests such as stem borer and plant hopper [39]. Silicon alleviates many abiotic stresses including chemical stress (salt, metal toxicity, nutrient imbalance) and physical stress (lodging, drought, radiation, high temperature, freezing, UV) and many others [38, 39, 42, 43]. In addition to the role of Si in alleviating various stresses, Si improves light interception by keeping leaves erect, thereby stimulating canopy photosynthesis in rice [39, 44].

It has been reported that Si availability in soil has a large influence on As uptake by rice [45]. Arsenic uptake may also be affected by silicic acid as shown by Guo et al. [46, 47]. This is not surprising because of the shared uptake pathway between Si and arsenite [48]. Two Si transporter proteins, Lsi1 and Lsi2, respectively, mediate the entry of arsenite into root cells and the efflux from cells to apoplast in the direction of stele for the translocation to the shoots [49, 50].

The source or form of P fertilizer application in As contaminated growth medium to reduce As contents in plants is not documented yet. Besides, the application of Si fertilizer has not been yet applied in growing vegetables in As contaminated aerobic soil. So, the present study was undertaken with aiming at the following objectives: to examine the effect of P application, supplied from both organic and inorganically derived fertilizers, in reducing As concentration in JMS plant from As containing soil-plant system; and, to investigate the effects of Si fertilizer application on the As content of JMS plant grown in As contaminated aerobic soil-plant system.

## **2. MATERIALS AND METHODS**

### **2.1 Soil Collection and Preparation**

The experiment was carried out in the glasshouse of Iwate University, Morioka, Japan. The soil employed in this experiment was made by mixing two soils. One of the soils was highly As contaminated soil collected from Gunma Prefecture in Japan by composite soil sampling method [51]. The other soil was commercially obtained (Trust, Tochigi) which had low concentration of As. The soils were air-dried, ground and sieved through a 2-mm sieve. The pH value of the 1<sup>st</sup> soil was around 8.0 and As of the soil was too high ( $242 \text{ mg kg}^{-1}$ ) to cultivate crops. Therefore, by mixing the other soil, pH value and As concentration were adjusted to the usual level of the soil in the field. An aliquot of the soil sample was stored for analysis. Characteristics of the mixed soil that was used in the present experiment are shown in the Table 1.

**Table 1. Characteristics of the soil used in the experiment**

Parameters	Values
pH	5.22
EC (dS m <sup>-1</sup> )	0.85
As (mg kg <sup>-1</sup> )	30.6
1N HCl Extractable As (mg kg <sup>-1</sup> )	1.19
Total P (%)	0.0587
Total Fe (%)	3.22
Total Na (%)	0.070
Total K (%)	0.604
Total Ca (%)	0.956
Total Mg (%)	0.397
CEC meq/100g soil	52.5
Available P <sub>2</sub> O <sub>5</sub> (mg/100g)	0.272
Exchangeable Na <sub>2</sub> O (mg/100g)	16.2
Exchangeable K <sub>2</sub> O (mg/100g)	81.7
Exchangeable CaO (mg/100g)	46.2
Exchangeable MgO (mg/100g)	87.7

## 2.2 Phosphorus or Silicon Fertilizers and Their Properties

A total of four P fertilizers, two of which were derived from organic sources viz. chicken manure (CM), and bone powder (BP), and two other from inorganic sources viz. fused magnesium phosphate (FMP), and super phosphate (SP), were used in this experiment. CM was produced by Ise Farms Tohoku Inc. (Miyagi) and contained citrate soluble P<sub>2</sub>O<sub>5</sub> 4.7%, N 3.0%, citrate soluble K<sub>2</sub>O 3.0%, CaO 19.9%. BP was produced by Nissin Garden Mate Co. Ltd. (Tokyo) and contained citrate soluble P<sub>2</sub>O<sub>5</sub> 7.0%, N 1.0%, citrate soluble K<sub>2</sub>O 3.0%, citrate soluble MgO 1%, total P<sub>2</sub>O<sub>5</sub> 18%, water soluble K<sub>2</sub>O 2%. FMP was produced by Seiwa Fertilizer Ind. Co. Ltd. (Osaka) and contained citrate soluble P<sub>2</sub>O<sub>5</sub> 20.0%, citrate soluble MgO 12.0%, soluble SiO<sub>2</sub> 20.0% and alkali (CaO+MgO) 40%. SP was produced by Nittofc Co. Ltd. (Nagoya) and contained citrate soluble P<sub>2</sub>O<sub>5</sub> 18.0%, CaSO<sub>4</sub> 15%.

A total of three Si fertilizers were applied in the experiment. These three different fertilizers were viz. potassium silicate (PS), magnesium trisilicate pentahydrate (MTP) and Iherzolite (LE). Silicon was applied at the rate of 1,050 kg SiO<sub>2</sub> ha<sup>-1</sup>. PS (K<sub>2</sub>SiO<sub>3</sub>) was produced by Kaihatuhiryu Co. Ltd. (Tokyo, Japan) and contained citrate soluble SiO<sub>2</sub> 34%, K<sub>2</sub>O 20%, MgO 4% and B 0.1%. MTP (Mg<sub>2</sub>Si<sub>3</sub>O<sub>8</sub>.5H<sub>2</sub>O) was bought as chemicals marketed by WAKO, Osaka, Japan. LE was produced by Miyamori Saiseki Corp. (Iwate, Japan) and contained total silicate SiO<sub>2</sub> 39% and MgO 36%. It is reported that LE has the characteristics to be applied for phytoremediation of Cd contaminated soil [52, 53].

## 2.3 Plant Cultivation

One of the common leafy vegetable plant viz. Japanese mustard spinach (JMS) (*Brassica rapa* var. *perviridis*) was selected. Seeds collected from Sakata seed corporation, Japan, were grown in the experiment. There were two rates of P application (50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) along with a control. These two rates of applied amount of P were calculated based on the citrate soluble P content of the P fertilizers. There were a total of 36 pots [4 (control) + 4 (P source) × 4 (replication) × 2 (P rate)]. The N was applied on the basis

of the total amount of N in the CM that was required to meet the amount of P. Because CM contained the highest amount of N (3%) among the four P fertilizers and this N was applied as urea. In the pots of 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, amount of applied N was adjusted to 0.753 g pot<sup>-1</sup>. In the pots of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, amount of applied N was adjusted to 1.51 g pot<sup>-1</sup>. In a plastic bowl, As contaminated soil, required amount of P fertilizers, and urea were mixed then put in the plastic pot of 0.5 L size, watered and kept for 7 days.

In the Si experiment, there was only one rate of Si application (1,050 kg SiO<sub>2</sub> ha<sup>-1</sup>) along with control. In each pot, 1.42 g of PS, 0.94 g of MTP and 1.25 g of LE was added in powder form. For better growth of the plant 0.1 g of chemical fertilizer [10:10:10, N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O (Taki Chemicals Co. Ltd, Kakogawa)] were added in each pot.

In both of the experiments of P and Si; there were four replications for each application. Seven days after the preparation of the pot, about 8–10 seeds of the plants were sown in each pot. Seven days after seed sowing, the plants were thinned remaining three plants in each pot. During the growing period, all visible symptoms were observed and recorded. Only the edible part (shoots) of the plant was harvested 21 days after seed sowing by cutting about 1 cm above from the soil level which included both stems and leaves.

## 2.4 Plant Sample Preparation

The harvested plant part (shoot) was washed with deionized distilled water to remove adhering soil particles on the plant surface. The collected plant samples were air dried followed by oven dried at 70°C for 48 hours. Both the fresh weight (FW) and the dry weight (DW) of the plant samples were measured and recorded. The dried plant samples were then ground and preserved for further analysis.

## 2.5 Measurement of the Elements

The soil and plant samples were digested with a mixture of concentrated nitric acid and perchloric acid [HNO<sub>3</sub>:HClO<sub>4</sub>, 2:1, (v/v)]. Arsenic and other elements in the digested soil and plant samples were analyzed by employing atomic absorption spectrophotometer (AA-6200, Shimadzu, Kyoto) by following the prescribed laboratory methods [54, 55]. Reagent blanks and internal standards were used to ensure the accuracy and precision in the analysis. The arsenic concentration was expressed as mg kg<sup>-1</sup> DW whereas the arsenic accumulation was expressed as µg plant<sup>-1</sup>. Arsenic accumulation was calculated by multiplying the arsenic concentration with the DW of the edible part of the respective plant.

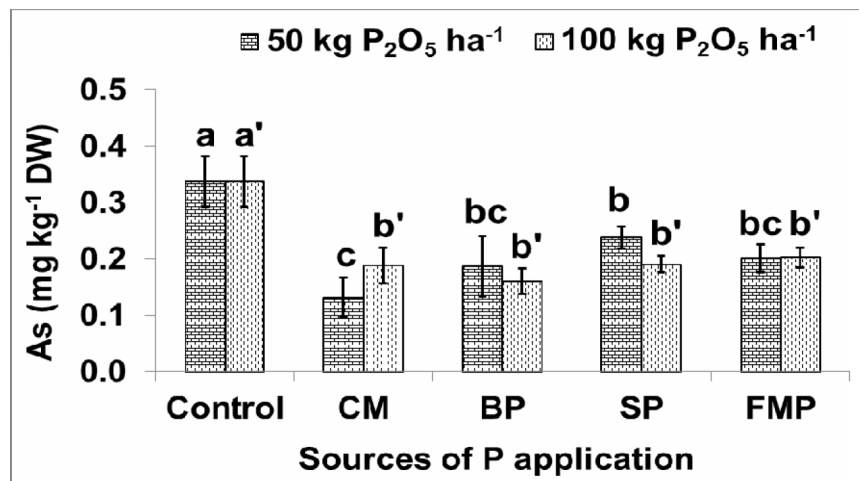
## 2.6 Statistical Analyses

The results were expressed as the averages of four replications. The data were subjected to an ANOVA [56] using the computer-built in SAS software program in Iwate University, Japan. Differences between means were statistically analyzed by using a Ryan-Einot-Gabriel-Welsch multiple range test (*P* = .05).

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of the Application of P Fertilizers

The As concentration ( $\text{mg kg}^{-1}$  DW) in the edible part of the plant (JMS) reduced significantly with the application of P fertilizers irrespective of the fertilizer source and fertilizer application rate (Fig. 1). The reduced As concentration in the plant part was significantly different from the As concentration in the control plant in all the individual fertilizer source and fertilizer application rate (Fig. 1). Among the four P fertilizers CM reduced the As concentration in the edible part of the plant mostly at  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  application rate, whereas at  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  application rate, As was reduced mostly by BP application. The other P fertilizers also reduced As concentrations in the edible part of the plant. But there was no significant difference in the reduction of As in the edible part of JMS among the fertilizer sources. Considering the reduced As concentration in the edible part of the plant, the effectiveness of the P fertilizer followed the order:  $\text{CM} > \text{BP} > \text{FMP} > \text{SP}$  at  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  application rate and it was followed the order:  $\text{BP} > \text{CM} > \text{SP} > \text{FMP}$  at  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  application rate. The As concentration ( $\text{mg kg}^{-1}$  DW) in the edible part of the plant reduced 61, 45, 41 and 30% from the control plant with the application of  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  from CM, BP, FMP and SP fertilizer sources, respectively. Whereas, the As concentration in the edible part of the plant reduced 53, 45, 44 and 40% from the control plant when  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  was applied from BP, CM, SP and FMP fertilizer sources, respectively. There was no significant effect of the rate of P application in the reduction of As concentration in the edible part of the plant except in the case of SP.

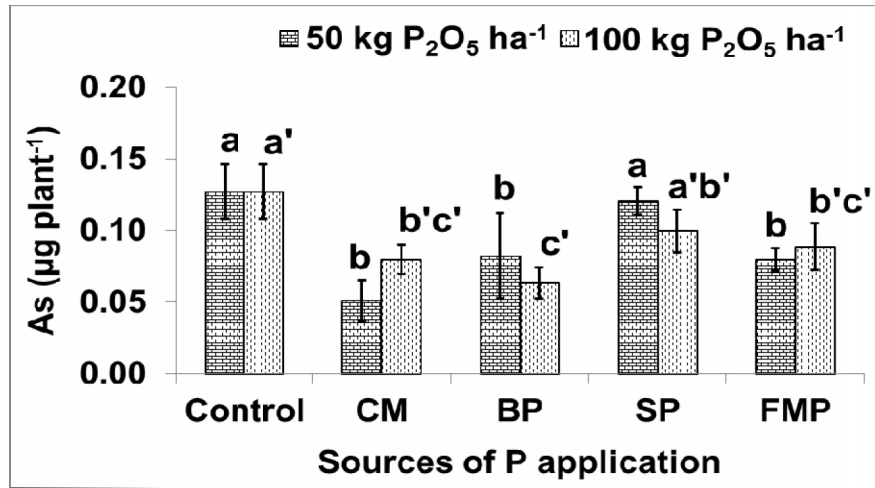


**Fig. 1. Arsenic concentration ( $\text{mg kg}^{-1}$  DW) in the edible part of Japanese mustard spinach after the application of P fertilizers**

CM, BP, SP and FMP represents chicken manure, bone powder, super phosphate and fused magnesium phosphate, respectively. Different letters above the bars indicate the significant differences ( $P = .05$ ). Error bars are the standard deviations (SDs)

The As accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of JMS also reduced with the application of P fertilizer irrespective of fertilizer source and fertilizer application rate (Fig. 2). Similarly to the As concentration, As accumulation was also reduced mostly with the application of CM at  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  application rate whereas at  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  application rate BP reduced

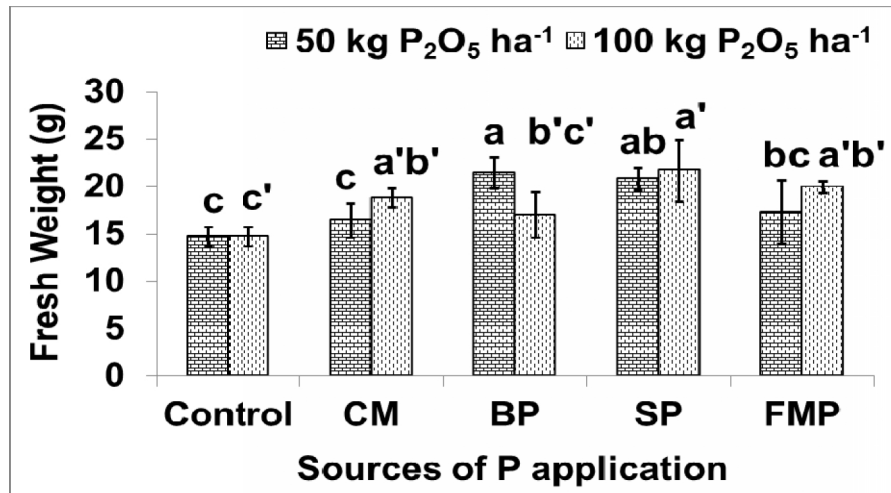
the most. Considering the reduced amount of As accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of the plant, the effectiveness of the P fertilizer followed the order: CM>FMP>BP>SP at 50 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  application rate and it was followed the order: BP>CM>FMP>SP at 100 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  application rate. The As accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of the JMS reduced 61, 39, 37 and 7% from the control plant due to the application of 50 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  from CM, FMP, BP and SP fertilizer sources, respectively. Whereas, the As accumulation in the edible part of JMS reduced 51, 39, 32 and 23% from the control plant due to the application of 100 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  from BP, CM, FMP and SP fertilizer sources, respectively.



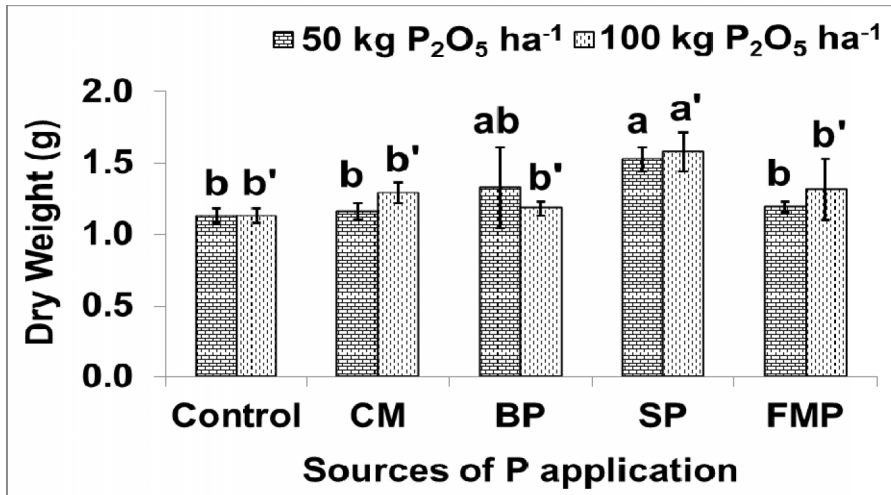
**Fig. 2. Arsenic accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of Japanese mustard spinach after the application of P fertilizers**

CM, BP, SP and FMP represents chicken manure, bone powder, super phosphate and fused magnesium phosphate, respectively. Different letters above the bars indicate the significant differences ( $P=0.05$ ). Error bars are the standard deviations (SDs).

A better growth of the plant was observed visually with the application of P fertilizers as compared to the control plant. Both the fresh weight (FW) and the dry weight (DW) of the plant increased with the application of P fertilizers but it was not statistically different from the control plant except in case of SP application for both the P application rate (Fig. 3a,b). The P concentration in the plant part also increased significantly with the application of P fertilizer (Table 2). There were no significant changes in the concentration of other nutrient elements (Fe, Na, K, C and Mg) in the edible part of JMS (Table 2).



(a)



(b)

**Fig. 3. Weight of the edible part of JMS (a) Fresh weight (g), and (b) Dry weight (g)**  
 CM, BP, SP and FMP represents chicken manure, bone powder, super phosphate and fused magnesium phosphate, respectively. Different letters above the bars indicate the significant differences ( $P = .05$ ). Error bars are the standard deviations (SDs)



**Table 2. Nutrient elements concentrations in the edible part of Japanese mustard spinach after the application of P fertilizers**

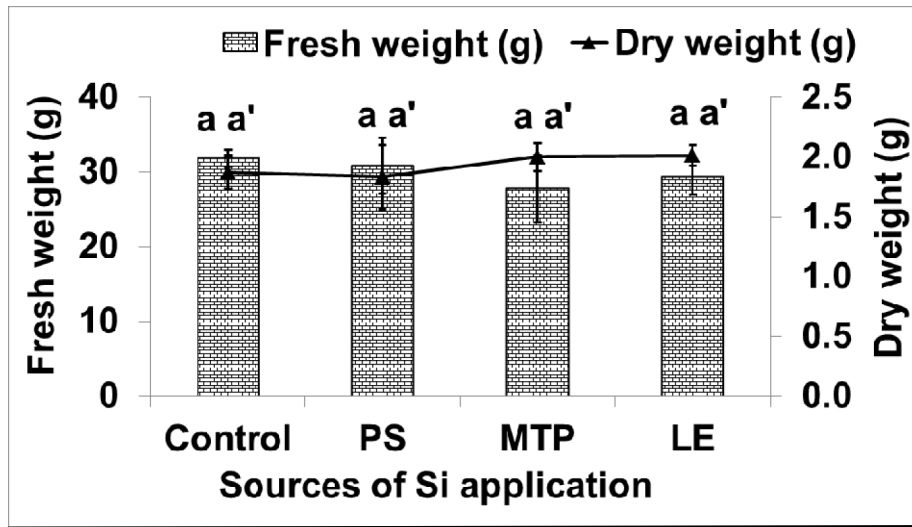
		<b>Fe (mg kg<sup>-1</sup>)</b>	<b>P (%)</b>	<b>K (%)</b>	<b>Ca (%)</b>	<b>Mg (%)</b>	<b>Na (%)</b>
<b>50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup></b>	Control	57.4 <sup>a</sup> (±5.51)	0.95 <sup>b</sup> (±0.09)	3.60 <sup>a</sup> (±0.34)	2.02 <sup>a</sup> (±0.15)	0.47 <sup>a</sup> (±0.02)	0.42 <sup>a</sup> (±0.01)
	CM	59.6 <sup>a</sup> (±6.66)	1.13 <sup>ab</sup> (±0.06)	3.62 <sup>a</sup> (±0.50)	2.01 <sup>a</sup> (±0.08)	0.46 <sup>a</sup> (±0.04)	0.45 <sup>a</sup> (±0.02)
	BP	58.6 <sup>a</sup> (±11.2)	1.10 <sup>ab</sup> (±0.07)	2.85 <sup>a</sup> (±0.50)	1.86 <sup>a</sup> (±0.13)	0.42 <sup>a</sup> (±0.03)	0.43 <sup>a</sup> (±0.04)
	SP	57.3 <sup>a</sup> (±7.59)	1.18 <sup>a</sup> (±0.05)	2.37 <sup>a</sup> (±0.93)	1.81 <sup>a</sup> (±0.13)	0.45 <sup>a</sup> (±0.03)	0.39 <sup>a</sup> (±0.02)
	FMP	66.1 <sup>a</sup> (±15.6)	1.17 <sup>a</sup> (±0.14)	2.81 <sup>a</sup> (±0.89)	2.04 <sup>a</sup> (±0.17)	0.46 <sup>a</sup> (±0.03)	0.41 <sup>a</sup> (±0.04)
<b>100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup></b>	CM	54.4 <sup>a</sup> (±6.30)	1.22 <sup>ab</sup> (±0.08)	4.04 <sup>a</sup> (±0.59)	1.83 <sup>a</sup> (±0.13)	0.43 <sup>a</sup> (±0.02)	0.43 <sup>ab</sup> (±0.02)
	BP	62.7 <sup>a</sup> (±5.73)	1.28 <sup>a</sup> (±0.03)	3.21 <sup>ab</sup> (±0.95)	1.75 <sup>a</sup> (±0.23)	0.49 <sup>a</sup> (±0.09)	0.44 <sup>a</sup> (±0.07)
	SP	62.9 <sup>a</sup> (±23.7)	1.18 <sup>ab</sup> (±0.03)	2.65 <sup>b</sup> (±0.68)	1.65 <sup>a</sup> (±0.13)	0.41 <sup>a</sup> (±0.02)	0.37 <sup>b</sup> (±0.02)
	FMP	54.5 <sup>a</sup> (±14.5)	1.13 <sup>b</sup> (±0.08)	2.95 <sup>ab</sup> (±0.35)	2.02 <sup>a</sup> (±0.22)	0.44 <sup>a</sup> (±0.02)	0.37 <sup>b</sup> (±0.02)

Results are expressed as mean values of four replicates (mean±SD). CM, BP, SP and FMP represents chicken manure, bone powder, super phosphate and fused magnesium phosphate, respectively.

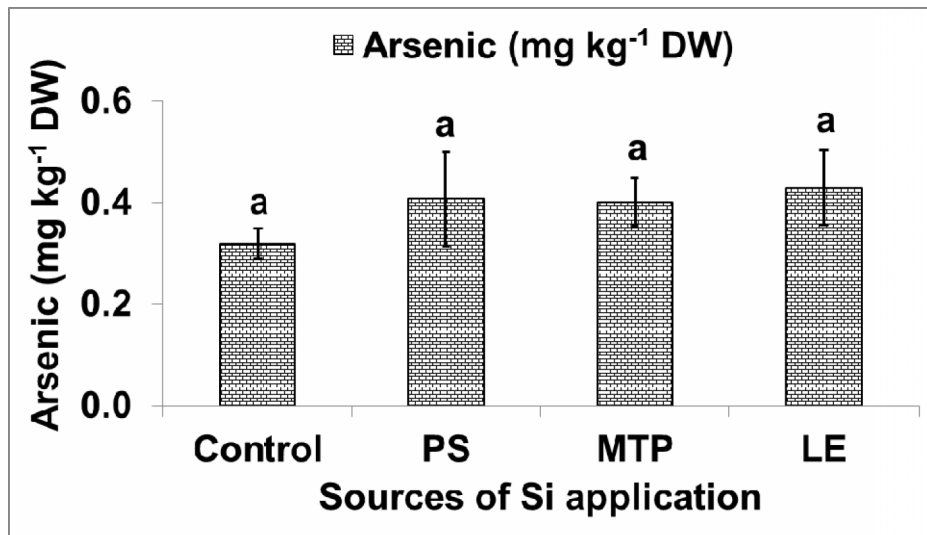
Different letters after the values in the table indicate significant differences ( $P = .05$ ). Numbers in the parentheses are the standard deviations (SDs)

### 3.2 Effect of the Application of Si Fertilizers

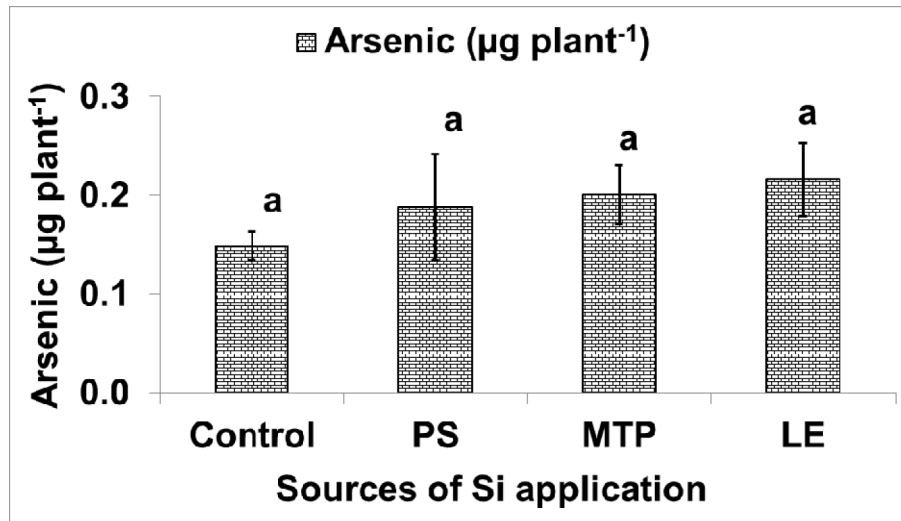
No distinct changes were observed in the growth of the plant JMS with the application of Si fertilizers in the As contaminated soil. All of the individual Si fertilizer statistically had the similar effect on the growth of the plants (Fig. 4). The As contents, both As concentration (mg kg<sup>-1</sup> DW) and As accumulation (µg plant<sup>-1</sup>), in the edible part of JMS increased with the application of Si fertilizers irrespective of Si source, but there was no significant difference in the increased As contents in Si applied plant with the As contents in control plant (Figs. 5, 6). All of the applied Si fertilizer had the similar effect on the As contents in JMS grown on As contaminated soil. Among the nutrient elements concentrations in the plant part, only the concentration of Mg was increased significantly when MTP and LE was applied in As contaminated soil (Table 3). The concentrations of the other nutrient elements were not affected by the application of Si fertilizers. It is reasonable that the Mg concentrations were increased by MTP and LE, because they contained Mg. It seemed that application of Si fertilizer did not have an effect on the absorption of essential elements shown in this experiment.



**Fig. 4. Weight (both fresh weight and dry weight) of the edible part of JMS**  
 PS, MTP and LE represents potassium silicate, magnesium trisilicate pentahydrate and Iherzolite, respectively. Same letters above the bars indicate the similar effect (statistically insignificant,  $P = .05$ ). Error bars are the standard deviations (SDs)



**Fig. 5. Arsenic concentration (mg kg<sup>-1</sup> DW) in the edible part of Japanese mustard spinach after the application of Si fertilizers**  
 PS, MTP and LE represents potassium silicate, magnesium trisilicate pentahydrate and Iherzolite, respectively. Same letters above the bars indicate the similar effect (statistically insignificant,  $P = .05$ ). Error bars are the standard deviations (SDs)



**Fig. 6. Arsenic accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of Japanese mustard spinach after the application of Si fertilizers**

PS, MTP and LE represents potassium silicate, magnesium trisilicate pentahydrate and Iherzolite, respectively. Same letters above the bars indicate the similar effect (statistically insignificant,  $P = .05$ ). Error bars are the standard deviations (SDs)

**Table 3. Nutrient elements concentrations in the edible part of Japanese mustard spinach after the application of Si fertilizers**

	Fe ( $\text{mg kg}^{-1}$ )	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
<b>Control</b>	195 <sup>a</sup> ( $\pm 56.5$ )	0.984 <sup>a</sup> ( $\pm 0.050$ )	1.90 <sup>a</sup> ( $\pm 0.092$ )	2.07 <sup>a</sup> ( $\pm 0.089$ )	0.444 <sup>b</sup> ( $\pm 0.028$ )	0.419 <sup>a</sup> ( $\pm 0.054$ )
<b>PS</b>	132 <sup>a</sup> ( $\pm 28.7$ )	0.977 <sup>a</sup> ( $\pm 0.065$ )	1.99 <sup>a</sup> ( $\pm 0.318$ )	2.09 <sup>a</sup> ( $\pm 0.262$ )	0.468 <sup>b</sup> ( $\pm 0.019$ )	0.458 <sup>a</sup> ( $\pm 0.070$ )
<b>MTP</b>	139 <sup>a</sup> ( $\pm 42.7$ )	1.137 <sup>a</sup> ( $\pm 0.073$ )	1.55 <sup>a</sup> ( $\pm 0.326$ )	1.85 <sup>a</sup> ( $\pm 0.206$ )	0.564 <sup>a</sup> ( $\pm 0.032$ )	0.444 <sup>a</sup> ( $\pm 0.011$ )
<b>LE</b>	184 <sup>a</sup> ( $\pm 55.7$ )	1.071 <sup>a</sup> ( $\pm 0.113$ )	1.97 <sup>a</sup> ( $\pm 0.582$ )	2.24 <sup>a</sup> ( $\pm 0.276$ )	0.524 <sup>a</sup> ( $\pm 0.025$ )	0.438 <sup>a</sup> ( $\pm 0.031$ )

Results are expressed as mean values of four replicates (mean $\pm$ SD). PS, MTP and LE represents potassium silicate, magnesium trisilicate pentahydrate and Iherzolite, respectively. Different letters after the values in the table indicate significant differences ( $P = .05$ ). Numbers in the parentheses are the standard deviations (SDs)

In the P application experiment, it was found that the As concentration ( $\text{mg kg}^{-1}$  DW) as well as As accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of JMS reduced with the application of P in the growth medium (Figs. 1, 2). The As contents in the plant part reduced significantly with the application of P irrespective of the fertilizer source and application rate. It is known that when the plant grows well then the concentration dilutes and reduces the concentration of the element in the plant. In our experiment, the growth of the plant increased due to the application of P in the growth medium which might dilute the As concentration in plant. But the "dilution effect" was not only the reason of the reduction of As concentration in the plant part. Some other phenomenon was involved in the reduction of As. Because, though the growth of the plants increased (Fig. 3a,b) due to the application of P, the As accumulation

( $\mu\text{g plant}^{-1}$ ) was also reduced (Fig. 2), which revealed that the other effect than “dilution effect” functioned in reducing the As concentration in the plant part. The reduced amount of As accumulation ( $\mu\text{g plant}^{-1}$ ) suggested that the As in the plant was reduced with the biochemical competition of absorption transporter between As and P, not only due to the dilution effect. Both As and P behave similarly in the soil-plant system because they belong to the V family on the periodic table of the elements and have the similarities in their chemical properties. Previous results suggested that phosphate can decrease or increase the uptake of As by plants, depending on the As species, the plant species, and the growth medium [24]. In our experiment, P applied in the As containing growth medium reduced the As concentration ( $\text{mg kg}^{-1}$  DW) and As accumulation ( $\mu\text{g plant}^{-1}$ ) in the edible part of JMS. High concentrations of P in the soil favor uptake of P rather than As, because P is absorbed through the transporter more efficiently than As [25, 27, 28].

Though all the individual P fertilizer reduced both As concentration and As accumulation in the edible part of JMS, the reduction was a little more, when organically derived P fertilizer CM and BP was applied, than inorganic P fertilizer FMP and SP. But these reductions were statistically similar among the applied fertilizers. So, the results showed that the supplied P was not source specific. All the sources of fertilizer from both organic and inorganic had the similar effect in reducing As contents in the edible part of JMS. That small higher reduction of As by organically derived fertilizer sources might be due to the As adsorption by biosolid organic substances which reduced the As phytoavailability. It was also found that the  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  was sufficiently enough to reduce the As contents in the edible part of JMS plant grown on  $30.6 \text{ mg As kg}^{-1}$ . This amount may vary depending on the soil type, soil As concentration and the plant species.

In the Si application experiment, different result was observed from that observed in the case of P fertilizer application. It was found that the As concentration and As accumulation both were increased, but not significantly, in the edible part of JMS with the application of Si fertilizers. In the previous report by other researchers, the addition of Si fertilizer markedly decreased As accumulation in rice shoots and, to a lesser extent, the concentration of As in the rice grain in greenhouse study [45, 47, 57]. Our results, however, did not support the previous results. The reason of the difference might be due to the fact that, though arsenate is absorbed through phosphate transporter, arsenite is absorbed mainly through the Si transporter in rice roots [48]. Our experiments were conducted on aerobic soil condition where arsenate was dominated than arsenite, so the inhibitory effect of As accumulation in plant was observed with the application of P fertilizer but it was not observed with the application of Si fertilizer.

From our experiments, it is suggested that for the repression of As absorption by plants, grown in aerobic soil, P fertilizer should be applied. The application of P fertilizer may help to safeguard the food security for human populations in regions with As contamination in soil. But in case of Si fertilizer application in the aerobic soil, the increased As accumulation in plant due to the application of Si fertilizer was not significantly different. Probably the reason of no reduction in As concentration and accumulation in plant is due to the difference in the form of As. It is considered that application of Si fertilizer to the crops grown on aerobic As contaminated soil may not have the effect to reduce As concentration of the plant part. More detail investigation needs to be conducted in considering the soil characteristics, soil As concentration and plant species in future.

#### 4. CONCLUSION

The application of P in the As containing growth medium significantly reduced the As concentration ( $\text{mg kg}^{-1}$  DW) and arsenic accumulation ( $\mu\text{g plant}^{-1}$ ) in Japanese mustard spinach. All the individual P fertilizer significantly reduced As contents in the edible part of JMS as compared to the control at both the rate of P applications. The application of P fertilizer at the rate of  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  in Japanese andosol containing  $30.6 \text{ mg As kg}^{-1}$ , was seemed to be enough to reduce the As contents in the edible part of Japanese mustard spinach. On the contrary, the application of Si fertilizer in the same soil had no effect in reducing As contents in JMS.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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