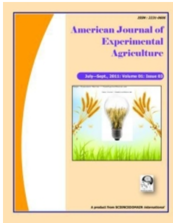

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# Phosphate Rich Soil Additive Baked Pig Manure Effectively Reduces Arsenic Concentration in Japanese Mustard Spinach (*Brassica rapa* var. *perviridis*) Grown with Arsenic Contaminated Irrigation Water

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***Authors' contributions***

*This work was part of a PhD thesis of the lead author JCJ. The second author SK was the principal supervisor. 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

A pot experiment was conducted to assess the efficiency of baked pig manure (BPM) application in Japanese andosol to reduce the arsenic (As) concentration in Japanese mustard spinach (JMS) (*Brassica rapa* var. *perviridis*) grown with As-contaminated irrigation water. Irrigation water was artificially spiked with As to 0.5 mg $L^{-1}$ dissolving di-sodium hydrogen arsenate heptahydrate ($Na_2HAsO_4.7H_2O$). BPM was applied to soil at the rate of 1, 2, and 3%; each treatment had four replications. The plant was grown for 30 days. Plant samples were analyzed for As and other elements. Plant As concentration decreased significantly with BPM application and based on the plant dry weight (DW) the As concentration reduced by 39, 52 and 66%, with the application of 1, 2 and 3% BPM, respectively, compared with those of control plant. There was no significant change in the As uptake (µg $plant^{-1}$) after the application of BPM. Plant FW and DW increased significantly with increasing amounts of BPM, which might function to decrease the As concentration in plants as 'dilution effect'. The phosphorus (P) contents of JMS increased

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significantly with BPM application, whereas the Fe, K and Mg contents decreased. The decreased As concentration and increased P concentration in plant indicated the competitive absorption of As and P in plant but this effect was not so strong because the As uptake (μg plant$^{-1}$) was not significantly reduced. Moreover, BPM might have properties that enable the adsorption of As because BPM contained charcoal due to the baking treatment. It is possible that the surface of BPM might adsorb As and thereby hindered the As absorption by the plant root. It is suggested that the phosphate rich BPM could be an environment friendly, cost effective and non-toxic soil additive for reducing As concentration in vegetable plant grown with As-contaminated irrigation water.

*Keywords: Irrigation water; arsenic; contamination; baked pig manure (BPM); phosphate; Japanese mustard spinach (JMS).*

## 1. INTRODUCTION

Groundwater is the main source of drinking water in most of the countries including Bangladesh. Approximately one third of the world's population use groundwater for drinking purpose [1]. But unfortunately, high concentrations of As in groundwater have been reported in Argentina, Bangladesh, Chile, China, India, Japan, Mexico, Mongolia, Nepal, The Philippines, Poland, Taiwan, Thailand, Vietnam, and some parts of the United States [2–6]. About 90% of the total population in Bangladesh use groundwater for drinking purpose because much of the surface water of Bangladesh is microbially unsafe to drink [7]. It is widely accepted that ingestion of As-contaminated groundwater is the major cause of As poisoning in areas affected by As contamination in Bangladesh. A nationwide survey suggested that 27% of all shallow-tube wells (STWs) were likely to have As contamination above the Bangladesh standard of 0.05 mg L$^{-1}$, with 46% in excess of the provisional World Health Organization (WHO) guideline value of 0.01 mg L$^{-1}$ [7, 8]. Another study that screened approximately 5 million STWs in 271 affected upazilas showed that approximately 1.4 million STWs (~29%) had As concentrations in excess of the Bangladesh standard (0.05 mg L$^{-1}$), and more than 8,000 villages had As contamination in 80% of all STWs [9]. But only a small portion (~16%) of the extracted groundwater is used for drinking purposes; the majority (~84%) is used for agricultural irrigation [10]. More than 80% of the population depends on agriculture for their livelihood, for that reason people use groundwater for irrigation especially during the dry season and during drought periods at the beginning and end of the rainy season. In Bangladesh, the total area under irrigation is 4 million ha of which 75 percent is covered by groundwater resources: 2.4 million ha via 924,000 STWs and 0.6 million ha via 23,000 deep tube-wells (DTWs) [11].

It is demonstrated that the Bangladesh groundwater is naturally contaminated with As concentrations ranging from <1–1,500 μg L$^{-1}$. Various survey and research studies reported different ranges of As concentrations in irrigation water. Imamul Huq et al. [12] reported that the irrigation water As concentration varied from 0.14–0.55 mg L$^{-1}$. Another study showed that 87 percent of irrigation DTWs contained As more than 0.05 mg L$^{-1}$ and the average As concentration in those DTWs was 0.21 mg L$^{-1}$ [13]. Ross et al. [14] estimated that 76 percent of the boro rice is grown in areas where STWs usually contain less than 0.05 mg L$^{-1}$, 17 percent in areas with 0.05–0.10 mg L$^{-1}$, and 7 percent in areas with more than 0.10 mg L$^{-1}$. Concentrations of As exceeding 1.0 mg L$^{-1}$ in STWs were also reported from 17 districts in Bangladesh [15].

Arsenic is naturally present in soil all over the world, with a concentration that varies depending on the origin of the soil [16]. The background As concentration in soil is approximately 5 mg $kg^{-1}$ [17]. Soil As concentrations ranging between 0.1–10 mg $kg^{-1}$ are considered as non-contaminated soils [18] and plants grown on non-contaminated soils are not toxic to plants and ordinary crops do not accumulate enough arsenic to be toxic to man. Researchers have paid their attention to the risks of the use of contaminated groundwater for irrigation. Irrigating with As contaminated water resulted in causing loss of yield and/or decreased growth of the plants [12, 19, 20] as well as higher levels of As in the edible parts of crops (rice as well as vegetables) [21–24] thus posing human health risks [12, 25]. Many researchers reported that long-term irrigation with As-contaminated water also resulted in As accumulation in the surface soil [21, 26–28].

There are several strategies for removing As from drinking water. But it is difficult to clean or remove As from As-contaminated irrigation water on a large scale. There are currently no alternatives to the use of As-contaminated irrigation water in Bangladesh, because no other water resources are available for agricultural irrigation during the dry season. There are no cost-effective methods or technologies to reduce As uptake by plants growing in As-contaminated soil and/or irrigated with As-contaminated water. Our goal was to find out a cost-effective, non-toxic, environmentally friendly soil additive that could reduce the As concentration in plants grown in As contaminated medium.

Arsenic is chemically similar to phosphorus (P); they have similar electron configurations, chemical properties, and compete for the same sorption sites in the root apoplastic route and for the same uptake and transport carriers in the root plasmalemma [29–34]. Several studies on the interactions between As and P indicated that P attenuated As uptake in plants [31, 35–39]. Therefore, we hypothesized that application of material with high concentrations of P to As-contaminated growth medium could reduce the plant As concentration. We selected high-P rich material *viz.* baked pig manure (BPM). BPM is an organic material could be used as organic fertilizer that contained high levels of P (13%). BPM is light and easy to work with in agricultural settings. In Bangladesh, vegetables are grown in winter season under irrigation facilities and 0.27 million ha of land is used for vegetable crops [11]. The present study examined the efficiency of BPM to reduce the As concentration in vegetable plant *viz.* Japanese mustard spinach grown with As-contaminated irrigation water.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Design

A pot experiment was carried out in the glasshouse where Japanese mustard spinach was grown in Japanese andosol irrigating with As contaminated water. The experiment was performed at Iwate University, Morioka, Japan.

### 2.2 Soil Collection and Characteristics of Soil

The Japanese andosol used in the experiment was commercially obtained (Trust, Tochigi). The soil was air-dried, ground, and sieved through a 2-mm sieve. This soil was used in the experiment. A small portion of the soil sample was stored for analysis. The characteristics of the soil are presented in Table 1.

**Table 1. Characteristics of soil**

| Parameters | Values |
|---|---|
| **pH** | 5.17 |
| **EC (dS $m^{-1}$)** | 0.17 |
| **Total As (mg $kg^{-1}$)** | 11.4 |
| **1N HCl Extractable As (mg $kg^{-1}$)** | 0.120 |
| **Total P (%)** | 0.260 |
| **Total Fe (%)** | 2.38 |
| **Total Na (%)** | 0.836 |
| **Total K (%)** | 0.356 |
| **Total Ca (%)** | 0.453 |
| **Total Mg (%)** | 0.313 |
| **CEC meq/100g soil** | 56.19 |
| **Available $P_2O_5$ (mg/100g)** | 0.292 |
| **Exchangeable $Na_2O$ (mg/100g)** | 9.95 |
| **Exchangeable $K_2O$ (mg/100g)** | 33.8 |
| **Exchangeable CaO (mg/100g)** | 30.7 |
| **Exchangeable MgO (mg/100g)** | 42.6 |

## 2.3 Properties of Baked Pig Manure

Several material forms of BPM were commercially available in Japan. The BPM used in this experiment was produced by Sanken Soil Corporation, Hachimantai, Japan. The BPM was produced by baking at 600–700°C for 10 min in a dedicated oven (SMK-800-S, Soil Farm, Kouchi). The raw materials contained ~21% moisture; the baked product contained 10% moisture and the particle size was <10mm. The BPM was ground into smaller sizes using a motor, sieved through a 0.5-mm sieve, and used for the experiment and chemical analyses. The pH and electrical conductivity (EC) of BPM were 11.7 and 3.4 dS $m^{-1}$, respectively. The cation-exchange capacity (CEC) was measured as 31.3 meq/100g using the Schollenberger method [40]. The amounts of $NH_4$-N and $NO_3$-N were 7.0 and 7.0%, respectively. Exchangeable cations, such as K, Ca, Mg, and Na, were 24.4, 4.4, 0.70, and 4.2 mg $g^{-1}$, respectively. The contents of total N and total P were 1.5 and 13%, respectively. The concentrations of Cu and Zn were 916 and 5,530 mg $kg^{-1}$, respectively. Arsenic was not detected in BPM.

## 2.4 Plant and Plant Cultivation

Japanese mustard spinach (JMS) (*Brassica rapa* var. *perviridis*) was grown in the present experiment. The irrigation water was artificially contaminated with As concentrations up to 0.5 mg $L^{-1}$ using di-sodium hydrogen arsenate heptahydrate ($Na_2HAsO_4.7H_2O$). Three different rate of BPM (1, 2 and 3%) were applied to the soil along with control (no BPM). Each treatment had four replications. The soil, the required amount of BPM, and 1 g of chemical fertilizer [10:10:10, N:$P_2O_5$:$K_2O$ (Taki Chemicals Co. Ltd, Kakogawa)] were mixed in a plastic bowl, transferred to a 1-L plastic pot, and watered. After preparation of the pot, approximately 8–10 seeds of the selected plant were sown in each pot. The irrigation with 0.5 mgAs$L^{-1}$ water was done according to the necessity of the plant but at equal volume for each pot on the same day. Seven days after seed sowing, the plants were thinned to four plants per pot. During the growing period, all visible symptoms were observed and recorded.

Pesticide was sprayed according to the necessity. The plants were harvested 30 days after seed sowing and fresh weight of the plant was recorded.

## 2.5 Plant Sample Preparation

Harvested plant was washed with deionized distilled water and then with Milli-Q water to remove any adhering soil particles. The collected plant samples were air-dried, followed by oven drying at 70°C for 48 h. The dry weight (DW) of the plant samples was measured and recorded. The dried plant samples were then ground and preserved for further analysis.

## 2.6 Measurement of Arsenic and Other Elements

The soil, plant and BPM samples were digested with a mixture of concentrated nitric acid and perchloric acid. [$HNO_3$:$HClO_4$, 2:1, (v/v)]. The digested plant, soil, and BPM samples were analyzed for As and other elements using an atomic absorption spectrophotometer (AA-6200, Shimadzu, Kyoto) according to the previously published protocols [41, 42]. Reagent blanks and internal standards were used to ensure the accuracy and precision of the analyses.

## 2.7 Statistical Analyses

The results were expressed as the averages of four replications. The data were subjected to ANOVA. Differences between means were statistically analyzed using a Ryan-Einot-Gabriel-Welsch multiple range test ($P$ =.05) performed with the SAS software program [43] at Iwate University, Japan.

# 3. RESULTS AND DISCUSSION

Japanese mustard spinach grew better in Japanese andosol grown with As-contaminated irrigation water when BPM was applied. Both the fresh weight (FW) and the dry weight (DW) increased significantly with the increasing rate of BPM application (Fig. 1). The As concentration (mg $kg^{-1}$) in the edible part of the plant JMS decreased significantly with the application of BPM (Fig. 2). However, the accumulation or uptake of As (µg $plant^{-1}$) in the edible part of JMS was statistically similar with the application of BPM (Fig. 3). The concentrations of other mineral nutrient elements in the plant are presented in Table 2. The P concentration in the plant part increased significantly whereas Fe, K and Mg concentration was decreased with the application of BPM.

**Table 2. Mineral nutrient concentrations in the edible part of Japanese mustard spinach grown with As-contaminated irrigation water in Japanese andosol**

| | P (%) | Fe (mg $kg^{-1}$) | Na (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|
| No BPM | $1.14^b$(±0.05) | $294^a$(±29) | $0.54^a$(±0.09) | $5.84^a$(±0.54) | $2.48^a$(±0.21) | $0.65^a$(±0.06) |
| 1% BPM | $1.50^a$(±0.16) | $253^a$(±71) | $0.55^a$(±0.08) | $5.23^{ab}$(±0.25) | $2.83^a$(±0.09) | 0.54ab(±0.05) |
| 2% BPM | $1.58^a$(±0.14) | $228^{ab}$(±22) | $0.46^a$(±0.09) | $4.12^b$(±0.84) | $2.39^a$(±0.41) | $0.42^b$(±0.05) |
| 3% BPM | $1.71^a$(±0.14) | $148^b$(±32) | $0.33^a$(±0.11) | $2.61^c$(±0.81) | $2.14^a$(±0.25) | $0.42^b$(±0.09) |

*Results are expressed as mean values of four replicates (mean±SD). Different letters after the values in the table indicate significant differences (P =.05). Numbers in the parentheses are the standard deviations (SDs)*

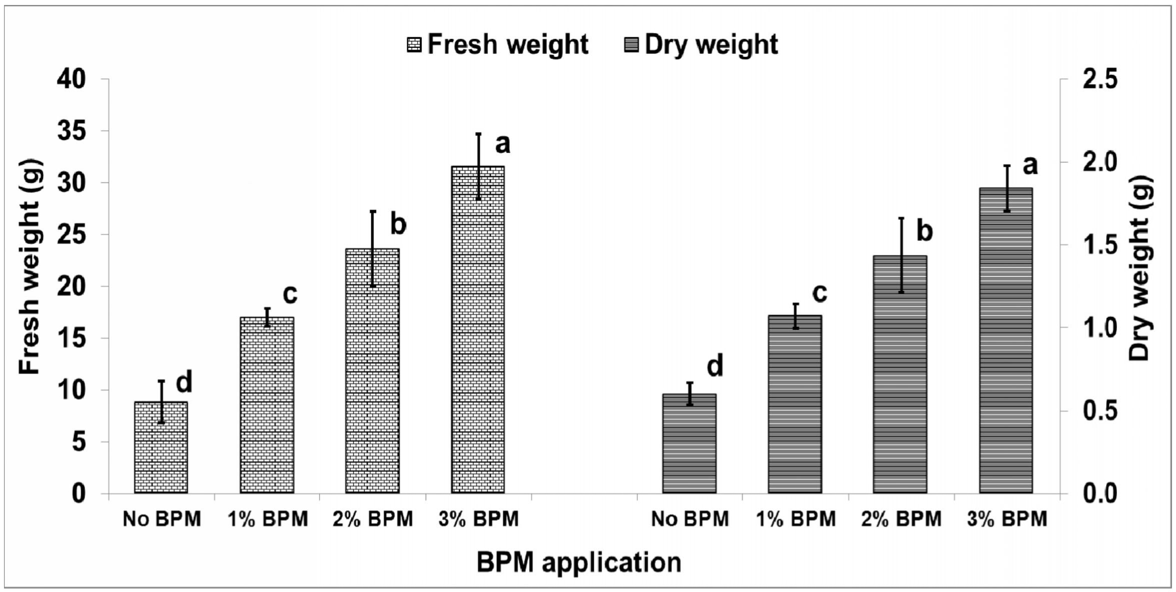


**Fig. 1. Fresh weight (g) and dry weight (g) of Japanese mustard spinach grown with As-contaminated irrigation water in Japanese andosol**

*Different letters on the bars indicate significant differences (P =.05). Error bars represent the standard deviations (SDs).*

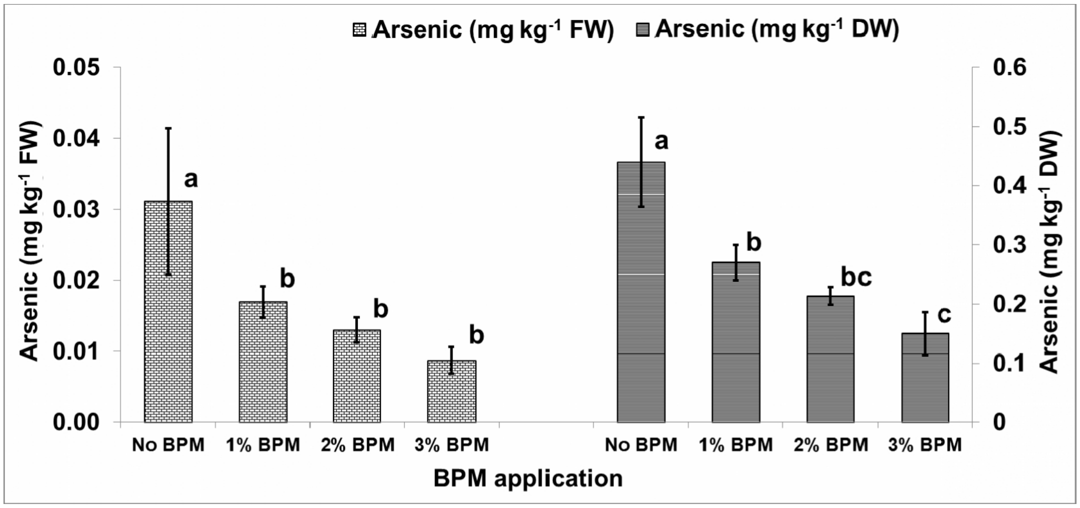


**Fig. 2. Arsenic concentration (mg kg$^{-1}$ FW and/or DW) in the edible part of Japanese mustard spinach grown with As-contaminated irrigation water in Japanese andosol**

*Different letters on the bars indicate significant differences (P =.05). Error bars represent the standard deviations (SDs)*

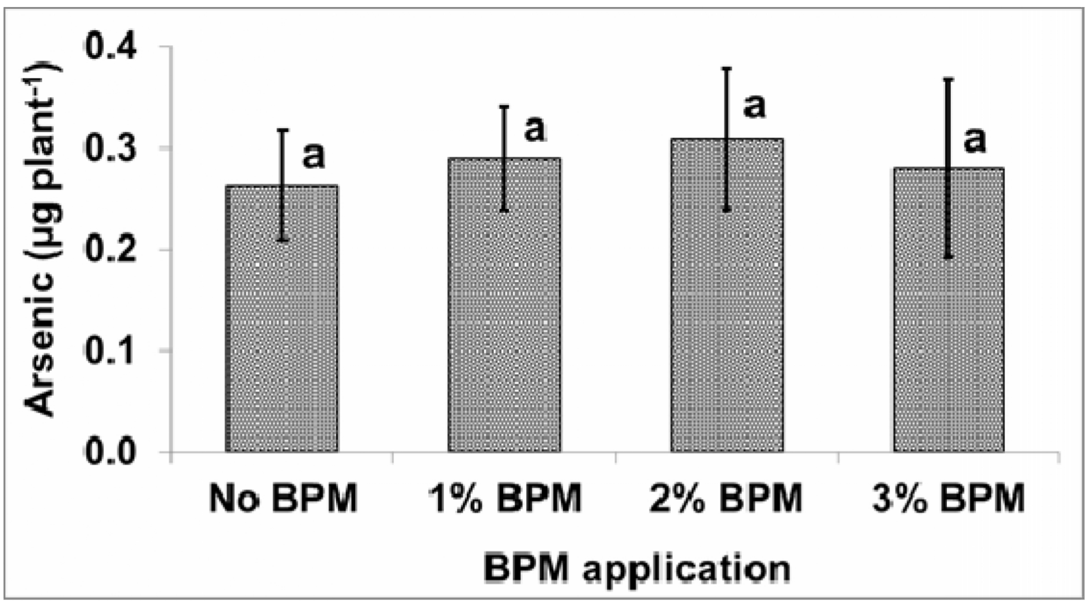


**Fig. 3. Arsenic accumulation/uptake (µg plant$^{-1}$) in Japanese mustard spinach grown with As-contaminated irrigation water in Japanese andosol**

*Different letters on bars indicate significant differences (P =.05). Error bars represent the standard deviations (SDs)*

In the present study we examined the efficiency of BPM in reducing As concentration in JMS grown with As-contaminated irrigation water. The results showed that the application of BPM to soil reduced the As concentration in the edible part of the plant JMS grown with As-contaminated irrigation water. Since BPM contained higher amounts of P and BPM is an organic material, so plant grew better in BPM-containing soil than that of control. Plant growth was significantly higher in BPM-containing soil than that of the control (Fig. 1). This result might be due to the presence of higher amounts of P in BPM-mixed soil, which could increase the plant growth. In fact, BPM is an organic fertilizer that can increase plant growth too.

Application of BPM in soil significantly reduced the As concentration in the edible part of JMS compared to the As concentration in control plant. The As concentration in the edible part of the JMS was reduced by 39, 52, and 66% (DW basis) with the application of 1, 2, and 3% BPM, respectively, compared to that of the control plant (Fig. 2). The reduction of As concentration in the edible part of JMS was significantly different from that of the control (Fig. 2). However, the As uptake (µg plant$^{-1}$) or As accumulation into the edible part of JMS was statistically similar as compared to the control (Fig. 3). Therefore, it was primarily considered that the decrease in As concentration in the edible part of JMS was due to "dilution effect" because of the increased plant growth with the application of BPM. The Japanese andosol is known to be a P-deficient soil that is low in available P (Table 1). Thus, the effect of high P provided by BPM on plant growth may be more significant depending on the soil. The P concentration in the plant increased with the application of BPM. The factors other than increased P supplied by BPM might also be involved in the reduction of plant As concentration.

One of the possible factors involved in the reduction of plant As concentration would be the biochemical competition between As and P in soil-plant system. 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 [35,37,38]. Because As and P belong to the V family on the periodic table of the elements, and are similar in their chemical properties, so they behave similarly in the soil-plant system. 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 [44]. In our experiment, the P supplied by BPM application decreased the As concentration (mg kg$^{-1}$) in Japanese mustard spinach but the accumulation of As (µg plant$^{-1}$) was not reduced (Fig. 3). This result revealed that the competition effect was not so strong in the present study, when As was applied with irrigation water to soil. Another one of the possible factor that might be involved in the attenuation of As concentration in plant was the characteristic of BPM. BPM might have properties that enable the adsorption of As because BPM contains charcoal due to the baking treatment. It is possible that the surface of BPM might adsorb As and thereby hindered the As absorption by the plant root. The mechanism of this effect on plants needs to be investigated further to understand the characteristics and interactions among soils, additives, and plants. This study showed that BPM was a cost-effective, environmentally friendly, non-toxic soil additive that could alleviate the As contamination in food material through irrigation with As-contaminated water. We hope that this strategy of plant growth may help to safeguard the food security for human populations in regions where As-contaminated water is used for irrigation.

## 4. CONCLUSION

This study showed that As concentration in the edible part of Japanese mustard spinach was attenuated grown with As-contaminated irrigation water by the application of high concentrations of P supplied with BPM. The application of BPM to soil also increased plant growth and P concentration. The results suggested that BPM was effective due to the "dilution effect" induced by the P supplied by BPM on andosol. Competition effect did not largely affect As absorption by roots. Further studies should be needed to assess other plant species, soil types, and additional parameters that could increase the effectiveness of BPM to reduce the plant As concentration.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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