Application of Baked Pig Manure Reduces Arsenic Concentrations in Plants Growing in Arsenic-Contaminated Soils

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Abstract*- Two experiments were conducted to determine the efficiency of application of baked pig manure (BPM) to reduce the arsenic (As) concentration in Japanese mustard spinach (JMS) (*Brassica rapa var. perviridis*) and Bangladesh spinach (BS) (*Spinacia oleracea*) that were grown in arsenic-contaminated Japanese andosol and Bangladesh alluvial soil, respectively. Soil As was artificially raised to 50 mg/kg. BPM was applied to soil at a concentration of 1%, 2%, and 3%; each treatment had four replications. The plants were grown in As-contaminated soil for 30 days. Soil and plant samples were analyzed for As and other elements. Plant As concentrations decreased significantly with BPM application compared to that in control plants in both experiments. The plant As concentrations based on plant dry weight (DW) were reduced by 61% and 49% in Japanese andosol and Bangladesh alluvial soil, respectively, compared with those of control plants. The total As uptake (µg/plant) was higher in JMS and lower in BS than that in controls. Plant DW increased significantly with increasing amounts of BPM, which might function to decrease the As concentration in plants. The phosphorous (P) contents of both JMS and BS increased significantly with BPM application, whereas the calcium content decreased. The decreased plant As concentration might be due to P supplied by BPM, which might competitively suppress As uptake. We conclude that BPM could be a cost-effective, environmentally friendly, non-toxic soil additive for reducing As concentrations in edible plants. This strategy will ultimately safeguard the food supply from As contamination.

***Keywords*- Arsenic; Soil; Contamination; Baked Pig Manure (BPM); Vegetable; Phosphate**

I. INTRODUCTION

Arsenic is one of the most dangerous global environmental toxicants and pollutants. Currently, As has been declared as a serious health risk in many countries. High concentrations of As in groundwater have been reported in Argentina, Bangladesh, Chile, China, India, Japan, Mexico, Mongolia, Nepal, Philippines, Poland, Taiwan, Thailand, Vietnam, and some parts of the United States [1–5]. Bangladesh tops the global list in terms of the severity of the problem, because approximately half of the total population is at risk of drinking As-contaminated water from tube wells. In one estimate, consumption of As-contaminated drinking water in Bangladesh resulted in approximately 9,100 deaths and 125,000 disability-adjusted life years (DALYs) in 2001 [6]. 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 arsenic contamination above the Bangladesh standard of 0.05 mg/L, with 46% in excess of the provisional World Health Organization (WHO) guideline value of 0.01 mg/L [7]. 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), and more than 8,000 villages had As contamination in 80% of all STWs [8]. Only a small portion (~16%) of the extracted groundwater is used for drinking purposes; the majority (~84%) is used for agricultural irrigation [9], especially during the dry season and during drought periods at the beginning and end of the rainy season.

Many researchers reported that long-term irrigation with As-contaminated water resulted in As accumulation in the soil [10–18]. This As accumulation in soil varied depending on the As concentration in irrigation water. It was estimated that 0.9–1.36 million kg As per year was introduced into arable land by irrigation with As-contaminated groundwater [19, 20]. It was also reported that if the irrigation water contained 0.1 mg/L As, it would cause an increase in soil As concentration of 1 mg/kg/year [18]. If the As concentration in irrigation water varied between 0.136–0.55 mg/L, the loading of As in agricultural fields for boro rice, which requires 1,000 mm of irrigation water per season, was between 1.36–5.5 kg/ha/year; the equivalent field loading for winter wheat, which requires 150 mm of irrigation water per season, was between 0.12–0.82 kg/ha/year [21]. It is now well-established that As-contaminated irrigation water is causing increases in soil As concentrations due to long-term irrigation.

Arsenic is naturally present in soil all over the world, with a concentration that varies depending on the origin of the soil [22]. The background As concentration in soil is approximately 5 mg/kg [23]. Soil As concentrations ranging between 0.1–10 mg/kg are considered as non-contaminated soils [24]. The soil As concentration in Bangladesh is higher than this value and it varies depending on the location. The average As concentration in soil in Bangladesh is 12.3 mg/kg. Numerous studies documented different As concentration ranges in Bangladesh soil as follows: from 0.3–49 mg/kg [25]; from below the detection limit to 56.7 mg/kg [26]; from 46 mg/kg to less than 10 mg/kg in areas with low concentrations of As in the irrigation water [18]; and from 3.2–27.5 mg/kg [16]. In areas where irrigation water did not contain As, the soil As concentration varied from 0.10–2.75 mg/kg.

There are several strategies for removing As from drinking water. However, if soil is contaminated with As, it is difficult to clean it and remove the As 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 irrigated with As-contaminated water. Our goal was to develop a cost-effective, non-toxic, environmentally friendly soil additive that could reduce the As concentration in plants.

Arsenic is chemically similar to phosphorus (P); they have similar electron configurations, chemical properties, and compete for the same sorption sites in the root apoplast and for the same uptake and transport carriers in the root plasmalemma [27–31]. Several studies on the interactions between As and P indicated that P attenuated As uptake in plants [29–34]. Therefore, we hypothesized that application of material with high concentrations of P to As-contaminated soil could reduce the plant As concentration. We found and selected a high-P material in the form of baked pig manure (BPM), because BPM is an organic material that contained high levels of P (13%). BPM is light and easy to work with in agricultural settings. It can be produced in quantity in areas where livestock husbandry is prosperous. The present study examined the efficiency of BPM to reduce the As concentration in plants grown in As-contaminated soil.

II. MATERIALS AND METHODS

A. Experimental Design

Two pot experiments were carried out in the glasshouse under two experimental conditions with two vegetables grown in two types of soils (Japanese andosol and Bangladesh alluvial soil). The first experiment was performed at Iwate University, Morioka, Japan. The second experiment was performed at Khulna University, Bangladesh.

B. Soil Collection and Preparation

The experimental Bangladesh alluvial soil was collected from Khulna, Bangladesh, using the composite soil sampling method developed by the soil survey staff of the USDA [35]. The Japanese andosol used in the experiment in Japan was commercially obtained (Trust, Tochigi). The soils were air-dried, ground, and sieved through a 2-mm sieve. These soils were used in the experiments. A small portion of the soil samples was stored for analysis. The characteristics of the soils are presented in Table 1.

TABLE 1 CHARACTERISTICS OF SOILS

Parameters	Japanese Andosol	Bangladesh Alluvial Soil
pH	5.17	7.43
EC (dS/m)	0.17	1.25
As (mg/kg)	11.40	6.90
As (mg/kg) 1N HCl extracted	0.12	0.29
P (%)	0.26	0.32
Fe (%)	2.38	3.44
Na (%)	0.84	1.12
K (%)	0.36	0.20
Ca (%)	0.45	0.65
Mg (%)	0.31	1.01
CEC (meq/100g)	56.19	37.92
Available P (mg/kg)	1.25	2.92

C. 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 ℃ 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 experiments and chemical analyses. The pH and electrical conductivity (EC) of BPM were 11.7 and 3.4 dS/m, respectively. The cation-exchange capacity (CEC) was measured as 31.3 meq/100g using the Schollenberger method [36]. 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, 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, respectively.

D. Plants and Plant Cultivation

Two common leafy vegetable plants were used for these studies. Japanese mustard spinach (JMS) (*Brassica rapa var. perviridis*) was grown in Japan and Bangladesh spinach (BS) (*Spinacia oleracea*) was grown in Bangladesh. The background As concentrations in soils were measured and used to calculate the total soil As concentration. The soils were artificially contaminated with As concentrations up to 50 mg/kg soil using di-sodium hydrogen arsenate heptahydrate ($Na_2HAsO_4.7H_2O$). This high level of As was selected because higher concentrations of soil As in Bangladesh have been reported [25, 26]. Three different amounts of BPM (1%, 2%, and 3%) were applied to As-contaminated soil along with control (no BPM). Each treatment had four replications. The As-contaminated 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, watered, and kept for 7 d. Seven days after preparation of the pot, approximately 8–10 seeds of the selected plants were sown in each pot. 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. The plants were harvested 30 days after seed sowing.

E. Plant Sample Preparation

Harvested plants were 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 ℃ 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.

F. Measurement of As and Other Elements

The plant samples were digested with a mixture of concentrated nitric acid and perchloric acid. The soil and BPM samples were digested with aqua regia [HCl:HNO_3, 3:1, (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 [37, 38]. Reagent blanks and internal standards were used to ensure the accuracy and precision of the analyses. The arsenic concentration was expressed as mg/kg DW whereas the arsenic uptake was expressed as µg/plant. Arsenic uptake was calculated by multiplying the arsenic concentration with the DW of the edible part of the respective plant.

G. 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=0.05) performed with the SAS software program [39] at Iwate University, Japan.

III. RESULTS AND DISCUSSIONS

In Japanese andosol, plants clearly grew better in As-contaminated soil after the application of BPM. The plant DW (g) is shown in Figure 1. The DW increased significantly with the application of BPM. The As concentration (mg/kg DW) in the edible part of the plant is shown in Figure 2. The As concentration in the edible part of the plant decreased significantly with application of BPM to As-contaminated soil. However, the total uptake of As (µg/plant) in the edible part of JMS increased with increasing concentrations of BPM (Fig. 3). The concentrations of other mineral nutrient elements in the plant are presented in Table 2.

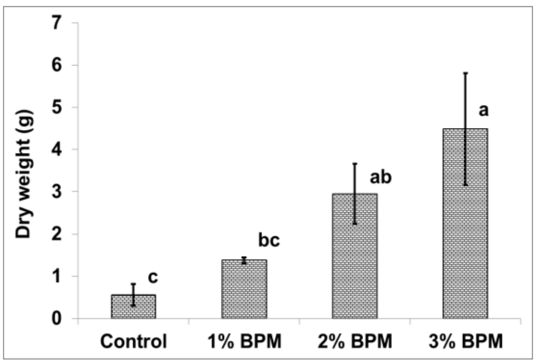

Fig. 1 Dry weight (g) of Japanese mustard spinach growing in As-contaminated soil with different concentrations of baked pig manure. Different letters on bars indicate significant difference (P<0.05). Error bars represent the standard deviations (SDs)

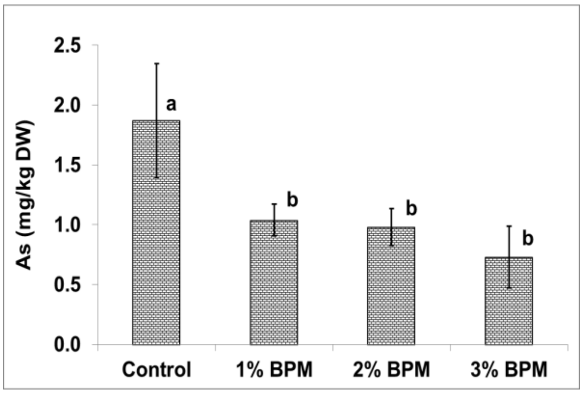

Fig. 2 Arsenic concentration (mg/kg DW) in Japanese mustard spinach growing in As-contaminated soil with different concentrations of baked pig manure. Different letters on bars indicate significant difference (P<0.05). Error bars represent the standard deviations (SDs)

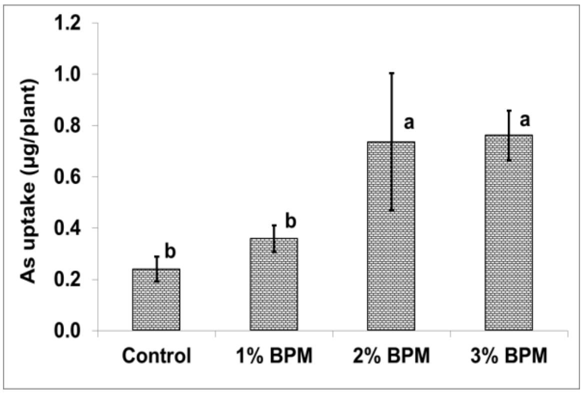

Fig. 3 Arsenic uptake (μg/plant) in Japanese mustard spinach growing in As-contaminated soil with different concentrations of baked pig manure. Different letters on bars indicate significant difference (P<0.05). Error bars represent the standard deviations (SDs)

TABLE 2 CONCENTRATION OF MINERAL NUTRIENTS IN JAPANESE MUSTARD SPINACH

	P (%)	Fe (ppm)	Na (%)	K (%)	Ca (%)	Mg (%)
Control	0.72 ± 0.21^{c}	88.81 ± 23.27^{a}	0.27 ± 0.19^{a}	6.57 ± 1.93^{ab}	1.51 ± 0.32^{a}	1.05 ± 0.21^{a}
1% BPM	1.21 ± 0.01^{b}	74.76 ± 4.45^{a}	0.16 ± 0.10^{a}	5.88 ± 0.87^{b}	0.90 ± 0.14^{b}	0.92 ± 0.09^{a}
2% BPM	1.76 ± 0.13^{a}	80.18 ± 16.61^{a}	0.40 ± 0.13^{a}	9.62 ± 0.41^{a}	0.75 ± 0.11^{b}	1.25 ± 0.10^{a}
3% BPM	1.82 ± 0.28^{a}	77.51 ± 20.33^{a}	0.39 ± 0.04^{a}	9.38 ± 1.56^{a}	0.66 ± 0.20^{b}	1.15 ± 0.13^{a}

Results are expressed as mean values of four replicates (mean ±SD). Different letters after the values in the table indicate significant difference (P<0.05). SD=standard deviation

In Bangladesh alluvial soil, BS grew better in As-contaminated soil that contained 3% BPM. The plant DW (g) is shown in Figure 4. The As concentration (mg/kg DW) in the edible part of the plant is shown in Figure 5. The As concentration in the edible part of the plant decreased significantly with the presence of BPM. The total uptake of As (μg/plant) in the edible part of the plant decreased significantly with increasing concentrations of BPM (Fig. 6). The concentrations of other mineral nutrient elements in the plants are shown in Table 3.

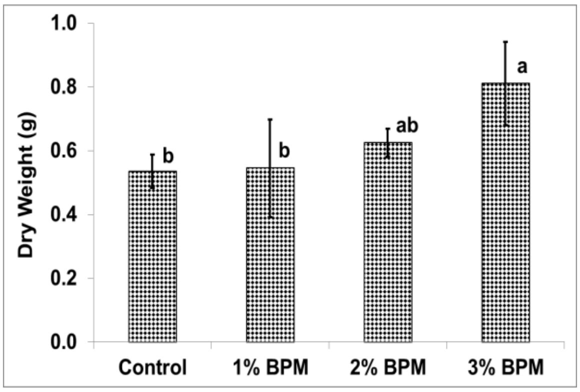

Fig. 4 Dry weight (g) of Bangladesh spinach growing in As-contaminated soil with different concentrations of baked pig manure. Different letters on bars indicate significant difference (P<0.05). Error bars represent the standard deviations (SDs)

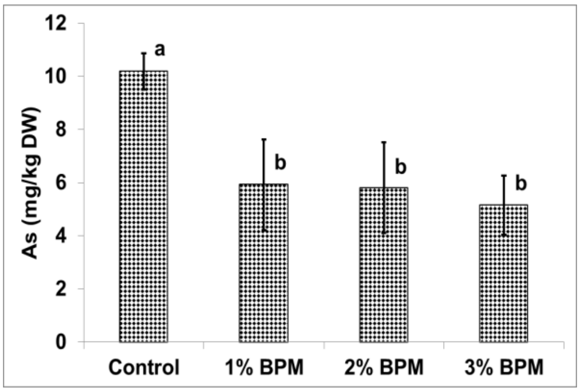

Fig. 5 Arsenic concentration (mg/kg DW) in Bangladesh spinach growing in As-contaminated soil with different concentrations of baked pig manure. Different letters on bars indicate significant difference (*P*<0.05). Error bars represent the standard deviations (SDs)

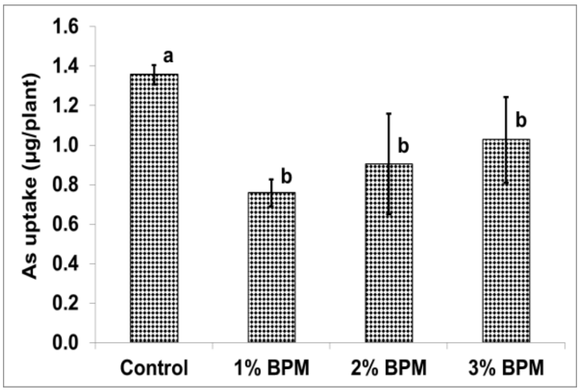

Fig. 6 Arsenic uptake (µg/plant) in Bangladesh spinach growing in As-contaminated soil with different concentrations of baked pig manure. Different letters on bars indicate significant difference (*P*<0.05). Error bars represent the standard deviations (SDs)

TABLE 3 CONCENTRATION OF MINERAL NUTRIENTS IN BANGLADESH SPINACH

	P (%)	Fe (ppm)	Na (%)	K (%)	Ca (%)	Mg (%)
Control	0.92 ± 0.06^{b}	467.98 ± 67.05^{c}	4.10 ± 0.41^{a}	0.55 ± 0.01^{c}	1.30 ± 0.24^{a}	0.80 ± 0.03^{a}
1% BPM	2.20 ± 0.26^{a}	730.89 ± 58.37^{ab}	3.70 ± 0.29^{ab}	2.30 ± 0.39^{b}	0.94 ± 0.16^{b}	0.76 ± 0.13^{a}
2% BPM	2.37 ± 0.22^{a}	758.50 ± 118.05^{a}	3.09 ± 0.33^{b}	2.72 ± 0.53^{ab}	1.00 ± 0.08^{ab}	0.91 ± 0.14^{a}
3% BPM	2.50 ± 0.21^{a}	604.84 ± 62.39^{bc}	3.33 ± 0.46^{b}	3.01 ± 0.31^{a}	0.91 ± 0.17^{b}	0.96 ± 0.08^{a}

Results are expressed as mean values of four replications (mean ±SD). Different letters after the values in the table indicate significant difference (*P*<0.05). SD=standard deviation

The two experiments were conducted under two experimental conditions, and two different vegetables were grown in two types of soils. The BPM soil additive was the same in both experiments. The focus of the research was to test if BPM can reduce the As concentration in plants. In both experiments, we found that application of BPM to As-contaminated soil could reduce the As concentration in the edible part of the plants. In the experimental conditions of Japan, JMS grew well in As-spiked andosol that contained BPM. BPM contained high amounts of P and was an organic material, so plants grew better in BPM-containing soil than that of controls. Plant growth in terms of DW was significantly higher in BPM-containing soil than that of the control plants (Fig. 1). In the experimental conditions of Bangladesh, BS grew well in the alluvial soil, but growth was significantly greater in soil containing 3% BPM (Fig. 4). In both the experimental conditions, application of BPM to soils yielded better growth of plants. These results might be due to the presence of higher amounts of P in BPM-treated soils, which could increase plant growth. In fact, BPM is an organic fertilizer that can increase plant growth.

Application of BPM significantly reduced the As concentration in the edible part of both JMS and BS compared to the As concentration in control plants. The As concentration in the edible part of the JMS was reduced by 44%, 48%, and 61% (DW) with the application of 1%, 2%, and 3% BPM, respectively, compared to that of the control plants (Fig. 2). The reduction of As concentration in the edible part of JMS was significantly different from that of the control; however, among the BPM treatments (1%, 2%, and 3%), the As concentrations were statistically similar (Fig. 2). The As concentration in the edible part of BS was reduced significantly with the application of BPM. The As concentration in the edible part of BS was reduced by 42%, 43%, and 49% (DW) with the application of 1%, 2%, and 3% BPM, respectively, compared to that of the control plants (Fig. 5). The As concentration in the edible part of BS was significantly lower than that of the control; however, among the BPM treatments (1%, 2%, and 3%), the As concentrations were statistically similar (Fig. 5). These results show that under the two different experimental conditions, BPM significantly reduced the As concentrations in the edible parts of both plants.

The total As uptake (μg/plant) into the edible parts of the plants increased significantly in JMS plants growing in soils containing 2% and 3% BPM compared to that of control plants (Fig. 3). In BS, the total As uptake (μg/plant) was reduced significantly in soil containing BPM compared to that of the control (Fig. 6). In JMS grown in As-contaminated Japanese andosol containing BPM, the As concentration in plants decreased but the As uptake into plants increased. Therefore, the decrease in As concentration in the edible part of plants was primarily due to a "dilution effect" because of the increased plant growth. The Japanese andosol is known to be a P-deficient soil that is low in available P. Thus, the effect of high P provided by BPM on plant growth may be more significant depending on the soil. However, in plants grown in the Bangladesh soil, the As concentration and As uptake both decreased in soils containing BPM. The result in Bangladesh soil is not simply explained by the "dilution effect" on plant growth. Bangladesh soil has a higher availability of P, and plant growth was not substantially improved by the addition of BPM. Therefore, it appeared that factors other than increased P supplied by BPM were involved in the reduction of plant As concentration.

One of the possible factors involved in the reduction of plant As concentration is the biochemical competition between As and P. Previous results suggest that P attenuates the absorption of As. Both As and P belong to the V family on the periodic table of the elements. Due to the similarities in their chemical properties, they behave similarly in the soil-plant system. Phosphate can decrease or increase the uptake of As by plants, depending on the As species, the plant species, and the growth medium [40, 41]. Our results are in good agreement with the results of many other reports [29, 30, 32–34, 42–44]. There are reports that demonstrated competition between P and As for the high-affinity phosphate transporter [33, 44–47]. 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. Competition between uptake of P and As was also reported in a vegetable crop [48].

This work showed that plant P concentration increased in soils containing BPM (Tables 2, 3). In Bangladesh soil, plant K concentration increased in soils containing BPM. Bangladesh alluvial soil may be deficient in K. The Ca concentration in the edible part of both JMS and BS decreased significantly when grown in soils containing BPM (Tables 2, 3). This might be due to Ca precipitation in combination with high P concentration in BPM. There were no significant effects of BPM on other elements.

BPM might have properties that enable the adsorption of As. BPM contains charcoal because of the baking treatment. It is possible that the surface of BPM might adsorb As and thereby attenuate 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 some of the dangers of As-contaminated soil. It may help to safeguard the food security for human populations in regions with heavy burdens of As contamination in the groundwater and soil. Future work will optimize parameters such as pH and soil moisture content to further reduce plant As concentration. Searches for more desirable materials to reduce plant As concentration should continue.

IV. CONCLUSIONS

This study showed that As accumulation in the edible part of plants was attenuated in As-contaminated soils by high concentrations of P supplied with BPM. The application of BPM to As-contaminated soils also increased plant growth. The results suggest that BPM was effective due to a "dilution effect" and/or a "competition effect" induced by the P supplied by BPM. Further studies are required 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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