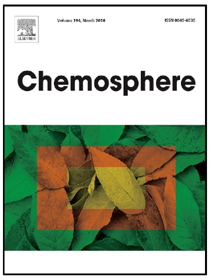

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Effect of Exogenous Phosphate on the Lability and Phytoavailability of Arsenic in Soils

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Abstract: The effect of exogenous phosphate (P, 200 mg·kg^{-1} soil) on the lability and phyto-availability of arsenic (As) was studied using the diffusive gradients in thin films (DGT) technique. Lettuce were grown on the As-amended soils following the stabilization of soil labile As after 90 day's incubation. Phosphate (P) application generally facilitated plant growth except one grown on P-sufficient soil. Soil labile As concentration increased in all the soils after P application due to a competition effect. Plant As concentration increased in red soils collected from Hunan Province, while decreases were observed in the other soils. Even though, an overall trend of decrease was obtained in As phytoavailability along with the increase of DGT-measured soil labile P/As molar ratio. The functional equation between P/As and As phytoavailability provided a critical value of 1.7, which could be used as a guidance for rational P fertilization, thus avoiding overfertilization.

Keywords: Diffusive gradients in thin films; phosphorus-arsenic interaction; labile P/As molar ratio

1. Introduction

Arsenic (As) contamination is ubiquitous in all environmental mediums and a worldwide concern. It is well-characterized as highly toxic, mutagenic and carcinogenic (Sun et al. 2011) to human, plants and microorganisms (Bolan et al. 2013, Hartley et al. 2008). Some areas in China, especially in Hunan province, have historical As contamination caused by realgar mine or tailing lagoon failure (Liao et al. 2005). Total As in soil contains large fractions unavailable to biota (Wang et al. 2014), therefore identifying the 'available' fraction of As in soils is urgently needed (McLaughlin et al. 2000, Mojsilovic et al. 2011). Manipulating the bioavailability of As in soils using chemical or biological means is an universal approach in remediation of As-contaminated soils (Bolan et al. 2013). Arsenate (AsO_4^{3-}) is the thermodynamically stable inorganic form of As under aerobic condition in soils (Masscheleyn et al. 1991, Signes-Pastor et al. 2007), and is strongly retained on soil mineral surfaces. As a chemical analogue of arsenate (Terwelle et al. 1967), phosphate has been commonly used to manipulate the mobilization of As in soils for soil remediation (Bolan et al. 2013), including phytoremediation (Jankong et al. 2007) and chemical washing.

Phosphorus (P) and As belong to the same family element and have similar external electronic structures. In environmental medium, P and As can form similar speciation phosphate (PO_4^{3-}) and arsenate (AsO_4^{3-}). Due to their similar chemical structures, ubiquitous competition could be found in sorption on both soil particles and plant root surfaces. Competition in sorption of phosphate and arsenate may vary greatly on different soils characterized by different mineralogy and chemical properties (Liu et al. 2001, Peryea 1991, Roy et al. 1986, Smith et al. 2002, Woolson et al. 1973). Generally, large P addition could facilitate As solubility in soil due to the stronger affinity of P to soil particles or mass action effect (Chen et al. 2002, Smith et al. 2002). As for the competition of P and As on root surfaces, plants adsorb arsenate *via* phosphate uptake channel (Meharg et al. 1991, Mojsilovic et al. 2011), and the uptake of As would be affected through the effect of P on root adsorption and translocation from root to shoot. Some studies suggested that P exhibited a significant ameliorative effect on the sensitivity of plant to soil As (Lei et al. 2012, Mojsilovic et al. 2011, Pigna et al. 2009). Wang et al. (2002) and Lou et al. (2010) found that increasing P supply could greatly decrease As uptake by Chinese brake (*P. vittata* L.). However, some other studies have suggested that addition of P increases As solubility and mobility, thereby increasing the plant uptake of As (Bolan et al. 2013). P addition would, on one hand increase As lability in soil, and on the other hand inhibit As phyto-availability. The contribution portion of P-As competition on soil particles and on plant roots may be the key factor that affects the availability of As in soil-plant system, which are still not clear.

In some studies, researchers tried to correlate soil P/As ratio with As phytotoxicity/phytoavailability. Available P/As ratios reflected different corn yields between two soils with similar available As levels, and adequate growth of corn was achieved with a soil available P/As weight ratios of 6.8 in a clay loam soil (Woolson et al. 1973). Increasing P/As ratios in the solution would reduced As uptake by 70% (Esteban et al. 2003), and alleviated the toxicity of As in corn plants (Vetterlein et al.

2007). Ognjen (2009) reported that the estimated EC50 based on the effective soil P/As molar concentration ratio ranged between 2.7 and 7.1, and on the soluble P/As molar concentration ratio ranged between 1.8 and 3.8. Increasing P/As ratio would reduce As phytotoxicity/phytoavailability according to previous literatures, however, no quantitative relationship between them were reported before. One deficiency in previous studies on interactions of P and As is the methodology. Chemical extraction methods were frequently used to monitoring the changes of P and As in soil. However, extraction procedures provided an unrealistic response to given chemical agents, and the extracted P(As) species are unlikely to represent the true available fraction, especially when the speciation or complexes of P(As) changed during the P-As interaction processes. A more precise reflection of the phytoavailable pool of As could be achieved when using the method of DGT (Wang et al. 2014). DGT method, which based on Fick’s first law of diffusion, is a dynamic in-situ technique for the determination of labile metal/metalloid in soil (Zhang et al. 2001). Besides, the kinetic process between soil solid and solution phases were taken into consideration in this method. Zhang et al. (1998) mixed ferrihydrite slurry into a polyacrylamide gel as a binding agent (FH-based DGT) for measurement of phosphate. FH-based DGT had been successfully used for measurement of labile P in soils. The correlations of DGT measured P with P in plant were better than that obtained by chemical extraction methods (Mason et al. 2010, Menzies et al. 2005, Six et al. 2012, Six et al. 2013). As an analogue of P, arsenic could also be measured by FH-based DGT in various medium including soils and waters (Fitz et al. 2003, Panther et al. 2008). Wang et al. (2014)

found that DGT measured soil labile As had a better correlation with plant As. It could reveal a more precise reflection of phytoavailable As pool than chemical extracted As. Mojsilovic et al. (2011) evaluated DGT on modelling As toxicity in wheat (*Triticum aestivum*), indicating that DGT- measured As/P ratio could be a promising phytotoxicity predictor. The objective of this study was to investigate the effect of exogenous P on the lability and phytoavailability of As in a set of As-amended soil samples, and to explorer the relationship between DGT-measured labile P/As molar ratio and As phytoavailability.

2. Materials and Methods

2.1 Soil collection and preparation

Eleven uncontaminated, cultivated topsoils (0-20 cm depth) were collected from nine sites in China; six soil samples collected from Beijing (BJ), Chongqing (CQ), Gansu (GS), Guizhou (GZ), Jilin (JL), Liaoning (LN), respectively, and other five samples from Hunan (HN). The soils were air-dried, and sieved to < 2 mm for pot experiments and DGT deployments. Subsamples of the soils were air-dried and ground (<0.149 mm) for chemical analysis. Soil pH was measured in a suspension of 1:2.5 soil/carbon dioxides–free water (PHS-3C, China) (Xu et al. 2018). Total organic matter content was estimated using the potassium dichromate volumetric method (He et al. 2017). Soil total N content was determined using semi-micro Macro Kjeldahl method (Calvo-Fernández et al. 2018). Soil total P content was determined using UV spectrophotometry following alkali fusion (Meena et al. 2018). Soil total K content was determined using flame photometry following alkali fusion (Xu et al. 2018). Soil Alkaline N content was determined using alkaline hydrolysis diffusion method (Bremner et al. 1966). Soil Olsen P content was determined using UV spectrophotometry following 0.5 M $NaHCO_3$ extraction (Egan et al. 2018). Soil rapid available K content was determined using flame photometry following 1 M NH_4OAc extraction (Ji et al. 2014). Soil available Fe content was determined using atomic absorption spectrometry (AAS) following DTPA extraction (Chatzistathis et al. 2017). Soil available Mn content was determined using AAS following EDTA extraction (Huang et al. 2017). The physical and chemical properties of soils used in this study were shown in Table 1.

Separate subsamples (2 kg) were amended with Na_2HAsO_4 solution at 60 mg $As{\cdot}kg^{-1}$ soil. All amended soils were stored in plastic boxes in dark with soil moisture of 30 % maximum water holding capacity (MWHC) at 20 ± 4 °C. DGT technique was used to monitor the changes of labile As in soils during aging. After 90 days incubation, the change of labile As concentration in all soils became less marked (data not shown). The soils were used for pot experiments afterwards.

2.2 Bioassay

Pot experiments were conducted to study the effect of P application on the lability and phytoavailability of As in soil. Each soil was divided in half for two different treatments. Treatment A: the soil was applied with 200 $mg{\cdot}kg^{-1}$ P, where nutrients (N, NH_4HCO_3; P, $NH_4H_2PO_4$; K, K_2SO_4) were added into the soils according to the ratio

N: K_2O = 0.15:0.15 g/kg soil to achieve 200 mg·kg^{-1} soil exogenous P in total. Treatment B: non-P application, where nutrients (N, NH_4HCO_3; K, K_2SO_4) were added into the soils with the ratio N: K_2O = 0.15:0.15 g/kg soil.

Lettuce was sown in pot containing 500 g of soil (dry mass, triplicate for each soil). Soil moisture was maintained at approximate 60% MWHC. Soils were left to equilibrate for 7 days before seeding. Once sown, all pots were placed in a glasshouse (20 ± 4 °C, natural light). To simulate the agricultural fertilization, macronutrient solution was added to the soils to achieve the amount mentioned above at 7, 14, and 21 days after seeding during plant growth. At six weeks after germination, the aboveground plant tissues were harvested, rinsed with deionized water, and oven-dried at 70°C for 72 h. Total P and As concentrations in the plant tissues were determined by inductively coupled plasma mass spectrometry (ICP-MS, Thermo X7) following microwave-assisted nitric acid digestion.

2.3 DGT preparation, deployment and calculation.

A DGT device consists of a plastic assembly containing a precipitated ferrihydrite binding gel overlaid by a layer of polyacrylamide diffusive gel, and a protective filter membrane through which ions can freely diffuse (Panther et al. 2008, Zhang et al. 2001). The binding gels and diffusive gels were prepared following a standard procedure (Luo et al. 2010).

DGT devices were deployed after plant harvest. The soils in each pot were wetted to 80%-100% MWHC, then the surface soil were mixed into slurry and left to equilibrate for 24 h at room temperature. A DGT device was placed on each pot and pressed down gently to ensure complete contact between the filter membrane of the device and the soil, and then left for 24 h. Temperature were recorded during the deployment. Upon retrieval of DGT devices, the binding gels were removed and eluted with 0.25 M H_2SO_4 for at least 24 h prior analysis by ICP-MS.

After a certain period of deployment, the time-averaged concentration of solute (C_{DGT}) at the interface of DGT device and soil can be calculated using Eq. (1) (Zhang et al. 2001):

$$C_{DGT} = M\Delta g/(DAt) \qquad (1)$$

where Δg is the total thickness (0.81 mm) of the diffusive gel layer and the filter membrane, D is the diffusion coefficients of solute (P and As) in the diffusive gel (Luo et al. 2010, Zhang et al. 1998), A is the surface area (3.14 cm^2) of the DGT sampling window, t is the deployment time (24 h), and M is the total amount of accumulated solute (P and As) in the binding gel, which was calculated using Eq. (2):

$$M = C\,(V_{acid} + V_{gel})/f_e \qquad (2)$$

where C expresses the solute concentration in the elution sample as measured by ICP-MS, V_{acid} is the volume of acid used for elution (1 mL) and V_{gel} is the volume of the binding gel (0.25 mL). f_e, the elution factor, is 100% for P (Zhang et al. 1998) and 0.78 for As (Luo et al. 2010) when 0.25 M H_2SO_4 is used as the extracting solution.

2.4 Quality Control (QC)

To control accuracy of heavy metal determination, reagent blanks, triplicate samples, and standard reference materials (GSS-1 soils and GSB-6 spinach, obtained from Center of National Standard Reference Material of China) were inserted with every batch of samples. The reference material recoveries for P and As in soil were 85.4±7.6 and 90.1±1.9%, while for P and As in spinach were 90.6±5.0 and 98.3±2.7%.

3. Results and discussion

3.1 Effect of exogenous P on DGT-measured concentrations of P and As.

Concentrations of labile P and As measured by DGT in soils with/without exogenous P addition were shown in Table 2. After addition of large amount of exogenous P of high lability, labile P concentrations in P applied soils increased clearly. Due to the chemical similarity of phosphate and arsenate, the addition of P would compete the adsorption sites with As on soil particles. In the present study, DGT-measured concentrations of the labile As increased with the increasing concentrations of labile P in P applied soils, and the percentage (PI) of increased labile P concentration compared to the blank were significantly correlated (R = 0.85, $p < 0.01$) with that of increased concentration of labile As. The increase of As desorption in soils after P addition was due to the stronger affinity of P to soil particles (Feldmann et al. 2002, Zhao et al. 2009).

3.2 Effect of exogenous P on plant growth.

The fresh weights of above ground part of lettuce under two different treatments of exogenous P were shown in Fig. 1. The biomass of lettuce grown in 200 mg·kg^{-1} P applied soils were generally higher than those in non-P applied soils. In soils BJ, GS, GZ, HN-1, HN-2, HN-4 and HN-5, the biomass of lettuce grown in P applied soils were significantly ($p < 0.05$) higher than those in non-P applied soils, wherein the most significant increase (554.0 %) was found in soil GZ. In the rest of soils, slight increases ($p > 0.05$) were found in soils CQ, LN and HN-3, while an unexpected significant ($p < 0.05$) decrease was found in soil JL.

The stepwise multiple regression analysis between fresh weights (FW) and some possible influential factors, including total accumulated mass of P and As in plant tissues (M_P and M_{As}), and soil physical and chemical properties, were listed in Table 3. The results showed that the biomass of lettuce depends on the accumulated mass of P, and further depends on the available P status in the soil. The most significant increase in biomass after P addition was found in soil GZ, while the significant decrease was found in soil JL. From Table 1 we could observe that the content of Olsen P in soil GZ and JL before P addition were 0.1 and 127.5 mg·kg^{-1}, respectively, which were the lowest and the highest values among all the collected soils. For soil GZ, the lowest Olsen P content resulted in a lowest biomass, while for soil JL, the highest Olsen P content resulted in a highest biomass. After addition of exogenous P, the nutrients significantly facilitated the growth of lettuce in soil GZ. For soil JL, P sufficiency made the extra applied P had little effect on plant growth, and on the other hand, the addition of P would facilitate the increase of soil labile As (see in Table 2), which resulted in a negative effect on plant growth. The growth of lettuce mainly depends on the P supply ability of soil and plant needs for P nutrient.

The general increase of plant biomass after exogenous P application could be attributed to P-induced growth response, indicating that plant growth was mainly depended on the supply of available P to the plant. However, this effect was not obvious in JL soil with largest P_{DGT} (26.3 $\mu g \cdot L^{-1}$). Exogenous P application would significantly increase soil labile P concentration, which would disturb the solution-solid phase distribution of As in soil, and consequently alter the lability of As. In this study, the increase of soil labile As concentration in all soils after exogenous P application were due to the competition of P and As on the sorption sites of soil particles. This P-induced As mobilization effect has also been reported previously (Bolan et al. 2013, Mkandawire et al. 2004). However, the effect of P on As phytoavailability does not equal to that on As desorption and lability in soil, and it depends on the extent of P-induced As mobilization in soils and P-induced competition with As for uptake by roots (Bolan et al. 2013).

3.3 Relationship between soil P(As) and plant P(As).

The Log-Log relationship between soil labile concentration and total concentration of P and As in plant tissues were shown in Fig. 2. Both P and As concentration in the plant tissues were significantly correlated (p<0.01) with DGT measured concentration of labile P and As in soil. Similar results were obtained previously for the relationship between P(As) concentration in plant and DGT measurement of P(As) concentration in soils (Menzies et al., 2005; Mason et al., 2010; Six et al., 2012; Six et al., 2013).

In a previous study from our research laboratory, DGT measured As concentration had a better correlation with plant As, and had a closer reflection of phyto-available As pool than chemical extracted As (Wang et al. 2014). For measurement of P in soil, numerous researches had demonstrated the feasibility and accuracy of DGT method (Mason et al. 2010, Six et al. 2012, Six et al. 2013), Six et al. (2014) indicated that DGT method relates best with yields compared to the established soil P tests Olsen and AEM (anion exchange membranes). DGT technique had also been adopted for simultaneous measurement of soil labile P and As (Mojsilovic et al. 2011). However, P and As have different affinities for ferrihydrite (Violante et al. 2002), which might lead to a competition of P and As on DGT binding gel.

3.4 Total P and As concentrations in aboveground tissues.

Total P and As concentrations in plant tissues under two different P treatments were shown in Fig. 3. After the addition of exogenous P, the total P concentration in plant tissues increased for all soils, compared to those in non-P applied soils. However, an interesting phenomenon was observed on the changes of total As concentration in plant tissues after P addition. For all the red soils collected from Hunan Province, the total As concentration in plant tissues increased after P application, while a decrease was observed in the other soils used in this study.

The increase of soil labile As after P addition did not result in increases of plant As in all the studied 11 soils, although significant positive relationship was obtained between soil labile As concentration and plant As concentration. Two opposite competition effect were observed on different types of soils. Facilitative effects on plant uptake As was observed in the red soils collected from Hunan Province, while an inhibition effect was observed in the other soils collected from rest areas.To explore the reason why As concentration in soils and plant tissues having different changing trends, the studied 11 soils were divided into two categories: red soils collected from Hunan Province (HN-soils), and the other soils collected from other areas (Other-soils). The stepwise multiple regression analysis were introduced to study the relationship between fresh weight (FW), As concentration in plant tissues (As_{plant}), labile As concentration (As_{DGT}) and soil physical and chemical properties.

As shown in Table 4, the stepwise multiple regression analysis showed that soil activated Al content (A-Al) was the main factor that would affect the plant As concentration on HN-soils, while As concentration on Other-soils would be affect not only by A-Al, but also by soil pH and CEC. Activated Al compound in soil provides sorption sites for As, P application would compete with As on these sorption sites, thereby alter the mobilization and speciation of As in soil. In addition, H^+ would be generated during the hydrolytic process of Al in soil, and the alteration of soil pH also had a significant influence on As lability and phytoavailability. However, A-Al was statistically the main fact that influence plant As uptake, the biodilution effects may be partly responsible for this phenomenon. After P application, the fresh weight of lettuce from soil BJ, GS, and GZ increased 2.8, 2.4, and 5.5 times, respectively, comparing with that under non-P application treatments, while the average increased times in Hunan soils was 1.3. The exact reason for the differences in different types of soils needs to be further explored using more soils.

From the above results, P application could increase As availability in some certain types of soils, in which P application could be used as a mobilization tool to facilitate plant uptake by hyperaccumlators (Tu et al. 2003). While for some other types of soils, P application will not only meet the demands of crop growth but will also be conducive to decreasing the As risk in soils (Zeng et al. 2012), thereby realizing safe agricultural production.

3.5 Implication of DGT measured soil labile P/As molar ratio.

The relationship between soil labile P/As molar ratio (P/As) and total As concentration in plant tissues (As_{plant}) was shown in Fig. 4. The plant As concentration decreased sharply along with the increase of soil labile P/As molar ratio. The curvilinear equation between P/As and As_{plant} was $y = 2.9073\ x^{-0.869}$ ($R = 0.70$, $n = 22$, $p < 0.01$). A (1.7, 1.8) was the subpoint of zero point on the curve, which could be considered as a critical value. Increasing soil labile P/As molar ratio would have a negative effect on plant uptake of As from soil, and this effect would be more significant when P/As was less than 1.7.

The ratio of P and As concentrations in soil/solution systems provides a simple analogue of the uptake interactions (Mojsilovic et al. 2011). Under solution culture conditions, increasing P/As was found to have a positive effect on wheat growth (Hurd-Karrer 1939). In the solution culture system, the effect of P on As phytoavailability only depends on the competition of P and As on plant roots uptake, and increasing P/As ratio would decrease As uptake by roots. However in soil system, the extent of P-induced As mobilization in soils and P-induced competition for As uptake by roots would both affect As phytoavailability in soil. Due to the complexity of soils, no consistent relationships between the plant growth and the soil P/As ratios across a range of soil types from previous literatures was (Benson 1953, Mojsilovic et al. 2011). Besides, the inaccuracy and uncertainty of extraction methods may lead to an imprecise representative of the true relationship between phyto-available P and As in soil. In this study, DGT technique was employed for the determination of soil labile P/As molar ratio. The application of exogenous P increased the DGT-measured labile P/As molar ratios (P/As) in all the experimental soils. Even though increases in plant As concentration in HN-soils after P application were observed, an overall trend of decrease of plant As concentration in all soils was observed with an increasing P/As. The functional equation between DGT-measured soil labile P/As molar ratio and plant As concentration provided a critical value of 1.7, which proposed an initial concept of the threshold effect of DGT-measured soil labile P/As molar ratio on plant accumulated As concentration. This value could be used as a guidance for rational P fertilization, thus avoiding overfertilization and a series of agro-environment problems that it posed.

An absence of a consistent effect in studies on soil P/As ratio and its relationships with plant growth and As phytoavailability could be observed. Morphological and biochemical alterations due to the changes of soil physical and chemical properties and plant physiology after exogenous P application could also be influence factors. For example, different soil P abundance would affect the growth, which would cause changes in soil exploration, along with secretion of organic acids and phosphatase enzymes in to the rhizosphere (Mojsilovic et al. 2011). Furthermore, fungal symbionts in the rhizosphere would also affect P-As interactions though exclusion of As, via detoxification and efflux. In this study, the overall trend of plant As along with increasing P/As was decreasing, however, As concentrations in plant tissues on HN-soils increased after P application. This variation was related to soil activated Al content from stepwise multiple regression analysis. However, the reason may be various. The soils used in this study were artificially contaminated soils, which were different from long-term contaminated soils in As lability and soil properties. Overall, the sensitivity of the association between DGT-measured soil labile P/As molar ratio and As phytoavailability on long-term contaminated soils and a great range of P and As fluxes of soil deserves further research.

4. Conclusion

The results obtained in this study showed that exogenous P application could generally facilitate plant growth, yet this facilitating effect was unconspicuous in P-suffcient soils. Competition was the main interaction between P and As in soil, and application of exogenous P would induce the mobilization of As in soil, thereby increase As lability in soil. Application of exogenous P facilitated As uptake by lettuce in red soils collected from Hunan Province, while P inhibited As uptake by lettuce in the other soils used in this study, which was conducive to decreasing the As risk in soils, thereby realizing safe agricultural production. Competition between P and As on plant uptake was more complicated, soil activated As content maybe an important influence factor, which still need to be further explored. Significant correlation was found between DGT-measured labile P/As molar ratio and As phytoavailability, which provided a critical value of 1.7, proposing an initial concept of the threshold effect of DGT-measured soil labile P/As molar ratio on plant accumulated As concentration.

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References

Benson, N. R. 1953. "Effect of Season, Phosphate, and Acidity on Plant Growth in Arsenic-Toxic Soils." Soil Science, 76 (3): 215-224.

Bolan, N., S. Mahimairaja, A. Kunhikrishnan and G. Choppala. 2013. "Phosphorus–Arsenic Interactions in Variable-Charge Soils in Relation to Arsenic Mobility and Bioavailability." Science of The Total Environment, 463–464 (0): 1154-1162.

Bremner, J. M. and D. R. Keeney. 1966. "Determination and Isotope-Ratio Analysis of Different Forms of Nitrogen in Soils: 3. Exchangeable Ammonium, Nitrate, and Nitrite by Extraction-Distillation Methods1." Soil Science Society of America Journal, 30 (5): 577-582.

Calvo-Fernández, J., Á. Taboada, A. Fichtner, W. Härdtle, L. Calvo and E. Marcos. 2018. "Time- and age-related effects of experimentally simulated nitrogen deposition on the functioning of montane heathland ecosystems." Science of The Total Environment, 613-614 (Supplement C): 149-159.

Chatzistathis, T., A. Papaioannou, D. Gasparatos and A. Molassiotis. 2017. "From which soil metal fractions Fe, Mn, Zn and Cu are taken up by olive trees (Olea europaea L., cv. 'Chondrolia Chalkidikis') in organic groves?" Journal of Environmental Management, 203 (Part 1): 489-499.

Chen, T., Z. Fan, M. Lei, Z. Huang and C. Wei. 2002. "Effect of Phosphorus on Arsenic Accumulation in As-Hyperaccumulator *Pteris Vittata* L. and Its Implication." Chinese Science Bulletin, 47 (22): 1876-1879.

Egan, G., M. J. Crawley and D. A. Fornara. 2018. "Effects of long-term grassland management on the carbon and nitrogen pools of different soil aggregate fractions." Science of The Total Environment, 613-614 (Supplement C): 810-819.

Esteban, E., R. O. Carpena and A. A. Meharg. 2003. "High-Affinity Phosphate/Arsenate Transport in White Lupin (*Lupinus Albus*) Is Relatively Insensitive to Phosphate Status." New Phytologist, 158 (1): 165-173.

Feldmann, J. and A. A. Meharg. 2002. "Uptake Kinetics of Arsenic Species in Rice Plants." Plant Physiology, 128 (3): 1120-1128.

Fitz, W. J., W. W. Wenzel, H. Zhang, J. Nurmi, K. Stipek, Z. Fischerova, P. Schweiger, G. Kollensperger, L. Q. Ma and G. Stingeder. 2003. "Rhizosphere Characteristics of the Arsenic Hyperaccumulator *Pteris vittata* L. and Monitoring of Phytoremoval Efficiency." Environmental Science & Technology, 37 (21): 5008-5014.

Hartley, W. and N. W. Lepp. 2008. "Effect of In Situ Soil Amendments on Arsenic Uptake in Successive Harvests of Ryegrass (*Lolium perenne cv Elka*) Grown in

Amended As-Polluted Soils." Environmental Pollution, 156 (3): 1030-1040.

He, S. R., Q. Lu, W. Y. Li, Z. L. Ren, Z. Zhou, X. Feng, Y. L. Zhang and Y. T. Li. 2017. "Factors controlling cadmium and lead activities in different parent material-derived soils from the Pearl River Basin." Chemosphere, 182 509-516.

Huang, Q.-n., H. An, Y.-j. Yang, Y. Liang and G.-s. Shao. 2017. "Effects of Mn-Cd antagonistic interaction on Cd accumulation and major agronomic traits in rice genotypes by different Mn forms." Plant Growth Regulation, 82 (2): 317-331.

Hurd-Karrer, A. M. 1939. "Antagonism of Certain Elements Essential to Plants Toward Chemically Related Toxic Elements." Plant Physiology, 14 (1): 9-29.

Jankong, P., P. Visoottiviseth and S. Khokiattiwong. 2007. "Enhanced Phytoremediation of Arsenic Contaminated Land." Chemosphere, 68 (10): 1906-1912.

Ji, W., Z. Shi, J. Huang and S. Li. 2014. "In Situ Measurement of Some Soil Properties in Paddy Soil Using Visible and Near-Infrared Spectroscopy." PLOS ONE, 9 (8): e105708.

Lei, M., X. M. Wan, Z. C. Huang, T. B. Chen, X. W. Li and Y. R. Liu. 2012. "First Evidence on Different Transportation Modes of Arsenic and Phosphorus in Arsenic Hyperaccumulator *Pteris vittata*." Environmental Pollution, 161 1-7.

Liao, X. Y., T. B. Chen, H. Xie and Y. R. Liu. 2005. "Soil As Contamination and Its Risk Assessment in Areas Near the Industrial Districts of Chenzhou City, Southern China." Environment International, 31 (6): 791-798.

Liu, F., A. De Cristofaro and A. Violante. 2001. "Effect of pH, Phosphate and Oxalate on The Adsorption/Desorption of Arsenate on/from Goethite." Soil Science, 166 (3): 197-208.

Lou, L. Q., Z. H. Ye, A. J. Lin and M. H. Wong. 2010. "Interaction of Arsenic and Phosphate on Their Uptake and Accumulation in Chinese Brake Fern." International Journal of Phytoremediation, 12 (5): 487-502.

Luo, J., H. Zhang, J. Santner and W. Davison. 2010. "Performance Characteristics of Diffusive Gradients in Thin Films Equipped with a Binding Gel Layer Containing Precipitated Ferrihydrite for Measuring Arsenic(V), Selenium(VI), Vanadium(V), and Antimony(V)." Analytical Chemistry, 82 (21): 8903-8909.

Mason, S., A. McNeill, M. J. McLaughlin and H. Zhang. 2010. "Prediction of Wheat Response to An Application of Phosphorus Under Field Conditions Using Diffusive Gradients in Thin-Films (DGT) and Extraction Methods." Plant and Soil, 337 (1-2): 243-258.

Masscheleyn, P. H., R. D. Delaune and W. H. Patrick. 1991. "Effect of Redox Potential and pH on Arsenic Speciation and Solubility in A Contaminated Soil." Environmental Science & Technology, 25 (8): 1414-1419.

McLaughlin, M. J., B. A. Zarcinas, D. P. Stevens and N. Cook. 2000. "Soil Testing for Heavy Metals." Communications in Soil Science and Plant Analysis, 31 (11-14): 1661-1700.

Meena, M. D., B. Narjary, P. Sheoran, H. S. Jat, P. K. Joshi, A. R. Chinchmalatpure, G. Yadav, R. K. Yadav and M. K. Meena. 2018. "Changes of phosphorus fractions in saline soil amended with municipal solid waste compost and mineral fertilizers in a mustard-pearl millet cropping system." CATENA, 160 (Supplement C): 32-40.

Meharg, A. A. and M. R. Macnair. 1991. "Uptake Accumulation and Translocation of Arsenate in Arsenate-Tolerant and Non-Tolerant *Holcus-Lanatus* L." New Phytologist, 117 (2): 225-232.

Menzies, N. W., B. Kusumo and P. W. Moody. 2005. "Assessment of P Availability in Heavily Fertilized Soils Using the Diffusive Gradient in Thin Films (DGT) Technique." Plant and Soil, 269 (1-2): 1-9.

Mkandawire, M., Y. V. Lyubun, P. V. Kosterin and E. G. Dudel. 2004. "Toxicity of Arsenic Species to *Lemna gibba* L. and the Influence of Phosphate on Arsenic Bioavailability." Environmental Toxicology, 19 (1): 26-34.

Mojsilovic, O., R. G. McLaren and L. M. Condron. 2011. "Modelling Arsenic Toxicity in Wheat: Simultaneous Application of Diffusive Gradients in Thin Films to Arsenic and Phosphorus in Soil." Environmental Pollution, 159 (10): 2996-3002.

Ognjen, M. (2009). Estimating Bioaccessibility, Phytoavailability and Phytotoxicity of Contaminant Arsenic in Soils at Former Sheep Dip Sites. master Master Dissertation, Lincoln University.

Panther, J. G., K. P. Stillwell, K. J. Powell and A. J. Downard. 2008. "Development and Application of the Diffusive Gradients in Thin Films Technique for the Measurement of Total Dissolved Inorganic Arsenic in Waters." Analytica Chimica Acta, 622 (1-2): 133-142.

Peryea, F. J. 1991. "Phosphate-Induced Release of Arsenic from Soils Contaminated with Lead Arsenate." Soil Sci. Soc. Am. J., 55 (5): 1301-1306.

Pigna, M., V. Cozzolino, A. Violante and A. A. Meharg. 2009. "Influence of Phosphate on the Arsenic Uptake by Wheat (*Triticum durum* L.) Irrigated with Arsenic Solutions at Three Different Concentrations." Water Air and Soil Pollution, 197 (1-4): 371-380.

Roy, W. R., J. J. Hassett and R. A. Griffin. 1986. "Competitive Coefficients for the Adsorption of Arsenate, Molybdate, and Phosphate Mixtures by Soils1." Soil Sci. Soc. Am. J., 50 (5): 1176-1182.

Signes-Pastor, A., F. Burló, K. Mitra and A. A. Carbonell-Barrachina. 2007. "Arsenic Biogeochemistry as Affected by Phosphorus Fertilizer Addition, Redox Potential and pH in a West Bengal (India) Soil." Geoderma, 137 (3–4): 504-510.

Six, L., P. Pypers, F. Degryse, E. Smolders and R. Merckx. 2012. "The Performance of DGT Versus Conventional Soil Phosphorus Tests in Tropical Soils - An Isotope Dilution Study." Plant and Soil, 359 (1-2): 267-279.

Six, L., E. Smolders and R. Merckx. 2013. "The Performance of DGT Versus Conventional Soil Phosphorus Tests in Tropical Soils-Maize and Rice Responses to P Application." Plant and Soil, 366 (1-2): 49-66.

Six, L., E. Smolders and R. Merckx. 2014. "Testing Phosphorus Availability for Maize with DGT in Weathered Soils Amended with Organic Materials." Plant and Soil, 376 (1-2): 177-192.

Smith, E., R. Naidu and A. M. Alston. 2002. "Chemistry of Inorganic Arsenic in Soils: II. Effect of Phosphorus, Sodium, and Calcium on Arsenic Sorption." Journal of

Environmental Quality, 31 (2): 557-563.

Sun, L., X. Yan, X. Liao, Y. Wen, Z. Chong and T. Liang. 2011. "Interactions of Arsenic and Phenanthrene on Their Uptake and Antioxidative Response in *Pteris Vittata* L." Environmental Pollution, 159 (12): 3398-3405.

Terwelle, H. F. and E. C. Slater. 1967. "Uncoupling of Respiratory-Chain Phosphorylation by Arsenate." Biochimica et Biophysica Acta (BBA) - Bioenergetics, 143 (1): 1-17.

Tu, C. and L. Ma. 2003. "Effects of Arsenate and Phosphate on Their Accumulation by an Arsenic-Hyperaccumulator *Pteris Vittata* L." Plant and Soil, 249 (2): 373-382.

Vetterlein, D., K. Szegedi, J. Ackermann, J. Mattusch, H.-U. Neue, H. Tanneberg and R. Jahn. 2007. "Competitive Mobilization of Phosphate and Arsenate Associated with Goethite by Root Activity." Journal of Environmental Quality, 36 (6): 1811-1820.

Violante, A. and M. Pigna. 2002. "Competitive Sorption of Arsenate and Phosphate on Different Clay Minerals and Soils." Soil Sci. Soc. Am. J., 66 (6): 1788-1796.

Wang, J. J., L. Y. Bai, X. B. Zeng, S. M. Su, Y. N. Wang and C. X. Wu. 2014. "Assessment of Arsenic Availability in Soils Using the Diffusive Gradients in Thin Films (DGT) Technique-A Comparison Study of DGT and Classic Extraction Methods." Environmental Science: Processes & Impacts, 16 (10): 2355-2361.

Wang, J. R., F. J. Zhao, A. A. Meharg, A. Raab, J. Feldmann and S. P. McGrath. 2002. "Mechanisms of Arsenic Hyperaccumulation in *Pteris Vittata*. Uptake Kinetics, Interactions with Phosphate, and Arsenic Speciation." Plant Physiology, 130 (3):

1552-1561.

Woolson, E. A., J. H. Axley and P. C. Kearney. 1973. "The Chemistry and Phytotoxicity of Arsenic in Soils: II. Effects of Time and Phosphorus." Soil Science Society of America Proceedings, 37 (2): 254-259.

Xu, S., Y. Zhao, M. Wang and X. Shi. 2018. "Comparison of multivariate methods for estimating selected soil properties from intact soil cores of paddy fields by Vis–NIR spectroscopy." Geoderma, 310 (Supplement C): 29-43.

Zeng, X., P. Wu, S. Su, L. Bai and Q. Feng. 2012. "Phosphate Has a Differential Influence on Arsenate Adsorption by Soils with Different Properties." Plant Soil and Environment, 58 (9): 405-411.

Zhang, H., W. Davison, R. Gadi and T. Kobayashi. 1998. "In Situ Measurement of Dissolved Phosphorus in Natural Waters Using DGT." Analytica Chimica Acta, 370 (1): 29-38.

Zhang, H., F. J. Zhao, B. Sun, W. Davison and S. P. McGrath. 2001. "A New Method to Measure Effective Soil Solution Concentration Predicts Copper Availability to Plants." Environmental Science & Technology, 35 (12): 2602-2607.

Zhao, F. J., J. F. Ma, A. A. Meharg and S. P. McGrath. 2009. "Arsenic Uptake and Metabolism in Plants." New Phytologist, 181 (4): 777-794.

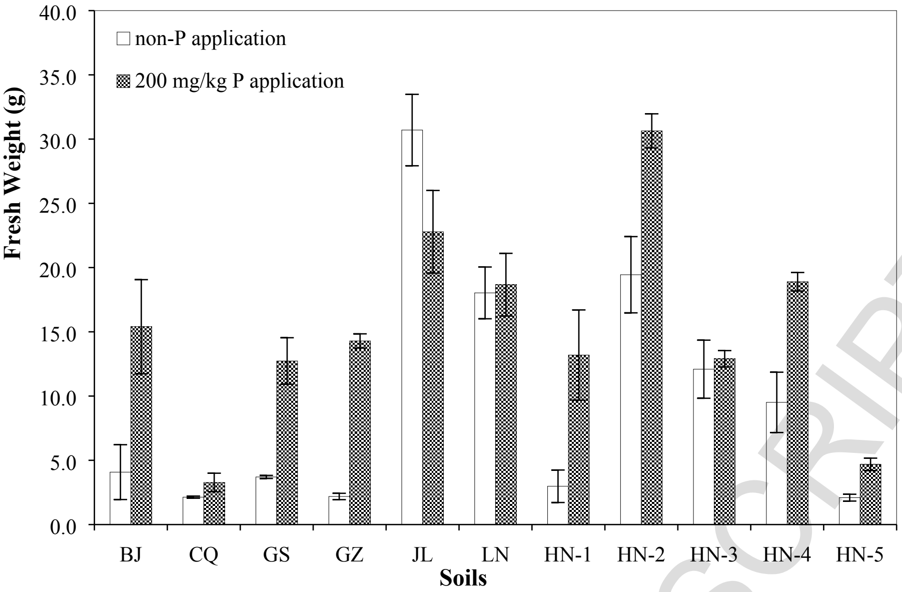

Fig. 1 Mean fresh weight (above ground, triplicate) of lettuce under non-P application treatment and 200 mg/kg P application treatment. Error bars: standard errors (n = 3).

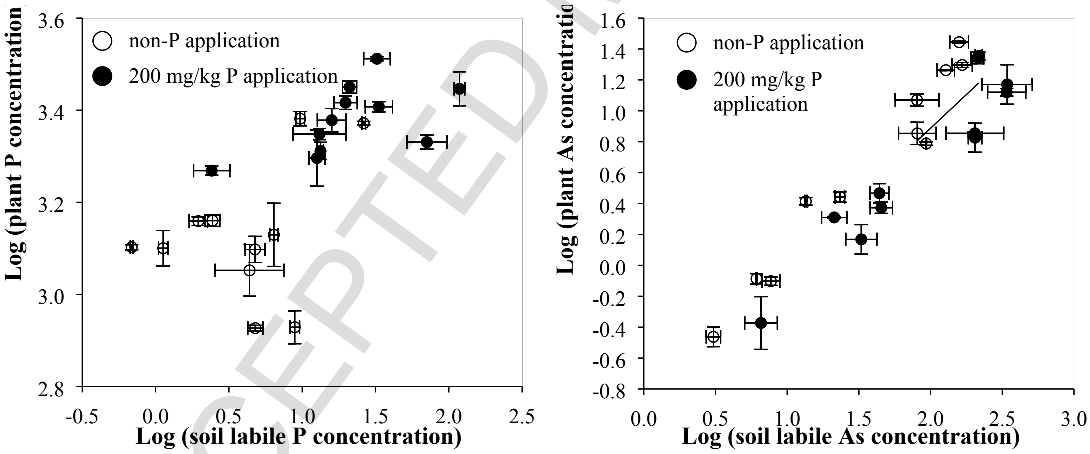

Fig. 2 Dependence of log concentrations of P and As in above-ground plant tissues on the log of DGT-measured concentrations of soil labile P and As. The liner regression equations and correlation coefficients for the logarithmically transformed data are shown. The error bars are standard deviations of the replicate pots.

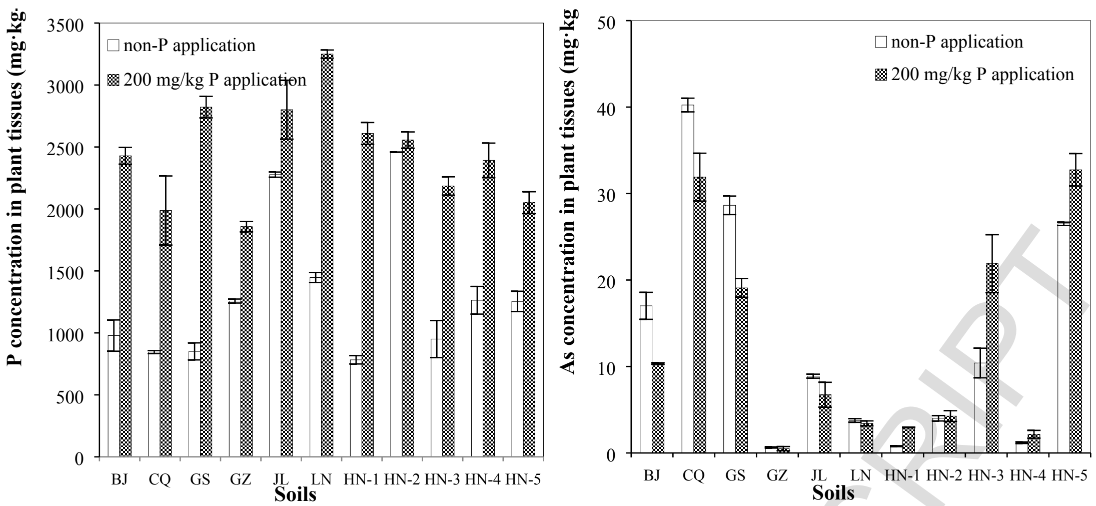

Fig. 3 Total P and As concentration accumulated in plant tissues

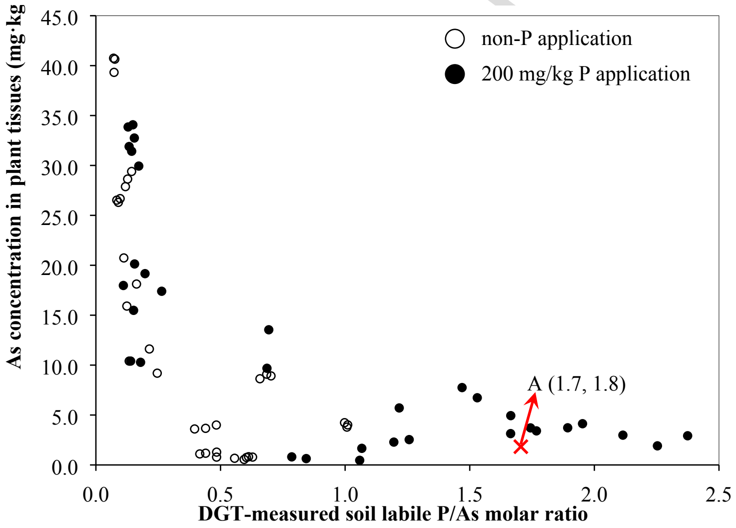

Fig. 4 Relationship between DGT measured soil labile P/As molar ratio and As concentration in plant tissues

Highlights:

- Application of exogenous P would induce the mobilization of As in soil, thereby increase As lability in soil.
- The relationship between DGT measured soil labile P/As molar ratio and As concentration in plant tissues provided a critical value of 1.7, proposing an initial concept of the threshold effect of DGT-measured soil labile P/As molar ratio on plant accumulated As concentration.

Table 1 Physical and chemical properties of the 11 studied soils

Soils	BJ	CQ	GS	GZ	JL	LN	HN-1	HN-2	HN-3	HN-4	HN-5
pH	8.26	8.74	8.10	5.72	6.70	5.48	4.98	7.01	5.10	7.28	8.88
OM ($g\cdot kg^{-1}$)	15.7	19.7	26.8	21.3	31.1	19.4	20.2	29.3	19.6	32.7	7.60
CEC ($cmol\cdot kg^{-1}$)	13.6	21.5	8.31	14.4	25.3	13.1	10.7	9.02	6.50	15.0	11.7
T-N ($g\cdot kg^{-1}$)	1.13	1.04	1.29	1.33	1.64	1.10	1.19	2.32	1.32	1.84	0.79
T-P ($g\cdot kg^{-1}$)	0.586	0.667	1.28	0.364	0.901	0.530	0.642	1.08	0.602	0.711	0.723
T-K ($g\cdot kg^{-1}$)	20.6	22.9	21.4	11.1	21.1	21.0	12.3	27.7	39.9	17.8	24.1
Alkeline-N ($mg\cdot kg^{-1}$)	109	113	108	282	135	136	108	223	148	149	42.2
Olsen P ($mg\cdot kg^{-1}$)	12.5	57.9	86.1	0.112	128	26.6	32.9	65.1	54.8	14.6	10.6
A-K ($mg\cdot kg^{-1}$)	102	173	317	84.4	199	137	190	118	58.2	182	87.1
A-Fe ($mg\cdot kg^{-1}$)	11.8	72.7	10.8	23.3	45.2	81.4	32.3	38.2	71.0	37.1	5.62
A-Mn ($mg\cdot kg^{-1}$)	18.7	33.1	17.5	11.6	54.5	49.8	194	94.4	34.2	23.2	13.3
A-Al ($mg\cdot kg^{-1}$)	2.13	0.84	1.30	2.92	3.10	2.29	2.58	3.36	1.75	3.05	n/a*
T-As ($mg\cdot kg^{-1}$)	8.86	6.37	14.2	17.0	14.7	10.2	22.0	22.6	20.8	23.7	10.7

T-N: soil total N content; T-P: soil total P content; T-K: soil total K content; A-K: soil rapid available K content; A-Fe: soil available Fe content; A-Mn: soil available Mn content; A-Al: soil activated Al content; T-As: soil total arsenic concentration (all the values are lower than the second level of environmental quality standard for soils (30 $mg\cdot kg^{-1}$), PR China (GB 15618-1995)); *n/a: not available

Table 2 Labile P and As concentrations in different soils measured by DGT with/without P treatments

Soils	labile P concentrations			labile As concentrations		
	P0 ($\mu g\cdot L^{-1}$)	P200 ($\mu g\cdot L^{-1}$)	PI (%)	P0 ($\mu g\cdot L^{-1}$)	P200 ($\mu g\cdot L^{-1}$)	PI (%)
BJ	3.2 ± 0.2	13.9 ± 5.5	332.0	65.7 ± 0.2	160.5 ± 15.9	144.2
CQ	4.8 ± 0.5	12.7 ± 1.5	163.5	172.5 ± 2.5	200.6 ± 6.4	16.3
GS	8.9 ± 0.7	21.1 ± 2.3	137.2	152.5 ± 11.5	284.6 ± 6.4	86.6
GZ	0.7 ± 0.0	2.5 ± 0.7	260.5	3.1 ± 0.4	5.7 ± 0.3	84.1
JL	26.3 ± 0.7	112.6 ± 2.3	328.6	93.2 ± 0.7	205.0 ± 22.1	120.1
LN	2.5 ± 0.3	36.5 ± 1.4	1386.7	13.5 ± 0.2	50.3 ± 7.8	272.9
HN-1	2.0 ± 0.3	21.9 ± 0.8	1013.7	7.1 ± 1.2	21.6 ± 4.3	204.4
HN-2	9.7 ± 0.7	29.6 ± 2.4	205.3	23.4 ± 1. 8	40.3 ± 6.9	72.5
HN-3	6.4 ± 0.4	59.6 ± 6.4	829.5	67.9 ± 0.5	288.5 ± 28.4	324.8
HN-4	1.1 ± 0.1	14.0 ± 3.8	1147.3	6.1 ± 0.0	33.5 ± 8.1	448.9
HN-5	4.8 ± 0.7	13.3 ± 0.6	177.6	117.9 ± 0.7	217. 1 ± 12.4	84.1

[a] P0: non-P application; P200: 200 $mg\cdot kg^{-1}$ P application; PI: Percentage of increase.

Table 3 Stepwise multiple regression analysis between fresh weight of plant tissue and total accumulated mass of P in plant tissue.

Treatments	Stepwise multiple regression equation	Correlation coefficient (R)
non-P application	$FW_0 = 2.661 + 0.401\ M_{P(0)}$	0.975
200 mg·kg^{-1} P application	$FW_{200} = 2.145 + 0.329\ M_{P(200)}$	0.962

[a] FW_0: fresh weight of aboveground tissues grown in non-P applied soils (g); FW_{200}: fresh weight of plant tissues grown in 200 mg·kg^{-1} P applied soils (g); $M_{P(0)}$: total accumulated mass of P in plant tissues grown in non-P applied soils (mg); $M_{P(200)}$: total accumulated mass of P in plant tissues grown in 200 mg·kg^{-1} P applied soils (mg).

Table 4 Stepwise multiple regression analysis

Soils	Stepwise multiple regression equation	Correlation coefficient (R)
HN-soils	FW = 2.457 + 4.741 A-Al	0.69
	As_{plant} = 27.581 – 8.163 A-Al	0.95
	As_{DGT} = 191.513 – 50.826 A-Al	0.66
Other-soils	FW = –7.575 + 6.602 A-Al + 0.101 Olsen-P	0.74
	As_{plant} = 2.109 – 10.459 A-Al + 3.639 pH + 0.515 CEC	0.97
	As_{DGT} = –238.685 + 42.934 pH + 0.932 Olsen-P	0.86