ISSN: 1394-7990
Malaysian Journal of Soil Science Vol. 23: 109-118 (2019) Malaysian Society of Soil Science

Effect of Triple Super Phosphate (TSP) and KH_2PO_4 at Different Application Rates on Biomass and Arsenic Uptake by *Pteris vittata* L.

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ABSTRACT

Pteris vittata L. has been demonstrated to absorb heavy metals, especially arsenic (As) from the soil. The interaction of As (V) and phosphate in the ferns that hyperaccumulate As is essential. Therefore, a glasshouse study was conducted to investigate the effect and interactions of phosphate in different forms at varying rates on biomass and uptake of As by *Pteris vittata* L. grown in naturally organic As-rich soil. *P.Vittata* was grown in soil subjected to 9 treatments including control (0 P treatment) and four different rates of P in solid form of Triple Superphosphate (TSP) and liquid form of potassium dihydrogen phosphate (KH_2PO_4) at 12.5, 25, 50 and 75 kg P ha^{-1}. Results showed a significant ($P \leq 0.0001$) increase in frond biomass compared to non-amended soil, whereas the biomass of roots showed no significant ($P > 0.05$) difference in all treatments. The concentration of As in the fronds ranged from 29 to 157 mg kg-1 with the highest uptake of As being 0.71% in TSP, while in KH_2PO_4, it was 0.331%, almost half the rate in TSP. The application of P has been shown to increase the phyto-availability of arsenic in soil, resulting in a positive response on P.vittata As uptake, that is, TSP performed better compared to KH_2PO_4 in terms of As uptake and plant growth. It is postulated that the increase in As uptake was accompanied or more likely a synergism by the addition of phosphate.

Keywords: Assisted Phyto-Remediation, Phosphate Forms, Hyper-accumulator Fern, Plant Uptake

INTRODUCTION

Soil contaminated by arsenic constitutes a fundamental problem to environmental and human health and this problem needs an effective and affordable technological solution (Raskin *et al.* 1997). Conventionally, the remediation of contaminated soil only focused on engineering-related methods rather than plant based techniques. Recently, phyto-extraction has been identified as a potential in-situ remediation option to traditional soil remediation methods (Watanabe 1997). Generally, there are several ways to enhance the phytoremediation process other than screening the plants that hyper-accumulate heavy metals. The first method is by increasing the biomass of hyper-accumulators or application of genetically engineered plants

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which can improve the biomass production to extract more metals from soil. The second method is by elevating the mobility of metals in soil. One example is by the application of amending reagents which enable plants to accumulate more contaminants.

The discovery of Chinese brake fern (*Pteris vittata* L.) to hyper-accumulate arsenic offers the potential for its use as a phyto-remediation agent of As contaminated soil. This plant was the first As hyper-accumulator to be identified (Chen and Wei 2000; Ma *et al.* 2001). Several studies have been carried out in other parts of the world, for example, United States of America (Wang *et al*. 2002) and China (Chen *et al*. 2002) on the effects of phosphate on As accumulation rate by hyper-accumulator, *Pteris vittata* L. However, there is no report on phosphate interaction with arsenic in Pteris vittata in Malaysia to remediate organic As-rich soil. The interaction of As (V) and phosphate in the ferns that hyper-accumulate As is essential since optimum phosphate fertilisation could be a key factor for optimum phyto-extraction of As (Rathinasabapathi *et al*. 2006). This study was conducted to investigate the effect and interactions of phosphate in different forms at varying rates on plant biomass and As uptake by *Pteris vittata* L. grown in naturally organic As-rich soil.

MATERIALS AND METHODS

Soil Sampling

The As-rich soil was sampled in Kampung Renal, Tangkak, Johor, Peninsular Malaysia (N 02.24438^{o}, E 102.55548^{o}). This soil is categorised as Histosol (Saprist) according to the USDA Soil Taxonomy (Soil Survey Staff 2014). The site is under guava and papaya cultivation. The soil sample was collected from the surface till 20 cm depth randomly. Then, the soil samples were placed in clean sacks and kept in a place which was not exposed directly to sunlight to retain moisture and to prevent change in the physico-chemical properties of the organic soil. The soil was then transferred to the laboratory and mixed well until it homogenised. The sample was then sieved through a 2-mm mesh to remove stones and plant materials (Jones 2001).

Plant Sampling

Forty young seedlings of Pteris vittata were obtained from the fern nursery of Universiti Kebangsaan Malaysia (UKM) which propagates *P. vittata* collected from the vicinity of the university.

Soil Preparation

The soil was sieved through the 2.0-mm sieve for routine analysis. The pot experiment was carried out in the glasshouse unit of Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The experiment consisted of nine treatments including control which was T0=Soil without P treatment and four different rates which were T1=12.5, T2=25, T3=50 and T4=75 kg P ha^{-1} in solid form of Triple

Superphosphate (TSP) at four different concentrations of T5=12.5, T6=25, T7=50 and T8=75 kg P ha^{-1} in liquid form of potassium dihydrogen phosphate (KH_2PO_4). Each polybag was fertilised with a basal dose of 75 kg N ha^{-1} (NH_4NO_3) and 105 kg K ha^{-1} (KCl). Before the start of the experiment, the treated soils were incubated for two weeks.

Plant Preparation and Maintenance

A seedling of healthy fern with 5 to 6 fronds each was transferred to each pot containing 16 kg of soil in the glasshouse and grown for 90 days. The ferns were kept under shady conditions and a ventilation fan in the glasshouse was used 24 h to maintain the temperature. Weeding was conducted manually. Soil moisture content was maintained close to field capacity level by adding deionised water periodically as required.

Harvesting

At the end of the experiment, whole plants were harvested. The plants were separated into two parts (fronds and roots including the rhizomes) for further analysis. Plant samples were washed with deionised water and kept in paper bags.

Soil and Plant Analyses

Basic properties of the soil were analysed for pH, electrical conductivity (EC), cation exchange capacity (CEC), total nitrogen (N), total carbon (C), available K, Ca, Mg and total As before the initiation of the experiment. After the plants were harvested, 50 g of soil in each of the polybags was mixed well and kept in a ziplock bag. They were brought to the laboratory for further analyses. The fresh weight of the plant sample was taken before drying in the oven at 65°C. Once constant weight was achieved, the dry matter was weighed, ground into powder to pass a sieve of 0.1 mm and dried in an oven at 105°C to eliminate moisture. Analysis of plant samples was done using dry ashing method to determine arsenic (As) and phosphorus (P) uptake in the fronds and roots of the ferns.

Statistical Analysis

The experiment was a factorial experiment with 4 blocks arranged in a Randomized Complete Block Design (RCBD). Statistical analysis was performed using SAS statistical software, Version 9.4 (SAS Institute Inc., Cary, NC, USA). Evaluation of statistical significance was computed using analysis of variance (ANOVA) and the means were compared using Tukey's Honestly Significant Different (HSD) test at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

The basic properties of the soil are shown in Table 1.

TABLE 1
Properties of soil (mean value ± standard deviation of mean, n=4)

Bulk density (g/cm^3)	0.30 ± 0.02
Organic matter (%)	77.73 ± 0.1
pH	6.24 ± 0.04
Organic carbon (%)	15.94 ± 0.023
Total N (%)	0.824 ± 0.005
Electrical conductivity (mS/cm)	1.68 ± 0.26
CEC ($cmol_c$/kg)	29.44 ± 0.88
Available P (%)	0.06 ± 0.0003
Exchangeable K ($cmol_c$/kg)	0.195 ± 0.04
Exchangeable Ca ($cmol_c$/kg)	32.29 ± 1.09
Exchangeable Mg ($cmol_c$/kg)	0.25 ± 0.05
Total Fe (%)	2.91 ± 0.18
Total As (mg/kg)	62.07 ± 1.37

Effect of Phosphate on Biomass in Fronds and Roots of Ferns

The dry aboveground biomass of fern is presented in Figure 1. The application rate of T3 and T4 in TSP form resulted in a significant ($P \leq 0.0001$) increase in the biomass of fern's fronds compared with control. *Pteris vittata* grew quite well with no apparent chlorosis and necrosis on the fronds. The plant's fronds were collected throughout the experimental period for further analysis to determine As accumulation in the aboveground part. Tu *et al.* (2002) stated that as the fronds aged, As concentration increased. However, Kertulis (2005) in her study concluded that the biomass of *P. vittata* fronds should be harvested before they senesce because As concentrations in live fronds were higher than in the senescing fronds. For the biomass of dry root, there was no significant ($P > 0.05$) difference between treatments and control as the range was 0.495 g to 2.658 g plant^{-1} with the highest rate of both phosphate treatments achieving the highest value. The insignificant results in this experiment could be attributed to the short period of planting (90 days); the response of phosphate towards the growth of root biomass could therefore not be determined.

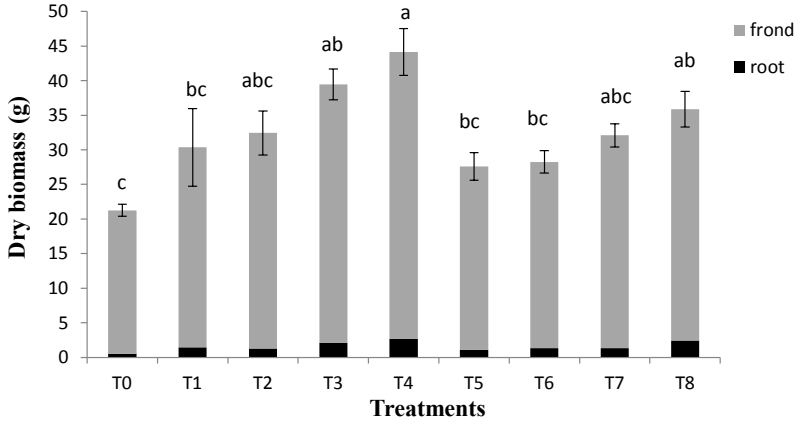

Fig. 1: Total biomass of fern from fronds and roots after 90 days of growing in a soil treated with phosphate.

Notes: T0=control (no P treatment added); T1, T2, T3 and T4 treated with12.5, 25, 50 and 75 kg P ha^{-1} of TSP treatment in solid form, respectively and for T5, T6, T7 and T8 with 12.5, 25, 50 and 75 kg P ha^{-1} of KH_2PO_4 in liquid form, respectively. Error bars represent the standard error of the mean (n=4)

Arsenic Uptake by Pteris vittata

Figure 2 shows As uptake by *Pteris vittata* in fronds after 90 days of planting; As uptake was found to significantly ($P \leq 0.0001$) increase at all rates of phosphate application. The fronds of plants treated with the highest treatment rates from TSP and KH_2PO_4 had the highest value of As uptake compared to the control and other treatments. The higher As uptake by fern applied with different phosphate sources indicates that these phosphates have potential to increase the availability of As in soil to be taken up by plants. However, TSP works better compared with KH_2PO_4. For phyto-extraction to be successful, the contaminants should be bioavailable or subjected to absorption by plant roots and translocated to the harvestable above-ground portions.

The intake of arsenic in roots of *Pteris vittata* is presented in Figure 3. Statistical analysis showed a significant increase ($P \leq 0.05$) in As intake by roots with the application of TSP and KH_2PO_4. The highest value of As intake was observed in soil amended with the highest application rate with the same amount of phosphorus.

Translocation and Bioconcentration Factor

The translocation factor (TF) was used to evaluate how much metal taken up was transported to the aboveground plant parts, while the bioconcentration factor (BCF) was used to indicate the ratio of metal moving from soil to fronds. The potential of a plant species can be assessed based on these numerical factors. Figure 4 shows there was no significant ($P=0.138$) difference in the translocation

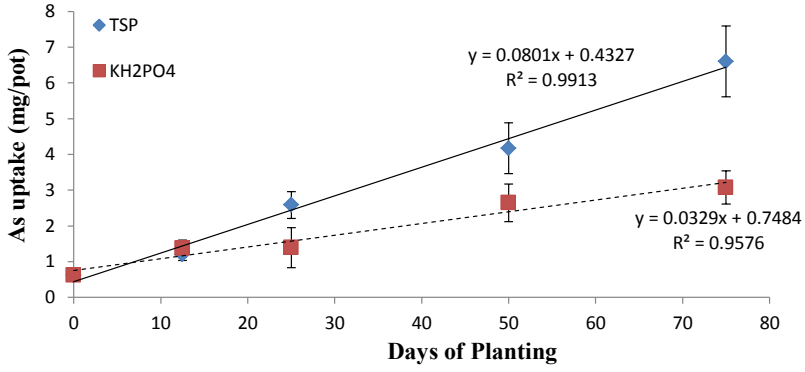

Fig. 2: Arsenic uptake in the fronds of the Pteris vittata. Error bars represent he standard error of the mean (n=4)

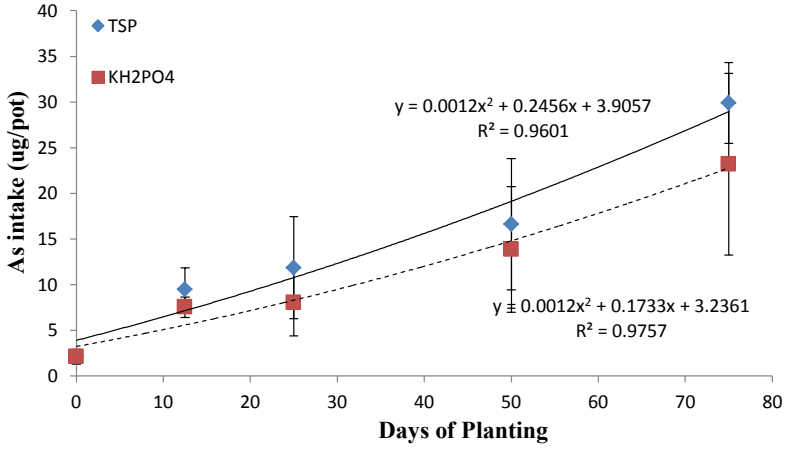

Fig. 3: Arsenic intake in the roots of the Pteris vittata. Error bars represent the standard error of the mean (n=4).

factor (TF) of Pteris vittata with the application of all rates of phosphate fertilisers compared with the control treatment. This showed that *P. vittata* is good in translocating As and can be classified as a hyper-accumulator plant as the value of TF exceeded 1. The contaminant was absorbed and then transported to the aboveground part of the plant making it practicable to harvest the aerial part containing the contaminant.

Figure 5 shows values obtained for BCF of Pteris vittata based on a ratio of As in the aerial part to total arsenic in soils which significantly ($P \leq 0.0001$) increased in all treatment rates compared with control. However, Prasetia *et al.* (2016) stated that a plant should be considered as a hyper-accumulator not only based on TF and BCF results, but also on the ability to accumulate the contaminant.

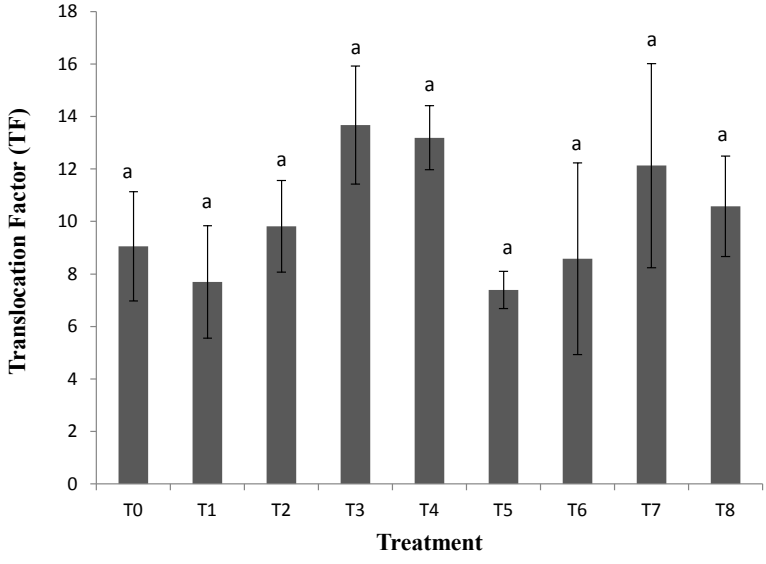

Fig. 4: Translocation factor of Pteris vittata grown in a soil treated with phosphate for 90 days. Error bars represent the standard error of the mean (n=4).

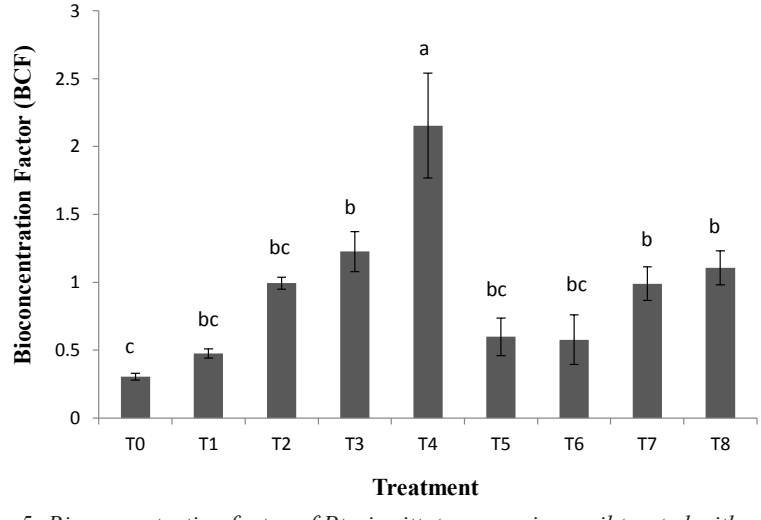

Fig. 5: Bioconcentration factor of Pteris vittata grown in a soil treated with phosphate for 90 days. Error bars represent the standard error of the mean (n=4).

Relationship between Phosphorus Treatment and Plant Uptake in P. vittata

Correlation coefficients between As uptake and phosphate uptake in fronds and roots of *P. vittata* are presented in Table 2. The arsenic uptake by fronds was found to be significantly correlated with phosphate uptake by fronds for both TSP (r = 0.7781***; $P \leq 0.0001$) and KH_2PO_4 (r = 0.8036***; $P \leq 0.0001$) treatments.

TABLE 2
Correlation coefficient between uptake/intake of As and phosphate in fronds and roots

Phosphate sources	Uptake / intake of Phosphate	Uptake / intake of Arsenic	
		Frond	Root
TSP	Frond	0.7781***	0.5870*
	Root	0.6706*	0.8057***
KH_2PO_4	Frond	0.8036***	0.4567*
	Root	0.4504*	0.8583***

Notes: *** significant at P≤0.0001; * significant at P≤0.05

This result is linked to the result of As intake by *P. vittata* roots which was also positively correlated to phosphate intake by roots (r = 0.8057***; P≤0.0001) in TSP and (r = 0.8583***; P≤0.0001) KH_2PO_4 treatments respectively.

Many studies have reported on the possibility of competition between phosphorus and arsenic uptake by plant. Arsenic (V) uptake is facilitated by the phosphate transporter, which means the mechanism of uptake is similar to phosphate. According to Koseki (1988) and Sharples *et al.* (2000), the initial phase of arsenate uptake occurred in an instantaneous action and evidently is an irreversible process, followed by a slower but much more stable and steady state phase. These two different phases of reaction were controlled and inhibited by phosphate. When the concentration of phosphate was extremely high, PO_4^{3-} acts as a competitor with AsO_4^{-3} in the plant membrane. Studies on competition between these two elements in other plants have been published. Khattak *et al.* (1991) demonstrated that As concentration of alfalfa in shoot decreased with phosphorus treatment while another study also showed results of a reduction in As concentration with an increase in phosphorus concentration (Meharg and Macnair 1990). Meanwhile, Zhao *et al.* (2002) pointed out that the lack of interaction between As and P could be due to the phosphate system in plant roots. As the affinity for phosphate is higher than for arsenate, phosphate is less able to compete with arsenate.

Nevertheless, the outcome from this experiment showed a significantly (P≤0.0001) positive correlation between As uptake and P uptake in frond for TSP at (r = 0.7781; P≤0.0001) and KH_2PO_4 at (r =0.8036; P≤0.0001). These results clearly show that there is no competition between phosphorus and arsenic uptake in *P. vittata* (Chen *et al*. 2002). The concentration of As in soil used in this experiment might have contributed to this condition. Our findings are in agreement with research conducted by Chen *et al*. (2002), which found that the addition of phosphate increased in As uptake. This indicates that phosphorus and arsenic are in synergism and based on our results, it is hypothesised that As (V) and phosphate are not taken up via the same uptake channel in *P. vittata*. It is therefore concluded that phosphate and arsenic uptake mechanism in *P. vittata* should be further investigated.

CONCLUSION

In conclusion, P.vittata has the potential to hyper-accumulate arsenic from a moderate concentration of naturally As contaminated soil assisted by phosphate. Efficiency of uptake by *P. vittata* can be judged by considering the production of plant biomass, translocation factor and bioconcentration factor. The application of TSP to the soil which was in solid form performed better compared to KH_2PO_4 in terms of As uptake and plant growth of P.vittata. Further studies are needed on the mechanisms of synergism between arsenate and phosphate on P. vittata, especially in naturally As-enriched soil.

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