Effect of Soil Water Contents on Arsenic Accumulation in Phytoliths of *Pteris Multifida* and *Phragmites Australis*

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Research Article

Keywords: Pteris multifida, phytoliths, metaloids, soil water content, P. australis

Posted Date: December 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1158274/v1

Abstract

Purpose: The encapsulation of toxic metal(loid)s in phytoliths represents a new area of research. The accumulation of metal(loid)s in phytoliths can alter the fate and toxicity of soil metal(loid). *Pteris multifida* is a well-known As hyperaccumulator which also harbors phytoliths. However, As accumulation in phytoliths has not yet been studied. Soil water content is considered the main factor influencing phytolith accumulation and also remains unexplored with respect to As accumulation in phytoliths. In this study, As concentration in the phytoliths of *P. multifida* was compared with that in *Phragmites australis* phytoliths as a function of the soil water content.

Methods: *P. multifida* and *P. australis* were grown under different soil water contents. The As concentration in phytoliths, roots, and shoots of plants was then determined.

Results: The range of As concentration in the phytoliths of *P. multifida* was 414.70 - 1610.74 mg kg^{-1}, and that for *P. australis* phytoliths was 41.67 - 126.54 mg kg^{-1}. In *P. multifida,* higher soil water content increased As accumulation in phytoliths but did not affect phytolith content in the plant. In *P. australis,* the higher soil water content increased phytolith content in the plant but decreased As concentration in phytoliths.

Conclusion: This study suggests that *P. multifida* has higher As content in phytoliths than *P. australis*, and this accumulation can be affected by soil water content. The current findings provide insight into the accumulation of As in phytoliths and provide a theoretical basis for our understanding on the fate of As in the environment.

1. Introduction

Silicon is a non-essential yet possibly helpful element in plants (Epstein, 1994). Si enhances the resistance of plants to various biological and non-biological stresses (Ma, 2004). A critical role of Si in plants is to reduce the uptake of As from roots to the shoot and stimulate the antioxidant system (Tripathi et al., 2013).

Silicon deposits within cell walls of the plant endodermis and epidermis, forming amorphous opals and phytoliths (Sharma et al., 2019). Phytoliths consist mainly of Si, have strong resistance against weathering, and are considered to act as carbon sinks (Song et al. 2017). The encapsulation of metal(loid)s within phytoliths represents a new field of research, and there are two opposing theories regarding phytolith-encapsulated metal(loid)s. The first one states that phytoliths can be considered as stabilized metal(loid) sinks because the phytolith decomposes more slowly than plant organic matter (Fernandes-Horn et al., 2016). The second considers metals stored within phytoliths as a possible leaching source, thus necessitating management for reducing the rate of runoff (Nguyen et al., 2021, 2019). Another hypothesis suggests that phytoliths resistant to degradation would sequester heavy

metals inside plants, thereby reducing heavy metal-associated toxicity (Delplace et al., 2020; Farnezi et al., 2020). While previous research has been inconclusive on the matter, it is agreed that phytolith-encapsulated metal(loid)s could affect the fate of metal(loid)s and, consequently, the environment at contaminated sites.

Most previous studies on metal(loid) accumulation in phytoliths were conducted with Gramineae species (Delplace et al., 2020; Farnezi et al., 2020; Fernandes-Horn et al., 2016; Nguyen et al., 2021). These are well-known phytolith accumulators and are appropriate models for studying phytoliths. However, the ability of different plant species to accumulate metal(loid)s also needs to be considered, particularly for hyperaccumulators of specific metal(loid)s, which would improve our understanding of phytolith-encapsulated metal(loid)s. *Pteris multifida* is a well-known As hyperaccumulator (Du et al., 2005) that is grown and researched in Korea (Han et al., 2014; Kim et al., 2017). *P. multifida* phytolith accumulation has also been previously studied (Sundue, 2009). Further, *P. multifida* is expected to have the potential of storing large amounts of arsenic inside phytoliths. Higher soil water content and plant water uptake can increase Si uptake into plants as well as its accumulation in phytoliths (Madella et al., 2009), potentially affecting metal(loid) accumulation in phytoliths. The differences in phytolith amount and shape were considered in ancient agricultural irrigation (Rosen and Weiner, 1994). *P. multifida* is a well-known As hyperaccumulator, with the relationship between soil water and As accumulation being well established (Wan et al., 2015). Understanding the differences in As accumulation within *P. multifida* phytoliths with regard to different soil water contents is of interest for understanding how phytolith-encapsulated As may affect the environment.

In this study, As accumulation in *Phragmites australis* and *P. multifida* was compared for different soil water contents. *P. australis* was grown in soil at 30%, 60%, 100%, and 130% water holding capacity. As *P. multifida* cannot survive in water-saturated soil, it was grown in soil of 30% and 60% water holding capacity. Soil characteristics (available As and Si, pH), plant characteristics (water content, water potential, and phytolith accumulation), and As accumulation from soil to phytoliths were analyzed in order to understand the effect of soil water content on As accumulation in the phytoliths of the two investigated plant species.

2. Materials And Methods

2.1. Experimental design

Soil for the experiment was collected near the Gilgok gold mine, which is mainly contaminated with As (Kim et al., 2020). The collected soil was 10 mm sieved and dried. Seedlings of *P. australis* and *P. multifida* were purchased, and all shoot parts were cut. The seedlings were grown in a greenhouse for 30 days, and the newly grown shoots were divided from the rhizome for use in the experiment. The plant root part was clearly washed, and transplanted into soil from the Gilgok gold mine. Soil with *P. australis* was irrigated at 30%, 60%, 100%, and 130% water holding capacity. In *P. multifida*, all plants grown under 100% and 130% of water holding capacity died within the first ten days. Thus, only those grown under

30% and 60% of water holding capacity were analyzed. Five plants were planted for each treatment. The water holding capacity of the soil was 34%. All transplanted plants were grown in a greenhouse for 90 days.

2.2. Sample analysis

2.2.1. Soil analysis

Control soil before plant cultivation and the soil after cultivation were air-dried and sieved through a 2 mm sieve. Soil pH was measured using a pH meter (Thermo Orion 920A, Thermo Fisher Scientific, Waltham, MA, USA). Soil was mixed with distilled water (1:5) and shaken for 1 h before measurement. Soil texture was determined via the pipette method (NIAST, 2000). The soil was dispersed using 5% sodium hexametaphosphate solution and sampling aliquot solution according to Stoke's law. Loss on ignition (LOI) was used to determine the organic matter (OM) content at 400°C over 16 h (Nelson and Sommers, 1996). Available silicon was extracted via the method described by Imaizumi and Yoshida (1954). The soil was mixed with extraction solution (1:10) and shaken in a water bath for 5 h at 40°C. The extraction solution was made of 0.18 M NaOAc + 0.87 M acetic acid and adjusted to pH 4. Analysis of arsenic (As) was conducted using the microwave aqua-regia method (Tighe et al., 2004). Soil (0.5 g) was mixed with aqua regia (HNO_3:HCl = 1:3) and digested in a microwave for 30 min at 180°C. Arsenic in soil was also analyzed via Mehlich-3 extraction (Mehlich, 1984). All digested and extracted soil samples were filtered and analyzed using ICP-OES (Agilent, Santa Clara, CA, USA).

2.2.2. Plant analysis

Cultivated plant shoot parts were rinsed with distilled water, and the moisture was wiped. The wiped plant shoot parts were weighed and dried in an oven at 105°C and weighed again to calculate the plant water content and plant dry weight. Dried plant samples were digested with 5 ml HNO_3 and 3 ml H_2O_2 in a microwave at 180°C for 30 min (Gulz et al., 2005). As the research focused on phytoliths, the digested solution was centrifuged, and the pellet was further digested with 10 M NaOH (Li et al., 2014) until the solution was clean. The two solutions were combined, filtered, and analyzed via ICP-MS (Agilent, Santa Clara, CA, USA).

2.2.3. Phytolith extraction and analysis

Phytoliths were extracted from plants as per the dry ashing method (Parr et al. 2001; Delplace et al. 2020). The plant material was dried in an oven at 50°C and moved to a porcelain crucible. The samples were digested in a furnace at 700°C for 12 h and then transferred to 50 ml polyethylene tubes. HCl (10%) was added to each tube, shaken, and heated in a water bath at 70°C for 20 min. The tubes were centrifuged at 4000 rpm for 15 min, and the supernatant was discarded. The precipitated pellets were rinsed with distilled water and centrifuged three times. 15% H_2O_2 was put into a rinsed pellet, shaken, heated in a water bath at 70°C for 20 min, followed by rinsing with distilled water. The remaining pellet was dried and weighed to determine the ratio of phytoliths in the plant. The extracted phytoliths were mixed with 10 M NaOH (Li et al., 2014) and digested in a microwave at 180°C until the solution was

clean. The digested solution was acidified with nitric acid, filtered, and analyzed via ICP-MS (Agilent, Santa Clara, CA, USA). The shape and composition of the phytolith surfaces were analyzed using a ZEISS SUPRA 55VP SEM (Zeiss, Oberkochen, Germany) at 10 kV as well as via energy-dispersive X-ray spectroscopy (EDS).

2.2.3. Arsenic accumulation from soil to phytoliths

The accumulation ratio of As from soil to phytoliths can explain how phytoliths affect the fate of soil As. Factors were calculated as the ratio between As concentration in plant shoot and soil obtained via Mehlich-3 extraction (EF_{root}).

EF_{root} = As in plant roots / As in soil (1)

As concentration in plant shoot and As concentration in soil obtained via Mehlich-3 extraction (EF_{shoot}),

EF_{shoot} = As in plant shoot / As in soil (2)

As concentration in phytolith and As concentration in soil obtained via Mehlich-3 extraction (EF_{lith}),

EF_{lith} = As in phytolith / As in soil (3)

Translocation factor of As from root to shoot (TF),

TF = As in shoot / As in root (4)

As concentrations in phytoliths and As concentrations in the plant shoot ($EF_{lith/shoot}$).

$EF_{lith/shoot}$ = As in phytolith / As in shoot (5)

2.3. Statistical analysis

All analyses were performed for five replicates. Significant differences were determined via Duncan's test, and a mean $P<0.05$ indicated statistical significance. Plant As concentration and amount were log-transformed (log(x+1)) due to the large-scale difference between the two species. All data were analyzed using statistical analysis software (SAS 9.4, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Soil characteristics

Soil characteristics are described in Table 1. Control soil before the experiment was analyzed via aqua regia extraction, and the As concentration was 3258.72 ± 131.53 mg kg^{-1}. The soil texture of control soil was sandy loam, and the OM was 5.71 ± 0.07%. The range of soil As concentration via Mehlich-3 extraction was 237.45 - 284.58 mg kg^{-1}. All experimental soils had significantly higher As concentrations

through Mehlich-3 extraction than the control soil. Soils with 100% and 130% water holding capacity had significantly higher As concentrations through Mehlich-3 extraction than soils with lower water content. Available Si and pH were lowest in the control soil, but sections with no statistically significant difference were overlapping, and no trend according to soil water content was observed.

Table 1
Soil As, Si concentration and pH*,**

Treatments		Results					
Plant species	Soil water content[a]	Soil available As[b]		Soil available Si[c]		Soil pH[d]	
Control [e]		237.45	d	203.43	c	8.51	c
Pteris multifida	30%	256.03	bc	220.50	abc	8.63	abc
	60%	258.77	b	213.64	bc	8.58	bc
Phragmites australis	30%	249.86	c	228.76	ab	8.71	a
	60%	255.88	bc	233.74	a	8.63	abc
	100%	284.58	a	223.59	ab	8.67	ab
	130%	280.67	a	216.93	abc	8.58	bc

* Pseudo-total As concentration in control soil with aqua regia extraction was 3258.72 ± 131.53 mg kg^{-1}.

** Different letter in the same row indicates significant differences at the 5% level by Duncan's test.

[a] Soil water content indicates the percentage of water holding capacity. [b] Soil available As is As concentration with Mehlich-3 extraction. [c] Soil available Si was extracted with acetic acid buffer. [d] Soil pH was extracted via 1:5 D.I. water extraction. [e] Control is composite soil before plant growth experiments.

3.2. Plant water condition and phytoliths

SEM images and EDS spectra of phytoliths are shown in Fig. 1. The shape of phytoliths was similar to that of previously imaged phytoliths obtained via high-temperature extraction (Nguyen et al., 2019). Phytoliths were mainly composed of Si and O.

Fig. 1 SEM images and EDS spectra of phytoliths from (a) *P. multifida* at 60% water holding capacity soil and (b) *P. australis* at 60% water holding capacity soil

Plant water conditions and phytolith contents are described in Table 2. The phytolith content of *P. australis* in 30% of water holding capacity was 58642.31 mg kg^{-1}, which was significantly lower than for all other analyzed samples. There was no significant difference in the water content of plant shoots between all samples. Higher soil water content increased the leaf water potential in *P. multifida.* The water potential in *P. multifida* with 30% water holding capacity was -11.47 bar and -7.00 bar at 60% of water

holding capacity. The water potential of *P. australis* leaves increased with higher soil water content. The water potential of *P. australis* leaves at 30% water holding capacity was significantly lower than that of other *P. australis* leaves. The range of water content for plant roots was 70.49 - 85.78%. *P. multifida* had a significantly lower root water content than *P. australis*. The root water content of *P. australis* with 30% water holding capacity was 79.36%, the lowest root water content among *P. australis* samples. There was no significant difference between the other *P. australis* samples.

Table 2
Plant water content water potential and phytolith content*

Treatments		Results					
Plant species	Soil water content [a]	Phytolith content ($mg\ kg^{-1}$)	Water content in plant shoot (%)	Water potential in plant leaf (bar)		Water content in plant root (%)	
Pteris multifida	30%	114157.51 a	64.41 a	-11.47	cd	70.49	c
	60%	104516.49 a	69.78 a	-7.00	a	72.06	c
Phragmites australis	30%	58642.31 b	69.61 a	-13.60	d	79.36	b
	60%	137370.92 a	67.78 a	-10.73	c	84.32	a
	100%	116530.55 a	68.28 a	-10.40	bc	85.08	a
	130%	103573.27 a	69.15 a	-8.87	ab	85.78	a

* Different letter in same row indicates significant differences at the 5% level determined via Duncan's test.

[a] Soil water content indicates percentage of water holding capacity'.

3.3. Concentration and amount of As in plants

The concentration and amount of As in plants and phytoliths are shown in Fig. 2. Plant roots accumulate less As under unsaturated conditions (soil water content < 100%) than saturated conditions (soil water content ≥ 100%) (Fig. 2 (a)). There was no significant difference between *P. multifida* and *P. australis* when the soil moisture content of the cultivated soil was the same.

In the case of *P. multifida*, As concentration in the shoot increased with higher soil water content (Fig. 2 (a)). When the soil water contents were 30% and 60% of the water holding capacity, the As concentrations of the *P. multifida* shoot were 144.54 mg kg^{-1} and 528.67 mg kg^{-1}, respectively. *P. australis* did not exhibit

a significant difference in shoot As concentration based on soil water content. All As concentrations in the shoot of *P. australis* were significantly lower than those determined for *P. multifida*.

In all treatments, As concentration in phytoliths was higher than that in plant shoots (Fig. 2 (a)). *P. multifida* in 60% of water holding capacity had the highest As concentration in phytoliths (1610.74 mg kg^{-1}), while the second highest *was observed in* 30% of water holding capacity (414.70 mg kg^{-1}). All phytoliths of *P. australis* had significantly lower As concentrations than those of *P. multifida.* The range of As in *P. australis* phytoliths was 41.67 - 126.54 mg kg^{-1}. *P. australis* in 30% of water holding capacity had a significantly higher As concentration in phytoliths than other *P. australis*.

Accumulation of As in the shoot and phytolith parts was described as the total amount in a pot (Fig. 2 (b)). *P. multifida* with 60% water holding capacity had the largest amount of As in the plant shoot (698.67 μg pot^{-1}), followed by *P. multifida* with 30% water holding capacity (171.98 μg pot^{-1}). The different soil water content of *P. australis* did not affect the amount of As in plant shoots, and there was no significant difference. As amount in the plant shoot of *P. australis* was in the rage of 9.87 - 17.25 μg pot^{-1}.

P. multifida with 60% of water holding capacity had the largest amount of As in phytoliths (218.78 μg pot^{-1}), while *P. multifida* with 30% of water holding capacity had the second-largest amount (57.47 μg pot^{-1}). The soil water content of *P. australis* did not affect the amount of As in phytoliths, with no significant differences observed. The range of As content in the phytoliths of *P. australis* was 8.06 - 4.91 μg pot^{-1}.

Fig. 2 Arsenic in *Pteris multifida* and *Phragmites australis* as a function of the water content. (a) As concentration in plant shoot, phytolith, and roots. As in the shoot and roots was calculated based on plant dry weight, and As in phytoliths was calculated based on phytolith weight. (b) The amount of As in plant shoots and phytoliths for each pot. Water content means the percentage of water holding capacity. Different letters in the same color bar indicate significant differences at the 5% level determined via Duncan's test after log-scale transformation

3.4. Accumulation of As from soil to phytolith

The accumulation ratio of As from soil to phytolith is shown in Fig. 3. *P. australis* with 100% and 130% of water holding capacity had higher EF_{root} than the other samples. All EF_{root} values of *P. australis*were greater than 1, while those for *P. multifida* were lower than 1. In the case of EF_{shoot},only *P. multifida* with 60% of water holding capacity had a value over 1. There was a large difference in EF_{shoot} between the two species. The range of EF_{shoot} for *P. multifida* was 0.54 - 2.04, while that for *P. australis*was 0.03 - 0.06. Therange of EF_{lith} for *P. multifida* was 1.61 - 6.23, and that for *P. australis*was 0.15 - 0.51.The range of *TF* for *P. multifida* was 0.92 - 5.77, and that for *P. australis*was 0.01 - 0.04. The range of $EF_{lith/shoot}$, except for *P. australis*with 30% of water holding capacity, was 3.11 - 5.56. The $EF_{lith/shoot}$ for *P. australis*with 30% of water holding capacity was 11.83.

Fig. 3. Arsenic accumulation ratio from soil to plant in *Pteris multifida* and *Phragmites australis* as a function of the water content. Soil water content means the percentage of water holding capacity. The EF_{root} is As concentration in root per As concentration in soil determined via Mehlich-3 extraction. EF_{shoot} is As concentration in shoot per As concentration in soil determined via Mehlich-3 extraction. EF_{lith} is As concentration in phytolith per As concentration in soil determined via Mehlich-3 extraction. [e] *TF* is As concentration in plant shoot per As concentration in plant root. $EF_{lith/shoot}$ is As concentration in phytolith per As concentration in soil determined via Mehlich-3 extraction

4. Discussion

4.1. Soil characteristics

When the soil water content exceeded the water holding capacity, the available As increased (Table 1). In water-saturated conditions, the availability of As was reported to increase faster than under unsaturated conditions (Onken and Adriano, 1997). The increase in As availability was due to the effects of anaerobic and redox conditions. Arsenic is reduced to arsenite, increasing plant availability under saturated conditions (Ascar et al., 2008). The higher As availability at 100% and 130% of water holding capacity can thus be attributed to the effect of soil saturation.

4.2. Plant water condition and phytolith

Although the soil water content did not affect the water content of plant shoots, the water potential of plant leaves and the water content of plant roots indicated that soil water content affected plant water conditions, with major differences observed between 30% and 60% of the soil water holding capacity (Table 2). Soil water content is a major factor in increasing plant water uptake, but increases in the latter cease beyond the limit point, and the same water potential value is maintained even when soil moisture increases (Teuling et al., 2006). In this study, only the phytolith accumulation in *P. australis* increased with high soil water content, while that in *P. multifida* was not affected. Previous research reported that higher soil water content increases phytolith accumulation in plants, describing the change as "more water - more phytoliths" (Katz et al., 2013). However, in most previous studies, the relationship between soil water and phytoliths was analyzed in Gramineae (Jenkins et al., 2016; Katz et al., 2013; Madella et al., 2009; Meunier et al., 2017; Webb and Longstaffe, 2003), with a major focus on the water management of food crops and phytolith accumulation (Jenkins et al., 2016; Madella et al., 2009; Meunier et al., 2017). In contrast, no previous research on the relationship between soil water content and phytolith accumulation was available for Pteridaceae species. In the present study, we determined that soil water content did not affect phytolith accumulation in *P. multifida* as much as in *P. australis.*

4.3. Concentration of As in plant

In the soil with 100% and 130% of water holding capacity, plant roots had the highest As concentrations and EF_{root} values (Fig. 2 (a); Fig. 3). This difference can be explained by increased soil As availability, which was obtained via Mehlich-3 extraction (Table 1). However, the As concentration of *P. australis*

shoots did not differ significantly between soil water content conditions (Fig. 2 (a)). In the case of *P. australis*, EF_{root} increased while *TF* decreased, and the As concentration in shoots did not show a significant difference. In previous research, the *TF* of *P. australis* was lower than 1, with some reported values even being lower than 0.1 (Castaldi et al., 2018; Ghassemzadeh et al., 2008a; Ghassemzadeh et al., 2008b; Huyen Nga, 2018; Štrbac et al., 2014). Lee et al. (2014) compared the metal accumulation of fern and Graminae, with the latter having a lower *TF*. Prica et al. (2019) concluded that *P. australis* is an As excluder and reduces root-to-shoot translocation as a defense mechanism. It is believed that the high As accumulation in the roots was not reflected in the shoot due to the characteristics of *P. australis*.

In the case of *P. australis*, phytoliths with 30% of water holding capacity had a significantly higher As concentration than other soil water contents, and the $EF_{lith/plant}$ of *P. australis* with 30% of water holding capacity was highest (Fig. 2 (a); Fig. 3). The mechanisms of phytolith formation and As accumulation in phytoliths have not yet been identified. One possible explanation for the concentration difference is that the phytolith content in *P. australis* decreased at 30% of water holding capacity. The As in phytoliths might have been condensed as a result of this decrease in plytolith content.

Although there was no difference in As availability in the soil, the *TF* of *P. multifida*, unlike *P. australis*, increased more than five times when soil water content increased from 30–60% of water holding capacity (Fig. 3). Transpiration is one of the major factors affecting As accumulation in *Pteris* (Wan et al., 2015). Higher soil water content can increase plant transpiration (Gardner and Ehlig, 1963), and As accumulation differences in *P. multifida* may be due to differences in soil water contents.

This study aimed to compare the arsenic accumulation capacity of phytoliths in *P. multifida* and *P. australis.* In the plant shoot, *P. multifida* accumulated much more As than *P. australis. P. multifida* is recognized as an As hyperaccumulator (Du et al., 2005), which can explain the observed difference in As concentration between the shoots of *P. multifida* and *P. australis.* As accumulation in phytoliths has not yet been studied in fern species and thus the mechanisms and capacity of accumulation remain unclear. However, the As concentration in *P. multifida* phytoliths was higher than that in shoots, and the $EF_{lith/shoot}$ was more than 1 (Fig. 2 (a); Fig. 3). It can thus be assumed that *P. multifida* accumulates As inside phytoliths, similarly to *P. australis*.

The concentration of As in *P. multifida* shoots was much higher than that in *P. australis* shoots (Fig. 2). Even though the accumulation ratio from shoot to phytolith was similar, the As concentration of *P. multifida* phytolith was higher than that of *P. australis.* The soil water content did not affect the $EF_{lith/plant}$ of *P. multifida* (Fig. 3), and the increase in As concentration in *P. multifida* shoots at higher soil water contents directly affected the As concentration in phytoliths. Finally, *P. multifida* with higher soil water content was in better condition for accumulating As in phytoliths.

4.4. Amount of As in plant

Both *P. multifida* and *P. australis* are perennial plants that grow in rhizomes. In this study, the shoot part was cut down, and the experiment was conducted with newly grown shoots for 90 days. Rhizomes store

carbohydrates from old shoots and supply them to newly grown ones (Sakamaki and Ino, 2006). Because plant shoots could not obtain nutrients from the old shoots and the growing time was not enough to represent total As accumulation in plants, it is difficult to determine the actual As accumulation. Nevertheless, we can compare the relative values.

Both *P. multifida* with 30% and 60% water holding capacity had a greater amount of As in the shoots and phytoliths than *P. australis* (Fig. 2 (b)). EF_{lith} and $EF_{lith/shoot}$ of *P. multifida* were higher than 1 for all soil water contents. In a previous study, the biomass of *Pteris* was 13.02 t ha^{-1}, and that of *Phragmites* was 19.26 t ha^{-1} (Ashraf et al., 2013). Although the biomass of *Phragmites* is usually larger than that of fern species, the difference is not large enough to influence As accumulation, and *P. multifida* may act as a better As sink through its phytoliths.

The amount of As in the phytoliths of *P. multifida* increased with soil water content. A large amount of As accumulation suggests that *P. multifida* may play a role as a hyperaccumulator in the phytolith, and *P. multifida* might affect As fate in soil with phytolith-occluded As. Moreover, the effect on As fate may be greater with higher soil water content. The soil water content of the *P. australis* pot affected the As concentration in phytoliths but did not affect that in shoots (Fig. 2 (a)). However, the amount of As in the phytoliths and shoots was not significantly different (Fig. 2 (b)). This is because the phytolith increase was offset by the decrease in phytolith content inside the plant, thus eliminating the change in total amount.

5. Conclusion

The effect of soil water content on As accumulation in phytoliths varied depending on the plant species. Higher soil water content increased the As concentration in the shoots of *P. multifida*. As the ratio of As in shoots and phytoliths did not change with soil water content, we concluded that As accumulation in the phytoliths of *P. multifida* increased in parallel to soil water content. In the case of *P. australis*, phytolith As was highest under low water content conditions. However, since phytolith content decreased with higher phytolith As concentrations, the total amount of As stored in phytoliths was not affected by soil water content. *P. multifida* is an As hyperaccumulator and accumulated As in phytoliths to a greater extent than in plant shoots. The amount of phytolith-encapsulated As was the largest for *P. multifida* under high soil water content. It is not yet clear whether phytolith-encapsulated As can act as a sink of As in soil or a releasing fraction. However, some studies have suggested that trace element accumulation in phytoliths may occur for a significant fraction of soil trace elements. The results of this study suggest that As is highly accumulated in phytoliths under certain conditions, thus contributing to our understanding of As fate in the environment.

Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Funding This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R1F1A1051997) and partly by Korea university.

Author Contributions *All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by* Hyun-Gi Min*,* Min-Suk Kim *and* Jeong-Gyu Kim*. The first draft of the manuscript was written by* Hyun-Gi Min *and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.*

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Figures

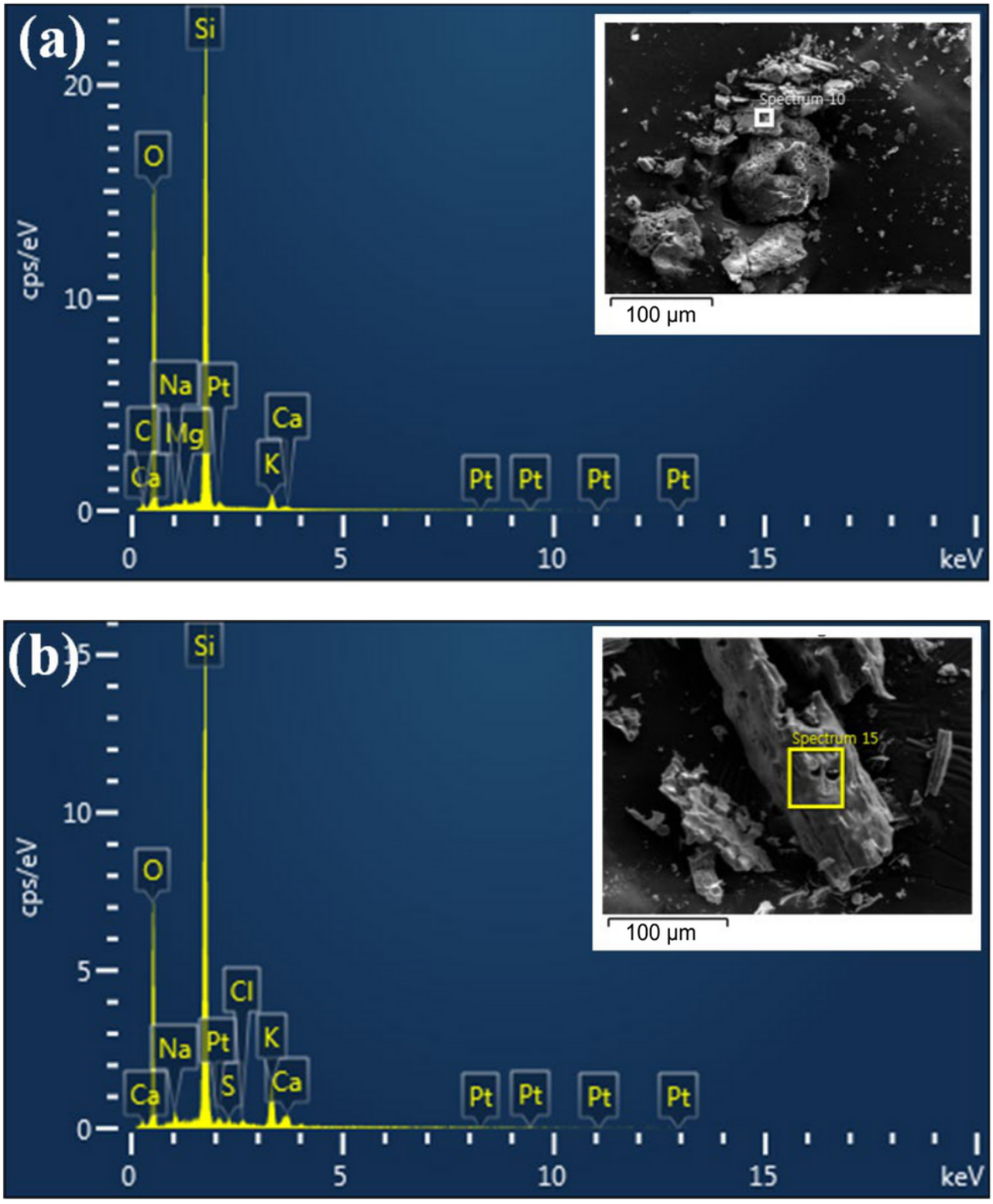

Figure 1

SEM images and EDS spectra of phytoliths from (a) *P. multifida* at 60% water holding capacity soil and (b) *P. australis* at 60% water holding capacity soil

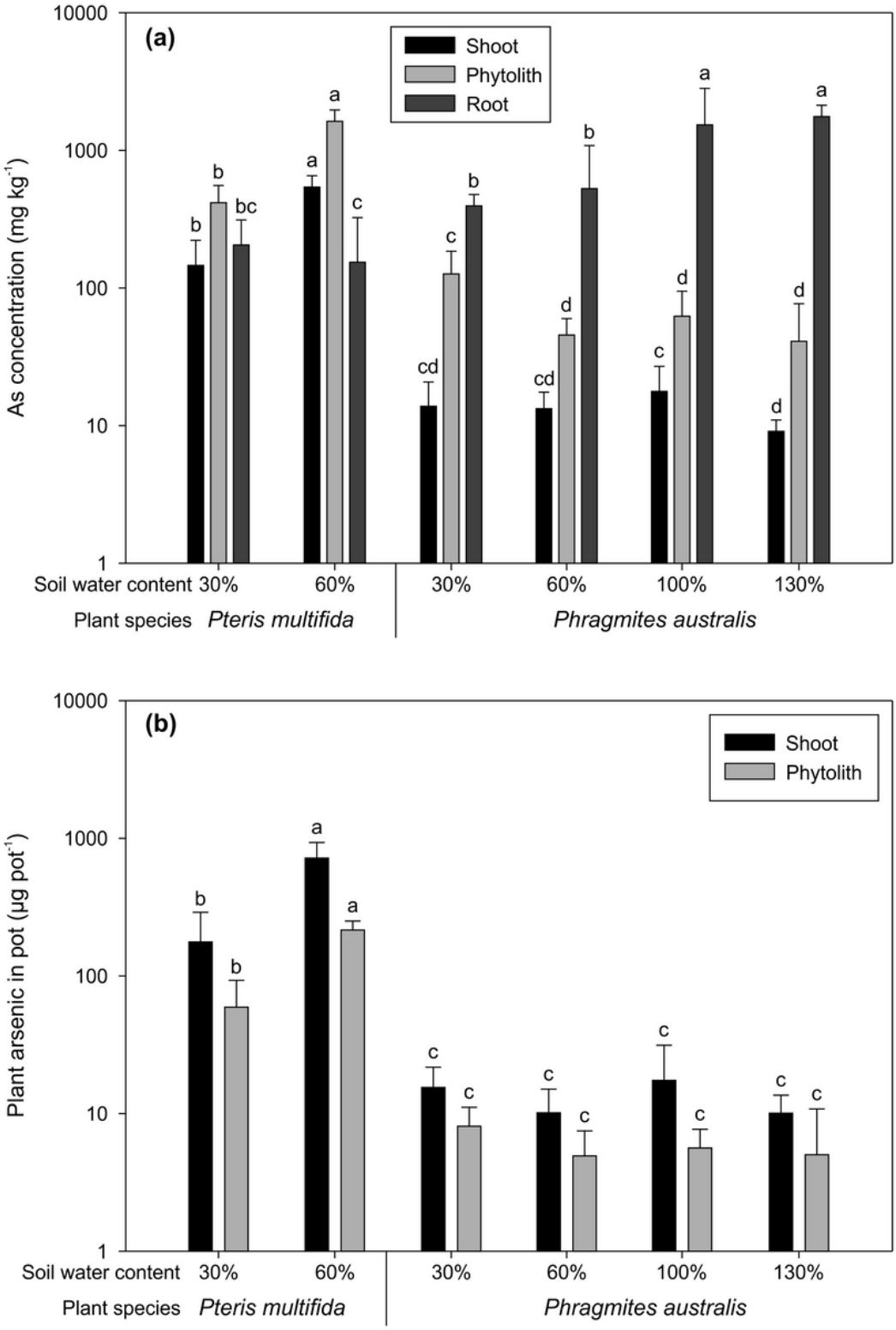

Figure 2

Arsenic in *Pteris multifida* and *Phragmites australis* as a function of the water content. (a) As concentration in plant shoot, phytolith, and roots. As in the shoot and roots was calculated based on plant dry weight, and As in phytoliths was calculated based on phytolith weight. (b) The amount of As in plant shoots and phytoliths for each pot. Water content means the percentage of water holding capacity.

Different letters in the same color bar indicate significant differences at the 5% level determined via Duncan's test after log-scale transformation

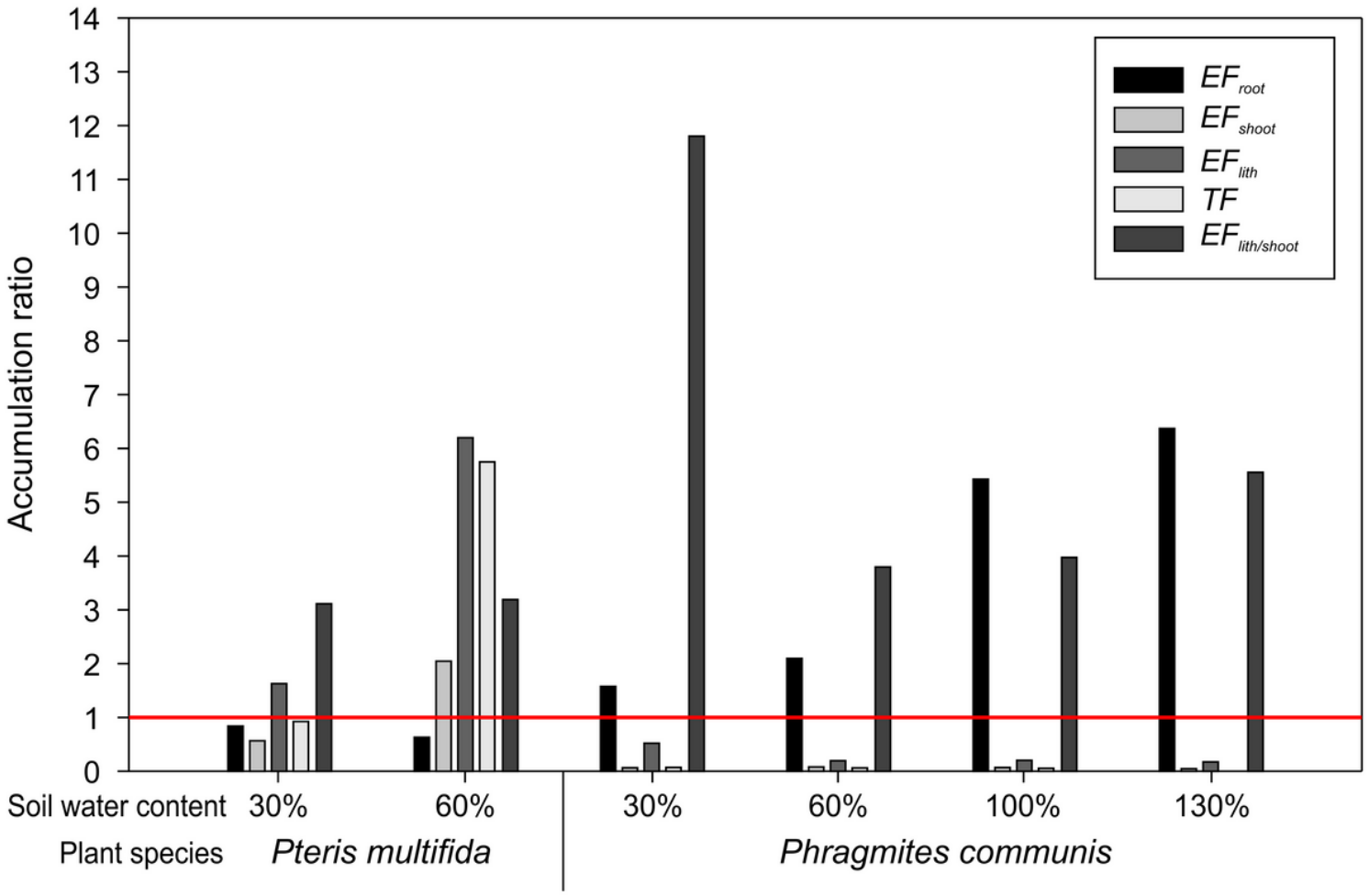

Figure 3

Arsenic accumulation ratio from soil to plant in *Pteris multifida* and *Phragmites australis* as a function of the water content. Soil water content means the percentage of water holding capacity. The EF_{root} is As concentration in root per As concentration in soil determined via Mehlich-3 extraction. EF_{shoot} is As concentration in shoot per As concentration in soil determined via Mehlich-3 extraction. EF_{lith} is As concentration in phytolith per As concentration in soil determined via Mehlich-3 extraction.[e] *TF* is As concentration in plant shoot per As concentration in plant root. $EF_{lith/shoot}$ is As concentration in phytolith per As concentration in soil determined via Mehlich-3 extraction