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Effect of Soil Characteristics on Arsenic Accumulation in Phytolith of Gramineae (*Phragmites japonica*) and Fern (*Thelypteris palustris*) Near the Gilgok Gold Mine

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Abstract: In South Korea, most metal mines were abandoned and caused contamination for more than 30 years. Even the soil is highly contaminated with trace elements, plants still grow in the area and can affect the contamination. Phytolith is amorphous silica in the plant body. Phytolith is resistant to decomposition, and the stabilization of carbon, nutrients, and toxic substances accumulated in the phytolith is being studied. In this study, the Gilgok gold mine, which is contaminated with arsenic was selected as the research site. We selected *Phragmites japonica* and *Thelypteris palustris* as targets for the analysis of arsenic accumulation in plants and phytolith. Plants accumulate more phytolith at the riverside. The higher water content of soil increased the Arsenic (As) concentration in the frond of the *T. palustris*. Soil available silicon (Si) did not affect phytolith accumulation but increased As accumulation in the plant and phytolith. The research result showed that *P. japonica* and *T. palustris* have the ability to accumulate As in phytolith and the accumulation can be changed with soil characteristics and plant species. This As accumulation in phytolith can affect plant tolerance in contaminated areas and change the As availability in the soil. The result of the research can be used as a database to build a sustainable environment.

Keywords: arsenic; metal mine; phytolith; South Korea; silicate

1. Introduction

Silicon (Si), the second-most abundant element of the earth's crust, is helpful to most plants [1]. Silicon endows plants with resistance to biological and non-biological stresses such as pests, diseases, climate change, water stress, heavy metals, and salt [2]. Si alleviates metal stresses in higher plants by facilitating the following: (1) antioxidant system induction in plants, (2) metal-Si complexing or co-precipitation, (3) toxic metal immobilization in growth media, (4) uptake processes, and (5) metal compartmentalization within plants [3].

Highly concentrated silicate in the plant body can form an amorphous opal called phytolith [4]. Phytolith has a high resistance to chemical weathering. With the characteristics of phytolith, it has been researched as a sustainable carbon sink for a long time [5], and also as an archaeological record [6]. With such characteristics of phytolith, research on trace element remediation by phytolith occlusion also exists [7–9]. Sequestration of trace elements in phytolith was researched in two ways. First, trace elements in phytolith are considered as detoxified trace elements from a plant defense mechanism. For this purpose, the plant needs to accumulate trace elements in the phytolith more than in the plant body [7,10,11]. Delplace et al. [7] collected reed from four mine areas and two control areas and analyzed the heavy metal concentration. The results showed that some heavy metal concentration in phytolith is higher than that of plant tissue and the research concluded that reed had the ability to occlude trace elements inside of phytolith. Second, phytolith is

highly resistant to decomposition in environmental conditions. Phytolith can accumulate a significant amount of trace elements and remain in the soil after plant organic matter is decomposed [9,12]. After phytolith returns to the soil, trace elements in phytolith are stable and do not decompose in various weather and water conditions [8]. The stabilization of trace elements by phytolith is called Si-phytolith remediation. For this purpose, a plant needs to accumulate trace elements in the plant body near the hyperaccumulator and the phytolith accumulates more trace elements than the plant body. In a different view, some research pointed that the solubility of phytolith was increased after burning and can be a source of toxic metal in soil [13,14]. Both types of research consider that phytolith can change the fate of trace elements in the soil. Understanding trace element accumulation in plants can help to understand the effect of wild plants on contaminated sites and choose a revegetation strategy. These studies can be used as basic data for research to increase environmental resilience and sustainability.

Arsenic is a toxic metalloid usually found around metal mine areas [15]. Arsenic has a structural similarity with silicate and phosphate. When plants uptake arsenic, arsenite passes through the transporter for silicic acid, and arsenate uses that of phosphates because of their similarity [16]. After plants uptake arsenic, most of the arsenate reduces to arsenite [16]. Such research can propose that arsenic is more likely to accumulate inside of phytolith than other elements.

To understand the ability of As accumulation inside of phytolith, the ratio of As concentration between phytolith, plant, and the soil is important. Delplace et al. [7] used enrichment factors to prove that phytolith can accumulate trace elements more than the plant body. Accumulation of As from plant to phytolith can explain how phytolith affects As toxicity in the plant body.

There have been previous researches focusing on the possible effect on environmental sustainability of metal accumulation in phytolith [7,8,14]. But the soil characteristics which can affect phytolith formation have not yet been considered with metal accumulation. Soil water and available silicate are major soil factors that change the content of phytolith [17]. The soil characteristics and environment around the rhizosphere can also affect the fate and plant uptake of As in plants [2,18–20] and the interaction of phytolith formation and As accumulation in phytolith needs to be considered.

Most of the research results on trace element accumulation in phytolith were from research on Graminae [7,9,11,14]. It is true that Graminae is a well-known phytolith accumulator but ferns can also accumulate similar amounts of phytolith [21]. Fern is also considered an As accumulator [22–24]. When As is a major pollutant, ferns can be good candidates to study the As accumulation in phytolith.

In this study, the Gilgok gold mine, a metal mine mainly contaminated with arsenic in South Korea [25], was selected as the research site. *Phragmites japonica* and *Thelypteris palustris* were selected as indigenous Gramineae and fern species in the mine area. Available soil water and silicate are major factors that affect phytolith accumulation [17]. To make the difference between the soil water content and arsenic concentration of the collected sample, the sample was collected at both riverside and non-riverside sites, which were divided by a dam. The dam was built to prevent the dispersal of contaminants from mines and was built along the river. Arsenic concentrations in soil (aqua-regia and Mehlich-3 extraction), plants, and phytolith were analyzed to find the possible role of phytolith for each species. Soil water content, available Si, pH, and electric conductivity (EC) were analyzed to find the environmental conditions that can change the soil's available water and silicate.

2. Materials and Methods

2.1. Sampling Site and Date

The Gilgok gold mine is an abandoned gold mine, which is located at Hongcheon, Gangwon-do, South Korea. Around the Gilgok gold mine, contaminated waste-rock and tailings from the mines are piled up along the riverside. To prevent pollutants from flowing into the river, a dam is blocking the waste-rock area from the riverside. The sampling position

was categorized as non-riverside (N) and riverside (R). *P. japonica* and *T. palustris* grew on the riverside and non-riverside. For sampling with little environmental changes during plant growth, field samples were taken in June, before the summer rainy season began, and soil near the root zone was also sampled (Figure 1). Information about the sampling sites is described in Table 1. Samples were taken at five points at non-riverside and at riverside sites. *P. japonica* leaf (PL), *P. japonica* stem (PS), and *T. palustris* frond (TF) were analyzed.

Table 1. Sampling points, sampling plant species, and their information.

Type of Site	Soil Code ^a	Coordinates (WGS 84, Decimal)		Plant Species	Plant Part	Plant Code ^b
		Latitude	Longitude			
Non-riverside	N1	37.61373	127.73419	<i>Phragmites japonica</i>	Stem	N1-PS
					Leaf	N1-PL
				<i>Thelypteris palustris</i>	Frond	N1-TF
	N2	37.61386	127.73405	<i>Thelypteris palustris</i>	Frond	N2-TF
	N3	37.61399	127.73386	<i>Phragmites japonica</i>	Stem	N3-PS
					Leaf	N3-PL
				<i>Thelypteris palustris</i>	Frond	N3-TF
	N4	37.61410	127.73372	<i>Phragmites japonica</i>	Stem	N4-PS
					Leaf	N4-PL
				<i>Thelypteris palustris</i>	Frond	N4-TF
Riverside	R1	37.61377	127.73433	<i>Phragmites japonica</i>	Stem	R1-PS
					Leaf	R1-PL
	R2	37.61394	127.73416	<i>Phragmites japonica</i>	Stem	R2-PS
					Leaf	R2-PL
	R3	37.61407	127.73401	<i>Phragmites japonica</i>	Stem	R3-PS
					Leaf	R3-PL
	R4	37.61418	127.73386	<i>Phragmites japonica</i>	Stem	R4-PS
					Leaf	R4-PL
	R5	37.61431	127.73373	<i>Phragmites japonica</i>	Stem	R5-PS
				<i>Thelypteris palustris</i>	Frond	R5-TF

^a Sampling position categorized to N (Non-riverside) and R (Riverside). ^b Sampling plants categorized to PS (*Phragmites japonica* stem), PL (*Phragmites japonica* leaf), and TF (*Thelypteris palustris* frond).

Figure 1. Sampling area near the Gilgok gold mine and soil, plant sampling points. Sampling points are categorized into N (Non-riverside) and R (Riverside).

2.2. Sample Analysis

2.2.1. Soil Analysis

The topsoil of the vegetation area was sampled for analysis. Some soils were collected by core sampler at the sampling site. After drying at 105 °C, the soil water content was analyzed by comparing the weight before and after. Another collected soil sample, not for soil moisture, was air-dried and 2 mm sieved. Soil pH was measured with a pH meter (Thermo Orion 920A, Thermo Fisher Scientific, Waltham, MA, USA), and electric conductivity (EC) was measured with a conductivity meter (CD-4322, Lutron). Soil pH and EC were measured from the filtered solution. Then, 5 g of soil was mixed with 25 mL distilled water and shaken for 1 h and filtered before it was measured. Available silicon was extracted with Imaizumi and Yoshida's [26] method; 3 g of soil was mixed with 30 mL extraction solution 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. The extracted solution was filtered and analyzed by ICP-OES (Agilent, Santa Clara, CA, USA) at 251.61 nm. Analysis of As was conducted with the microwave aqua-regia method [27] as a pseudo-total concentration. Then, 0.5 g of soil was mixed with aqua-regia (HNO₃:HCl = 1:3) with microwave (Milestone, Italy) digestion for 30 min, 180 °C at 2.45 Ghz and 12.25 cm. Soil As concentration was also analyzed with Mehlich-3 extraction [28] at the plant's available concentration. All digested and extracted soil samples were filtered and analyzed by ICP-OES (Agilent, Santa Clara, CA, USA) at 193.70 nm. The result of As was certified by NIST 2710a soil.

2.2.2. Plant Analysis

Sampled *P. japonica* shoot was divided into leaves and stems. The shoot part of *T. palustris* was sampled as frond without dividing. The plant shoot part was rinsed with distilled water and dried in an oven at 50 °C. Then, 0.5 g of plant samples were digested by 5 mL of HNO₃ and 3 mL of H₂O₂ in a microwave (Milestone, Italy) for 30 min, 180 °C at 2.45 Ghz and 12.25 cm [29]. Because the research focused on phytolith, the digested solution was centrifuged, and the pellet was digested with 10% KOH [30] until the solution was clean. The two solutions were combined, filtered, and analyzed by ICP-MS (Agilent, Santa Clara, CA, USA). The analyzed result with plant weight was based on the dry weight. The result of As in the plant was certified BCR-402, white clover.

2.2.3. Phytolith Extraction and Analyzation

Phytolith in plants was extracted by the dry ashing method [7,31]. First, 5 g of plant material was dried in a 50 °C oven and moved to a porcelain crucible. The samples were digested in a furnace at 700 °C for 12 h. The remaining ashes were transferred to 50 mL polyethylene tubes. Then, 10% HCl was added to each tube and 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 discharged. The precipitated pellets were rinsed with distilled water and centrifuged three times. Then, 15% H₂O₂ was put into the rinsed pellet and 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 discharged. The precipitated pellets were rinsed with distilled water and centrifuged three times. The remaining pellets were dried and weighed to know the ratio of phytoliths in the plant. The extracted phytoliths were digested with 10% KOH [30] by microwave (Milestone, Italy) digestion at 180 °C at 2.45 Ghz and 12.25 cm until the solution was clean. The digested solution was acidified with nitric acid, filtered, and analyzed by ICP-MS (Agilent, Santa Clara, CA, USA) at 75 amu.

2.2.4. Enrichment Factor

Accumulation of As from soil to phytolith can explain how phytolith affects As fate in soil. In this research, As concentrations in soil (aqua-regia extraction and Mehlich-3 extraction) and plant (Figure 2) were calculated.

Figure 2. Conceptual image for As enrichment from soil to phytolith.

Enrichment factors were calculated as the ratio between As concentration in plant shoots and As concentration in soil with aqua-regia extraction (EF_{plant}),

$$EF_{plant} = \text{As in plant shoot} / \text{As in soil with aqua-regia} \quad (1)$$

As concentration in plant shoots and As concentration in soil with Mehlich-3 extraction ($EF_{plant/avail}$),

$$EF_{plant/avail} = \text{As in plant shoot} / \text{As in soil with Mehlich-3} \quad (2)$$

As concentration in phytolith and As concentration in plant shoot part ($EF_{lith/plant}$),

$$EF_{lith/plant} = \text{As in phytolith} / \text{As in plant} \quad (3)$$

As concentration in phytolith and As concentration in soil with aqua-regia extraction (EF_{lith}),

$$EF_{lith} = \text{As in phytolith} / \text{As in soil with aqua-regia} \quad (4)$$

and As concentration in phytolith and As concentration in soil with Mehlich-3 extraction ($EF_{lith/avail}$).

$$EF_{lith/avail} = \text{As in phytolith} / \text{As in soil with Mehlich-3} \quad (5)$$

2.2.5. Statistical Analysis

All analysis was thrice replicated. Significant differences in ANOVA were tested with Tukey's test and mean $p < 0.05$. The Pearson correlation coefficient was calculated from the average values. All data were analyzed using a statistical analysis system program (SAS 9.4, SAS Institute Inc., Cary, NC, USA).

3. Result

3.1. Soil Characteristics

The characteristics of the soils are shown in Table 2. The soils at non-riverside sites had lower water content than those of riverside sites. Water content at the non-riverside sites was 8.05–16.36%, and water content at riverside sites was 21.62–33.30%. The available silicon concentration was not grouped based on the riverside. The available silicon concentrations at N3, N4, and N5 sites showed five times more concentration than other sites. The soil pH range of the researched area was 6.62–8.78. Soil samples at the lower side of the dam sites were neutral. Sampling sites, N1, N3, N4, and N5 were alkaline. Like the soil water content, the electrical conductivity of the soil was affected by whether it was collected from

the riverside or not. The EC at the non-riverside was in the 151–260 $\mu\text{m cm}^{-1}$ range. The EC at the riverside was in 83–111 $\mu\text{m cm}^{-1}$ range. The concentration of As in the soil with aqua-regia extraction had a wide range, 20.85–3560.90 mg kg^{-1} . Two non-riverside places, N1 and N2 had the highest soil As concentrations at 3560.90 and 3376.77 mg kg^{-1} . The As concentration in soil with the Mehlich-3 extraction fraction showed a similar tendency to the aqua-regia extraction.

Table 2. Soil characteristics at sampling sites.

Soil Code ^a	Water Content (%)	Available Silicon ^b (mg kg^{-1})	pH	EC ^c ($\mu\text{m cm}^{-1}$)	As Concentration in Aqua-Regia Extraction (mg kg^{-1})	As Concentration in Mehlich-3 Extraction (mg kg^{-1})
N1	13.27	166.82	8.07	195	3560.90	901.09
N2	16.36	153.53	6.99	194	3376.77	794.80
N3	8.59	1748.69	8.78	151	20.85	3.22
N4	8.05	1971.18	8.69	162	41.45	2.40
N5	3.75	1714.22	8.05	260	65.71	3.38
R1	22.31	234.60	7.34	99	54.48	8.84
R2	33.30	140.11	6.71	83	73.12	4.54
R3	26.90	132.93	6.62	87	97.23	5.74
R4	24.93	354.34	7.02	111	117.86	10.06
R5	21.62	202.87	7.30	100	1378.28	219.22

^a Sampling position categorized to N (Non-riverside) and R (Riverside). ^b Available Si extracted by pH 4 acetate buffer. ^c EC is electric conductivity.

3.2. Arsenic Concentration in Plant and Phytolith

The plant As concentrations (in dry weight, DW) are described in Figure 3a. Most of the total As concentration in the plants was not different significantly. Only N2-TF and R5-TF had a significantly higher value than other samples. The concentration of As in L2-TF was 3.82 mg kg^{-1} and that of R5-TF was 7.49 mg kg^{-1} . The concentrations of As in other plant samples were 0.29–1.28 mg kg^{-1} .

The concentration of As in phytolith is described in Figure 3b. The As concentration in the phytolith of N2-TF and R5-TF was 4.77 and 2.82 mg kg^{-1} , which was the first and second highest. At N1, N3, and R5, As in TF was significantly higher than PS and PL. At N3 and R2, PL was significantly higher than PS. The percentage of phytolith in the plants (DW) is described in Figure 3c. The proportion of phytolith inside the plants had different tendencies depending on sampling position. All plants at the riverside showed higher phytolith contents than that of non-riverside. At N1, N3 and N4, phytolith content in TF was significantly lower than PS and PL. At N5, R2, and R4, phytolith content in PS was significantly higher than PL.

3.3. Enrichment Factors of As

Enrichment factors for As are described in Table 3. The range of EF_{plant} was 0.0001–0.0331. Only 8 out of 23 samples were greater than 0.01. The range of $EF_{\text{plant/avail}}$ was 0.004–0.2361. The range of $EF_{\text{lith/plant}}$ was 0.3765–3.2262. At R5, the largest difference of EF_{plant} and $EF_{\text{plant/avail}}$ between two plants appeared. EF_{plant} and $EF_{\text{plant/avail}}$ of TF at R5 was more than 10 times higher than that of PS and PL. For $EF_{\text{lith/plant}}$, 18 out of 23 samples exceeded 1, and 7 of them exceeded 2. The range of EF_{lith} was 0.0003–0.0947. Only 9 out of 23 samples were greater than 0.01. $EF_{\text{lith/plant}}$ of TF at R5 was the lowest and only the $EF_{\text{lith/plant}}$ of TF was less than 1. The range of EF_{lith} was 0.0014–0.5190. The range of $EF_{\text{lith/avail}}$ was 0.0014–0.6139. The largest difference of EF_{lith} and $EF_{\text{lith/avail}}$ between two plants appeared at R5 but the difference was less than 10 times.

3.4. Correlation Coefficient

The Pearson correlation coefficients between soil characteristics are described in Table 4. Soil water content was significantly correlated with most of the soil factors except the As concentration in aqua-regia extraction and As concentration in Mehlich-3 extraction. The correlation coefficient between As in soil and As in soil mobile fraction was 0.99,

and they were not significantly correlated with other factors. Soil water content was significantly correlated with available Si, pH, and EC. Soil pH was significantly correlated with EC.

Table 5 describes the correlation coefficients between the analyzed factors of the *P. japonica* stems and soil characteristics. Correlations between As concentration in *P. japonica* stems and soil characteristics were not significantly correlated. The percentage of phytolith in *P. japonica* stems had significant correlation coefficients with pH, EC, and water content. The concentration of As in phytolith was correlated with EC positively and water content negatively. The As concentration of phytolith was positively correlated with EC, and water content was negatively correlated. Soil available Si was significantly correlated with $EF_{plant/avail}$, EF_{lith} , and $EF_{lith/avail}$. In the case of $EF_{lith/plant}$, only EC had a positive correlation. Soil pH negatively correlated with soil water content.

Table 6 describes the correlation coefficients between the analyzed factors of *P. japonica* leaves and soil characteristics. The As concentration of *P. japonica* leaves was positively correlated with the As concentration in aqua-regia extraction and the As concentration in Mehlich-3 extraction. Water content was negatively correlated with the As concentration in *P. japonica* leaves. The percentage of phytolith in the plant was negatively correlated with pH and EC. $EF_{plant/avail}$ was positively correlated with soil available Si.

Table 7 describes the correlation coefficients between analyzed factors of the *T. palustris* fronds. The As concentration of *T. palustris* frond was positively correlated with water content. $EF_{plant/avail}$ had a positive correlation coefficient with available silicon in the soil and a negative correlation coefficient with As concentration in aqua-regia extraction. $EF_{lith/plant}$ had a negative correlation coefficient with soil water content. The correlation coefficient between $EF_{lith/avail}$ and available silicon in the soil was positive.

Table 3. As enrichment factors between sampled soil, plant, and phytolith.

Sampling Site ^a	Plant Species	Plant Part	EF_{plant} ^b	$EF_{plant/avail}$ ^c	$EF_{lith/plant}$ ^d	EF_{lith} ^e	$EF_{lith/avail}$ ^f
N1	<i>Phragmites japonica</i>	Stem	0.0001	0.0004	3.2262	0.0003	0.0014
		Leaf	0.0003	0.0010	1.5898	0.0004	0.0016
		Frond	0.0004	0.0014	1.5798	0.0006	0.0022
N2	<i>Thelypteris palustris</i>	Frond	0.0012	0.0049	1.2178	0.0014	0.0060
N3	<i>Phragmites japonica</i>	Stem	0.0189	0.1224	1.6619	0.0314	0.2034
		Leaf	0.0331	0.2144	1.5141	0.0501	0.3246
		Frond	0.0322	0.2088	2.9403	0.0947	0.6139
N4	<i>Phragmites japonica</i>	Stem	0.0109	0.1889	2.2267	0.0244	0.4206
		Leaf	0.0137	0.2361	0.9333	0.0128	0.2203
		Frond	0.0133	0.2288	2.2685	0.0301	0.5190
N5	<i>Phragmites japonica</i>	Stem	0.0051	0.0991	2.9104	0.0148	0.2884
		Leaf	0.0060	0.1162	2.3754	0.0142	0.2761
R1	<i>Phragmites japonica</i>	Stem	0.0105	0.0646	0.4566	0.0048	0.0295
		Leaf	0.0101	0.0623	0.5012	0.0051	0.0312
R2	<i>Phragmites japonica</i>	Stem	0.0043	0.0696	1.2111	0.0052	0.0843
		Leaf	0.0045	0.0729	2.7467	0.0124	0.2003
R3	<i>Phragmites japonica</i>	Stem	0.0050	0.0847	1.1536	0.0058	0.0977
		Leaf	0.0043	0.0724	1.3033	0.0056	0.0944
R4	<i>Phragmites japonica</i>	Stem	0.0025	0.0295	1.9294	0.0049	0.0569
		Leaf	0.0027	0.0312	1.6555	0.0044	0.0517
R5	<i>Phragmites japonica</i>	Stem	0.0002	0.0015	1.2416	0.0003	0.0018
		Leaf	0.0004	0.0026	0.9333	0.0004	0.0024
		Frond	0.0054	0.0342	0.3765	0.0020	0.0129

^a Sampling position categorized into N (Non-riverside) and R (Riverside). ^b EF_{plant} is As concentration in plant per As concentration in soil with aqua-regia extraction. ^c $EF_{plant/avail}$ is As concentration in plant per As concentration in soil with Mehlich-3 extraction. ^d $EF_{lith/plant}$ is As concentration in phytolith per As concentration in plant. ^e EF_{lith} is As concentration in phytolith per As concentration in soil with aqua-regia extraction. ^f $EF_{lith/avail}$ is As concentration in phytolith per As concentration in soil with Mehlich-3 extraction.

Table 4. Pearson's Correlation coefficient between soil characteristics.

	Available Si (mg kg ⁻¹)	pH	EC ^a (μm cm ⁻¹)	Water Content (%)	As Concentration in Aqua-Regia Extraction (mg kg ⁻¹)	As Concentration in Mehlich-3 Extraction (mg kg ⁻¹)
Available Si (mg kg ⁻¹)	1					
pH	0.84 *	1				
EC (μm cm ⁻¹)	0.52	0.54 *	1			
Water content (%)	−0.80 *	−0.86 *	−0.85 *	1		
As concentration in aqua-regia extraction (mg kg ⁻¹)	−0.43	−0.06	0.37	−0.12	1	
As concentration in Mehlich-3 extraction (mg kg ⁻¹)	−0.40	−0.02	0.40	−0.27	0.99 *	1

^a Electric conductivity. Values with * represent significance at $p < 0.05$.

Figure 3. Result of plant analysis. (a) is As concentration in plant shoot part (in plant dry weight); (b) is As concentration in phytolith (in phytolith weight); (c) is the percentage of phytolith in plant shoot weight (in plant dry weight). Different letters mean significant differences at the 5% level by Tukey's test. Sampling position categorized into N (Non-riverside) and R (Riverside). Value with * means two plant species in same site had significant different but stem and leaf of *Thelypteris palustris* did not have significant difference. Value with ** means all plant parts in same site had significant difference. When leaf and stem of *Phragmites japonica* had significant difference in same site, *Thelypteris palustris* frond also had significant difference with *Phragmites japonica*. Analyzed plant parts categorized into PS (*Phragmites japonica* stem), PL (*Phragmites japonica* leaf), and TF (*Thelypteris palustris* frond).

Table 5. Pearson's Correlation coefficients between soil characteristics and analyzed factors of *Phragmites japonica* stem.

	As Concentration in Plant (mg kg ⁻¹)	Percentage of Phytolith (%)	As Concentration in Phytolith (mg kg ⁻¹)	EF _{plant} ^b	EF _{plant/avail} ^c	EF _{lith/plant} ^d	EF _{lith} ^e	EF _{lith/avail} ^f
Available Si (mg kg ⁻¹)	−0.00	−0.64	0.46	0.65	0.81 *	0.40	0.91 *	0.92 *
pH	0.08	−0.87 *	0.63	0.57	0.52	0.53	0.76 *	0.63
EC ^a (μm cm ⁻¹)	−0.19	−0.78 *	0.81 *	0.04	0.22	0.85 *	0.35	0.51
Water content (%)	−0.16	0.84 *	−0.72 *	−0.50	−0.45	−0.54	−0.68 *	−0.57
As concentration in aqua-regia extraction (mg kg ⁻¹)	−0.16	−0.21	0.51	−0.50	−0.57	0.51	−0.47	−0.46
As concentration in Mehlich-3 extraction (mg kg ⁻¹)	−0.11	−0.47	0.57	−0.48	−0.42	0.55	−0.42	−0.42

^a EC is electric conductivity. ^b EF_{plant} is As concentration in plant per As concentration in soil with aqua-regia extraction. ^c EF_{plant/avail} is As concentration in plant per As concentration in soil with Mehlich-3 extraction. ^d EF_{lith/plant} is As concentration in phytolith per As concentration in plant. ^e EF_{lith} is As concentration in phytolith per As concentration in soil with aqua-regia extraction. ^f EF_{lith/avail} is As concentration in phytolith per As concentration in soil with Mehlich-3 extraction. The first row represents plant analysis values and the first column represents soil factors. Values with * represent significance at $p < 0.05$.

Table 6. Pearson's Correlation coefficients between soil characteristics and analyzed factors of *Phragmites japonica* leaf.

	As Concentration in Plant (mg kg ⁻¹)	Percentage of Phytolith (%)	As Concentration in Phytolith (mg kg ⁻¹)	EF _{plant} ^b	EF _{plant/avail} ^c	EF _{lith/plant} ^d	EF _{lith} ^e	EF _{lith/avail} ^f
Available Si (mg kg ⁻¹)	0.09	−0.48	0.13	0.67	0.88 *	0.05	0.65	0.82 *
pH	0.61	−0.68 *	0.39	0.64	0.68 *	−0.16	0.57	0.52
EC ^a (μm cm ⁻¹)	0.31	−0.80 *	0.56	0.10	0.27	0.29	0.18	0.41
Water content (%)	−0.67 *	0.73 *	−0.32	−0.57	−0.60	0.31	−0.46	−0.37
As concentration in aqua-regia extraction (mg kg ⁻¹)	0.75 *	−0.46	0.61	−0.41	−0.54	−0.06	−0.39	−0.55
As concentration in Mehlich-3 extraction (mg kg ⁻¹)	0.76 *	−0.48	0.66	−0.37	−0.49	−0.03	−0.35	−0.50

^a EC is electric conductivity. ^b EF_{plant} is As concentration in plant per As concentration in soil with aqua-regia extraction. ^c EF_{plant/avail} is As concentration in plant per As concentration in soil with Mehlich-3 extraction. ^d EF_{lith/plant} is As concentration in phytolith per As concentration in plant. ^e EF_{lith} is As concentration in phytolith per As concentration in soil with aqua-regia extraction. ^f EF_{lith/avail} is As concentration in phytolith per As concentration in soil with Mehlich-3 extraction. The first row represents plant analysis values and the first column represent soil factors. Values with * represent significance at $p < 0.05$.

Table 7. Pearson's Correlation coefficient between soil characteristics and analyzed factors of *Thelypteris palustris* frond.

	As Concentration in Plant (mg kg ⁻¹)	Percentage of Phytolith (%)	As Concentration in Phytolith (mg kg ⁻¹)	EF_{plant}^b	$EF_{plant/avail}^c$	$EF_{lith/plant}^d$	EF_{lith}^e	$EF_{lith/avail}^f$
Available Si (mg kg ⁻¹)	−0.66	−0.39	−0.67	0.80	1.00 *	0.83	0.77	0.98 *
pH	−0.81	−0.52	−0.88	0.73	0.84	0.87	0.74	0.87
EC ^a (μm cm ⁻¹)	−0.61	−0.85	0.21	−0.27	−0.20	0.32	−0.14	−0.11
Water content (%)	0.96 *	0.81	0.61	−0.63	−0.79	−0.96 *	−0.67	−0.83
As concentration in aqua-regia extraction (mg kg ⁻¹)	0.24	−0.09	0.62	−0.81	−0.92 *	−0.54	−0.73	−0.87
As concentration in Mehlich-3 extraction (mg kg ⁻¹)	0.11	−0.21	0.56	−0.77	−0.86	−0.43	−0.67	−0.80

^a EC is electric conductivity. ^b EF_{plant} is As concentration in plant per As concentration in soil with aqua-regia extraction. ^c $EF_{plant/avail}$ is As concentration in plant per As concentration in soil with Mehlich-3 extraction. ^d $EF_{lith/plant}$ is As concentration in phytolith per As concentration in plant. ^e EF_{lith} is As concentration in phytolith per As concentration in soil with aqua-regia extraction. ^f $EF_{lith/avail}$ is As concentration in phytolith per As concentration in soil with Mehlich-3 extraction. The first row represents plant analysis values and the first column represent soil factors. Values with * represent significance at $p < 0.05$.

4. Discussion

4.1. Soil Characteristics

The soils at non-riverside and riverside sites were determined based on the dam. With the dam, the sampling position exhibited two main distinctive characteristics. First, the dam blocked the movement of arsenic from the upper end to the lower end. Second, the dam was built along the river flow, so the soil under the dam was adjacent to the river, and it was submerged in water during the summer rainy season. Not all of the sampling points at the non-riverside site showed higher As concentrations in aqua-regia extraction than those at the riverside, especially at points higher than other points such as N1 and N2 appearing at the non-riverside, so the dam restricted the dispersion of highly polluted soil (Table 2). The concentration of arsenic at the R5 point was about half of the N1 and N2 points, which is believed to be due to the outflow of some highly polluted soil (Table 2).

With the analysis described in Table 4, water content was significantly correlated with soil available Si, pH, and EC. In many studies, soil pH and EC can be changed by the soil water content [32–34]. It was pH that had the highest correlation with soil available silicon (Table 4). The higher pH value can cause higher silicate solubility [35,36]. The correlation between pH and available Si can be caused by the dissolution of soil minerals with high pH. Soil As with aqua-regia extraction and Mehlich-3 extraction were not affected by other soil factors.

4.2. Arsenic in Plant

The indigenous fern species around the Gilgok gold mine, *T. palustris*, was not considered a hyperaccumulator of As. However, many ferns could accumulate arsenic [22–24], and *T. palustris* also has the potential. The concentration of arsenic in R5-TF was higher than that in R5-PS and R5-PL by more than 10 times (Figure 3a). The concentration of arsenic in N2-TF had the second-highest value. Those results can represent that *T. palustris* can accumulate As at higher concentrations than *P. japonica*.

N1, N3, and N4 were the sites where *P. japonica* and *T. palustris* grew together. The concentration of As in the phytolith inside *T. palustris* was higher than in the *P. japonica* at all points where *T. palustris* and *P. japonica* were growing together, and statistical significance was shown in N3 and R5 (Figure 3b). *T. palustris* at the N2 site had the highest As in phytolith (Figure 3b). When comparing *T. palustris* and *P. japonica* at the same spot, *T. palustris* showed higher or similar $EF_{lith/plant}$ values to *P. japonica* at most points except R5, and EF_{lith} values were higher than *P. japonica* at all spots. There are research results that show the ability of trace element accumulation in the phytolith of Graminae [7,8], but fern species were not researched with phytolith and trace elements. The research results demonstrate that *T. palustris* has an equivalent or higher ability to accumulate As inside phytolith compared with that of *P. japonica*.

When comparing the percentage of the phytolith inside the plant, it was found that the phytolith ratio of the *P. japonica* was higher than that of the *T. palustris* at most points (Figure 3b). In most of the research results that compare phytolith content within plant species, Graminae had more phytolith than other species [37–39].

In the research of Delplace et al. [7], enrichment factors under the value of 0.01 were unconsiderable. In the case of high soil pollution, where the concentration of arsenic in the soil exceeded 1000 mg kg^{-1} , EF_{plant} and EF_{lith} in all plants were less than 0.01 (Table 3). When the As concentration in the soil was less than 1000 mg kg^{-1} , the enrichment factors were relatively high but did not exceed 0.1. In the case of our researched plants, As accumulation in phytolith cannot be considered the factor that affects soil As availability. Of the 23 plant samples, $EF_{lith/plant}$ of four samples was less than 1, and seven samples exceeded 2 (Table 3). It is believed that both varieties can concentrate the As into the phytolith, and this characteristic helps the detoxification of As in plants with sequestration of As inside of phytolith.

4.3. Effect of Soil Characteristics on Plants

Even though phytolith is concentrated silicate in the plant body, phytolith content in plants was not significantly correlated with soil available Si. Water content, pH, and EC were significantly correlated with phytolith content in the *P. japonica* stem and leaf (Tables 5 and 6). In the case of *T. palustris*, the plant could be sampled only at five points, and most of the factors were not significantly correlated with soil, but water content and EC were the factors that had the highest correlation coefficient with phytolith content (Table 7). R5 was the only riverside place where *T. palustris* grew and the place had the highest soil water content and percentage of phytolith in *T. palustris* among the growing points of *T. palustris* (Table 2; Figure 3c). The results from the work of Katz et al. [17] show that water availability had more of an effect generating phytolith than available silicate. Madella et al. [40] also found that water availability can profoundly affect phytolith content.

In view of these results, the reason that phytolith formation inside plants did not have a significant correlation with the available silicon in soil and had a significant correlation with soil water content was that soil water content had a greater effect on the formation of phytolith than available silicon. Besides, because available silicon had the highest correlation with soil pH, which had a negative correlation with soil water content, it was judged that available silicon had a negative correlation with the formation of phytolith. Water content in soil can be considered a major factor increasing the amount of phytolith and it could affect the amount of As occluded in phytolith which was a major topic of the research. However, soil water content was negatively correlated with As concentration in phytolith and the EF_{lith} of the *P. japonica* stem (Table 5) and the $EF_{lith/plant}$ of the *T. palustris* frond (Table 7). A higher soil water content can increase the accumulation of phytolith in a plant but also has a possibility to reduce the accumulation of As in phytolith.

Different from PS and PL, the As concentration in TF was higher at N1, N2, and R5 than other sites. It could be the characteristics of the metal accumulator but the ranking of arsenic concentrations in plants is opposite to that of soils at the three sites. Soil water content was positively correlated with As concentration in *T. palustris* frond. Higher water contents can increase As accumulation in plant and the accumulation can be affected by the plant's accumulation capacity [41]. In this research, the As concentration of *T. palustris* was higher than that of *P. japonica* (Figure 3b) and this can be a reason why only the As accumulation of *T. palustris* was affected by soil water content. Metal accumulators have high metal translocation from root to shoot and save toxic metals in a detoxified form [42], and the translocation of metal accumulators can be enforced by high transpiration [18]. With the tendency of a metal accumulator, *T. palustris* may show more sensitivity to soil water content than soil As concentration.

Available Si in soil was significantly correlated with EF_{plant} in the case of the *P. japonica* leaf and *T. palustris* frond. Available Si in soil was significantly correlated with EF_{lith} in all plant samples. In the soil, Si and As have competitive adsorption, which can increase the uptake of As [43]. The mechanism in which arsenic is absorbed into the phytolith has not been studied compared to other plants. However, since $EF_{lith/plant}$ and silicon in soil did not have a significant correlation, the correlation between EF_{lith} and silicon in soil is estimated to be the effect of EF_{plant} .

4.4. Possible Role of Phytolith with As Contamination

The adverse effect of environmental pollutants appears when the concentration of pollutants in a compartment of the ecosystem is higher than the threshold value [44]. Elimination of pollutants from the compartment and retardation of the material cycle and of the velocity of adding the pollutant into the compartment can be useful to prevent environmental pollution and to sustain the feedback and structure of an ecosystem [45]. Phytoremediation with metal(loid) is not only conducted as elimination of the pollutants in a compartment of the ecosystem but also decreasing the bioavailability of metal(loid). Immobilization and stabilization are some of the phytoremediation techniques [45]. Revegetation can also reduce metal(loid) availability and dispersion [46]. Arsenic sequestration

in phytolith can be considered as As immobilization with its high stability. In plants, As sequestration in phytolith can reduce As toxicity and it can help revegetation in contaminated areas.

This study focused on two roles of phytolith: the role of sequestering arsenic in the phytolith and reduce toxicity inside of plant body and the role of arsenic availability change, when As sequestered in phytolith returned to the soil. Both *P. japonica* and *T. pilsuensis* had low EF_{lith} and high $EF_{lith/plant}$ (Table 3), which means possible ability to reduce arsenic toxicity by arsenic sequestration by phytolith in plants but cannot stabilize As in soil by phytolith. In two plants phytolith can reduce As toxicity and can help the revegetation but did not have enough ability to have a role in Si-phytolith remediation. Even the most of $EF_{lith/plant}$ was higher than 1 the value was lower than that in Delplace et al. [7]'s research. The difference of species and environmental characteristics can exist. The sampling was conducted at end of the spring season and short growth date can be the reason for the low $EF_{lith/plant}$. *T. pilsuensis* was selected as possible As accumulator in the Gilgok gold mine area. *T. pilsuensis* had a higher ability to accumulate As in plant shoot and phytolith than *P. japonica* as expected (Figure 3, Table 3), but the value was much lower than other famous As hyperaccumulator fern species like *Pteris vittata* and *Pteris multifida* [47]. Even the research result about *T. pilsuensis* was not the same as expected, the result proved that *T. pilsuensis* also accumulate As in phytolith.

5. Conclusions

Arsenic concentration in soil, plant, phytolith in the Gilgok gold mine was compared. Soil As concentration, water condition, pH, EC, and available silicon were considered possible factors affecting the formation of phytolith and As accumulation in phytolith. Water condition affects As availability and phytolith formation. pH is a major factor affecting silicon and As availability. EC is an indicator of the total ionic strength which can be related to ion dynamics. Even though Si is the main component of phytolith, available Si did not affect phytolith concentration in all analyzed plants. The higher the available Si the higher As accumulation in phytolith. With soil water content increasing, phytolith content in *P. japonica* was increased but As concentration in phytolith was reduced in *P. japonica*'s leaf so the increased phytolith contents can not guarantee the increase of phytolith occluded As. In the case of *T. pilsuensis* frond, soil water content was the only factor that had a positive correlation with plant As concentration significantly. Phytolith contents in *T. pilsuensis*'s frond were increased at the riverside where had highest soil water content but had lowest As accumulation from frond to phytolith. The result of this study was to analyze the correlation of complex factors in the field, and there was a limit to revealing the clear effect of each factor. However, the research results and correlations show that phytolith and arsenic accumulation in the phytolith in abandoned metal mines is greatly influenced by soil moisture and silicon availability, and the tendency is dependent on plants.

Arsenic concentration in soil, plant, phytolith in Gilgok gold mine was compared. Arsenic in soil was analyzed in two ways, aqua-regia extraction and Mehlich-3 extraction. Two soil As concentration was not correlated with other soil characteristics and highly correlated with each other. Soil As availability in Gilgok gold mine was only affected by total As concentration. In most of the cases, As concentration of phytolith was higher than that of the plant body. Two researched plants, *P. japonica* and *Thelypteris pilsuensis* had the ability to concentrate As in phytolith. The percentage of phytolith in plants was highly affected by the accessibility of the sampling position to the river. In the case of both species, the plant sample from the riverside had a higher amount of phytolith than the plant sample from non-riverside sites. Sampling position was significantly correlated with soil pH, EC, water contents, and such soil characteristics considered the main factors changing the amount of phytolith in the plant body. Arsenic accumulation in *Thelypteris pilsuensis* was positively correlated with soil water content and negatively correlated with As accumulation from plant body to phytolith. The research result showed that As accumulation in plant and

phytolith can be diversified by soil As concentration and water content and the effect of the soil factors can be different depends on plant species.

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