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Pteris cretica as a Potential Biomarker and Hyperaccumulator in an Abandoned Mine Site, Southwest Japan

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Abstract: This study aimed to determine the potential of naturally occurring Cretan brake fern (*Pteris cretica*) as a biomarker and hyperaccumulator in an abandoned mine in Southwest Japan. This species is a known hyperaccumulator of As. Total concentrations of heavy metals and As were determined in the shoots and roots of plants collected from inside and outside of the mine area. The results indicate that As and Pb in the shoots of *P. cretica* reached 1290 and 3840 mg/kg dry weight, respectively, which is classified as hyperaccumulation. The metal uptake intensity in the shoots indicates that *P. cretica* is a biomarker for As, Pb, and Zn. Furthermore, the metal concentrations, and bioconcentration and translocation factors indicate that *P. cretica* is a good candidate for phytoremediation of sites that are contaminated with As and Pb.

Keywords: hyperaccumulator; biomarker; bioconcentration factor; translocation factor

1. Introduction

Environmental pollution is a worldwide problem. Anthropogenic activities that are a source of environmental pollution contribute to heavy metal contamination of soil, water, and air at levels that are harmful to human health. The toxicity of heavy metals is a serious problem for the integrity of environmental, ecological, and nutritional systems [1]. The biomarker concept [2] can be used as an early warning of heavy metal toxicity at the sub-organism level.

A species (in this case a plant) can be defined as a biomarker (biological marker) from measurements of heavy metals in different parts of the plant, such as its shoots and roots [2]. In ecological risk assessments, biomarkers offer solutions for toxicity assessment at different concentrations that avoid the limitations of extrapolation [3]. Following Depledge and Fossi [4], biomarkers can be viewed as an attempt to identify the biological response to changes in ecosystem structure and function. The effectiveness of the biomarker can be determined by the amount of heavy metal uptake from the soil to the shoot and the nature of the correlations between the concentrations in the shoots and roots. Biomarker data can help in selecting the optimum remediation technique, especially the phytoremediation approach.

Phytoremediation is the removal of pollutants from contaminated soil and water [5]. This technique is well known as a cost-effective and environmentally friendly technology. The contaminant in soil or water is degraded, extracted, contained, or immobilized during phytoremediation [6]. A hyperaccumulator is a plant that can accumulate extremely high

concentrations of heavy metals in its shoots [7], and can therefore be used for phytoremediation. The potential of a plant to act as a hyperaccumulator can be determined by the plant's bioconcentration and translocation factors, known as BCF and TF, respectively. Plants with BCF and TF values greater than one are suitable for phytoremediation, and are particularly suitable for phytoextraction [8].

Certain plants can tolerate heavy metals in soil and water. Nirola et al., [9] reported that *Acacia pycnantha* is a tolerant plant of copper. Based on the bioavailability of metals, plants can be categorized as hyperaccumulators, accumulators, indicators, and excluders [10]. The bioavailability of metals in plant tissues is influenced by the properties and mobility of each metal [11]. Metal transport involves several processes, such as movement in the xylem and phloem, storage, accumulation, and immobilization within the plant tissue and organelles [12].

The Cretan brake fern, *P. cretica*, is a well-known accumulator plant of the *Pteris* L genus [13], which comprises approximately 280 species worldwide [14]. The Pteridaceae family is ancient and makes up ~10% of extant fern diversity [15]. It is a large and widely distributed family, and while it has a preference for tropical areas [14], it can also exist in, among others, aquatic, epiphytic, terrestrial, xeric-adapted, and desert habitats [16]. *P. cretica* is widely distributed throughout the study site. The aim of this study is to determine the potential of *P. cretica* as a biomarker and hyperaccumulator at an abandoned mine site, and for phytoremediation applications.

2. Materials and Methods

We carried out a field survey and laboratory analyses to determine the potential of *P. cretica* as a phytoremediation solution at an abandoned mine site in Southwest Japan.

2.1. Sampling Plots

We completed two field surveys at an abandoned mine site in Southwest Japan during 4–7 October and 1–2 November 2013. We sampled *P. cretica* from randomly selected sites that were inside and outside of the mining area. *P cretica* grows naturally in this abandoned mine site on a wide range of substrates, including debris flow sediment, tailing waste material, river sediment, and humic soil, and is mainly found under pine trees and on tailing waste disposal areas. We collected samples of live *P. cretica* plants along with their soil from 31 sampling plots along a transect distance of 1440 m (Figure 1).

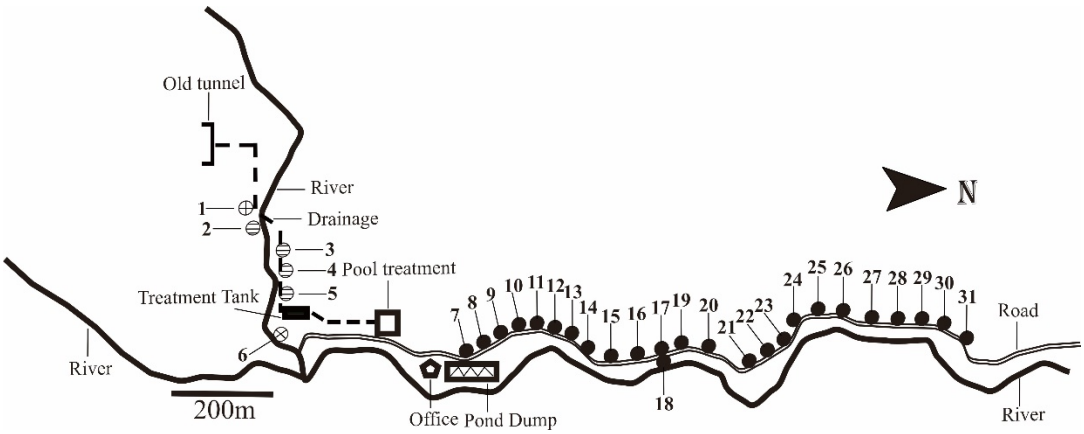

Figure 1. Location of sampling plots in an abandoned mine site; (⊕) Debris flow sediment; (⊖) Tailing waste; (⊗) River sediment; (●) Humic soil.

2.2. Analytical Methods

We analyzed the collected plant and soil samples. The fern samples were first washed with tap water and then separated into shoots, stems, and roots. After separation, they were rinsed in

an ultrasonic frequency cleaner with ultrapure water for 5 min until they were clean. The samples were then dried at ~80 °C for two days in a ventilated oven. Once dry, the samples were crushed to fine powder using a powder mill (Varian PM-2005 m, Osaka Chemical Co., Ltd, Osaka, Japan). The shoot powder (30 mg of each sample) was digested with a mixture of In:HNO_3 at a ratio of 3:100 before determination of the heavy metal concentrations by particle induced X-ray emission (PIXE) [17] at Cyclotron Center of Iwate Medical University, Iwate, Japan. Then, 20 mg of both the roots and shoots were digested with a solution of H_2O_2, HF, and HNO_3 at a ratio of 2:5:10 before heavy metal concentrations were determined by inductively coupled plasma–optical emission spectrometry (ICP–OES) (Varian Optima^{TM} 8300 series, PerkinElmer, Kanagawa, Japan) at the Integrated Centre for Sciences, Ehime University, Matsuyama, Japan. The wavelengths of the metals were determined by standard samples.

Soil samples were dried at ~120 °C for three days in a ventilated oven. Soil samples (50 g of each dry soil) were assumed to be homogenous. After drying, the soils were crushed in a vibrating mill (Varian TI-100, CMT Co., Ltd, Fukusima, Japan) and 3.3 g of the powder soil was mixed, pressed with polyethylene (C:H:O at a ratio of 13:14:6), and dried at ~120 °C for two hours in a ventilated oven. The heavy metal concentrations in the pressed soil were determined by X-ray fluorescence (Epsilon 5, PANalytical, Almelo, Netherlands) at Ehime University, Matsuyama, Japan.

2.3. Bioconcentration Factor (BCF)

The bioaccumulation in the plant can be evaluated using the BCF [18]. The BCF is defined as the ratio of metal concentrations in the shoot to those in the soil [19,20]. The BCF shows the capacity of the plant species to remove metals from soil and accumulate them in the shoot [18] and is calculated as follows [21,22]:

$$BCF = C_{shoot}/C_{soil} \quad (1)$$

where C_{shoot} is the metal concentration in the shoot and C_{soil} is that in the soil.

2.4. Translocation Factor (TF)

The TF is calculated from the heavy metal concentrations and is used to evaluate the phytoextraction ability of the plant, in particular the plant's ability to translocate heavy metals from the roots to the shoots [23]. The TF is defined as the ratio of the heavy metal concentration in the shoots to that in the roots [20,24], as follows [21,23]:

$$TF = C_{shoot}/C_{root} \quad (2)$$

where C_{shoot} is the metal concentration in the shoot and C_{root} is that in the root.

2.5. Heavy Metal Uptake Intensity

Plant responses to heavy metal contamination in soil and water vary among species, and different plants accumulate different contaminants in their tissues at different rates [25]. The uptake intensity can be used to determine the biomarker ability of plants. The metal concentrations in biomarker plants are positively correlated with metal concentrations in soil.

2.6. Statistical Analysis

Statistical analyses were carried out using OriginPro 9.0 for Windows. The Shapiro–Wilk test was used to check the normality of the heavy metal concentrations in the shoots, roots, and soil. The data were log-normally distributed, so the Kruskal–Wallis ANOVA test was used to test for significant differences, with $\rho < 0.05$ considered statistically significant.

3. Result

This is a preliminary study which focuses on *P. cretica* screening in an abandoned mine site. This preliminary study can be used to evaluate the phytoremediation and bioindicator approach for environmental assessment. There are several types of substrate in this study, including debris flow sediment, tailing waste, river sediment, and humic soil. The lack of laboratory observations causes the limitation of grain size information in this study. The As total concentrations in the plant shoots ranged from not-detected (ND) to 1300 mg/kg dry weight (DW) (Tables 1 and 2). In this study, ND data is likely due to the leaching process. The As total concentrations in the roots and soil ranged from 24.2 to 3275, and from 15.0 to 15100 mg/kg DW (Tables 1 and 2), respectively. The results suggest that *P. cretica* can accumulate high concentrations of As and is a hyperaccumulator of As (Figure 2) at this abandoned mine site.

The shoots of *P. cretica* accumulated Pb at total concentrations ranging from 2.40 to 3840 mg/kg DW (Tables 1 and 2).The Pb concentrations in roots and soils ranged from ND to 430.3, and from 33.4 to 1610 mg/kg DW (Tables 1 and 2), respectively. The results suggest that *P. cretica* can accumulate high concentrations of Pb and is a hyperaccumulator of Pb (Figure 2). Ranges of Zn concentrations in the shoots, roots, and soil were 25.0–296, 18.6–1742, and 113–10010 mg/kg DW, respectively. The results suggest that *P. cretica* can accumulate Zn and is a biomarker of Zn (Figure 2). The results indicate that *P. cretica* has an ability to remediate heavy metal contamination by phytoremediation using an environmental assessment approach.

Table 1. Heavy metal concentrations of *P. cretica* in shoot and soil; BCF of *P. cretica*.

Sample	As			Zn			Pb		
	Shoot (mg/kg DW) ± SD	Soil (mg/kg DW) ± SD	BCF	Shoot (mg/kg DW) ± SD	Soil (mg/kg DW) ± SD	BCF	Shoot (mg/kg DW) ± SD	Soil (mg/kg DW) ± SD	BCF
1	147 ± 13.5	370 ± 5.72	0.40	96.7 ± 6.89	2730 ± 10.0	0.04	440 ± 31.4	584 ± 16.6	0.75
2	274 ± 27.2	1476 ± 16.8	0.19	168 ± 14.2	4600 ± 20.0	0.04	835 ± 70.6	1610 ± 26.1	0.52
3	ND	250 ± 2.90	ND	117 ± 7.70	795 ± 9.50	0.15	2.40 ± 1.86	273 ± 4.77	0.01
4	1300 ± 89.9	15100 ± 150	0.09	65.0 ± 4.72	834 ± 14.1	0.08	3840 ± 258	874 ± 3.19	4.39
5	46.5 ± 8.60	161 ± 6.00	0.29	147 ± 13.1	10000 ± 50.0	0.01	125 ± 13.2	420 ± 2.19	0.30
6	91.1 ± 12.8	47.3 ± 0.63	1.92	70.8 ± 6.54	246 ± 6.79	0.29	300 ± 27.9	113 ± 2.81	2.65
7	241 ± 24.8	234 ± 1.05	1.03	177 ± 15.2	1200 ± 29.1	0.15	820 ± 70.3	143 ± 3.07	5.73
8	37.4 ± 5.88	92.0 ± 3.05	0.41	98.5 ± 6.94	720 ± 13.5	0.14	103 ± 8.60	89.2 ± 1.06	1.15
9	38.1 ± 5.98	333 ± 0.77	0.11	100 ± 7.06	562 ± 8.95	0.18	105 ± 8.75	636 ± 14.6	0.16
10	153 ± 16.0	355 ± 9.04	0.43	249 ± 19.1	1900 ± 25.1	0.13	520 ± 41.0	790 ± 7.15	0.66
11	48.5 ± 7.86	27.2 ± 2.95	1.78	48.3 ± 4.04	210 ± 2.48	0.23	174 ± 15.0	64.0 ± 2.19	2.73
12	96.2 ± 10.3	91.0 ± 0.40	1.06	95.1 ± 6.77	1100 ± 2.95	0.09	305 ± 22.3	141 ± 2.70	2.16
13	42.0 ± 4.86	76.0 ± 2.56	0.55	62.0 ± 3.80	377 ± 7.98	0.16	120 ± 8.02	83.0 ± 1.28	1.41
14	25.3 ± 4.92	758 ± 7.21	0.03	146 ± 9.87	983 ± 11.6	0.15	90.6 ± 7.58	443 ± 8.09	0.20
15	85.3 ± 8.43	106 ± 0.98	0.81	99.0 ± 6.45	933 ± 10.2	0.11	250 ± 17.1	105 ± 2.50	2.37
16	105 ± 11.3	61.0 ± 0.88	1.73	66.0 ± 5.05	1010 ± 2.97	0.07	304 ± 23.5	85.4 ± 1.36	3.56
17	110 ± 12.0	95.3 ± 4.36	1.14	234 ± 17.7	2100 ± 0.00	0.11	303 ± 24.3	133 ± 6.56	2.27
18	40.0 ± 6.1	39.4 ± 1.60	1.01	43.1 ± 3.17	291 ± 8.38	0.15	155 ± 11.8	49.0 ± 3.73	3.18
19	23.1 ± 5.52	32.0 ± 2.38	0.72	52.3 ± 4.33	196 ± 8.28	0.27	116 ± 10.7	59.2 ± 3.10	1.96
20	11.0 ± 3.87	17.2 ± 2.18	0.62	54.3 ± 4.08	173 ± 8.10	0.31	40.3 ± 4.53	39.3 ± 4.53	1.02
21	21.0 ± 3.85	49.3 ± 3.24	0.42	44.0 ± 2.87	337 ± 5.11	0.13	66.9 ± 5.36	88.0 ± 1.00	0.76
22	38.0 ± 6.02	19.0 ± 0.23	1.99	48.0 ± 3.62	200 ± 1.86	0.24	115 ± 9.56	45.0 ± 3.29	2.56
23	40.0 ± 7.63	24.2 ± 1.14	1.65	54.1 ± 4.84	128 ± 1.87	0.42	85.0 ± 9.12	43.0 ± 2.44	1.98
24	20.2 ± 3.87	104 ± 2.20	0.20	74.4 ± 4.80	533 ± 4.64	0.14	70.0 ± 5.79	91.4 ± 1.41	0.76
25	204 ± 18.2	78.3 ± 2.28	2.61	164 ± 11.8	910 ± 3.58	0.18	696 ± 50.1	270 ± 4.28	2.58
26	31.0 ± 5.63	30.5 ± 0.80	1.00	34.3 ± 2.77	238 ± 4.71	0.14	100 ± 8.80	52.4 ± 0.40	1.91
27	12.4 ± 4.63	21.1 ± 2.07	0.59	296 ± 20.2	540 ± 8.94	0.55	57.1 ± 5.64	40.0 ± 2.83	1.44
28	3.10 ± 0.00	27.1 ± 2.90	0.11	65.3 ± 5.06	220 ± 7.15	0.30	17.5 ± 3.05	46.1 ± 2.67	0.38
29	0.40 ± 0.00	15.0 ± 1.04	0.03	33.3 ± 2.45	113 ± 1.97	0.29	8.32 ± 2.06	33.4 ± 0.20	0.25
30	ND	19.0 ± 2.79	ND	25.0 ± 2.30	202 ± 3.66	0.12	8.53 ± 2.95	37.0 ± 1.40	0.23
31	5.84 ± 2.89	20.0 ± 1.36	0.30	65.0 ± 4.58	282 ± 8.34	0.23	16.3 ± 2.50	43.3 ± 1.60	0.38

DW: Dry weight; SD: Standard Deviation; ND: Not detected.

Table 2. Heavy metal concentrations of *P. cretica* in shoot and root; TF of *P. cretica*.

Sample	As			Zn			Pb		
	Shoot (mg/kg DW) ± SD	Root (mg/kg DW) ± SD	TF	Shoot (mg/kg DW) ± SD	Root (mg/kg DW) ± SD	TF	Shoot (mg/kg DW) ± SD	Root (mg/kg DW) ± SD	TF
1	147 ± 13.5	2819 ± 1185	0.05	96.7 ± 6.89	1171 ± 22	0.08	440 ± 31.4	385 ± 167	1.14
2	274 ± 27.2	2909 ± 123	0.09	168 ± 14.2	1447 ± 32	0.12	835 ± 70.6	264 ± 132	3.16
3	ND	1232 ±459	ND	117 ± 7.70	325 ± 947	0.36	2.40 ± 1.86	196± 103	0.01
4	1300 ± 89.9	3275 ± 763	0.40	65.0 ± 4.72	464 ± 3.00	0.14	3840 ± 258	ND	ND
5	46.5 ± 8.60	1865 ± 175	0.03	147 ± 13.1	293 ± 7.00	0.50	125 ± 13.2	139 ± 124	0.90
6	91.1 ± 12.8	2186± 477	0.04	70.8 ± 6.54	197 ± 646	0.36	300 ± 27.9	58.0 ± 249	5.15
7	241 ± 24.8	2395± 9455	0.10	177 ± 15.2	402 ± 10.0	0.44	820 ± 70.3	ND	ND
8	37.4 ± 5.88	2861± 3.05	0.01	98.5 ± 6.94	431 ± 16.0	0.23	103 ± 8.60	ND	ND
9	38.1 ± 5.98	2427± 654	0.02	100 ± 7.06	428 ± 25.0	0.23	105 ± 8.75	25 ± 607	4.28
10	153 ± 16.0	2524 ±1065	0.06	249 ± 19.1	1742 ± 39.0	0.14	520 ± 41.0	219 ± 13.0	2.37
11	48.5 ± 7.86	2210 ± 1220	0.02	48.3 ± 4.04	19.0 ± 29.6	2.58	174 ± 15.0	183 ± 235	0.95
12	96.2 ± 10.3	2163 ± 703	0.04	95.1 ± 6.77	451 ± 26.0	0.21	305 ± 22.3	ND	ND
13	42.0 ± 4.86	2227 ± 2131	0.02	62.0 ± 3.80	278 ± 57.0	0.22	120 ± 8.02	ND	ND
14	25.3 ± 4.92	2847 ± 248	0.01	146 ± 9.87	370 ± 24.0	0.39	90.6 ± 7.58	32.0 ± 404	2.86
15	85.3 ± 8.43	1569 ± 415	0.05	99.0 ± 6.45	442 ± 16.0	0.22	250 ± 17.1	15.0 ± 200	16.8
16	105 ± 11.3	380 ± 1623	0.28	66.0 ± 5.05	307± 18.0	0.21	304 ± 23.5	430 ± 331	0.71
17	110 ± 12.0	480 ± 113.5	0.23	234 ± 17.7	384 ± 28.0	0.61	303 ± 24.3	108 ± 94.0	2.80
18	40.0 ± 6.1	24.0 ± 1676	1.65	43.1 ± 3.17	27.0 ± 18.0	1.58	155 ± 11.8	195 ± 176	0.79
19	23.1 ± 5.52	1148± 673	0.02	52.3 ± 4.33	266 ± 15.0	0.20	116 ± 10.7	111 ± 145	1.05
20	11.0 ± 3.87	140 ± 559	0.08	54.3 ± 4.08	37.0 ± 14.0	1.47	40.3 ± 4.53	63.0 ± 78.0	0.63
21	21.0 ± 3.85	777 ± 1251	0.03	44.0 ± 2.87	144 ± 11.00	0.31	66.9 ± 5.36	117 ± 315	0.57
22	38.0 ± 6.02	201 ± 1043	0.19	48.0 ± 3.62	183 ± 46.0	0.26	115 ± 9.56	17.4 ± 255	6.61
23	40.0 ± 7.63	1212 ± 564	0.03	54.1 ± 4.84	171 ± 68.0	0.32	85.0 ± 9.12	92.6 ± 109	0.92
24	20.2 ± 3.87	902 ± 430	0.02	74.4 ± 4.80	138 ± 38.0	0.54	70.0 ± 5.79	260 ± 109	0.27
25	204 ± 18.2	400 ± 1108	0.51	164 ± 11.8	246 ± 11.0	0.67	696 ± 50.1	88.0 ± 180	7.90
26	31.0 ± 5.63	1380 ± 1306	0.02	34.3 ± 2.77	198 ± 25.0	0.17	100 ± 8.80	74.0 ± 98.0	1.36
27	12.4 ± 4.63	998 ± 1968	0.01	296 ± 20.2	479 ± 51.0	0.62	57.1 ± 5.64	ND	ND
28	3.10 ± 0.00	630 ± 479	0.01	65.3 ± 5.06	94.0 ± 16.0	0.69	17.5 ± 3.05	104 ± 244	0.17
29	0.40 ± 0.00	812 ± 818	0.00	33.3 ± 2.45	54.0 ± 20.0	0.61	8.32 ± 2.06	16.1 ± 103	0.50
30	ND	463 ± 555	ND	25.0 ± 2.30	232 ± 22.0	0.11	8.53 ± 2.95	ND	ND
31	5.84 ± 2.89	431 ± 218	0.01	65.0 ± 4.58	65.0 ± 28.0	1.00	16.3 ± 2.50	211 ± 405	0.08

DW: Dry weight; SD: Standard Deviation; ND: Not detected.

4. Discussion

4.1. Heavy Metal Uptake Intensity from Soil to Shoot of P. cretica

The uptake intensity data show that the uptake ability of *P. cretica* varies with the substrate. The As and Pb absorption was very high in the shoots of *P. cretica* growing in the tailing waste substrate (Figure 2). With the exception of three data points, the As uptake intensity of *P. cretica* was positively correlated, in a logarithmic diagram, with the soil concentrations (Figure 2). Previous field observations showed that the tailing waste substrate contained sand grain. It probably caused one data point of tailing waste substrate to have a low concentration, and heavy metal leached out from the shoot. The distance to the contamination source has an influence on heavy metal accumulation, as shown by the two data points of the humic soil's low concentration, in the shoot. This is shown in Tables 1 and 2, and Figure 2. The As uptake and accumulation were highest in the shoots from the tailing waste and lowest in the humic soil (Figure 2).

The mechanism of As uptake in plants is thought to be via phosphate absorption, given the similarity in their structures [26]. The As concentrations in the plants studied here decreased as the phosphate concentration increased [27], which suggests that excess phosphate in the humic soil caused low As uptake in the shoots of this study (Figure 2). The soil substrate may influence heavy metal absorption by plant tissue. Differences between the soil substrates may influence the uptake ability, as suggested by the low concentrations of As in plants grown in the tailing waste at the study site (Figure 2). *P. cretica* has been previously described as an As accumulator [13].

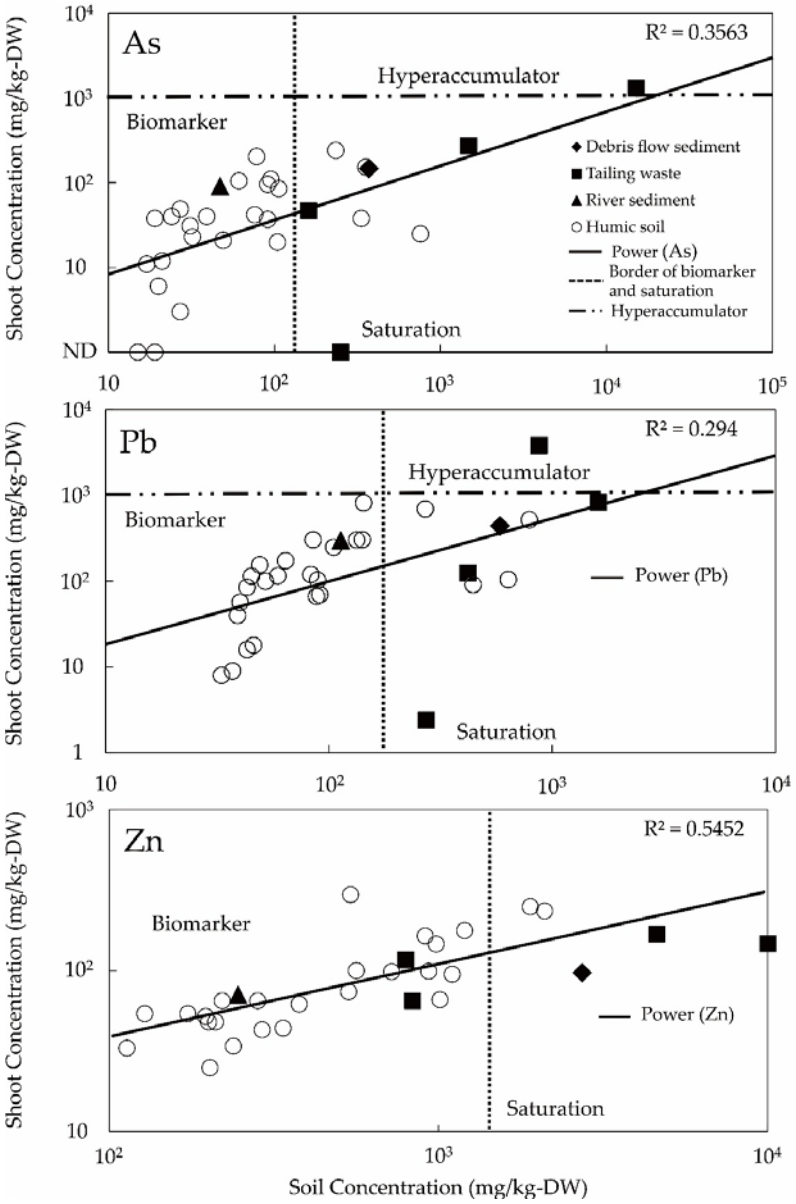

Figure 2. Heavy metal uptake intensity of As, Pb, and Zn from soil to shoot of *P. cretica*; Not Detected; DW: Dry Weight.

The shoot total concentrations in the previous study ranged from 149 to 694 mg/kg DW [13], which indicates that *P. cretica* is relatively tolerant of As contamination. Pyrite (FeS_2), which was found at the second sampling plot, is thought to be a source of sulphate at mine sites, which in turn is thought to be a source of As contamination [28] and may cause high As concentrations in plant shoots and soils. Other studies have reported that As accumulation in *Pteris vittata* is influenced by soil properties [13]. The different substrate types may therefore influence As accumulation in the shoots. As concentrations exceeding 1000 mg/kg have been reported in the shoots of As hyperaccumulator plants [29]. The As concentrations in the shoots in this study reached 1300 mg/kg DW (Tables 1 and 2). This indicates that As hyperaccumulates in the shoot tissue of *P. cretica* (Tables 1 and 2; Figure 2). The positive correlation between As uptake and soil concentrations indicates that *P. cretica* has potential as a biomarker at the individual level, such as shoots and roots (Figure 2).

Of all the metals considered in this study, *P. cretica* accumulates mainly Pb and Zn (Figures 2 and 3). The uptake intensity of Pb is similar to that of As, and with the exception of one data point being

positively correlated, on a logarithmic diagram, with soil concentrations (Figure 2). The positive correlation between soil concentrations and Pb uptake indicates that *P. cretica* has potential as a biomarker and hyperaccumulator of Pb at the individual level in a biomonitoring system (Figure 2).

Lead is highly mobile in the soil and accumulates at depths of up to 20.32 cm [30]. The soil substrate may influence the absorption of Pb from soil to the plant tissue (Figure 2). Concentrations of Pb were highest in *P. cretica* shoots from the tailing waste, and the high concentrations most likely reflect the high Pb concentrations in the tailing waste substrate (Table 1). The Pb uptake intensity, similar to As uptake, tended to increase and stabilize at high concentrations in the humic soil (Figure 2).

The amount of Pb absorbed by the plant is controlled by the pH, organic matter, and phosphorus concentration in the soil [30]. The shoot morphology of *P. cretica* controls Pb absorption into shoot tissues [31] and has an influence on Pb accumulation. Carbonate and phosphorus precipitation control the solubility of Pb [31]. In this study, the phosphorus concentrations in the soil may have influenced As and Pb accumulation in the shoots of *P. cretica.* Davies [32] reported that Pb uptake was influenced by soil particle size, cation exchange capacity, mycorrhizae, root exudates, and the rate of transpiration. Baker [7] reported that Pb concentrations in hyperaccumulator plants exceeded 1000 mg/kg in above-ground biomass. In this study, Pb accumulation in the shoots reached 3840 mg/kg DW (Tables 1 and 2), which exceeds the hyperaccumulator level. *P. cretica* is therefore a hyperaccumulator of Pb, a finding that has not been reported in previous studies, and can therefore be used for the phytoremediation of Pb contamination.

The Zn uptake intensity in *P. cretica* was positively correlated, in a logarithmic diagram, with soil Zn concentrations (Figure 2). The positive correlations between soil concentrations and Zn uptake indicate that *P. cretica* has potential as a biomarker at the individual level in biomonitoring systems (Figure 2). Zn uptake is controlled by plant metabolism [12]. The Zn accumulation in the plant shoot is less than the hyperaccumulation level, which is less than the 10,000 mg/kg Zn accumulated in the above-ground biomass [7]. The Zn uptake intensity tends to increase from the soil to the shoot because of the high concentrations in the humic soil (Figure 2). The results of this study indicate that *P. cretica* could be used as a biomarker for Zn at the individual level in a biomonitoring system.

Gaur and Adholeya [33] reported that some heavy metals, such as Zn, Cu, Mg, Ni, and Co, are micronutrients and are needed for plant growth, while other metals, such as Cd, Pb, and Hg, have unknown biological functions. The Zn concentrations in contaminated soil exceed the levels of nutrients required and may cause phytotoxicity [1]. Because it is a micronutrient, in this study, some accumulation and absorption of Zn is needed in the shoots of *P. cretica*. The soil and plant samples were taken directly from natural resources. There is a possibility that leaching occurred during sampling. This probably caused data limitation with respect to a very wide concentration range concentration in this study. The logarithmic diagram, due to the wide concentration range, was used to determine the correlation between soil and plant and root and shoot.

4.2. Bioconcentration Factor (BCF)

The BCF may be used to identify hazards. As a quantitative measure, the BCF indicates the potential toxicological impact of substances. The ranges of BCF values for As, Pb, and Zn were ND to 2.61, 5.73–0.01, and 0.55–0.01 (Table 1; Figure 2), respectively. Other studies have reported BCF values of between 1.34 and 6.62 for *P. cretica* [13]. Plants with BCFs that exceed one are hyperaccumulator plants [24]. The BCF results for this study indicate the hyperaccumulation of As and Pb in *P. cretica* (Figures 2 and 3).

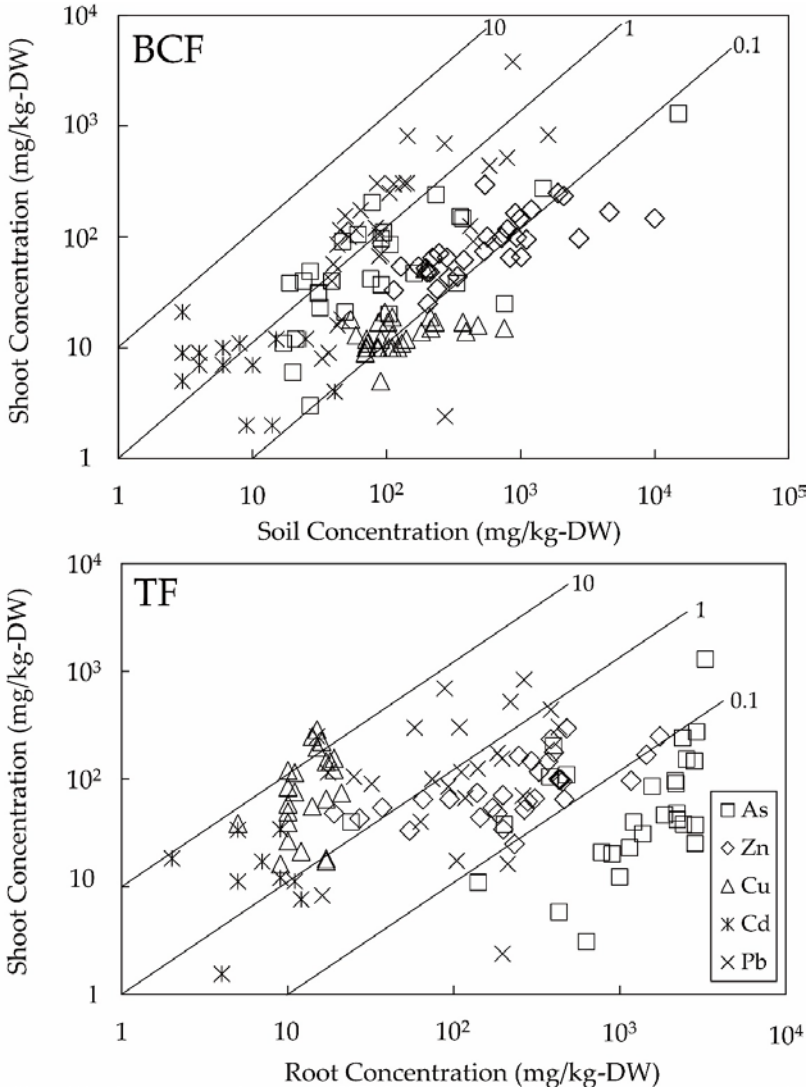

Figure 3. BCF and TF of heavy metals in shoot accumulation of *P. cretica*; ND: Not Detected; DW: Dry weight.

4.3. Translocation Factor (TF)

The TF is a measure of a plant's ability to translocate heavy metals from the root to the shoot. While some species are able to accumulate heavy metals in their roots, most plants have low BCF and TF values, which indicates they have limited ability to translocate and accumulate heavy metals. In this study, the TF values of As, Pb, and Zn ranged from ND to 1.65, ND to 16.8, and 0.08 to 2.58 (Table 2), respectively. A TF value that exceeds one indicates a hyperaccumulator plant. Mycorrhizal fungi may influence the TF value [34]. The TF values in this study indicate the hyperaccumulation of As, Pb, Zn, Cu, and Cd (Figure 3). However, TF results differ from the accumulation results (Tables 1 and 2), especially for Zn accumulation. This finding indicates that a plant should not be classified as a hyperaccumulator based solely on TF and BCF results, but that accumulation results should be considered in the classification, and vice versa.

5. Conclusions

The results of this study show that *P. cretica* has steady As and Pb accumulation, and accumulates high concentrations of As and Pb in its shoots. The uptake intensities of As, Pb, and Zn indicate that *P. cretica* is a biomarker at the individual level in soils with low concentrations, such as the humic

soil in this study, but it is a hyperaccumulator of As and Pb at the individual level in soils with high concentrations, such as the tailing waste substrate. The results of this study indicate that the hyperaccumulator classification should reflect the combination of shoot accumulation, BCF, and TF. These factors indicate that *P. cretica* has potential for phytoextraction of As and Pb at abandoned mine sites. The Kruskal–Wallis ANOVA test, used to determine significance differences between accumulation, translocation, and substrates, indicated that translocation and accumulation in plant shoots were influenced by substrate type.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PIXE	Particle Induced X-ray Emission
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
BCF	Bioconcentration Factor
TF	Translocation Factor
DW	Dry Weight
SD	Standard Deviation
ND	Not Detected

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