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Arsenic, lead and cadmium removal potential of *Pteris multifida* from contaminated water and soil

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ABSTRACT

The main threats to the environment from heavy metals are associated with arsenic (As), lead (Pb) and cadmium (Cd). In this study, the potential of *Pteris multifida* for removing As, Pb and Cd from hydroponic solution and pot soil was evaluated for the first time. Short-term (5 day) experiments were conducted to assess phytofiltration efficiency of temperate zone fern *P. multifida* and to compare it with mostly studied tropical zone fern *P. vittata*. Within 5 days, *P. multifida* accumulated 33% of As(III), whereas *P. vittata* could not accumulate that most toxic arsenic species As(III) at all. Long-term hydroponic results showed that 90% of Pb, 50% of As and 36% of Cd were removed by *P. multifida*. Concentration of As in the frond (22 mg/kg dw) was comparatively higher than other parts of plant and significantly higher concentration of Cd and Pb were stored in root and rhizome. Pot soil experiment of *P multifida* confirmed the comparative uptake and translocation of As(V), Pb and Cd from soil. Therefore, from the assessment of heavy metal accumulation capacity, translocation and healthy survival for long time, *P. multifida* was identified as an excellent species for the treatment of multi-metal contaminated water and soil.

KEYWORDS

Pteris multifida; heavy metal; phytoremediation; multi-accumulation; translocation

1. Introduction

Heavy metal pollution is a great threat worldwide. This type of pollution has accelerated rapidly since the onset of the industrial revolution (Gisbert *et al.* 2003). Retention of heavy metals at high concentrations in the environment exerts toxic effects on fauna and flora (Xue *et al.* 2010). Increased metal concentrations in the soil or water up to toxic levels is also an severe environmental problem (Briat and Lebrun 1999).

Arsenic (As), lead (Pb) and cadmium (Cd) are heavy metals of particular interest because of their unique toxic and carcinogenic effects. It was reported that 42.7 million people in West Bengal, India and 79.9 million people in Bangladesh suffered from groundwater contamination by As, with As levels in groundwater above the World Health Organization maximum permissible limit of 50 μg/L (Chowdhury *et al.* 2000). Most of the previous studies of As focused on As(V) remediation (Mandal *et al.* 2012; Natarajan *et al.* 2011; Tu and Bondada 2002; Ma *et al.* 2001), but in this research, we have used As(III) for hydroponic culture which is 10 times more toxic than As(V) (Van Herreweghe *et al.* 2003). In case of cadmium (Cd) poisoning, itai–itai disease is the most severe form of chronic poisoning caused by prolonged oral Cd ingestion which developed in numerous inhabitants of the Jinzu River basin in Toyama Prefecture of Japan (Inaba *et al.* 2005). Severe exposure to Pb can induce badly damage of the central nervous system (Kaul *et al.* 1999; Hertz-Picciotto 2000). According to Cui *et al.* (2004), soil and vegetables were heavily polluted by Pb and Cd near a smelter producing Pb in a suburb of Nanning, the capital of Guangxi Province in southern China.

For removal and recovery of toxic heavy metals, the use of biological methods has been recommended because they can be cheap, effective and environmentally-friendly compared with other conventional (physical and chemical) methods (Hanif, Bhatti and Hanif 2009). Phytoremediation is one of the most promising biological technologies for the removal of environmental pollution caused by the limitations of traditional technologies (Rahman *et al.* 2008). Recently, heavy metal accumulation by plants, such as *Cynodon dactylon* (Wu *et al.* 2010), *Salvinia natans* (Dhir and Srivastava 2011), *Melastoma malabathricum* L. (Selamat, Abdullah and Idris 2014), Switchgrass (Jeke, Zvomuya and Ross 2016) and *Pteris vittata* (Ronzan *et al.* 2017) has been tested and the effectiveness of phytoremediation by these plants has been demonstrated. The use of fern for this removal technique has developed considerable interest since the initial report of a Chinese brake fern *(Pteris vittata)* as an arsenic hyperaccumulator (Ma *et al.* 2001). Although *P. vittata* is the most studied fern, it is limited in its cold-tolerance. In case of Japan, *P. vittata* naturally grows in very south part of Japan, belongs to sub-tropical zone (average temperature: 7–15°C in winter; 25–30°C in summer). Being a tropical zone fern, germination and growth rates of *P. vittata* were also limited at around 25°C (Wan *et al.* 2009). So it is of interest to find an alternative cold-tolerant heavy metal-accumulating fern.

Pteris multifida (spider brake fern) is an As hyperaccumulating fern that can accumulate 1145 mg/kg of As in the fronds from soil (Du *et al.* 2005). Actually this is one of the world's common plants, distributed almost all areas in Japan and China

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including **temperate areas**. *P. multifida* has already shown some ability to tolerate low temperatures from **5°C to −4.6°C** (measured by thermometer in the field) during field experiments (Sugawara, Chein and Inoue 2015), which is an essential property for large scale phytoremediation in temperate zones such as the northern part of Japan. There is currently limited knowledge on the accumulation and translocation of heavy metals by *P. multifida* and to date there is no published information on the potential for accumulation of As, Pb and Cd by *P. multifida* from solution. Therefore, the aims of the present study were: (I) to determine the potential of *P. multifida* for heavy metal accumulation from both water and soil media and to compare that with *P. vittata*; (II) to investigate the long-term accumulation of heavy metals by *P. multifida and P. vittata*; and (III) to investigate the translocation of heavy metals from roots to aerial tissues. Hopefully the results of this study will provide novel information on the ability of *P. multifida* to accumulate and translocate As, Pb and Cd from both water and soil. These results will contribute to the application of *P. multifida* for phytofiltration or phytoremediation of multi-metal-contaminated soil and contaminated water.

2. Materials and methods

2.1. Plant materials

P. multifida and *P. vittata* were obtained from Fujita Co. (Tokyo, Japan). Fujita company prepared those Pteris from spore. Almost 7/8 months had passed to prepare Pteris sporophytes from spores. We received 4/5 frond containing fern at the age of 7/8 months. The average height and fresh weight was around 23 cm and 3.5 g respectively.

Efforts were made to ensure the uniformity and similarity of the size of plants used in the experiments. The ferns were used without sterilization for hydroponic cultivation, but sand was washed with tap water to remove the adhering compost from the roots, which is relevant to real treatment conditions (Natarajan ei al 2008; Huang *et al.* 2004). Then plants were transplanted to 500 mL opaque containers where they were acclimatized in 400 mL 5 times diluted Hoagland's solution (Hoagland and Arnon 1950) for two weeks in a growth chamber with the following conditions: 16-h light period with a light intensity of around 280 μmol m^{-2} s^{-1}; 25°C daytime; 20°C night; and 70% relative humidity. All of the experiments of this study were done with the same growth conditions as mentioned above. After adapting to the hydroponic environment, the plants were used for heavy metal accumulation experiments. The experiments were set up with five plants of approximately equal size and after analysis, the average of best three plants was selected for results.

2.2. Comparative hydroponic accumulation trial of P. multifida and P. vittata

To determine the As(III), Pb and Cd accumulation potential of *P. multifida* and to establish its efficiency compared with *P. vittata*, 5-day hydroponic trials were conducted with both *P. multifida* and *P. vittata*. In this study, the more toxic form of As, As(III), was used during the hydroponic experiments to obtain the responses of *Pteris multifida* and *Pteris vittata* to As(III). Plants were precultivated in 5 times diluted Hoagland's nutrient solution. The nutrient solution was composed of: 8 mM of KNO_3; 4 mM $Ca(NO_3)_2$; 2 mM $MgSO_4$; 1 mM $NH_4H_2PO_4$; 50 lM H_3BO_3; 9 lM $MnSO_4$; 1 lM $ZnSO_4$; 0.2 lM $CuSO_4$; 0.1 lM Na_2MoO_4; and 60 lM Fe(III)-EDTA. The acclimatized plants were incubated in 0.1 M 2-morpholinoethanesulfonic acid monohydrate (MES) buffer solution for three days to allow them to adjust to the buffer solution. Then *P. multifida* and *P. vittata* plants were transplanted to mixed metal solution where they have been growing for 5 days. Before plantation, initial concentrations were about 21 μg/L for As(III) (**$NaAsO_2$**) and 24 μg/L for both Cd (**$Cd(NO_3)_2.4H_2O$**) and Pb (**$Pb(NO_3)_2$**) in mixed metal solution during short term hydroponic experiment of *P. multifida*. Metal solutions were prepared by using 0.1 M MES buffer. In case of *P. vittata*, initial concentrations of As(III) and Cd were about 30 μg/L and Pb was 20 μg/L before transplantation. Every day, 1 mL of sample solution was collected at a fixed time for analysis and initial solution level (400 mL) was supplemented very carefully by adding MilliQ water. pH was kept at around 6.0 by adding 0.1 M HNO_3 or 0.1 M NaOH initially and was not adjusted again during the experiment. The buffer solution maintained the pH within the range of 5.98 to 6.04 during the 5 day experiment.

To investigate the long-term accumulation and translocation ability of *P. multifida*, a separate long-term hydroponic experiment was conducted over 24 days. Initial concentrations were measured before plantation when concentrations of Pb ($Pb(NO_3)_2$), Cd ($Cd(NO_3)_2.4H_2O$) and As(III) ($NaAsO_2$) in mixed metal solution were 27 μg/L, 30 μg/L and 33 μg/L respectively. All methods were the same as described above for the 5-day experiment but this time, 5 times diluted Hoagland's solution was used initially and solution was continuously aerated to ensure survival and health of the plant during the longer incubation period (24 days). All experiments were kept in the growth chamber.

2.3. Pot soil experiment for As(V), Pb and Cd accumulation and translocation

Pot soil experiments were conducted to investigate As(V), Pb and Cd uptake and translocation by *P. multifida* and *P. vittata* from pot soil medium from July to October, 2015. During the 3-month pot soil experiment, it was not possible to prevent the oxidation of As, so As(V) was spiked into the soil instead of As (III). During pre-cultivation, plants were grown in pot soil for 2 weeks to allow them to acclimatize to the pot conditions, where each pot contained about 3 kg mixture of sand and peat moss (4:1, v/v). According to Zheng *et al.* 2003 high concentration Pb reacted with As in soil solution to form a stable mineral. To avoid those possibilities, individual metal solutions of As(V) ($Na_2HAsO_4.7H_2O$), Pb ($Pb(NO_3)_2$) and Cd ($Cd(NO_3)_2.4H_2O$) were injected into separate pots so that in each pot metal concentration was maintained at 0.5 mg heavy metal/kg soil. Initial metal concentration of pot soil (0.5 mg/Kg) was higher than that of hydroponic solution (about 30 μg/L) because usually, in soil most of the metals are absorbed as soon as possible and would like to form some insoluble compounds like minerals or hydroxides. So the availability of metals in soil is always comparatively lower than that of metal solution (containing metals as ions). Plants were watered every day and changes in the physical appearance of plants were recorded. Each metal was injected into triplicate separate pots for both *P. multifida* and *P. vittata* where each pot contained one plant only. After 3 months' incubation, plant samples were digested for inductive coupled plasma mass spectroscopy (ICP-MS) analysis.

2.4. Sample preparation and chemical treatment

After the incubation period was **completed**, plant roots and rhizomes were immersed in a solution containing 1 mM K_2HPO_4, 0.5 mM $Ca(NO_3)_2$, and 5 mM Mes (pH 6.0) for 10 min and then briefly rinsed in distilled water. Fronds were also washed three times with distilled water and then dried in an oven for 3 days. The dried plant samples (10 mg) were digested in 3 mL of concentrated HNO_3 on a heating block (ALB-121, Acinics, Japan) at 130°C for 90 min. The digests were subsequently cooled and diluted 10-fold using Milli-Q water (Merck Millipore Corporation, Darmstadt, Germany), filtered through a PTFE 0.45 μm filter membrane (Merck Millipore Corporation) and then stored for analysis in 15 mL polypropylene tubes. As(III) and As(V) were separated by using an As speciation cartridge (Metal Soft Center, Highland Park, NJ, USA) that retains As(III) (Meng *et al.* 2001). As species in the solution were analyzed by the process described previously (Huang, Hatayama and Inoue 2011)

Inductively coupled plasma mass spectrometry (ICP-MS; ELAN 9000, Perkin Elmer, SCIEX) was used for quantitative analysis of As, Pb, and Cd. The internal standard used during analysis was 10 μg/L indium (In). Standard reference materials were standard solution of As, Pb, and Cd (Wako chemicals, Japan) and recovery rates were more than 100%. All glassware were washed five times with detergent solution, 3% HNO_3 and Milli-Q water. All reagents were of analytical grade.

2.5. Data analysis

The values reported in both text and figures are the mean ± SE (standard error of the mean). The statistical significance (at 95% confidence) was tested using analysis of variance, where appropriate, and the numbers of replicates are included in the figure legends.

3. Results and discussion

3.1. As, Pb and Cd uptake from hydroponic mixed metal solution

P. multifida has the potential to accumulate and store As, Pb and Cd from multi-metal solution, although the accumulation efficiency varied for each individual metal. The concentrations of As, Pb and Cd in the mixed metal solution decreased with increasing exposure time. To compare the efficacy of *P. multifida* and *P. vittata* for accumulating As(III), Pb and Cd, a short-term (5 day) hydroponic experiment was conducted. A long-term (24 day) hydroponic experiment was also conducted to clarify the long-term accumulation ability of those two ferns.

3.1.1. As(III) was removed by P. multifida, but not by P. vittata during short-term incubation

During the short-term (5 day) hydroponic experiment, a mixed metal solution was used where the form of As was As (III). To the best of our knowledge, the As(III) removal potential of *P. multifida* has not yet been tested, even though As(III) is almost 2 to 10 times more toxic than As(V) (Goyer 2001). During this experiment, the hydroponic conditions were strictly maintained to prevent the oxidation of As(III). Nutrients were not added to the metal stock solution to avoid the interactions between nutrients and metals. According to Su *et al.* (2008), in hydroponic solutions without nutrient, the possibility of oxidation from As(III) to As(V) is very low, which agrees with our results. Speciation tests for As in the mixed metal solution showed that even on the last day of the experiment, almost 93% of As was measured as As(III) (supplementary data Fig. S1(a)).

During this experiment, 63%, 44% and 33% of Pb, Cd and As (III), respectively, were accumulated by *P. multifida* within 5 days (Fig. 1(a)). *P. vittata* accumulated 79% Pb and 45% Cd respectively, but no As(III) at all from the mixed metal solution (Fig. 1(b)). As(III) accumulation by *P. multifida* was significantly

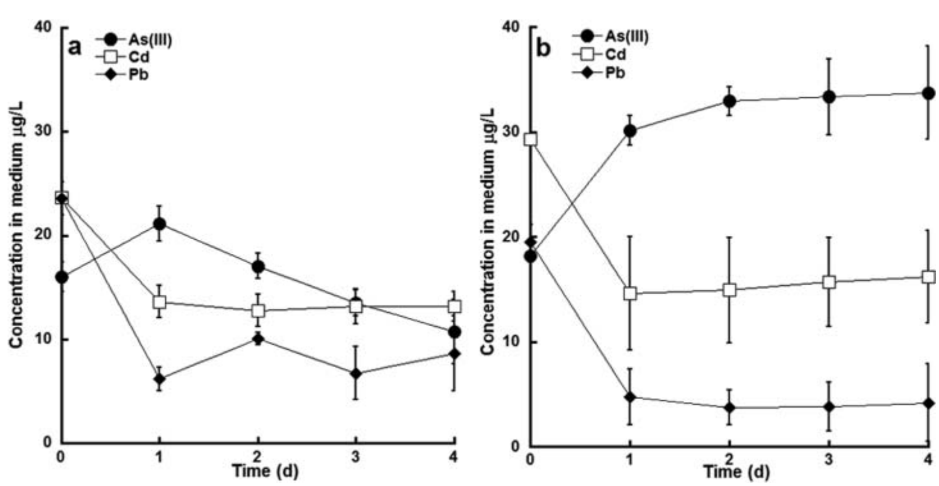

Figure 1. Concentrations of arsenic (III) [As(III)], cadmium (Cd) and lead (Pb) in hydroponic solution during 5 days' incubation with *Pteris multifida* (a) and *Pteris vittata* (b) ($n = 3$)

higher than that of *P. vittata* ($P = 0.005$). Cd and Pb accumulation by *P. multifida* and *P. vittata* were almost similar ($P = 0.41$ and $P = 0.94$ respectively) during short term. The first sampling (0 h) in this experiment was done after transplantation of the ferns. It is likely that at first, some of the As(III) was adsorbed by the root surface but was released back into solution within day 1 of the experiment; *P. multifida* then started to absorb up to 33% of the As(III) within 5 days but *P. vittata* could not accumulate As(III) at all. This unique result suggests that *P. multifida* is better than *P. vittata* for As(III) removal. In some plants, As(III) can be transported by aquaglyceroporin channels (Hatayama *et al.* 2011; Bienert *et al.* 2008). Further investigations are needed to clarify the mechanism of As(III) accumulation and transportation by Pteris species.

3.1.2. Translocation of heavy metals during hydroponic experiments

For successful application of phytoextraction or phytofiltration, accumulation and translocation of heavy metals is very important (Rozas, Alkorta and Garbisu 2006); if the metals are not translocated to aerial plant parts but stay attached to the root then there is a high possibility they can be released again to the media. To investigate the translocation of As, Pb and Cd from the root to other plant organs in *P. multifida*, concentrations of As, Pb and Cd in fronds, rhizomes and roots after 24 days' incubation (long-term) were analyzed. As shown in Fig. 2(a), concentration of As in the frond (22 mg/kg dw) was comparatively higher than root (4 mg/kg dw) or rhizome (7 mg/kg dw) but not significant ($P = 0.08$ and 0.13 respectively). Within 24 days' significantly higher concentration of Cd and Pb were stored in the root and rhizome ($P \leq 0.01$) (Fig. 2(a)) rather than frond. Long term translocation pattern of *P. multifida* can be compared with *P. vittata* which was incubated in mixed metal solution (100 μM As +60 μM Cd) for 15 days (Ronzan *et al.* 2017). Significantly higher concentration of As was detected in frond ($P < 0.05$) rather than root of *P. vittata* and significantly higher Cd was detected in root and rhizome than fronds ($P < 0.01$) which was similar as *P. multifida* of this research. According to Wu et al 2009 *P. vittata* could accumulate and store Pb mainly in root but some concentration was also detected in frond which consistent with our result of Pb accumulation by *P. multifida*.

During the short-term experiment, similar translocation of heavy metals was observed to that in the long-term experiment although the accumulated concentrations were lower (Fig. 2(b)). The translocation of *P. vittata* showed in (Fig. 2(c)) was compared with *P. multifida*. After 5 days' hydroponic cultivation, As translocation of *P. vittata* to the frond was significantly lower than *P. multifida* ($P = 0.005$). Concentration of Pb and Cd in the frond of *P. multifida* was also significantly higher than that of *P. vittata* ($P = 0.001$ and 0..004 respectively) within 5 days.

3.1.3. Long-term (24 days) accumulation from hydroponic solution

During the long-term experiment, 90%, 36% and 50% of Pb, Cd and As, respectively, were accumulated by *P. multifida* from mixed metal solution, as shown in Fig 3(a). Removal potential of *P. multifida* was compared in case of three target metals. Within 24 days, amount of Pb (9.5 ± 1.2 μg) that removed from hydroponic solution, was significantly higher than that of Cd (4.2 ±

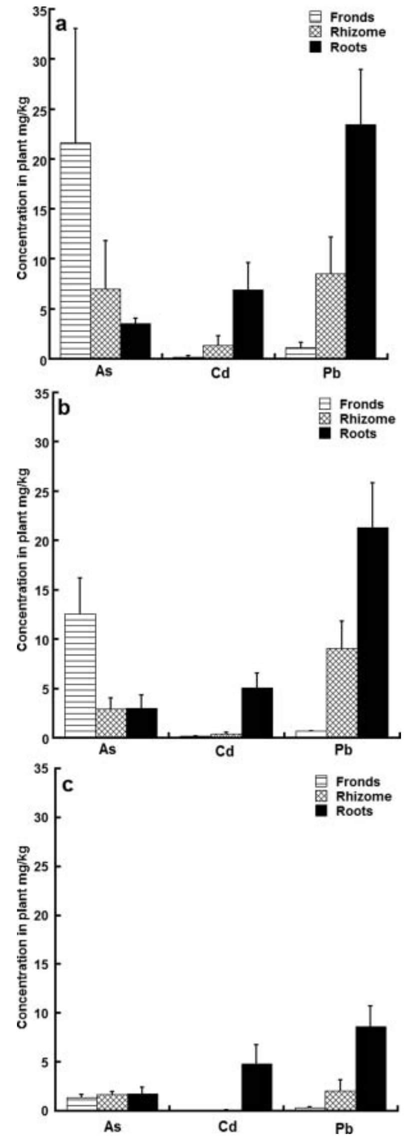

Figure 2. Translocation of arsenic (As), cadmium (Cd) and lead (Pb) in different parts (fronds, rhizome and roots) of *Pteris multifida* after the 24-day hydroponic experiment (n = 3) (a) after the 5-day hydroponic experiment (n = 3) (b) and Translocation of arsenic (As), cadmium (Cd) and lead (Pb) in different parts (fronds, rhizome and roots) of *Pteris vittata* after the 5-day hydroponic experiment (n = 3)

0.1 μg) and As (6.6 ± 2.0 μg) ($P = 0.007$ and $P = 0.04$ respectively). *P. vittata* accumulated 98% As, 43% Cd and 47% Pb during 24 days (Fig. 3(b)). In case of long term incubation, significantly higher amount of As (12.3 ± 0.2 μg) was removed by *P. vittata* than that of Cd (5.5 ± 0.6 μg) and Pb (5.9 ± 2.0 μg) ($P < 0.0001$ and $P = 0.0003$ respectively). To avoid the initial adsorption and release observed in the short-term experiment, the first sampling (0 h) was undertaken before transplantation. The major difference between the long-term and short-term experiments was that essential nutrients were added initially to the mixed metal solution in the long-term experiment to ensure the health and survival of the plant, which might have caused some interaction between metals and nutrients. According to our hypothesis, the interaction between metals and nutrients during the long-term experiment resulted in slow accumulation of heavy metals during the first few days. In the case of the long-term (24 days) experiment with nutrient, it is most likely that As(III) was gradually oxidized to As(V) and finally

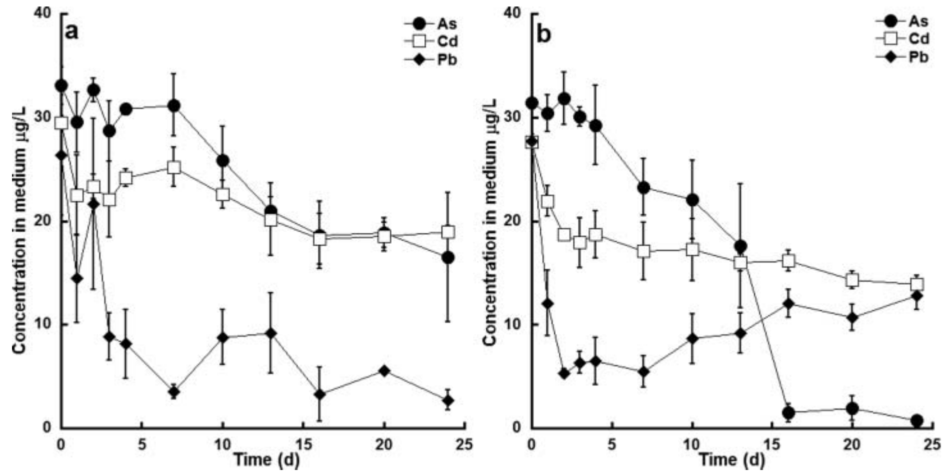

Figure 3. Concentrations of arsenic (As), cadmium (Cd) and lead (Pb) in hydroponic solution during 24 days' incubation with *Pteris multifida* (n = 3) (a) and *Pteris vittata* (n = 3) (b)

almost all As(III) was converted to As (V) (99.7%) (supplementary data Fig. S1(b)). According to Hatayama *et al.* 2011 in hydroponic condition, most of the arsenite was almost completely oxidized to arsenate (96%) within 48 h. After oxidation As(V) might be transported by the phosphate transporter (Wang *et al.* 2002; Hatayama *et al.* 2011). According to Hughes (2002), phosphate uptake can be replaced by As(V), which would interrupt many biochemical pathways. But as the initial phosphate concentration in the solution was high, that time phosphate might be transported mainly by the phosphate transporter for the first few days. As the phosphate concentration decreased within first few days, ferns might have started to accumulate As(V) by using the phosphate transporter. The decrease in phosphate concentration from the solution was analyzed to support the above possibilities (supplementary data Fig. S2).

3.2. As(V), Pb and Cd accumulation and translocation from pot soil

A long-term pot soil experiment confirmed the uptake and translocation of As(V), Pb and Cd from soil by *P. multifida.* After 3 months' assimilation in metal-injected pot soil at 0.5 mg/kg metals concentration, *P. multifida* contained 1.6×10^3, 2.4×10^2 and 99 mg/kg dw of As, Pb and Cd, respectively (Figs. 4(a–c)). Significantly higher concentration of As was stored in the rhizome (1.2×10^3 mg/kg) ($P < 0.05$) than other parts, similar to the results of Sugawara, Chein and Inoue (2015). The results of this study and those of Sugawara, Chein and Inoue (2015) indicated that, during long-term exposure to As-contaminated soil, *P. multifida* might have stored the maximum concentration of As in the rhizome, although the pathway of accumulation started with the root and was then translocated to the frond before finally ending up in the rhizome. The morphology of *P. multifida* is suited to the storage of nutrients and heavy metals for long time periods, as the rhizomes are relatively large in size. Control *P. multifida* plants also showed translocation of metals even at very low metal concentrations. Pb-infused *P. multifida* stored almost two times higher concentrations of Pb in the rhizome than in the root ($P < 0.05$). Rhizome and root concentrations of Cd were almost the same ($P = 0.93$) in *P. multifida.* Further investigations are needed to clarify the mechanism of accumulation and storage of heavy metals over long time periods.

P. vittata accumulated significantly higher concentrations of As (5.0×10^3 mg/kg) ($P < 0.01$) and Cd (1.8×10^2 mg/kg) ($P < 0.01$) but lower Pb (88 mg/kg) ($P < 0.05$) than *P. multifida* (Figs. 4(a–c). The translocation pattern was also different for *P. vittata* than for *P. multifida*. After the 3-month experiment, rhizomes and fronds of *P. vittata* showed significantly higher concentrations of As than root ($P < 0.05$). Concentration of Pb was not significantly varied in root and rhizome ($P = 0.37$) but Cd was translocated to the rhizome significantly ($P < 0.01$). Translocation of As, Pb and Cd in *P. multifida* and *P. vittata* compared with the control plants was shown in Figs. 4(a–c).

3.3. Responses of P. multifida after three months' exposure to As, Pb and Cd

Even after 3 months' growth in As, Pb and Cd injected soil, *P. multifida* plants still looked healthy. Natural spontaneous changes in plant condition, like the formation of new fronds, was also noticed which can be compared with *P. vittata* that grew healthily at sites highly contaminated by As and Pb (An *et al.*, 2006; Wu *et al.*, 2007). Kachenko *et al.* 2007 also demonstrated that pot trials.of *P. vittata* possesses a relatively high tolerance to Cd.

With respect to the control, the appearance of As spicked plants were not damaged by metal toxicity after three months exposure which is similar with the findings of Ronzon *et al.* 2017 who have done the details histological analysis of *P. vittata* on the apical, median and basal part after As exposure. The exposure of *P. multifida* to 0.5 mg Cd/Kg soil for three months did not cause any visual modification in comparison with the control. But according to Ronzon et al 2017, Cd caused a strong cell wall thickening in all the tissues around the midrib and epidermis when exposed to 60 μM Cd. Wu *et al.* 2009 also stated that besides having a high level of As tolerance, P. vittata also possesses considerable tolerance to Pb which is similar as the Pb tolerance of *P. multifida* in this experiment. Comparative dry biomass of metal injected plants and control plants confirmed that the growth and vitality of *P. multifida* had not been affected by

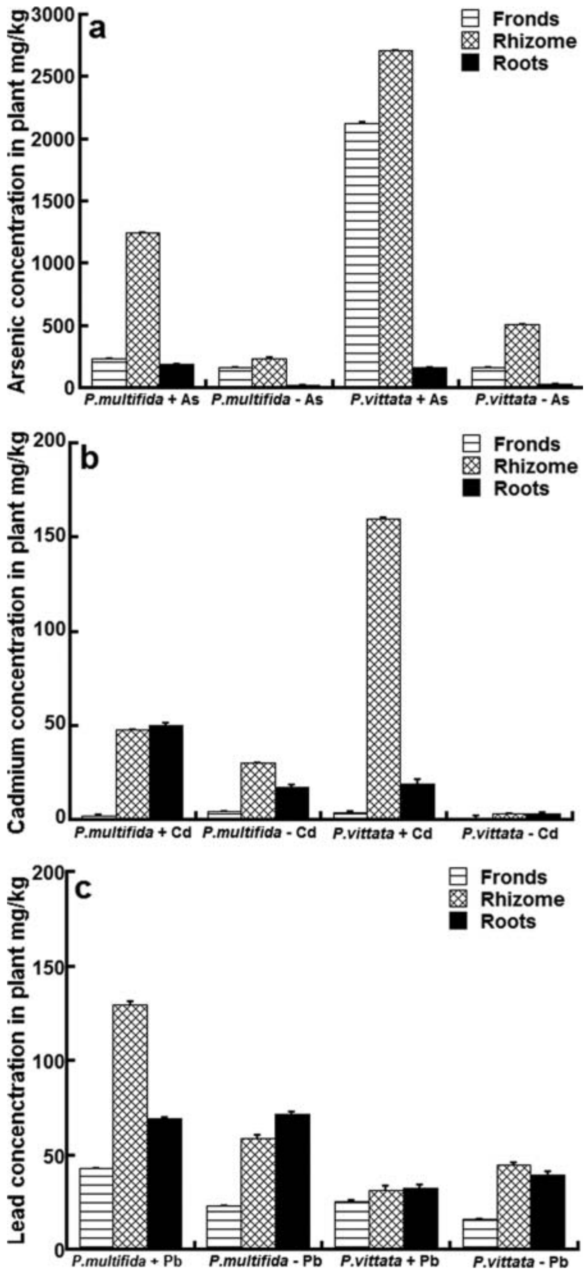

Figure 4. Concentrations of arsenic (As) (a), cadmium (Cd) (b) and lead (Pb) (c) in different parts (fronds, rhizome and roots) of *Pteris multifida* and *Pteris vittata* after 3 months' pot soil experiment with (+) or without (−) As, Cd and Pb exposure ($n = 3$)

the toxic metals (Table S1). The tolerance of *P. multifida* to 0.5 mg As, Pb and Cd/Kg soil for three months' provides evidence that this plant could be practically applied to toxic metal contaminated environments.

4. Conclusion

In the present study, *P. multifida* was chosen because of its high cold tolerance in the field. The potential of *P. multifida* to remove As(III), Pb and Cd from solution and comparison with *P. vittata* was reported here for the first time. Our results showed that during the hydroponic experiment, heavy metal concentrations in the solution decreased continuously with time. *P. multifida* accumulated 90%, 36% and 50% of Pb, Cd and As, respectively, from the mixed metal solution within 24 days. The hydroponic experiment comparing *P. multifida* and *P. vittata* revealed that 33% of As(III) was removed by *P. multifida*, whereas *P. vittata* could not accumulate As(III) at all within 5 days. As was translocated to the frond and some Pb and Cd was also translocated to the rhizome from the root during the hydroponic experiment. The results of the long-term (3 months) pot soil experiment provided confirmation of the As(V), Pb and Cd uptake and translocation potential of *P. multifida.* After 3 months, *P. multifida* grown in metal-spiked soil stored significantly higher concentrations of As, Pb and Cd compared with the control plants ($P < 0.01$). The heavy metal (As, Pb and Cd) accumulation capacity and translocation ability of *P. multifida* were demonstrated in this study, providing evidence that it could be applied for the treatment of water and soil that are not only contaminated with As but also co-contaminated with Pb and/or Cd.

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