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Application of arsenic hyperaccumulator *Pteris vittata* L. to contaminated soil in Northern China

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ABSTRACT

Phytoextraction of arsenic (As) from contaminated soil using hyperaccumulator *Pteris vittata* L. has been applied to several contaminated areas in China and USA; however, most of them are located in Southern China. Low temperature during winter is the main reason limiting the application of *P. vittata* in Northern China. The aim of the current study is to explore the possibility of using *P. vittata* to remediate As-contaminated soil in Northern China and other areas with similar climate conditions and to obtain appropriate measures to aid in the survival of *P. vittata* in winter. Four different cover materials were used in the current study; by comparing growth conditions and remediation efficiency, the most suitable cover material for *P. vittata* was obtained. Results showed that using the combination of plastic film and soil or maize straw significantly improved soil temperature. While, the use of a single layer plastic film was the best choice when considering As accumulation.

1. Introduction

Arsenic is a universal element in our environment and its toxicity has become a global issue given its increasing contamination in water, soil, and crops in various countries like China, India, Bangladesh, Vietnam and some parts of Europe (Madeira et al., 2012; Nguyen and Lee, 2015; Pandey and Singh, 2015; Robson et al., 2013; Yu et al., 2015). Phytoremediation is an emerging technology well-adapted to the remediation of large scales of slightly or moderately contaminated soil. *Pteris vittata* L. is an arsenic hyperaccumulator being used to repair arsenic-contaminated soils on farmlands and residential areas (Chen et al., 2002; Ma et al., 2001).

Because of the lack of successful field trials of phytoextraction, some researchers are suggesting that phytoextraction may be still impractical at present (Robinson et al., 2015). However, our group conducted several field trials in China, all indicating successful applications of phytoextraction to contaminated farmlands (Wan et al., 2016). An annual removal rate of 17.5% has been reached in several remediation sites. A phytoremediation project using *P. vittata* has also been successfully conducted in a residential site in the US (Ebbs et al., 2009).

During the application process of phytoremediation technology to the As contaminated sites, some problems were encountered. The practical application of phytoremediation requires an integrated understanding and management of soil ecosystem processes, including microbes and decomposers (Barcelo and Poschenrieder, 2011).

One of the most difficult issues we have encountered is that *P. vittata* is mainly distributed in tropical and subtropical areas, whereas contamination is not limited in these areas. According to an investigation on the distribution of *P. vittata* in China, it can well grow under the following weather conditions: an annual average temperature of above 10 °C, average temperature in January (the coldest month in China) above 0 °C, average temperature in July above 20 °C, and the effective accumulated temperature $>$3800 °C, with frost-free period not $<$200 days and annual precipitation $>$500 mm. Low temperature in winter is the main restrictive factor for further distribution of *P. vittata* to higher latitudes (Chen et al., 2005). On the one hand, low temperature can disable the germination of spores (Wan et al., 2010). On the other hand, it can freeze plants to death when temperature is lower than 0 °C. This limitation is the reason that phytoextraction can only be conducted in southern parts of China.

Based on existing regulatory criteria, 19.4% of agricultural lands in China are polluted; 82.4% of which are contaminated by metals and metalloids Cd, Zn, Cu, Pb, Ni, Cr, Hg, and or As. As-contaminated soil is distributed not only in Southern China where mining activities are active but also in Northern China where irrigation of wastewater and mine production frequently occur (Gao et al., 2015; Heimann et al., 2015; Li et al., 2015; Tang et al., 2015). Similar condition also exists in other countries. Regarding the potential As accumulating ability of *P. vittata*, Stefan Outzen has tried to plant *P. vittata* in Demark, North Europe, but hardly survive (personal communication).

Therefore, the aim of this study is to explore the possibility of using *P. vittata* to remediate As-contaminated soil in Northern China and other areas with similar climate conditions, and to obtain appropriate

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measures to aid in the survival of *P. vittata* in winter. Covering the soil surface with certain special materials to improve soil temperature is one of the main technologies used to help plants survive the cold winter weather (Li et al., 2014). In Northern China, soils and crop straws are easily obtained as cover materials. Four different cover materials were used in the current study. By comparing growth conditions and remediation efficiency, the most suitable cover material for *P. vittata* in North China was obtained.

2. Materials and methods

2.1. Description of the experimental site

The experimental site is located in Jiyuan, northwest of Henan province (35°07′49.5″N, 112°32′50.7″E). Jiyuan has a temperate continental monsoon climate featuring four distinct seasons. Due to the low temperature in winter, there is no natural distribution of *P. vittata* in Jiyuan. The annual temperature is 14.3 °C and annual rainfall is 600–650 mm. Metallurgy is one of the main industries in Jiyuan. The lead (Pb) smelter industry started in 1950s, with the annual Pb output reaching 8,000,000 t in 1980. Henan Yuguang Au & Pb Co., located in the suburb of Jiyuan city, is the biggest Pb smelter company in China. According to the survey conducted by Henan Environment Monitoring Stations, the soil around Yuguang Au & Pb Co. has been seriously contaminated, with main contaminants being As, Pb, and Cd. The remediation site, with an area of approximately 10,000 m^2, is in the southwest of Yuguang Au & Pb Co. (Fig. 1), which is mainly contaminated by As and Cd (Table 1).

2.2. Experimental setup

To test the possibility of using *P. vittata* to remediate contaminated soils in Northern China, with an average temperature in the coldest month lower than 0 °C, the experiments were designed as follows.

Table 1
Soil properties.

Index	Value or concentration
pH	8.0 ± 0.2
Organic matter (g/kg)	17.0 ± 1.0
Total N (N, g/kg)	0.89 ± 0.07
Total P (P, g/kg)	0.51 ± 0.02
Available P (P, mg/kg)	8.97 ± 2.93
Total K (K, g/kg)	18.8 ± 0.9
Available K (K, mg/kg)	128 ± 8
NH4-N (mg/kg)	0.77 ± 0.06
NO3-N (mg/kg)	8.51 ± 1.98
As (mg/kg)	33.7 ± 1.5
Pb (mg/kg)	241 ± 20
Cd (mg/kg)	2.25 ± 0.21
Ni (mg/kg)	29.9 ± 3.4
Cr (mg/kg)	44.6 ± 0.5
Hg (mg/kg)	0.36 ± 0.07

At the start of November, when temperature at night began to drop below 0 °C, all the aboveground biomass of *P. vittata* were removed, leaving only ~5 cm stubble. Different kinds of cover materials were placed on *P. vittata*. A total of five treatments were performed: 1) one layer of non-woven fabric was covered; 2) one layer of plastic film was covered; 3) soil with 10 cm was covered, on top of which one layer of plastic film was covered; 4) maize straw of 10 cm was covered, on top of which one layer of plastic film was covered; 5) control with no cover material. Each treatment has four repeated trials. Temperature of the atmosphere and soil was recorded every day. In April, when plants began to grow, the cover materials were removed. Survival rate is defined as the ratio of the number of plants after winter to the number of plants before winter. Coverage was estimated and calculated by taking photos, and then processed by Adobe Photoshop 5.0 (Adobe Systems. Inc., CA, USA) (Sendo et al., 2010). In July, the survival rate and coverage were recorded. Afterward, *P. vittata* was harvested, to measure biomass, water content, and heavy metal concentrations. As

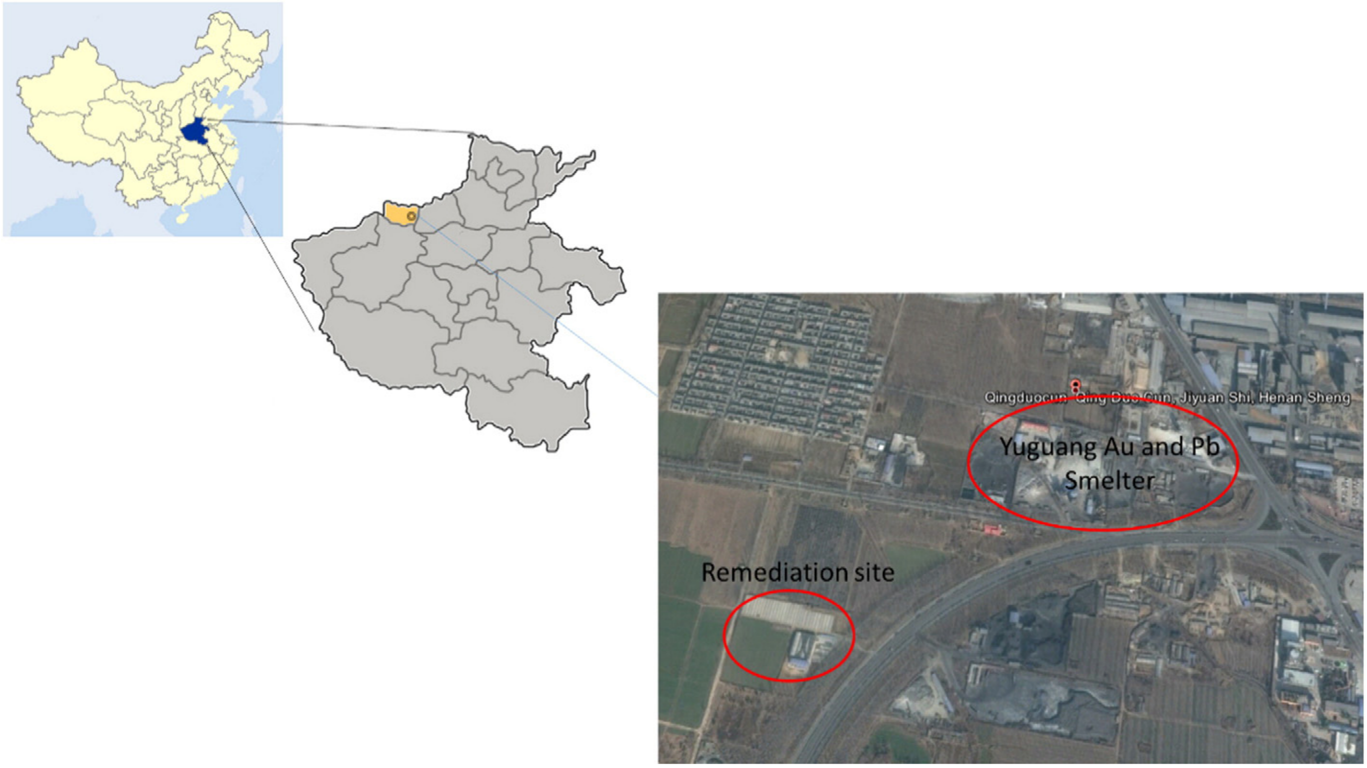

Fig. 1. Location of the remediation site.

concentrations were measured again in September when plants grow more leaves, for comparison between harvests. When harvesting plants, the leaves were washed using tap water and deionized water before drying.

2.3. Chemical analysis and data processing

To obtain soil properties, soil samples were air-dried, ground and sieved (to <2 mm). Soil pH was determined in a 1/2.5 soil/water mixture (Wei and Chen, 2006) and the total organic matter was calculated using the Walkley–Black method (Nelson and Sommers, 1982). Total P and total N in soil were determine using the titrimetric and gravimetric method with ascorbic acid (John, 1970) and Kjeldahl method (Anantakrishnan and Srinivasa Pai, 1952), respectively. To determine total K concentration, soil samples were digested using HNO_3-$HClO_4$, followed by HF, and then detected using atomic absorption spectrophotometry, according to the national standard for the Method for determination of total potassium in soil (GB 9836-88). Whereas the available K was extracted by NH_4OAC and detected by atomic absorption spectrophotometry. NH_4-N was extracted by KCl and determined by Kjeldahl method (Trehan and Wild, 1993). NO_3-N was measured by dual-wavelength spectrophotometry (Dong et al., 2014).

Heavy metals (HMs) in soils was determined through HNO_3-H_2O_2 digestion in accordance with the 3050B method of USEPA (1996). Plant samples were dried, ground, and digested with a mixture of HNO_3-$HClO_4$ (Chen et al., 2002), for the later analysis of As, Cd and Pb concentrations. To perform quality control, we simultaneously digested the samples of certified standard reference materials for soils (GSS-1) and plants (GSV-2 and GSV-3) from the China National Standard Materials Center with the experimental samples. There is no recommended value for Cd concentration of GSV-2, so reference material GSV-3 was included for the quality control of Cd measurement. As and Hg concentrations were determined using an atomic fluorescence spectrometer (Haiguang AFS-2202, Beijing Kechuang Haiguang Instrumental Co., Ltd., Beijing, China). The concentrations of Pb, Cd, Ni, and Cr were determined using an inductively-coupled plasma mass spectrometer (ICP-MS ELAN DRC-e, PerkinElmer, USA). The reference As, Hg, Pb, Cd, Ni and Cr concentrations of GSS-1 was 34 ± 4 mg kg^{-1}, 0.032 ± 0.004 mg kg^{-1}, 98 ± 6 mg kg^{-1}, 4.3 ± 0.4 mg kg^{-1}, 20.4 ± 1.8 mg kg^{-1}, and 62 ± 4 mg kg^{-1}, respectively; whereas the measured As, Hg, Pb, Cd, Ni and Cr concentrations of reference samples ($n = 3$) ranged from 30.5 to 37.0 mg kg^{-1}, from 0.027 to 0.035 mg kg^{-1}, from 93.3 to 103.1 mg kg^{-1}, from 4.0 to 4.62 mg kg^{-1}, from 19.1 to 22.7 mg kg^{-1}, and from 59.4 to 65.6 mg kg^{-1}, respectively. The reference As and Pb concentrations of GSV-2 was 1.25 ± 0.15 mg kg^{-1}, and 47 ± 3 mg kg^{-1}, respectively; whereas the measured As and Pb concentrations of reference samples ($n = 3$) ranged from 1.20 to 1.32 mg kg^{-1}, and from 44.2 to 49.6 mg kg^{-1}, respectively. Data were analyzed using SPSS version 11.0 (IBM SPSS, Armonk, NY, USA). One-way ANOVA was performed to determine the significance of treatment effects. Multiple comparisons were conducted using the least significant difference method. Curve fitting was conducted using SigmaPlot version 9.0 (Systat Software Inc., San Jose, CA, USA).

3. Results and discussion

3.1. Air and soil temperature at the experimental site

From November 2012 to March 2013, air temperature fluctuated. The highest temperature ranged from −1 °C to 29 °C, whereas the lowest temperature ranged from −10 °C to 13 °C. The gap between the highest temperature and the lowest can reach 22 °C.

The temperature from December to the next February was comparatively lower, during which, the lowest temperature was below 0 °C. A total of 94 days had the lowest temperatures lower than 0 °C and six days with the highest temperature lower than 0 °C. Continuous snow and frost threatened the growth of *P. vittata*.

The soil temperature fluctuated with less amplitude than air temperature. Soil temperature was in the order of blank < fabric < film < soil + film < maize straw + film. The difference between the blank and covered treatments was more obvious from November to February. In spring, all these five treatments showed almost the same soil temperature.

The blank treatment displayed the lowest soil temperature, all lower than 0 °C during late November to early February. The treatment covered by fabric improved soil temperature by <1 °C from November to February; whereas in spring, fabric improved soil temperature by 10 °C. The treatment covered by one layer of film performed better than fabric, improving temperature by an average of 1.3 °C. The combination of soil and film, and the combination of maize straw and film performed the best among these four treatments, improving soil temperature by an average of 2.5 °C and 3.5 °C, respectively. The lowest soil temperature of

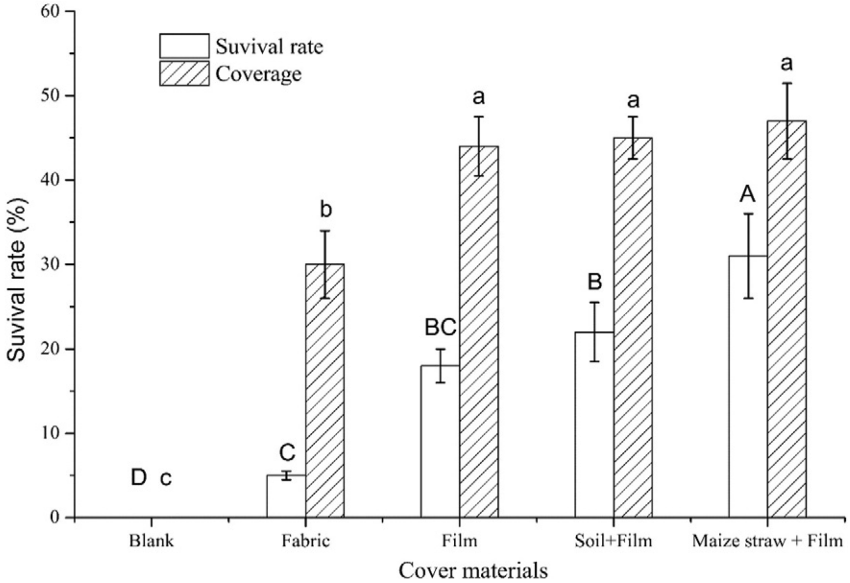

Fig. 2. Survival rate and coverage of *P. vittata* with different cover materials. Different capital letters indicate significant differences in survival rate among different treatments; different lowercase letters indicate significant differences in coverage among different treatments ($P < 0.05$).

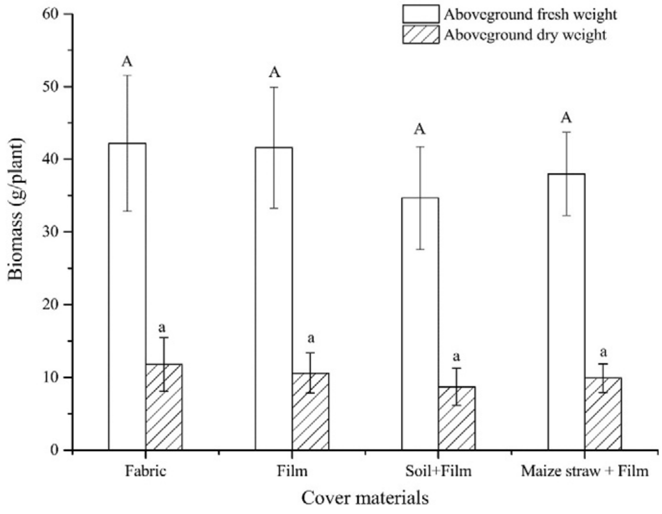

Fig. 3. Biomass with different cover materials.

maize straw + film treatment was all higher than 0 °C, whereas the lowest soil temperature of soil + film treatment was ~20% lower than 0 °C.

3.2. The effect of different cover materials in P. vittata survival

In accordance with soil temperature results, the survival rate and coverage were in the sequence of blank < fabric < film < soil + film < maize straw + film (Fig. 2). In the treatment with no cover material, no *P. vittata* survived, which indicates that without aiding measures, *P. vitatta* cannot survive in the winter of Northern China. The treatment of maize straw + film showed the highest survival rate and coverage, being 31% and 47%, respectively. The survival rate and coverage of the treatment of soil + film were slightly lower, being 22% and 45%, respectively. The survival rate and coverage of the treatment of film were lower than their combination, being 18% and 44%, respectively. The treatment of fabric displayed the lowest survival rate and coverage, being 18% and 30%, respectively.

Based on the survival rate and coverage of these four kinds of cover materials, the combination of film and soil or the combination of film and maize straw is a better cover material for *P. vittata* in Jiyuan.

3.3. The growth characteristics of P. vittata covered different materials

Both biomass and growth index results indicate that different cover materials have no significant effect on the growth of *P. vittata* (Fig. 3, Table 2). The biomass of individual *P. vittata* under different treatments was not significantly different (Fig. 3). The treatment of fabric and single layer of film had a slightly higher individual biomass than the other two treatments, being ~42.2 g/plant and ~41.6 g/plant, respectively. Similarly, dry biomass was also slightly higher in the treatment of fabric and film, being 11.8 g/plant and 10.6 g/plant, respectively. Similar to biomass results, growth indexes showed that growth of the treatment of fabric and film was slightly better than the other two treatments (Table 2), although the difference was not significant. The height and leaf area of *P. vittata* were the highest in the treatment of fabric, significantly higher than in the treatment of maize straw + film. No significant difference in frond number, pinnae number, or leaf thickness was observed among the treatments.

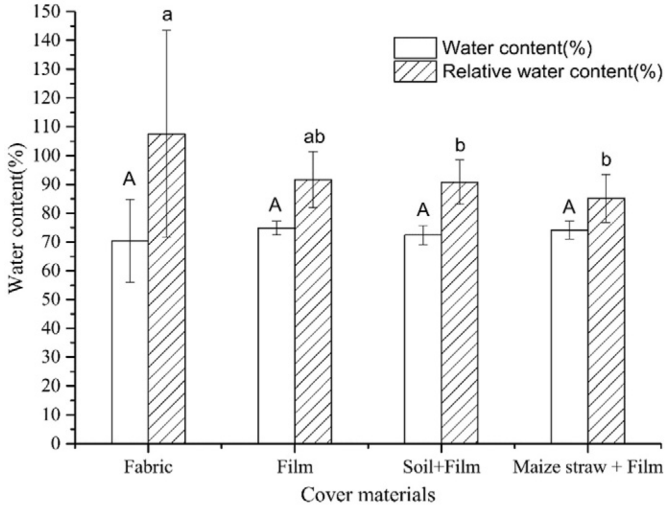

Fig. 4. Water content of *P. vittata* pinnae under different treatments. Different capital letters indicate significant differences in water content among different treatments; different lowercase letters indicate significant differences in relative water content among different treatments ($P < 0.05$).

Relative water content of pinnae is an important indicator of whether a plant is under cold stress (Thiagarajan et al., 2016). Results indicated no significant difference among the treatments (Fig. 4), showing that after several months of growth, all the overwintered plants developed well.

3.4. As concentration in P. vittata

The treatment of film showed the highest As concentration in the aboveground parts of *P. vittata* (Fig. 5). In July, As concentration in *P. vittata* ranged from 209 mg/kg to 321 mg/kg. The order of As concentration was film > fabric > soil + film > maize straw + film. The treatment of film had significantly higher As concentration than in the treatment of maize straw + film.

In September, As concentration apparently increased to 460–795 mg/kg. The order of concentration changed to film > soil + film > fabric > maize straw + film. The treatment of film had significantly higher As concentration than the treatment of fabric and the treatment of maize straw + film.

There were no significant difference in Cd and Pb concentration among different treatments (Fig. 6). It is interesting to find that with the extended time, the Cd concentration in *P. vittata* increased very slightly, whereas Pb in *P. vittata* increased significantly.

4. Discussion

4.1. Application potential of P. vittata in North China

Metal hyperaccumulator offers possibilities for a progressive phytoextraction of certain contaminant metals and metalloids from

Table 2
The growth indexes of *P. vittata* in experimental sites covered different materials.

Cover materials	Plant height (cm)	Frond numbers	Pinnae numbers	Leaf area (cm^2)	Leaf thickness (mm)
Fabric	52.7 ± 2.2 a	30 ± 9 a	51 ± 9 a	159.3 ± 12.1 ab	0.205 ± 0.021 a
Film	48.0 ± 4.2 ab	34 ± 9 a	54 ± 6 a	170.9 ± 10.4 a	0.208 ± 0.015 a
Soil + film	44.0 ± 4.5 b	34 ± 8 a	56 ± 5 a	153.2 ± 16.3 ab	0.213 ± 0.013 a
Maize straw + film	47.0 ± 3.2 ab	34 ± 7 a	49 ± 7 a	137.8 ± 14.2 b	0.203 ± 0.022 a

Note: the time of collecting and measuring samples was July 2013. The different letters represented the significant differences ($n = 10$, $P < 0.05$).

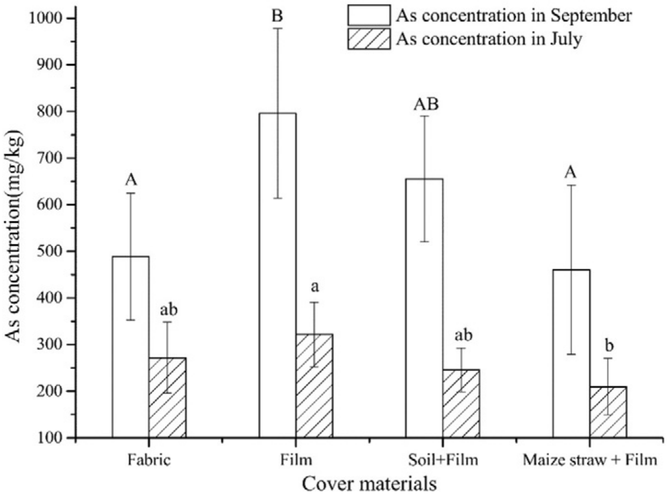

Fig. 5. As concentration of *P. vittata* covered by different materials. Different capital letters indicate significant differences in As concentration in September among different treatments, different lowercase letters indicate significant differences in As concentration in July among different treatments ($P < 0.05$).

soils (Lotfy and Mostafa, 2014; Polechonska and Klink, 2014). Present studies still focus on the selection of efficient hyperaccumulator plants or populations (Bech et al., 2012; Yang et al., 2015) and hyperaccumulation mechanisms (Bitterli et al., 2010; Higueras et al., 2003), with limited number of reports on field applications of phytoremediation technology. As far as we know, only As hyperaccumulator *P. vittata* (Ciurli et al., 2014; Jingqian et al., 2010; Niazi et al., 2012; Tongbin et al., 2010), and Cd hyperaccumulator *Sedum alfredii* (Tang et al., 2014) and *S. plumbizincicola* (Deng et al., 2016) have been demonstrated to be very effective for As and Cd removal, respectively. These plants were found able to strip out metal(loid)s in soil and transfer them to harvestable aboveground biomass.

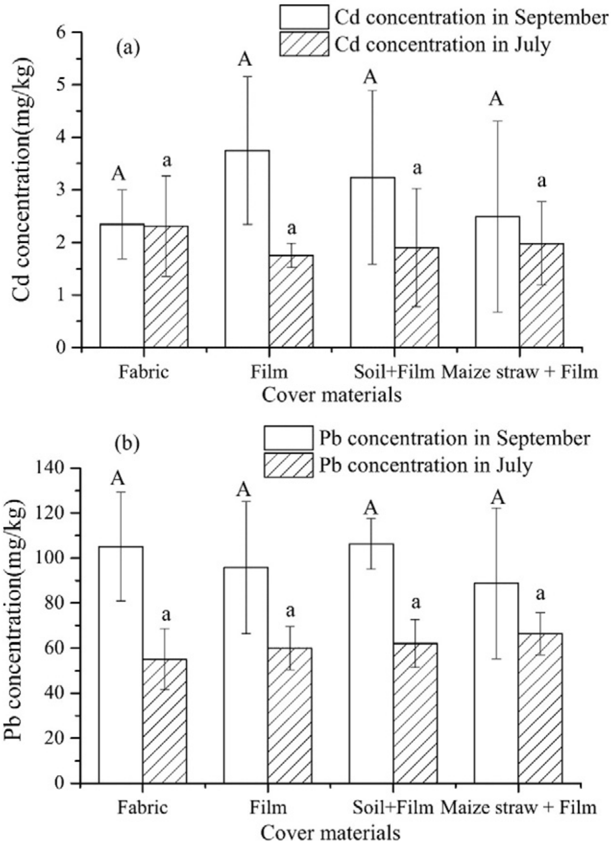

Fig. 6. Cd (a) and Pb (b) concentration of *P. vittata* covered by different materials.

Considering that phytoremediation greatly depends on the growth of plants, remediation efficiency is liable to changes in environmental conditions. Such uncertainty is the main issue scientists are trying to predict and control. The efficiency of the phytoremediation projects depend largely on the integrated ecosystem on the site (Barcelo and Poschenrieder, 2011). There has been some studies conducted aiming to optimize the ecosystem efficiency (Fayiga et al., 2008; Kertulis-Tartar et al., 2006; Mandal et al., 2012).

Temperature is one of the main uncertainties that limits the application of As hyperaccumulator in cold regions (Wan et al., 2010). Through the current study, the possibility of the application of *P. vittata* to As-contaminated soils in Northern China is confirmed. Although under natural conditions, *P. vittata* cannot survive in cold winter, the adoption of simple and cheap cover materials can efficiently and economically solve this problem. The cost for the cover material ranges from 5000–6000 $ per hectare soil, whereas the cost for the re-planting of *P. vittata* is as high as 30,000 $ per hectare soil. With the extended remediation period, the saved cost will further increase.

4.2. Comparison of cover materials

The current study found that the combination of plastic film and another layer of soil or maize straw can better improve soil temperature and survival rate. In terms of temperature boundary conditions for the growth of *P. vittata*, we propose that −2 °C is the lowest soil temperature that *P. vittata* can survive in.

However, treatments with higher soil temperature and higher survival rate do not promote growth and As accumulation by *P. vittata*. The reason behind is still unclear. Because cover materials were removed in April, when the highest temperature can be as high as 29 °C, better temperature retaining power of the combined treatments may have become a high-temperature stress at this time, which decreased growth and As accumulation of *P. vittata*. The inner reason still requires further investigation.

The final objective of phytoextraction is to remove As as much as possible. Therefore, the removal amount of As by each treatment was calculated. According to our plant density, the treatment of fabric, film, soil + film, and maize straw + film can remove 3.2, 16.7, 13.8, and 15.5 mg As from 1 m^2 soil. Therefore, film is the best cover material for As remediation in Jiyuan.

5. Conclusion

Phytoextraction using hyperaccumulator *P. vittata* L. to extract As from soil has been applied to some contaminated areas in China, with highly-efficient results. However, cold winter weather is the main obstacle for this technology to be implemented in Northern China. Through the application of *P. vittata* in Jiyuan, Northern China, and the comparison of four cover materials, *P. vittata* has a strong potential to be used in cold regions. Also, a single layer of plastic film is the best cover material to obtain the highest As removal amount from each unit of soil.

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