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ISOETES SINENSIS: A RARE AND ENDANGERED SPECIES, CAN ABSORB AND ACCUMULATE LEAD (PB)

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Abstract

Isoetes sinensis Palmer (Isoetaceae) is listed as a Wild Plant of National Protection Grade I for its endangered status. The limited population of *I. sinensis* is thought to be caused by difficulty breeding and habitat pollution (especially heavy metal pollution). This research used a lead nitrate solution to treat *I. sinensis* and to analyze the characteristics of uptake, transportation and accumulation of lead in *I. sinensis* by measuring the concentration of Pb^{2+} in the roots, rhizomes and leaves. The results showed that *I. sinensis* could absorb lead from both the root system and the leaves and upward or downward transport, and then accumulate lead in all organs, especially in the leaves. Comparatively, the ability of leaf to absorb and to accumulate lead was the strongest among organs. In the treatment I, the lead content of the organs direct contacted with lead solution were 1190 mg kg^{-1}, 3474 mg kg^{-1} and 95906 mg kg^{-1} in the leaves, roots and rhizomes, respectively. Although the ability of lead transportation between rhizome and leaf was stronger, the ability of lead translocation was weak on the whole in contrast to other heavy metals. *Isoetes sinensis* is a lead hyperaccumulating plant and can maintain the ability of leaf regeneration under 2000 mg kg^{-1} high concentration of lead stress after 30 days.

Key words: Lead pollution, *Isoetes sinensis* Palmer, Heavy metal, Accumulation.

Introduction

Quillwort (*Isoetes* L.) is an ancient relict plant widely distributed in the World (Hao *et al.*, 2000). It has an isolated systematic evolution (Pigg, 2001) and it has a special pathway of the Crassulacean acid metabolism (Keeley, 1998). It can also produce heteromorphosis spores (Kott and Britton, 1983) and can occupy a series of habitats from aquatic to terrestrial (Taylor and Hickey, 1992). Both aluminum toxicity and water acidification could affect growth of young *Isoetes* (Ctvrtlikova *et al.*, 2009). Quillwort in various habitats had different sensitivities to temperature (Liu, 2007). Additionally, the low content of rapidly available potassium in soil could also limit the growth of quillwort population (Zhu *et al.*, 2010).

So far, in China, only five species have been assigned to genus *Isoetes*: *I. hypsophila*, *I. yunguiensis*, *I. taiwanensis*, *I. orientalis* and *I. sinensis* (Liu *et al.*, 2008). They all belong to the National Key Protective Plant Grade I because of their habitat loss, agricultural land-use, and invasion by exotic species (Hao *et al.*, 2000). *Isoetes sinensis* is the most endangered among them in which currently only a few of them distributed in Jiande country in Zhejiang Province, Xiuning country in Anhui Province and Guilin country in Guangxi Province(Pang *et al.*, 2003).

Up to now, the researches on *I. sinensis* have mainly focused on the ecological geography, genetics and phylogeny (Liu *et al.*, 2003a; Li *et al.*, 2013). Some researchers suggested that the reason of the sharp decline in the population of *I. sinensis* may be related to the pollution of their original habitats and other factors, such as contamination of heavy metals, herbicides, and pesticides (Chen *et al.*, 1998; Liu *et al.*, 2003b). Our laboratory explored the physiological responses of *I. sinensis* to eight kinds of herbicides and confirmed that the *I. sinensis* was very sensitive to the herbicides (Sun *et al.*, 2013) and it could be one of the reasons for the endangered situation of *I. sinensis.*

Heavy metals are one of the sources of pollution in ecological environment, even worse, heavy metal pollution becomes more and more serious along with the rapid development of industry, which does great harm to ecological environment and seriously threat to human's health (Barona and Romero, 1996; Salazar *et al.*, 2012). Lead is one of the most abundant of the heavy metals in nature (Sengar *et al.*, 2008; Zhang *et al.*, 2014). Effects of lead on ecological environment, plants, animals, and especially on human have been explored (Krzesłowska and Woźny, 1996; Xiong, 1999), owing to strong accumulation and weak migration of lead in soils, plants and animals. But so far the properties of uptake, transportation and accumulation of lead in the *I. sinensis* have been rarely reported. In this study, using lead to treat the artificially propagated *I. sinensis* in the laboratory, we measured the lead concentration in soil as well as in various parts of the plants in order to explore feature of uptake, transportation and accumulation of lead in *I. sinensis*, especially to explore the further evidence of the endangering mechanism of *I. sinensis.*

Materials and Methods

Three-year-old *I. sinensis* plants from the Fern Research Office of Harbin Normal University were transplanted and then moved from the greenhouse to the laboratory in April 2011. The basic properties of the soil were as following: the pH value is 5.8, the organic matter is 36.17 g Kg^{-1}, the carbonate is 15.77 g Kg^{-1}, and the organic carbon is 16.74 g Kg^{-1}. Forty healthy plants about 20-cm high were planted into plastic containers (pot) of 11 cm long by 9 cm wide by 13 cm high. Each test plant was planted in a pot filled with 650 g of soil. Then the pots were placed into a 3,000 mL beaker with 2,200 mL of water submerging half of the plant.

Isoetes sinensis were cultivated for 30 days for acclimation period before lead treatment. $Pb(NO_3)_2$ solution was used to create a Pb stress environment. Three different lead treatments were designed to explore the different aspects of lead accumulation and transportation within *I. sinensis*.

For treatment I, we replaced the water inside the beaker with 2,200 mL of 2,000 mg L^{-1} $Pb(NO_3)_2$ (AR) solution as treatment group and same volume deionized water as the control group. The photoperiod was set at 12 hours of light per day, and the temperature was set at 20-25ºC during the photophase and 18 - 20ºC during the scotophase. The relative humidity was 60% to 70%. The deionized water was added each day to supply the lack of water and keep the concentration of the $Pb(NO_3)_2$ solution unchanged. Thirty five days after treatment, tested plants were harvested and separated into roots, rhizomes, upper part of the leaves (not submerged by the solution or water) and lower part of the leaves (submerged by the solution or water; see Fig. 1) for measuring the Pb^{2+} concentration. Three replicates were conducted.

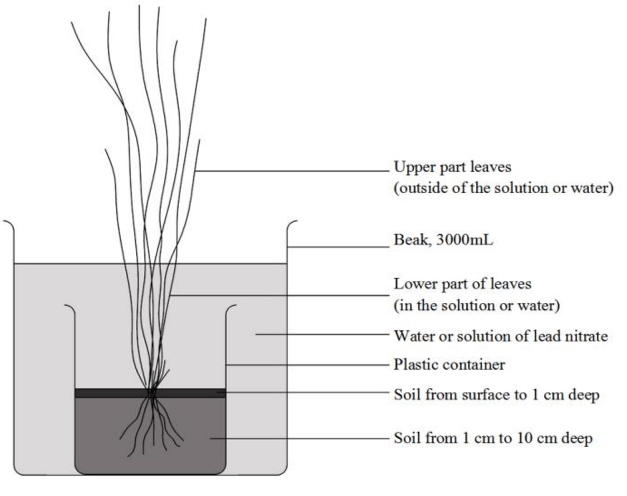

Fig. 1. The experimental device used in Treatment I. The lower parts of the leaves were submerged in the lead nitrate solution or water. The upper parts of the leaves were not submerged in the lead nitrate solution or water, so the lead in this part of the leaves could only be present through internal transport.

Treatment II aimed to investigate the ability of Pb^{2+} transported downward in leaves. The plants were tilted at a 30 degree angle and the upper part of the leaf dipped into a handmade pot filled with 1,300 mL of the $Pb(NO_3)_2$ solution (2000 mgL^{-1}) (see Fig. 2). All other growth conditions were the same as above. After 10 days these plants were harvested as above and the Pb^{2+} concentration was measured. Three replicates were conducted.

In order to evaluate the root's ability of transfer lead in *I. sinensis*, treatment III was designed. The quillworts were washed clean with tap water after dug out of soil, and were cultivated in tap water until new roots grew 5 - 8 cm. The quillworts were treated with 2000 mg L^{-1} $Pb(NO_3)_2$ solution to submerge the new roots but not to touch the rhizome in the slightest. All other growth conditions were the same as above. After 10 days these plants were harvested as above and the Pb^{2+} concentration was measured. Three replicates were conducted.

Preparation of the plant samples for metal analysis was as follow: The harvested plant samples were washed with deionized water and divided into roots, rhizomes, upper part of the leaves (not submerged) and lower part of the leaves. The plant part was then rinsed once with tap water and three times with deionized water. The washed material was oven-dried at 105ºC for 30 min, and then dried at 80ºC to constant weight. The dry plant samples were ground and passed through an 80-mesh sieve.

In this study, there were three soil samples. Based on our data (unpublished), Pb was deposited in soil surface within 1 cm. Two soil samples were collected from the treated group of treatment I, in which the soil was divided into two parts: 1) was collected from the surface to 1 cm deep and 2) was collected from 1 cm to 10 cm deep. Another soil sample was collected from the control group of the treatment I and the groups of the treatment II. The Chinese National Standard Reference Materials of soil (GBW07458) for quality control have gotten from the Institute of Geophysical and Geochemical Exploration CAGS (Beijing).

Digestion of the samples and measurement of Pb^{2+} concentration was as follow method: Randomly sampled 0.200 g of each sample and mixed with 5 mL of concentrated HNO_3 and 1 mL of H_2O_2 (30%) in a digestion tank. We used the CEM-MARS5 Microwave Digestion System (PYNN CORP, USA) to digest the sample, and then transferred the entire solution to a 100 mL beaker. The solution was heated at about 90°C until it was nearly dry. After cooled, 1mL of concentrated HNO_3 and then 99 mL of deionized water were added to bring the final volume up to 100 mL. The Pb^{2+} concentration was measured using an Inductively Coupled Plasma-Mass Spectrometer 7500CX (ICP-MS) (Agilent Technologies, USA) with following operating parameters: radio frequency power, 1500 W; gas flow rate of plasma, 15 L/min; carrier gas flow rate, 1 L/min; makeup gas flow rate, 1 L/min, sample injection rate, 0.8 L/min.

The bio-concentration factor (BCF) was calculated as formula (1) (Bu-Olayan & Thomas, 2009). Here the organs included roots, rhizome and leaves.

$$\mathrm{BCF} = \frac{\text{Metal Concentration in organs (mg kg}^{-1}\text{)}}{\text{Metal concentration in solution or soil (mg kg}^{-1}\text{)}} \qquad (1)$$

The translocation factor (TF) was calculated as formula (2) (Baker and Brooks, 1989; Zhou *et al.*, 2015). For example, $TF_{U/L}$ was used to estimate the transport ability of heavy metal from lower part of leaf to upper part of leaf. TF>1 indicates that the plant transports metals effectively from B organ to A organ (Baker and Brooks, 1989).

$$\mathrm{TF}_{A/B} = \frac{\text{Metal Concentration in A organs (mg kg}^{-1}\text{)}}{\text{Metal concentration in B organ (mg kg}^{-1}\text{)}} \qquad (2)$$

Upper part of leaf, lower part of leaf, leaf blade, rhizome and root were abbreviated to Up, Lp, Lb, Rh and Ro, respectively.

Separation was carried out using Fisher's LSD test at p<0.05 significance level. All statistical analyses were performed using SPSS software (SPSS 19.0, Chicago, IL, USA).

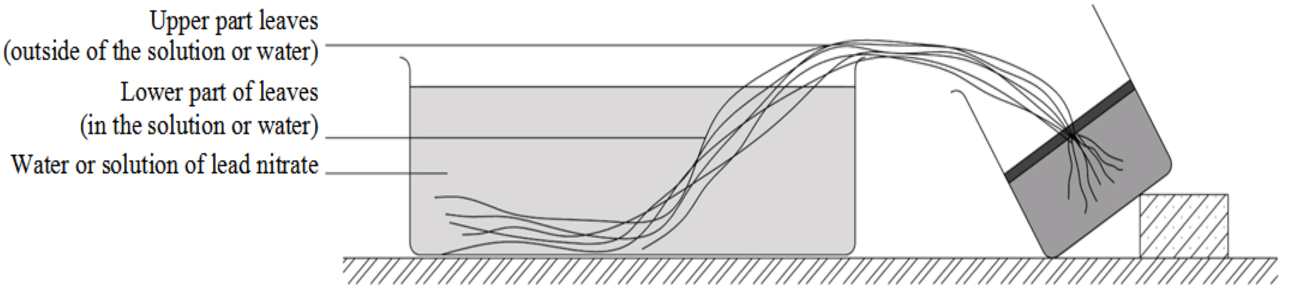

Fig. 2. The experimental device used in Treatment II. The lower parts of the leaves were not submerged in the lead nitrate solution, but the upper parts of the leaves were submerged in the lead nitrate solution. The lead in roots, rhizomes and the lower parts of the leaves could only be present through internal transport.

Table 1. Pb^{2+} concentration in soil samples (mg kg^{-1}).

	Control group	Treated group in treatment I	
		Surface to1cm	1cm below
Pb^{2+} concentration	11.32 ± 0.47 c	17134 ± 509.82 a	73.66 ± 4.91 b

Note: The data represents an average value ± standard deviation (n = 3); lowercase letters (a, b, c) represent the compared significant difference among the soil samples.

Table 2. Pb^{2+} concentration in each plant part of the control group and treatment groups (mg kg^{-1}).

	Root	Rhizome	Lower part of leaf	Upper part of leaf
Control group	27.88 ± 6.19a	8.38 ± 1.25b	4.55 ± 1.50b	6.00 ± 3.58b
Treatment I	1190.1 ± 378.60*c	3474 ± 668.43*c	95906.67 ± 8672.03*a	21033 ± 2204.73*b
Treatment II	46.73 ± 15.55c	2847 ± 213.19*b	3993.67 ± 1509.25*b	12833.67 ± 1452.05*a
Treatment III	129554.83 ± 11394.91*a	2447.53 ± 747.38*b	225.48±57.32*c	

Note: The data represents an average value ± standard deviation (n = 3); '*'represents significance between the treatment and control group at the 0.05 levels; lowercase letters (a, b, c) represent the compared significant difference among the organs within each group.

Results and Discussion

During five weeks, the growth status of *I. sinensis* was observed. Compared with CK (no Pb stress), the plant appears only on the tip of a small amount of the blade wilting and chlorosis in the first two weeks. From the third week, the wilting and chlorotic symptom was aggravated, but the plant as a whole looked like still well. By the end of the fourth week, the overwater part of nearly had half of the peripheral blade wilted and turned yellow. Until the fifth week, most of the mature leaves were presented wilting and chlorosis in the overwater part, but the core of young leaves can grow well and some new leaves were born.

It is suggested that the lead toxicity on the quillwort mainly can affected the periphery mature leaf, but less did the core young leaves. The plants appeared strong ability of regeneration under lead stress.

Table 1 showed the Pb^{2+}concentration of three soil samples, which were collected from the control group and treatment group in treatment I where is the third sample. We found that both of the soil samples of the treatment group with Pb^{2+} solution had a higher Pb^{2+} concentration than the control group with deionized water in treatment I, and the Pb^{2+} concentration in the surface soil (depth within 1 cm) was the highest in treatment I.

The result of lead accumulation suggested that the $Pb^{2+}$was mainly deposited on the soil surface, which was consistent with results of previous research (Chaney *et al.*, 1989; Covelo *et al.*, 2007). Pb^{2+} could easily be absorbed by soil because it could be combined with the top of the soil anion and formed low solubility compounds such as $Pb_3(PO_4)_2$, $PbSO_4$ and $PbCO_3$, that stayed on the soil surface and rarely migrated (Chaney *et al.*, 1989) to the bottom. But we still found that the Pb^{2+} concentration in the soil of depth 1-10 cm of the treatment group was significantly higher than that of the control group in the treatment I. Obviously a small amount of the Pb^{2+}could migrate into deepsoil before it was combined and precipitated, therefore, the Pb^{2+} in the deepsoil could be absorbed by roots of quillwort.

Table 2 showed the Pb^{2+} concentration in different organs. In the control group, the Pb^{2+} concentration varied in the different organs of the plants; the order of Pb^{2+} concentration in the organs from high to low was followed as root, rhizome, upper part of the leaf, and lower part of the leaf. There was no significantly different of Pb^{2+} concentration among the organs each other, but the Pb^{2+} concentration in the roots was significantly higher than that in the other organs.

In treatment I, The Pb^{2+} concentration in the treated group was significantly higher compared with the control group. The order of Pb^{2+} concentration in the organs from high to low as follow: the lower part of the leaf, the upper part of the leaf, the rhizome, the root, but there was no significant difference between the rhizome and the root. In treatment II, the order of the Pb^{2+} concentration in the organs from high to low as follow: the upper part of the leaf, lower part of the leaf, rhizome, the root, and there was no significant difference between the rhizome and lower part of leaf; there was also no significant difference between treated group and control group in roots. In treatment III, the Pb^{2+} concentration in the organs was root > rhizome > leaf (in the case the leaves were not divided upper and lower parts).

Table 3. Bio-concentration factors (BCF) of Pb^{2+} in plant parts of *Isoetes sinensis*.

Groups	Root	Rhizome	Lower part of leaf	Upper part of leaf
Control group	2.46	—	—	—
Treatment I	16.16	2.78	76.66	—
Treatment II	4.13	—	—	10.26
Treatment III	44.62	—	—	—

Table 4. Translocation factor ($TF_{A/B}$) of Pb^{2+} in *Isoetes sinensis*.

	Treatment I	Treatment II	Treatment III
$TF_{Up/Lp}$	0.22	—	—
$TF_{Lp/Up}$	—	0.31	—
$TF_{Rh/Lp}$	—	0.62	—
$TF_{Ro/Rh}$	—	0.02	—
$TF_{Rh/Ro}$	—	—	0.07
$TF_{Lb/Rh}$	—	—	0.08

Note: Upper part of leaf, lower part of leaf, leaf blade, rhizome and root were abbreviated to Up, Lp, Lb, Rh and Ro, respectively

The bio-concentration factor (BCF) can measure the ability of the plant to absorb Pb^{2+}. In the table 3, because the soils of the control group and treatment II group only had trace amounts of lead, the BCF was very low, it was 2.46 and 4.13; the soils of treatment I group and treatment III group had high concentration of lead, therefore the BCF were very high, it was 16.16 and 44.62. This seems to mean that the ability of root to absorb lead rise along with the increase of lead concentration of the solution contacted with root. From treatment I, we found that the ability of leaf to absorb lead was the strongest, and that of rhizome was the weakest. In treatment III, only root contacted with lead solution, its BCF even reached 44.26, which suggested that the roots had a high ability of uptake lead. The result indicated that *I. sinensis* rhizome had the lowest capability of accumulating Pb^{2+}.

Translocation factor (TF) can measure the ability of plants to transport heavy metals. In treatment I, both the soil and the solution contained Pb^{2+}, therefore, *Isoetes sinensis* was divided into a submerged part (contact with Pb^{2+}) and an unsubmerged part (not contact with Pb^{2+}) according to whether contacted with Pb^{2+}. In table 4, the $TF_{Up/Lp}$ of 0.22 in treatment I indicated that Pb^{2+} was upward transported from the submerged part to the unsubmerged part of the leaf blades, even though their lead contents were very low. In treatment II, only the solution contained Pb^{2+} (the lead concentration in the soil was very low and can be negligible), therefore only the submerged parts of the plant samples could contact with Pb^{2+}. The $TF_{Lp/Up}$ of 0.31 revealed that Pb^{2+} was able to be downward transported from the upper part of leaf to the lower part of leaf in *I. sinensis*. The $TF_{Rh/Lp}$ of 0.62 was the highest, and the $TF_{Ro/Rh}$ of 0.02 was the lowest, which means that the leads could easily transport from lower part of the leaves to the rhizome but difficultly transport from the rhizome to the root. In treatment III, only the roots contacted the leads in the solution, and both the $TF_{Rh/Ro}$ of 0.07 and $TF_{Lb/Rh}$ of 0.08 were so low, which means that the leads rarely transport from the roots to the rhizomes as well as from the rhizomes to the leaves. The fact that the content of leads was 129554.83 mg kg^{-1} in the roots, 2447 mg kg^{-1} in the rhizomes and 225.48 mg kg^{-1} in the leaves indicated that the leads mainly accumulated in the roots and the *I. sinensis* has weaker ability of transporting lead. In this study, we found that no matter which the treatments, the organs directly contacted with lead always have the highest content of lead.

The above data shows that roots, rhizomes and leaves can absorb Pb^{2+} and the Pb^{2+}can be transported both upward and downward the plant. Yet, the amount of transported lead from rhizome to root is the lowest.

This study found that Pb^{2+} could be transported in *I. sinensis* upward and downward, but the TF value indicate the ability of lead transfer was very weak, which means that the transport of lead within the plant is difficult (Xue *et al.*, 2000; Lu and Li, 2003). In this study, thus it mainly accumulated in the lower part of leaf if the plants grew in lead water (the treatment I), or mainly accumulated in the upper part of leaf if only upper part of leaf submerged in lead water (the treatment II), or mainly accumulated in the roots if only the roots grew in lead water (the treatment III). Similarly Yin also found that wheat leaves and roots were able to absorb Pb^{2+} and it could be transferred in plant, but only in a small extent (Yin *et al.*, 2010). Since Pb^{2+} is not an essential element required for plants growth, plants lack a Pb^{2+} transport mechanism, and Pb^{2+} has low mobility in the plant.

Conclusions

Isoetes sinensis is a lead hyperaccumulating plant. It can absorb Pb^{2+} through the root system or the leaves contacted lead, but transport Pb^{2+} weakly both upward and downward the plant. Its leaf has the strongest ability of absorption lead among organs. The submerged leaves appeared little wilting and chlorotic symptom under lead stress, especially new leaves can constantly regenerate.

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