

# Phytoremediation potential of *Pteris vittata* L. and *Eleusine indica* L. through field study and greenhouse experiments

*Triển vọng sử dụng thực vật Pteris vittata L. và Eleusine indica L. để xử lý ô nhiễm qua nghiên cứu tại hiện trường và trong phòng thí nghiệm*

Research article

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This study focused on determining Arsenic (As), Lead (Pb), Cadmium (Cd) and Zinc (Zn) concentrations of indigenous plants *Pteris vittata* L. and *Eleusine indica* L. in greenhouse experiment and some mining sites in Bac Kan province, Vietnam. The results showed that the soils of surveyed mining areas contained 378 – 6753 mgkg<sup>-1</sup> As, 3210 - 21312 mgkg<sup>-1</sup> Pb, 15.6- 312 mgkg<sup>-1</sup> Cd and 1280-25310 mgkg<sup>-1</sup> Zn depending on the characteristics of each mining site. In both greenhouse and field, *Pteris vittata* L. was As hyperaccumulators, containing more than 0.1% As in its shoots. *Eleusine indica* L. accumulate high concentrations of Pb (1234-4316 mgkg<sup>-1</sup>) and Zn (982-2352 mgkg<sup>-1</sup>) in their roots. None of two plant species had high Cd accumulating ability in the root and shoot. The amounts of heavy metals in two species under the field condition smaller than in the greenhouse experiment when they grew nearly in the same soil. Both two plants are good candidates for phytoremediation of these mining sites.

Nghiên cứu này nhằm đánh giá hàm lượng Asen (As), Cadimi (Cd), Chì (Pb) và Kẽm (Zn) của hai loài thực vật bản địa là *Pteris vittata* L. và *Eleusine indica* L. trong thí nghiệm nhà kính và tại một số điểm khai thác mỏ ở tỉnh Bắc Kạn, phía Bắc Việt Nam. Kết quả chỉ ra rằng, đất vùng mỏ khảo sát có chứa 378 – 6753 mgkg<sup>-1</sup> As, 3210 - 21312 mgkg<sup>-1</sup> Pb, 15,6- 312 mgkg<sup>-1</sup> Cd và 1280-25310 mgkg<sup>-1</sup> Zn phụ thuộc vào tính chất của từng điểm lấy mẫu. Trong cả thí nghiệm tại nhà kính và ngoài đồng ruộng, *Pteris vittata* L. đã được xác định là loài siêu tích lũy As, nó có chứa lượng As nhiều hơn 0.1% ở trong phần trên mặt đất của cây. *Eleusine indica* L. tích lũy cao lượng Pb (1234-4316 mgkg<sup>-1</sup>) và Zn (982-2352 mgkg<sup>-1</sup>) trong phần rễ cây. Hai loài thực vật nghiên cứu không có khả năng tích lũy cao lượng Cd ở phần thân và rễ cây. Hàm lượng kim loại nặng tích lũy trong thực vật nghiên cứu ở ngoài thực địa nhỏ hơn trong thí nghiệm khi cùng mọc trên một loại đất ô nhiễm như nhau. Cả hai loài thực vật nghiên cứu là đối tượng tốt cho xử lý ô nhiễm tại các điểm mỏ khảo sát.

**Keywords:** heavy metals; soil pollution; hyperaccumulator; phytoremediation

## 1. Introduction

Heavy metal contaminated soils at mining areas in Vietnam are a major ecological concern. Contaminated soils reduce the quality of both soil and ground water in many areas subjected to mining activities (Ha et al., 2011; Bui et al., 2011). There are many methods used for remediating this pollution but only phytoremediation is a cost effective, environmental friendly, aesthetically pleasing approach most suitable for developing countries like Vietnam (Baker et al., 1990; Baker et al., 1994; Chen et al., 2002; Bui et al.,

2011). It is important to use native plants for phytoremediation because such plants respond better to the stress conditions at the site than would plants introduced from other environments (Reeves et al., 2000; Ma et al., 2001; Ha et al., 2011; Bui et al., 2011).

Bac Kan has been identified as a province rich in Pb – Zn mines. Due to inadequate technology, e.g. sites lacking a treatment system or only having a preliminary treatment option, these sites have caused a serious environmental pollution problem, especially for soil and water (DARD, 2012; Ha et al., 2011).

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Studies of heavy metal contaminated soil are sparse in Vietnam, and have focused on traditional handicraft settlements and sites influenced by chemical and paint industries. The aims of the present study are to (1) determine the tolerance and accumulation of heavy metals in *P. vittata* L. and *Eleusine indica* L. growing near Pb – Zn mine sites, and (2) assess the feasibility of using these plant in the field through the experimental in laboratory.

## 2. Materials and Methods

### 2.1. Sampling site

The survey areas are located at Cho Don district, Bac Kan province in North of Vietnam (Fig. 1). It is in the monsoon tropical climate zone with two distinct seasons, the rainy season is from May to October, and the dry season is from November to April (DARD, 2012). Four Zn-Pb mining sites were selected for this study (Figure 1). A total of 40 plant samples (5 species per site  $\times$  2 species  $\times$  4 sites) and 40 soils samples were taken at the same place where the species were collected.

### 2.2. Experimental design

The plant species were collected from the mine sites. Three plants without shoot from each species were grown in plastic pots, contained 3 kg of soil. There were four treatments sets with three replicates for each. The tailings soil contained mean concentrations of As, Cd, Pb and Zn: 1420, 200, 14520 and 3267 mgkg<sup>-1</sup>, respectively. The experiments have four formulars: 25 % tailing soil + 75 % garden soil (F1), 50 % tailing soil + 50 % garden soil (F2), 75% tailing soil + 25 % garden soil (F3) and 100 % tailing soil (F4). The amount applied for N, P, and K nutrients, was 540, 180, and 360 mg, respectively in every plastic pot. The pots werekept moist by watering spray everyday. Sixteen weeks after the application, the greenhouse experiments

plants were harvested and then separated into shoot and root biomass. One sample of shoot and root of every replication from each treatment was put into plastic bags and immediately brought to the laboratory to prepare for analysis.

### 2.3. Soil and plant analysis

Approximately 100 g soil around the plant species were collected with a clean shovel, placed in plastic bags, and transported to the laboratory in Ha Noi. Soil samples were air-dried, crushed to pass through a 0.5 mm sieve and stored at 4°C in dark plastic bags until being analysed. Digestion of heavy metals was conducted using US EPA 3051 method (USEPA, 2007).

The plant samples were washed with tap water to remove soils and dust, rinsed with deionized water, and oven dried at 80°C for 2 days. Digestion of heavy metals in the plant samples was conducted following Bui et al., 2011.

The heavy metal concentration in soil and plant were measured by a Atomic Absorption Spectroscopy (AAS; AA-6800, Shimadzu, Japan). Reagent blanks with reference material standard from NIES CRM No. 1 (provided by the National Institute for Environmental Studies (NIES) of Japan) and SRM 1643e (provided by National Institute of Standards Technology, U.S.A) were used to verify the precision of the digestion procedure and the subsequent analysis.

### 2.4. Data analysis and statistical analysis

The Bioconcentration Factor (BF) of each plant species was determined by dividing the heavy metal content in plant shoots by the heavy metal concentration in the soil where the plant is growing. All the data in mean and standard deviation were performed using Microsoft excel for window program.

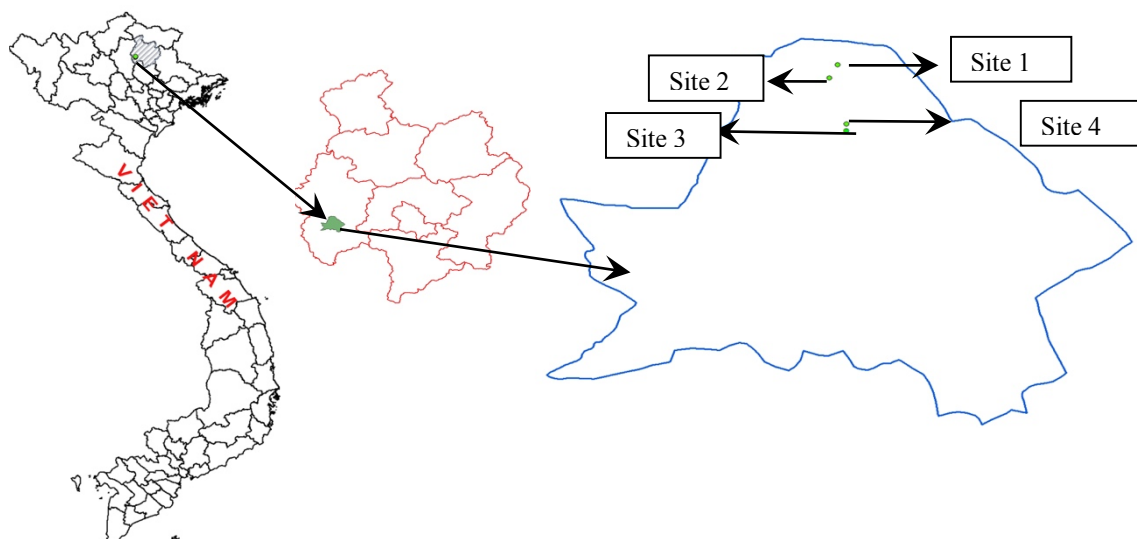


Figure 1. Map showing the location of the sampling sites

## 3. Results and Discussion

### 3.1. Heavy metal concentration in plants and soil of mine sites

A total of 20 different plants per each species were collected from four mining sites to identify the heavy metal concentration in their roots and shoots. All results were presented in Table 1 are average of 5 replications and all results are presented as a mean values  $\pm$  standard deviation. The results showed that two species *Pteris vittata* L. and

*Eleusine indica* L. can tolerate with very high heavy metal concentration in the soil. As concentration in shoot of *Pteris vittata* L. were  $1231 \pm 214$  -  $4539 \pm 213$   $\text{mg kg}^{-1}$ , indicating this plant is Asenic hyperaccumulator. Hyperaccumulators are defined as plants with leaves able to accumulate at least  $100 \text{ mg kg}^{-1}$  of Cd;  $1000 \text{ mg kg}^{-1}$  of As, Cu, Pb, Ni, Co, Se, or Cr; or  $10,000 \text{ mg kg}^{-1}$  of Mn or Zn (dry weight) when grown in a metal-rich environment [Tu et

al., 2002; Chao et al., 2006; Ha et al. 2011]. *Eleusine indica* L. can accumulate high concentration of Pb ( $1234 \pm 112$  -  $4316 \pm 321$   $\text{mg kg}^{-1}$ ) and Zn ( $982 \pm 67$  -  $2352 \pm 216$   $\text{mg kg}^{-1}$ ) in its root. The results in Table 1 showed that none of two plant species had high Cd accumulating ability in the root and shoot. The results demonstrated that *Pteris vittata* L and *Eleusine indica* L. could use for phytoremediate As, Pb and Zn in Bac Kan province.

**Table 1. Heavy metal concentration in the plants and soil at difference sites**

Mining sites	Elements	Heavy metal concentration (mgkg <sup>-1</sup> )				Soil
		Plant species				
		<i>Pteris vittata</i>		<i>Eleusine indica</i>		
		Root	Shoot	Root	Shoot	
Site 1	As	982±21	3796±231	728±57	547±16	4567± 132
	Cd	10.2±2.2	2.7±1.2	14.5±2.8	23.7±7.6	312±26
	Pb	1231±87	312±21	3215±137	671± 23	13450±935
	Zn	1034±52	215±13	2352±216	723± 71	25310±1126
Site 2	As	592±23	2467±134	567±42	642±54	1345±29
	Cd	11.3±1.4	4.8±1.2	20.5±2.9	13.4±2.6	110±17
	Pb	1165±234	278±31	4316±321	572± 32	21312±1324
	Zn	823±85	187±15	1320±78	567±41	4210±139
Site 3	As	324±31	1231±214	28±3	137±12	378±43
	Cd	2.5±0.9	2.6±0.7	6.7±2.1	6.8±3.1	32±3
	Pb	987±23	345±19	1234±112	584±26	3210±124
	Zn	421±35	146±12	982±67	326±15	1280±145
Site 4	As	726±123	4539±213	325±26	120±24	6753±126
	Cd	3.3±0.9	7.1±1.2	5.6±1.1	8.1±2.3	15.6±5.6
	Pb	1015±123	512±32	1674±123	726±34	4657±79
	Zn	561±56	237±41	1572±215	628±32	5124±117

Parallel to analysing heavy metal contents in shoots and roots of two plant species samples, heavy metal concentrations of soil samples around these plants were also analyzed. The relationship between the content of a heavy metal in soil near the plant growing showed in table 1. The result showed that the highest concentrations ( $\text{mg kg}^{-1}$ ) of As, Cd, Pb and Zn in the mining soil were  $6753 \pm 126$  (Site 4),  $312 \pm 26$  (Site 1),  $21312 \pm 1324$  (Site 2) and  $25310 \pm 1126$  (Site 1), respectively. All the soil samples collected from the mine sites exceeded Vietnamese standard limits for industrial soil many times. Normally, the plant species grows in soil contains high heavy metals concentration, they will uptake high heavy metals. In this study, metal concentrations in the plants were poorly correlated with total metal concentrations in the soil. This result was similar to other researches, they demonstrated that total metal concentrations are considered to be poor

indicators of metal availability to plants (Ha et al. 2011; Bui et al., 2013).

### 3.2. Heavy metal distribution in two plant species after the experiments

Heavy metal contents in frond and root of *Pteris vittata* L and *Eleusine indica* L. depend on each formular (Table 2 & 3). After 16 weeks experiment, *Pteris vittata* can accumulate As, Cd, Pb and Zn were  $1953 \pm 78$  -  $4789 \pm 67$   $\text{mg kg}^{-1}$ ,  $13.7 \pm 4.5$  -  $35.2 \pm 7.2$   $\text{mg kg}^{-1}$ ,  $23.7 \pm 5.6$  -  $402.3 \pm 23$   $\text{mg kg}^{-1}$  and  $567.2 \pm 45.3$  -  $810.2 \pm 97.2$   $\text{mg kg}^{-1}$  respectively, for the plant shoots; and  $673.4 \pm 67.1$  -  $818.9 \pm 37.6$   $\text{mg kg}^{-1}$ ,  $34.5 \pm 2.4$  -  $62.3 \pm 4.5$   $\text{mg kg}^{-1}$ ,  $712.1 \pm 9.5$  -  $1259.4 \pm 9.7$   $\text{mg kg}^{-1}$  and  $673.1 \pm 13.4$  -  $1298.8 \pm 36.4$   $\text{mg kg}^{-1}$ , alternatively, for the plant root.

**Table 2. Heavy metals concentration in *Pteris vittata* L. at difference formular**

Formular	Shoot (mg kg <sup>-1</sup> )				Root (mg kg <sup>-1</sup> )			
	As	Cd	Pb	Zn	As	Cd	Pb	Zn
<b>F1</b>	1953±78	13.7±4.5	23.7±5.6	567.2±45.3	673.4±67.1	34.5±2.4	712.1±9.5	673.1±13.4
<b>F2</b>	2895±85	24.1±4.9	52.6±8.7	658.9±67.8	698.3±48.2	42.7±5.2	878.7±8.6	732.5±25.8
<b>F3</b>	3350±46	35.2±7.2	402.3±23	720.9±120.3	716.5±34.5	52.1±6.1	970.6±8.3	820.6±28.7
<b>F4</b>	4789±67	30.5±8.6	398.4±16.2	810.2±97.2	818.9±37.6	62.3±4.5	1259.4±9.7	1298.8±36.4

**Table 3. Heavy metals concentration in *Eleusine indica* L. at difference formular**

Formular	Shoot (mg kg <sup>-1</sup> )				Root (mg kg <sup>-1</sup> )			
	As	Cd	Pb	Zn	As	Cd	Pb	Zn
<b>F1</b>	251±24	14.7±2.5	51.5±5.9	720.5±24.1	356.7±25.2	124.3±23.1	612.2±34.9	787.2±56.3
<b>F2</b>	345±38	28.5±5.7	67.3±6.7	789.7±45.2	579.2±38.6	135.5±26.4	928.5±29.8	1293.4±87.4
<b>F3</b>	413±31	34.7±7.9	152.6±8.3	880.6±54.7	615.8±32.8	147.9±22.8	1131.4±42.5	1898.6±79.2
<b>F4</b>	499±42	41.2±6.2	191.3±9.5	1010.3±67.2	791.8±33.7	160.7±31.5	1271.5±47.3	2798.2±92.1

*Eleusine indica* L. can accumulate As, Cd, Pb and Zn in the range of 251±24 - 499±42 mgkg<sup>-1</sup>, 14.7±2.5 - 41.2±6.2 mgkg<sup>-1</sup>, 51.5±5.9 - 191.3±9.5 mgkg<sup>-1</sup> and 720.5±24.1 - 1010.3±67.2 mgkg<sup>-1</sup> respectively, for the plant shoot and were 356.7±25.2 - 791.8±33.7 mgkg<sup>-1</sup>, 124.3±23.1 - 160.7±31.5 mgkg<sup>-1</sup>, 612.2±34.9 - 1271.5±47.3 mgkg<sup>-1</sup> and 787.2±56.3 - 2798.2±92.1 mgkg<sup>-1</sup>, alternatively, for the plant root.

At F4 (100 % tailing soil), the two plants accumulated highest heavy metal concentration in the root and shoot. Heavy metal concentrations in the plants at different formulas are in the order: F4> F3> F2> F1. The correlation between heavy metals uptaken in plants and the heavy metal concentration in the soil before the experiment was directly proportional.

However, the heavy metals concentration in the soil and plant have relatively correlation. In the actual, heavy metal concentration taken up by plants showing that the metal extraction from soil, possibly, not only depends on heavy metal accumulation in plant tissue but also influence by other processes, such as interaction with microorganisms in the rhizosphere or biovolatilisation of arsenic (Ronit Jakob, 2010).

*Pteris vittata* L. and *Eleusine indica* L. accumulate high concentration of heavy metal under green house and field conditions. However, the amounts of heavy metals in the frond and root of two species were smaller under field condition. For example, at site 2, heavy metal concentrations in the soil were close with the formular (F4), concentrations of As, Cd, Pb and Zn in the *Pteris vittata* root were 592, 11.3, 1165 and 823 mgkg<sup>-1</sup>, respectively in the field (site 2) and were 818.9, 62.3, 1259.4 and 1298.8 mgkg<sup>-1</sup>, alternatively, in the green experiment (F4). Concentrations of As, Cd, Pb and Zn in the *Pteris vittata* shoot were 2467, 4.8, 278 and 187 mgkg<sup>-1</sup>, respectively in the field (site 2)

and were 4789, 30.5, 398.4 and 810.2 mgkg<sup>-1</sup>, alternatively, in the green experiment (F4). So, the heavy metal accumulation in the plant may be due to difference parameters (soil properties, the presence of other metals, exposure time, plant age,...)

## 4. Conclusion

Both plant *Pteris vittata* L. and *Eleusine indica* L. are good candidates for phytoremediation of the mining sites contaminated with As, Pb and Zn in Bac Kan province. *Pteris vittata* is As hyperaccumulator. *Eleusine indica* L. is the best at keeping Pb, Zn concentration in its root. The studies in greenhouse experiment showed more phytoremediation potential of these plant species. The amounts of heavy metals in the shoot and root of two species under field condition were smaller indicated that agronomic requirements and optimal management practices are very important.

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