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Heavy Metal Accumulation in Soil and Some Fern Species at Phu Soi Dao National Park, Phitsanulok Province, Thailand.

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ABSTRACT

In Southeast Asia, Thailand is natural endowment of biodiversity in the forests. The varieties of genes, species, and ecosystems, such as ferns, are rich and naturally abundant. Therefore, some fern species have a good possibility for using in phytoremediation process. The objectives of this research were to determine the accumulation of heavy metals of some fern species and to study the ecological effects on heavy metals absorption. The study area was conducted at Phu Soi Dao National Park, Phitsanulok province, Thailand. The soil and fern samples were taken in December 2005 using line transect method along the pathway and applied square plots size of 1×1 m^2 for the sampling process. Soil properties and element concentrations were analysed and heavy metal accumulation in ferns were calculated. It was observed that the ferns had higher potentials of absorbing heavy metals than in the soil. From 19 terrestrial fern species, some fern species had high heavy metal accumulation due to the ecological effect. *Adiantum philippense* L. showed significantly highest levels of Pb and Ni concentration in their leaves, while *Adiantum caudatum* L. was the best Pb, Ni and Co absorption The result from this study can play an important role in bioremediation process to mitigate concentration of heavy metals from the environment.

Key words: Fern, Heavy metal, Phu Soi Dao, Thailand

INTRODUCTION

Thailand has a rich plant biodiversity, and hence it has good potential to provide suitable species for phytoremediation which is a promising new method. Phytoremediation uses green plants to assimilate or detoxify metals and organic compounds. The term was first coined in 1991 to describe the use of plants to accumulate metals from soil and groundwater (Licht *et al.*, 1995). The large scale phytoremediation of metal pollutants from soils require plant species that have high biomass, rapidly growing and accumulate metals. Plants that accumulate metals to high concentrations are sometimes referred as “hyperaccumulators” (Visoottiviseth *et al.*, 2002). Hyperaccumulation of heavy metals or metalloids is a rare phenomenon in terrestrial higher plants. To date, some 400 taxa of hyperaccumulator species have been identified (Zhao *et al.*, 2002). The distribution of heavy metal in soils varies with the parent materials from which the soil is derived. However, growing under exactly the same natural conditions each plant species displayed quite unique uptake characteristics (Reimann *et al.*, 2007). Although microorganisms have also been tested for remediation potential, plant have shown the greater ability to withstand and accumulate high concentrations of toxic metals and chemical substances. A wide range of plant species has been identified as being arsenic resistant. Many researches reported that ferns can highly absorb toxic and carcinogenic substances, heavy metals, from contaminated soils, that opened up the possibility for its use for remediation of soils. Scientists in the USA found that the fern (*Pteris vittata*), known as brake fern, can absorb arsenic from soils and translocate arsenic to its parts above the ground including leaves (fronds). This study demonstrated for other fern species which are hyperaccumulated other heavy metals.

METHODOLOGY

A. Soil sampling and analysis

The study was carried out in the Phu Soi Dao National Park, Phitsanulok Province, Thailand, located at 600-1,633 m.a.s.l. (17°41'-18°04' N and 100°56'-101°09' E) (Figure 1). Samples of soil were collected in December 2005, having 193 plots, each 1m wide by 1m long. Soil samples (0-15 cm, approx. 1000 g) were taken by using line transects method along the pathway. The soil was air dried for a week and then it was sieved (2-mm mesh) to remove stones and plant materials. After that soil chemical properties such as soil pH, organic matter (%OM), cation exchange capacity (CEC) were measured. Soil pH was determined with glass electrode in a 1: 5 soil water ratio (Rayment and Higginson, 1992). Organic matter was analysed according to the Walkley and Black method (Nelson and Sommers, 1996) by wet ashing with a $K_2Cr_2O_7$-H_2SO_4 solution and titrated with $FeSO_4$. Cation exchange capacity was analysed by ammonium saturation method (Kimble *et al.*, 1993). Heavy metals, Pb, Ni and Co were determined using DTPA 0.005 M extracting solution with a soil:extractant ratio of 1:5 (Norvell, 1984). All metals were analysed by flame atomic absorption spectrophotometry (FAAS model GBC Avanta PM 05593).

B. Plant sampling and analysis

The plant samples were collected from the same location as the soil samples. Some plant samples were kept in a plant press, and preserved by drying at 50-55°C for 4 days by making them into a dried form (Herbarium) in order to further classify

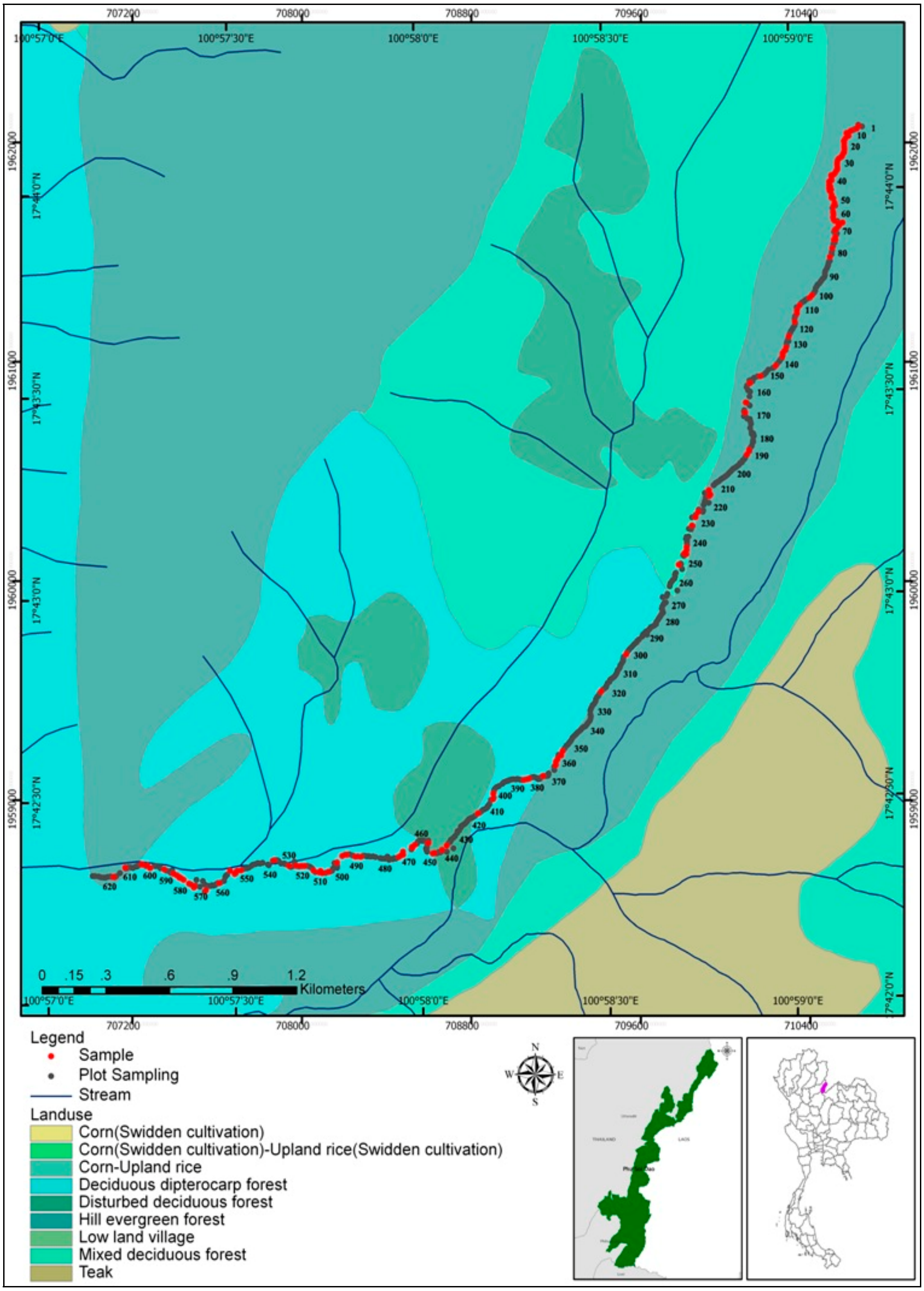

Figure 1 Topographic map of Phu Soi Dao National Park showing the location and plot sampling area. (Map ratio 1 : 20,000)

the fern types. Pteridophyte specimens were identified using keys and descriptions from taxonomic literature, such as flora, manual, monograph, as well as research papers. Botanical names of each specimen were verified by comparison to the voucher herbarium specimens deposited at the Forest Herbarium, Royal Forest Department (BKF) and the Herbarium of Queen Sirikit Botanic Garden (QSBG). After collection, each plant sample was washed with tap water, rinsed and cleaned with deionised water, then separated to part of leaves. The samples were dried at 70°C for 48 hours. The dried samples were then ground to a powder by mortar for heavy metal determination.

C. Determination of heavy metal in leaves

Plant samples were prepared for analysis using the digestion method (Zhao *et al*., 1994 ; Simmons *et al*., 2004). A portion (~ 0.2 – 0.5 g) of the dried powder was accurately weighed (± 0.001g) directly to a flask and mixed with 15 ml of mixed acid (HNO_3 and $HClO_4$, 80:20, v : v). The samples were digested at 120 °C on a hot plate. When digestion was completed, samples were removed and diluted to 50 ml by using deionised water and filtrated. This solution was analysed for Lead (Pb), Nickel (Ni) and Cobalt (Co) using frame atomic absorption spectrophotometry (FAAS, GBC Avanta PM 05593). Acid blanks were also analysed in order to possible contamination. The FAAS analyses were done at Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. Heavy metal concentrations in the samples were reported on a dried mass basis.

RESULTS AND DISCUSSION

Specific distribution

The specific distribution of fern is illustrated in Figure 2 and Table 1. The species *Blechnum orientale* L., *Pteridium aquillium*, *Lindsaea ensifolia* Sw., *Pteris ensiformis* Burm.f., *Pteris biaurita* L., *Thelypteris Terminans*, *Thelypteris interrupta* and *Adiantum philippense* L. were found in elevation between 1201 – 1600 m.a.s.l. The species found in elevation between 801 – 1200 m.a.s.l. were *Pteris venusta* Kunze., *Lygodium* sp., *Tectaria impressa*. and *Tectaria angulata*. Other fern species that found in elevation between 400 – 800 m.a.s.l. were *Colysis pothifolia, Tectaria polymorpha*, *Tectaria herpetocaulos* Holtt., *Diplazium esculentum*, *Adiantum caudatum* L., *Thelypteris nudata* and *Angiopteris evecta*. The species *Adiantum philippense* L., *Thelypteris interrupta, Thelypteris Terminans* and *Pteris biaurita* L. were found both upper and lower m.a.s.l. levels.

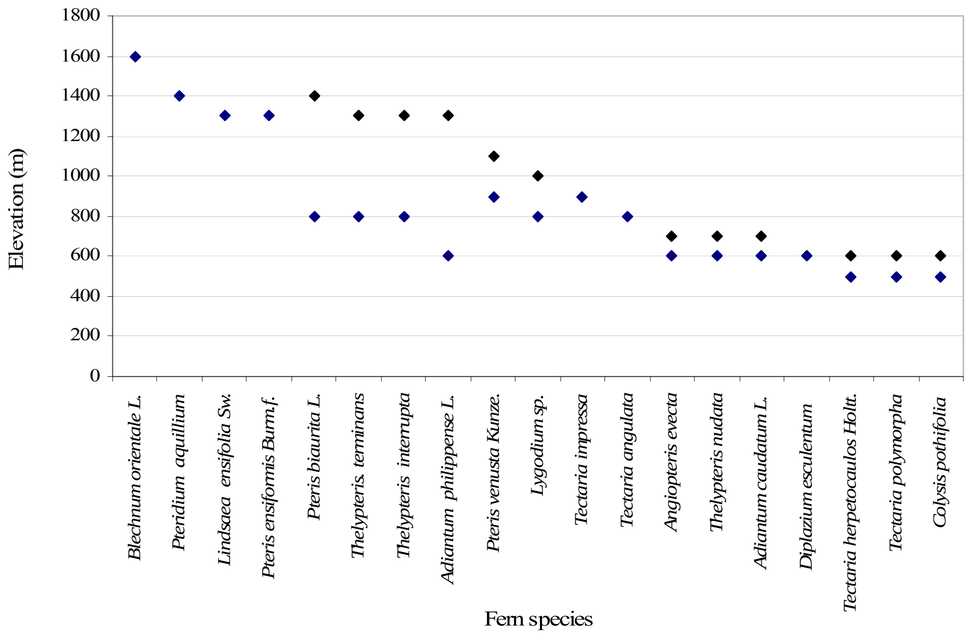

Figure 2 Specific distribution of elevation in each fern specie.

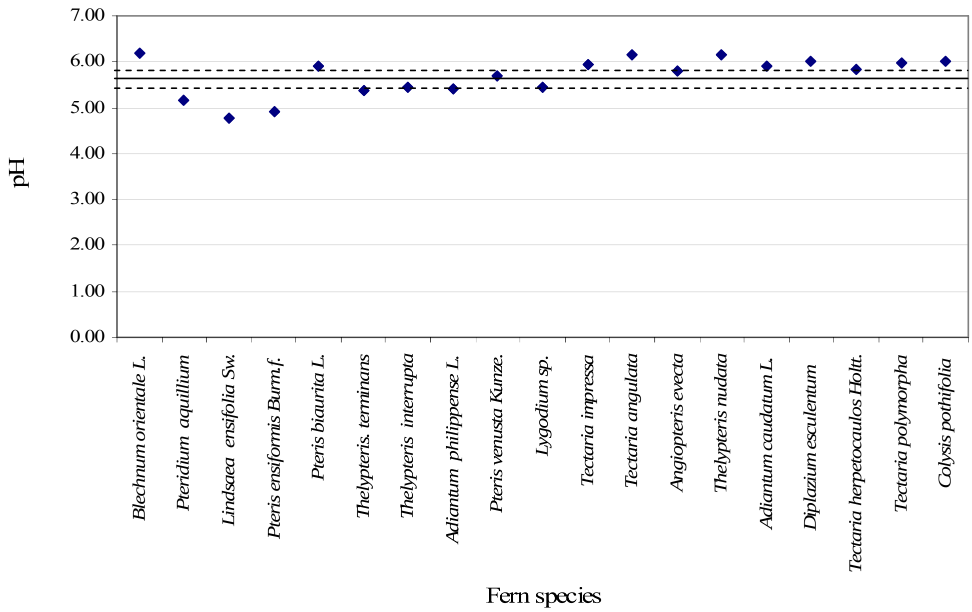

Figure 3 Specific distribution of pH in soil. Solid line indicates the mean value of all species and dot line shows the significant range with a critical value of $\alpha = 0.01\%$

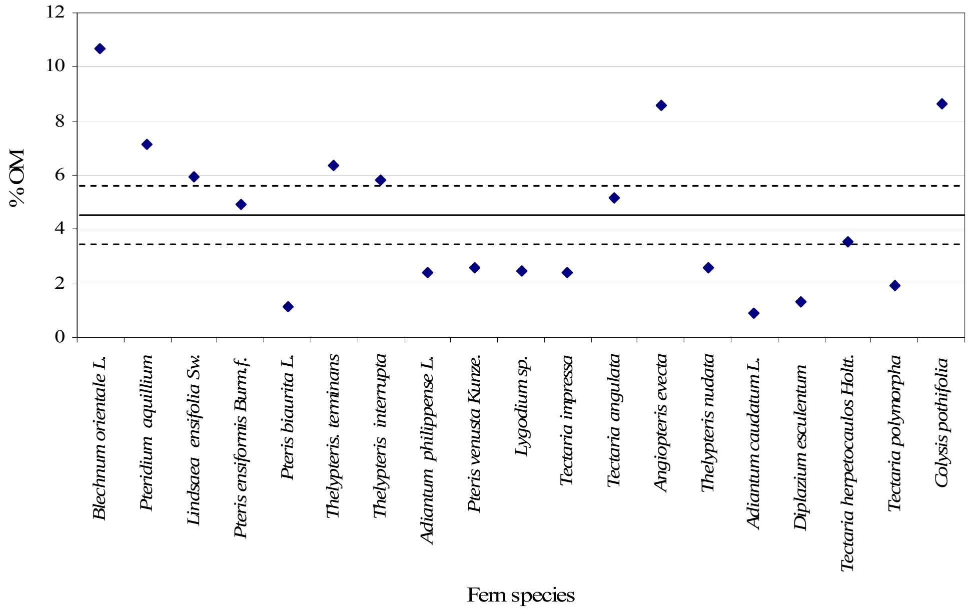

Figure 4 Specific distribution of OM in soil. Solid line indicates the mean value of all species and dot line shows the significant range with a critical value of α = 0.01%

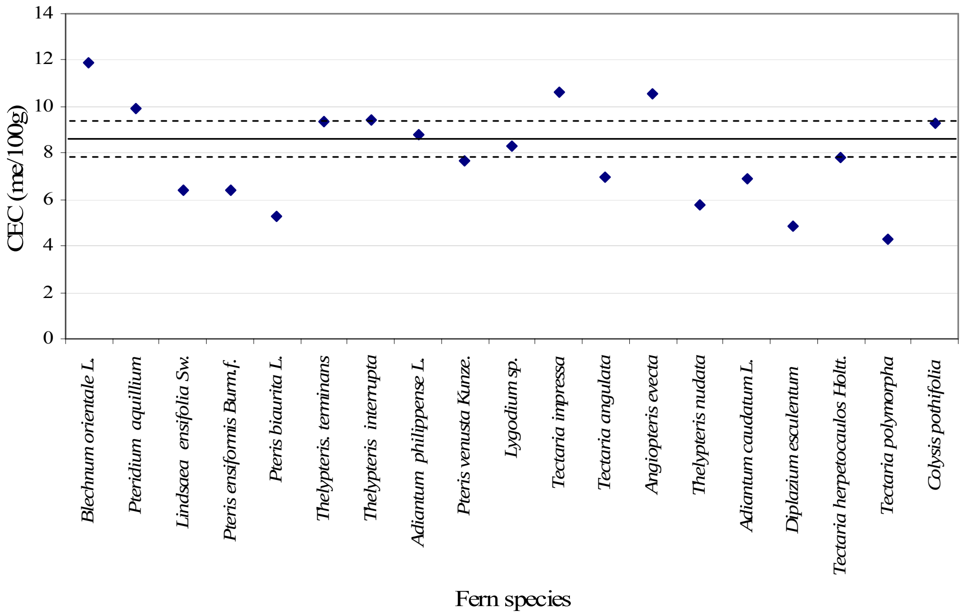

Figure 5 Specific distribution of CEC in soil. Solid line indicates the mean value of all species and dot line shows the significant range with a critical value of α = 0.01%

Table 1 Elevation and soil properties (pH, %OM and CEC) of some fern species.

Family	Species	Elevation (m)	pH	OM (%)	CEC me/100g
BLECHNACEAE	*Blechnum orientale* L.	1600	6.20 ± 0.28	10.70 ± 1.19	11.91 ± 1.36
DENNSTAEDTIACEAE	*Pteridium aquillium*	1400	5.15 ± 0.47	7.15 ± 4.85	9.89 ± 2.51
LINDSAEACEAE	*Lindsaea ensifolia* Sw.	1300	4.78 ± 0.09	5.91 ± 3.73	6.37 ± 1.33
PTERIDACEAE	*Pteris ensiformis* Burm.f.	1300	4.91 ± 0.10	4..93 ± 2.35	6.79 ± 1.93
	Pteris biaurita L.	800 - 1400	5.91 ± 0.13	1.17 ± 0.23	5.26 ± 0.55
	Pteris venusta Kunze.	900 - 1100	5.68 ± 0.38	2.61 ± 1.21	7.67 ± 3.11
SCHIZAEACEAE	*Lygodium* sp.	800 - 1000	5.46 ± 0.43	2.45 ± 1.08	8.32 ± 0.83
DRYOPTERIDACEAE	*Tectaria impressa*	900	5.94 ± 0.60	2.42 ± 1.02	10.61 ± 0.92
	Tectaria angulata	800	6.15 ± 0.19	5.18 ± 3.48	6.97 ± 0.63
	Tectaria herpetocaulos Holtt.	500 - 600	5.83 ± 0.37	3.54 ± 2.91	7.79 ± 2.64
	Tectaria polymorpha	500 - 600	5.97 ± 0.74	1.92 ± 0.03	4.31 ± 1.26
THELYPTERIDACEAE	*Thelypteris. terminans*	800 - 1300	5.39 ± 0.50	6.36 ± 4.46	9.34 ± 1.53
	Thelypteris interrupta	800 - 1300	5.46 ± 0.44	5.85 ± 4.32	9.44 ± 1.57
	Thelypteris nudata	600 - 700	6.14 ± 0.23	2.59 ± 1.01	5.80 ± 2.83
PARKERIACEAE	*Adiantum philippense* L.	600 -1300	5.41 ± 0.68	2.37 ± 1.51	8.77 ± 2.46
	Adiantum caudatum L.	600 - 700	5.92 ± 0.53	0.89 ± 0.67	6.91 ± 3.63
MARATTIACEAE	*Angiopteris evecta*	600 - 700	5.80 ± 0.38	8.57 ± 5.05	10.55 ± 1.60
ATHYRIACEAE	*Diplazium esculentum*	600	6.02 ± 0.03	1.29 ± 0.40	7.10 ± 3.16
POLYPODIACEAE	*Colysis pothifolia*	500 - 600	6.02 ± 0.03	8.65 ± 5.15	9.30 ± 3.69

Values of pH OM and CEC are reported in means ± SD

Soil properties

Geology is another important factor that influence element concentrations in the soil. The soil properties, pH, %OM and CEC, all played a role for the observed metal concentrations in leaves. The overall means of pH, %OM and CEC were calculated. In order to examine the deviation of any particular species from overall means, the critical intervals of student-t distribution were applied to examine the significance of deviation. Analytical results are summarized in Table 1 and distribution profiles were drawn as shown in Figures 3, 4 and 5.

pH

All soils contain acidic values in the ranged of 4.78 – 6.20. *Lindsaea ensifolia* Sw. were found lowest pH value (Table 1). *Blechnum orientale* L. was found the highest value of pH (Table 1). *Lindsaea ensifolia* Sw. and *Pteris ensiformis* Burm.f. showed significantly lowest levels of pH value in their soil at $p \leq 0.05$. *Diplazium esculentum*,*Colysis pothifolia*,*Thelypteris nudata*, *Tectaria angulata* and *Blechnum orientale* L. showed significantly highest levels of pH value in their soil at $p \leq 0.05$ (Figure 3).

OM

The organic matter are in the ranged of 0.89 – 10.70. *Blechnum orientale* L. was found the highest organic matter content (Table 1) and it showed significantly highest organic matter content in their soil at $p \leq 0.05$ (Figure 4). *Adiantum caudatum* L. was found the lowest of organic matter content (Table 1). However, the fern such as *Adiantum caudatum* L.,*Diplazium esculentum*,*Pteris biaurita* L., *Tectaria polymorpha*, *Adiantum philippense* L., *Tectaria impressa*, *Lygodium* sp., *Thelypteris nudata* and *Pteris venusta* Kunze. showed significantly low levels of organic content in their soil at $p \leq 0.05$.

CEC

The cation exchange capacity is in the ranged of 4.31 – 11.91 me/100g (Table 1). The lowest of cation exchange capacity was found in *Tectaria polymorpha* and it showed significantly lowest cation exchange capacity in their soil at $p \leq 0.05$ *Blechnum orientale* L. was found the highest value of cation exchange capacity and it showed significantly highest cation exchange capacity in their soil at $p \leq 0.05$ (Figure 5).

Heavy metal concentrations in soil and leaves of fern

Leaves are the physiologically active organs of a plant converting the inorganic matters into coordination compounds. Total samples of leaves were collected from terrestrial fern over 5 cm in height. Sampling ferns were identified as 19 species, 11 genera and 11 families. The overall means of the concentrations of metal (Pb, Ni and Co) were calculated. The means of particular species for the leaves were compared with the overall means in order to examine the deviation of any particular species. When the value of any particular species was found greatly deviated from the mean, the critical intervals of T-test distribution and DMRT were applied to examine the significance of deviation. When the concentration of certain elements was significantly high in certain species, the species was regarded as accumulator of the element (Pampasit, 1998). The mean of the concentration for heavy metals as Pb, Ni and Co in soil and leaves of some fern species are also given in Table 2. Some value showed the concentrations in leaves and soil along the transect reveals the high standard deviation, this reason displays by the location of the plot sampling was collected in different lithologies, typical of the greater area and different soil horizons along transect. Figure 6 is drawn by plotting the heavy metal concentration in leaves of different fern species.

Table 2 The mean heavy metal concentrations (Pb, Ni and Co) in soil and leaves of some fern species.

Family	Species	Pb (mg/kg)		Ni (mg/kg)		Co (mg/kg)	
		soil	leaves	soil	leaves	soil	leaves
BLECHNACEAE	*Blechnum orientale* L.	2.641 ± 0.30	13.586 ± 3.21	0.851 ± 0.33	5.403 ± 1.30	1.724 ± 0.57	ND
DENNSTAEDTIACEAE	*Pteridium aquillium*	15.617 ± 5.13	7.964 ± 4.00	1.169 ± 0.46	14.565 ± 3.79	1.273 ± 0.66	0.637 ± 0.37
LINDSAEACEAE	*Lindsaea ensifolia* Sw.	**22.343 ± 0.00**	38.581 ± 26.78	**3.632 ± 0.00**	23.470 ± 21.30	0.826 ± 0.00	1.214 ± 1.30
PTERIDACEAE	*Pteris ensiformis* Burm.f.	20.635 ± 2.42	30.370 ± 9.90	2.464 ± 1.65	8.550 ± 1.86	1.135 ± 0.44	ND
	Pteris biaurita L.	10.903 ± 2.91	19.966 ± 13.89	1.309 ± 1.13	10.440 ± 1.90	0.886 ± 0.05	0.682 ± 0.55
	Pteris venusta Kunze.	9.818 ± 6.11	5.729 ± 3.68	1.583 ± 0.48	15.652 ± 11.46	1.233 ± 0.70	0.219 ± 0.13
SCHIZAEACEAE	*Lygodium* sp.	12.633 ± 1.31	6.552 ± 2.64	1.457 ± 0.23	7.694 ± 2.11	1.574 ± 0.99	0.156 ± 0.13
DRYOPTERIDACEAE	*Tectaria impressa*	10.628 ± 1.75	8.245 ± 1.46	0.989 ± 0.09	11.955 ± 3.42	0.545 ± 0.50	0.835 ± 0.41
	Tectaria angulata	11.899 ± 0.00	46.186 ± 44.83	2.470 ± 0.69	9.900 ± 9.12	**2.409 ± 1.21**	ND
	Tectaria herpetocaulos Holtt.	6.010 ± 3.18	9.352 ± 3.52	1.491 ± 0.90	10.514 ± 2.53	1.300 ± 0.50	0.943 ± 0.31
	Tectaria polymorpha	3.159 ± 4.47	18.254 ± 3.48	1.479 ± 2.09	6.022 ± 1.29	0.875 ± 0.14	ND
THELYPTERIDACEAE	*Thelypteris. terminans*	18.417 ± 2.13	17.112 ± 3.78	1.543 ± 0.37	5.477 ± 1.70	0.951 ± 0.65	ND
	Thelypteris interrupta	18.081 ± 2.24	10.571 ± 3.73	1.577 ± 0.40	5.697 ± 1.54	1.125 ± 0.59	ND
	Thelypteris nudata	6.535 ± 1.54	4.447 ± 3.21	1.476 ± 0.47	18.564 ± 2.59	1.235 ± 0.73	ND
PARKERIACEAE	*Adiantum philippense* L.	9.439 ± 7.16	**90.786 ± 67.33**	2.023 ± 0.41	**28.892±22.27**	1.045 ± 0.50	ND
	Adiantum caudatum L.	1.822 ± 0.03	22.928 ± 16.74	0.868 ± 1.23	23.516 ± 15.54	1.144 ± 0.12	**1.971 ± 0.45**
MARATTIACEAE	*Angiopteris evecta*	16.047 ± 3.72	5.857 ± 2.23	1.499 ± 0.28	7.591 ± 3.43	1.300 ± 0.07	0.322 ± 0.08
ATHYRIACEAE	*Diplazium esculentum*	11.285 ± 3.45	15.863 ± 2.53	1.192 ± 0.96	3.742 ± 0.32	1.168 ± 0.35	ND
POLYPODIACEAE	*Colysis pothifolia*	1.604 ± 2.27	12.645 ± 6.01	1.292 ± 1.83	13.532 ± 2.33	0.575 ± 0.55	0.745 ± 0.17

ND : not detected, the lower limit of detection was 0.0044 ppm
Values are reported in means ± SD

Pb

Lead concentrations in soil of all species showed a weak positive correlation with OM and CEC (r = 0.107 and 0.173). *Lindsaea ensifolia* Sw. showed highest levels of Pb concentration in soil.The heavy metal concentration of some fern species found that Pb has the concentration in leaves ranged from 0.96 – 138.39 mg/kg. The specific distribution profile of Pb concentration in leaves is given in Figure 6. *Adiantum caudatum* L., *Adiantum philippense* L., *Lindsaea ensifolia* Sw., *Pteris ensiformis* Burm.f. and *Tectaria angulata* showed significantly higher concentration than the mean value. The fern species revealed significantly lower Pb concentration in such species as *Angiopteris evecta*, *Blechnum orientale* L., *Colysis pothifolia*, *Diplazium esculentum*, *Lygodium* sp., *Pteridium aquillium*, *Pteris venusta* Kunze., *Tectaria herpetocaulos* Holtt., *Tectaria impressa*, *Thelypteris interrupta*, *Thelypteris nudata* and *Thelypteris terminans*. The fern species *Adiantum philippense* L. showed significantly highest levels of Pb concentration in their leaves (90.79 mg/kg) at $p \leq 0.01$.

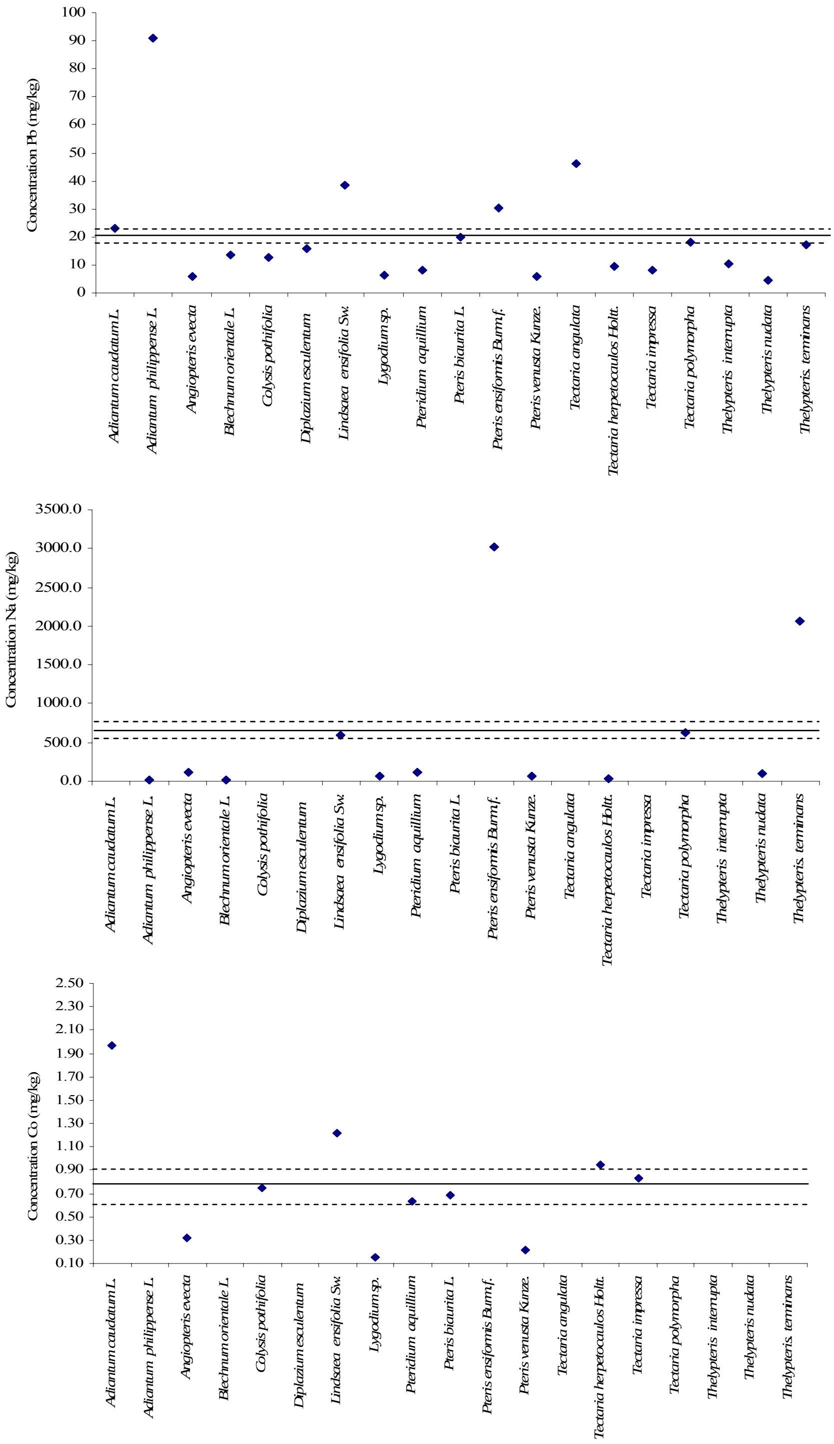

Figure 6 Specific distribution of Pb Ni and Co in leaves. Solid line indicates the mean value of all species and dot line shows the significant range with a critical value of α = 0.01%

Ni

Nickel concentrations in soil of all species showed a weak negative correlation with OM (r = -0.059). *Lindsaea ensifolia* Sw. showed highest levels of Ni concentration in soil. Ni concentration in leaves of fern ranged from 3.06 – 56.17 mg/kg, *Adiantum caudatum* L., *Adiantum philippense* L., *Lindsaea ensifolia* Sw., *Pteridium aquillium*, *Pteris venusta* Kunze. and *Thelypteris nudata* revealed significantly higher Ni concentration**.** *Adiantum philippense* L. showed significantly highest accumulated Ni. On the contrary *Angiopteris evecta*, *Blechnum orientale* L., *Diplazium esculentum*, *Lygodium* sp., *Pteris biaurita* L., *Pteris ensiformis* Burm.f., *Tectaria angulata*, *Tectaria polymorpha*, *Thelypteris interrupta* and *Thelypteris terminans* showed significantly lower concentration than the mean value in their leaves at $p \leq 0.01$ as shown in Figure 6.

Co

Cobalt concentrations in soil of all species showed a weak positive correlation with OM and CEC (r = 0.149 and 0.118). All fern species showed non significantly of Co concentration in soil. The concentration of Co in leaves of fern ranged from 0.10 – 2.32 mg/kg. The specific distribution profile of Co in leaves is illustrated in Figure 6. The fern species *Adiantum caudatum* L., *Lindsaea ensifolia* Sw. and *Tectaria herpetocaulos* Holtt. showed significantly higher concentration than the mean value. *Adiantum caudatum* L. showed significantly highest accumulated Co at $p \leq 0.01$ and the mean accumulate Co was about 1.97 mg/kg. Fern species that showed significant lower concentrations than the mean value were *Angiopteris evecta*, *Lygodium* sp. and *Pteris venusta* Kunze. Other fern species such as *Blechnum orientale* L., *Pteris ensiformis* Burm.f., *Tectaria angulata*, *Tectaria polymorpha*, *Thelypteris nudata*, *Thelypteris terminans*, *Thelypteris interrupta*, *Adiantum philippense* L. and *Diplazium esculentum* were not accumulate Co.

Absorption coefficients

The absorption coefficients of different fern species in their leaves and soil were also calculated. For an element, the absorption coefficient is defined as concentration in plant leaves to be divided by concentration in host soil (Zhenggui *et al*., 2001). Figure 7 is drawn by plotting the absorption coefficient of different fern species.

Absorption coefficients = concentration in plant leaves/ concentration in host soil

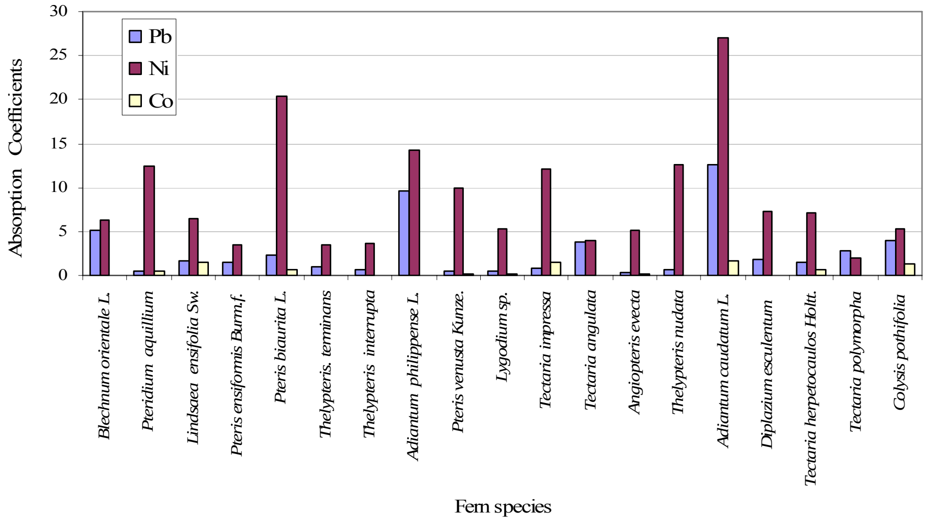

Figure 7 Absorption coefficients of Pb, Ni and Co in each fern species.

Each fern species has different level of heavy metal absorption, depending on area base, soil fertility and forest type. Although *Adiantum philippense* L. showed significantly highest levels of Pb concentration in their leaves, In the contrary fern species which has highest Pb absorption coefficients was *Adiantum caudatum* L.(12.58). The *Adiantum philippense* L. showed significantly higher Ni concentration in leaves. However, fern species which have higher Ni absorption coefficients were *Adiantum caudatum* L.(27.09) and *Pteris biaurita* L.(20.39), respectively. The *Adiantum caudatum* L. showed absorption coefficients of Co about 1.72. The Pteris sp. such as *P. ensiformis* Burm.f. did not have highest concentration of Pb, Ni and Co but it accumulated Pb rather high about 30.37 mg/kg. The *Pteris cretica*, *P. longifolia*, along with *P. vittata*, *Pityrogramma calomelanos* would be considered arsenic hyperaccumulators. These species have previously been recorded as hyperaccumulators by Zhao *et al*., (2002). With the identification, there are members of the Pteris genus that do not high accumulate all heavy metals. Plants have different ability to uptake metals. Cd, Ni, Zn and Cu were elements that plants can uptake more than Pb, Hg, and Mn. So, the bioaccumulation factor varied between soils (Caille *et al*., 2004). There are many factors that have influence on heavy metal accumulation in soil and plants such as pH, soil texture, soil parent material, type and quantity of soil, organic compounds, soil solution, soil moisture, temperature and microorganism activities. Soil acidity and soil quality may also exert a profound influence on the availability of elements to plants (Tyler and Olsson, 2001; Tyler, 2004; Reimann *et al*., 2007). The pH level has effect to heavy metal uptake by plants that the high pH values decreased metal uptake by plant (Siriratpiriya *et al*., 1985). Geology is the driving force behind the pattern observed for the plant leaves. It is not always easy to decide whether pH related to high concentration. It is most likely that several factors operate together (Reimann *et al*., 2007). The uptake rate is related by %OM values which occurred in this study that might be explaned for their different accumulation. Heavy metal uptake decreased when soil CEC was increased with more organic matter in soil (Chaney, 1982). The soil-plant barrier refers to that a general plant has a barrier for hindering the absorption of toxic metal ions, which is one of the resistance (tolerance) mechanisms of some metal ions by some plants (Zhenggui *et al*., 2001). Uptake of Pb into plant is independent of the transport mechanisms of any of the nutriens (Reimann *et al*., 2007). Although the reasons why certain species become and accumulator or excluder of the particular elements are not fully elucidated, it seems some species may accumulate the particular elements for their specific metabolic processes.

CONCLUSION

From 19 terrestrial fern species, *Adiantum philippense* L. showed significantly highest levels of Pb and Ni concentration in their leaves, while *Adiantum caudatum* L. was the best Pb, Ni and Co absorption. It was observed that the ferns had higher potential for heavy metals accumulation than in the soil. The uptake rate of metal is releated with organic matter content.

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