ELEMENT CONCENTRATIONS OF SOME FERN SPECIES AT PHU SOI DAO NATIONAL PARK, PHITSANULOK PROVINCE, THAILAND

Supaporn Pongthornpruek [1,*] and Savent Pampasit[2]

[1]Department of Environment and Energy, Faculty of Science and Technology, Uttaradit Rajabhat University, Uttaradit, Thailand 53000
[2]Department of Natural Resources Management and Environment, Faculty of Agriculture, Natural Resources Management and Environment, Naresuan University, Phitsanulok, Thailand 65000

*e-mail pongthornpruek@hotmail.com

Abstract:

The study on element concentrations cabability of some fern species was conducted at Phu Soi Dao National Park, Phitsanulok province, Thailand. The objectives of this research were to study on element concentration and accumulation in leaves of some ferns species and the capability of some ferns ecotypes in absorption the element, moreover to investigate the ecological effects on heavy metals absorption. The soil and fern samples were taken using line transect method and applied square plots size of 1×1 m^2 for the sampling process at the height ranging from 500 – 1,633 m above mean sea level. The survey has been conducted along existing a 6,500 m forest trail. Soil characterictics and element concentrations were analysed and metal accumulation in ferns were calculated. The result showed that from 19 terrestrial fern species, the fern species showed highest N and Ca concentration in leaves is *Colysis pothifolia*, *Diplazium esculentum* showed significantly higher P concentration.The species *Adiantum philippense L.* showed significantly higher concentration of K, Co, Cu, Cr, Fe, Ni and Pb. *Pteris ensiformis* Burm.f. showed highest concentrations of Mg, Na and Zn. *Lygodium* sp., showed significantly higher Mn concentrations. Cd are less concentration that was only found in *Lygodium* sp. and *Pteris venusta* Kunze. The uptake of metals by fern in this study depend on the plant physiology and soil characteristic.

Keywords: Fern, Element, 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 that 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 phyto-remediation 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). Although 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. There are the reports that a fern can absorb highly toxic and carcino-genic substance, heavy metal, 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 hyper-accumulated other metal and element.

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) (Fig.1). Samples of soil were collected 193 plots, each 1×1 m^2. 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 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). Soil texture was determined by hydrometer method (Jones, 2001). The moisture content, soil was accurately weight, dried at 105°C for 24 hours and then reweighed.

Total nitrogen content was analysed by kjeldahl digestion apparatus (VELP, DK42/26). Available phosphorus was extracted by BrayII (0.1N HCl+0.03 N NH_4F) and analysed by spectro-photometer (HACH, DR 4000U). Exchangeable K, Ca, Mg, Na were extracted by 1.0 N ammonium acetate and analysed by atomic absorption spectrophotometry. (FAAS model GBC Avanta PM 05593). Element and metal, Mn, Fe, Cu, Zn, Cd, Pb, Ni, Co and Cr 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 the fern types. 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 element and metal determination.

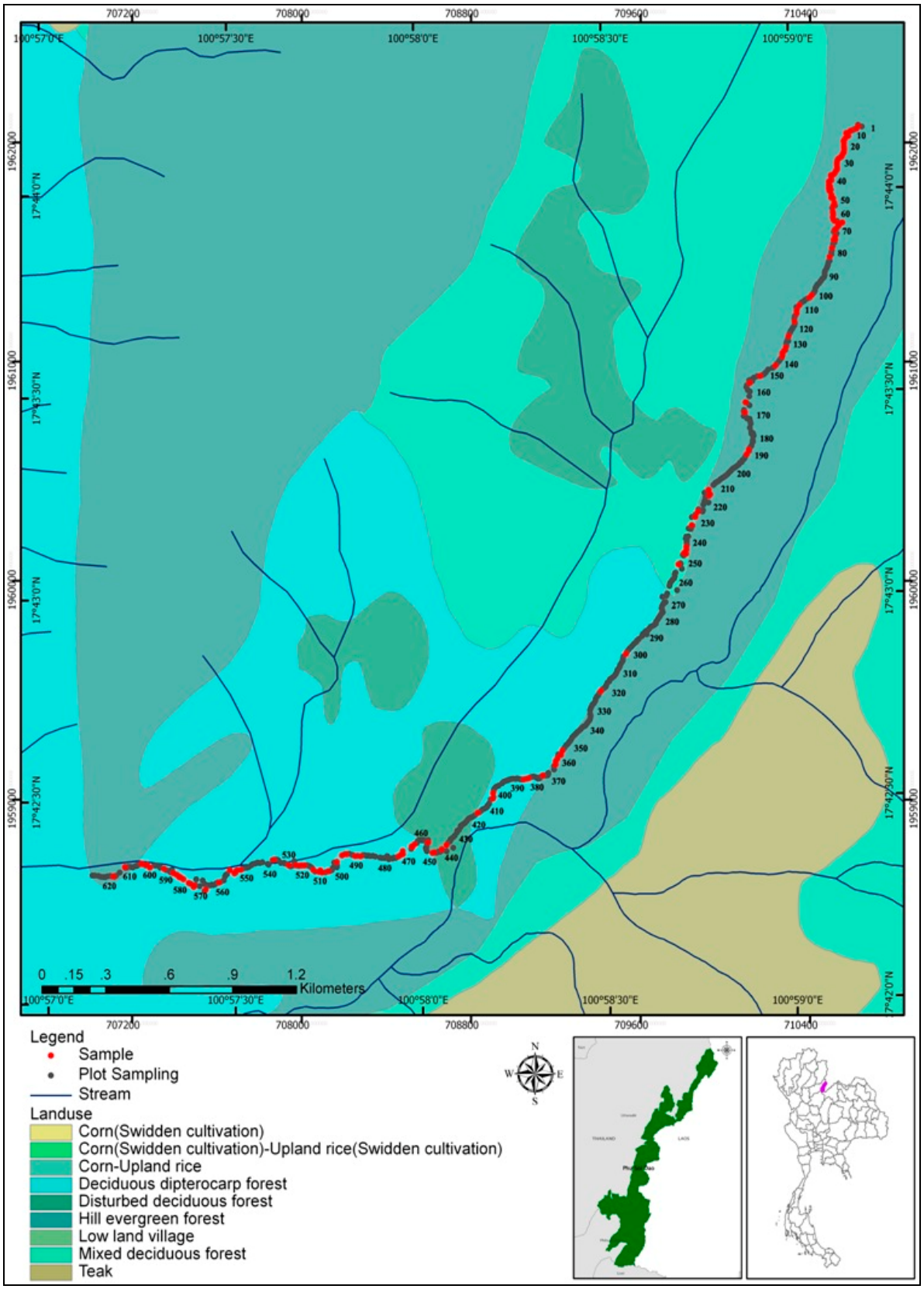

Figure 1 Topographic map of Phu Soi Dao National Park showing the location and plot sampling area. (Map ratio 1 : 20,000)

C. Determination of element and metal in leaves

Total nitrogen were analysed by kjeldahl digestion apparatus (VELP,DK42/26). Phosphorus was digested with mixture of HNO_3 and $HClO_4$ on hot plate and analysed by spectrophotometer (HACH, DR 4000U). Determination of element and metal, plant samples were prepared for elemental analysis using a wet digestion method (Zhao *et al*., 1994 ; Simmons *et al*., 2004). A portion (~ 0.2 – 0.5 g) of 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 then digested at 120 °C on a hot plate. When digestion was completed, samples were removed and diluted to 50 ml with deionised water then filtered. This solution was analysed for elements and heavy metal, K, Ca, Mg, Mn, Na, Fe, Cu, Zn, Cd, Pb, Ni, Co and Cr using frame atomic absorption spectrophotometry (FAAS, GBC Avanta PM 05593). Acid blanks were also analysed in order to detect possible contamination. The FAAS analyses were done at Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. All elements and metal concentrations in the samples were reported on a dried mass basis.

Results, Discussion and Conclusion:

A. Element concentration in leaves of fern

Leaves are the physiologically active organs of a plant converting the inorganic matters into organic compounds. The leaves are considered to reflect the significant plant physiology on mineral composition. The results of the chemical analysis for those elements as N, P, K, Ca, Mg, Cu, Fe, Mn, Na, Zn, Cd, Co, Cr, Ni and Pb of leaves were averaged as given in Table 1.

Table 1 The Characteristics of element concentration in leaves of each fern species.

Species	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn	Ni	Na	Co	Cd	Cr	Pb
Adiantum caudatum L.		--	--	---	-	+++				+		+++		-	
Adiantum philippense L.	+++	++	+++			--	+			++	--			+++	+++
Angiopteris evecta		--	---	-	--	--	--				--	--			
Blechnum orientale L.		--	-	--	-	---	-			---	--				
Colysis pothifolia	+++	--	---	++		--	+								
Diplazium esculentum		++			+	---			-	---					
Lindsaea ensifolia Sw.		--	++	-		+			+	+	-	++			+
Lygodium sp.		--	---	---	-	--			++		--	---			
Pteridium aquillium		-	---	--	--		-				--	-			
Pteris biaurita L.		++	+			--	-								
Pteris ensiformis Burm.f.		--	++		+++		++				+++				
Pteris venusta Kunze.		--	---	-	-	--	--				--	---			
Tectaria angulata		+	++	+	-									+	++
Tectaria herpetocaulos Holtt.		-	---	+	-	--					--	+			
Tectaria impressa		--	---	+	-	--									
Tectaria polymorpha				+		+				---	-				
Thelypteris interrupta		--		+		--				---					
Thelypteris nudata		--	---			--					--				-
Thelypteris terminans		-	+		++	++					+				

+++ α = 0.001 % --- α = 0.001 %

++ α = 0.01 % -- α = 0.01 %

+ α = 0.05 % - α = 0.05 %

The overall means of the concentrations of elements 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 in certain species, the species was regarded as accumulator of the element.

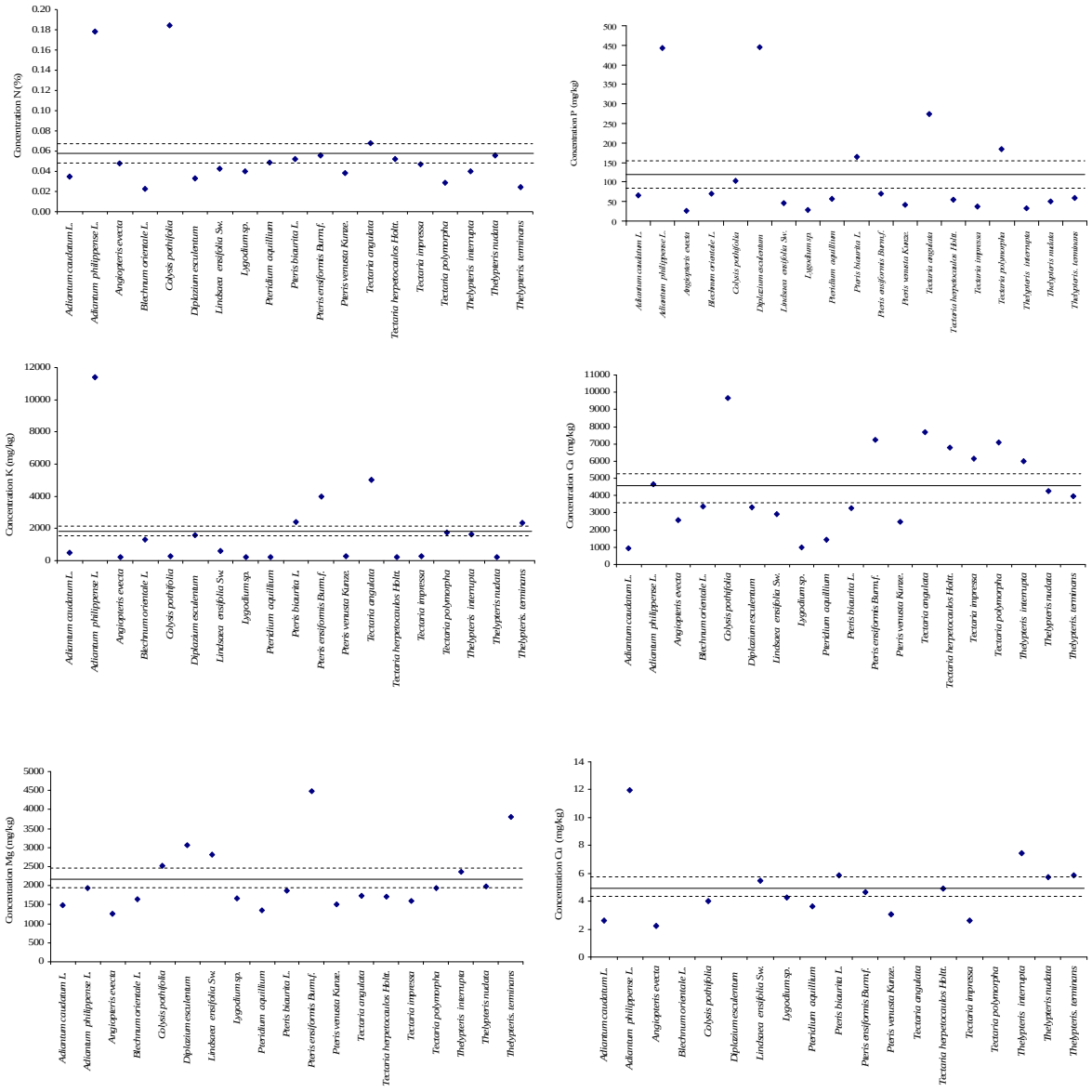

Figure 2 Specific distribution profiles of N, P, K, Ca, Mg, Cu, Fe, Mn in leaves. Solid line indicated the mean value of all species and dotted line showed the significant range with a critical value of $\alpha = 0.01$ %.

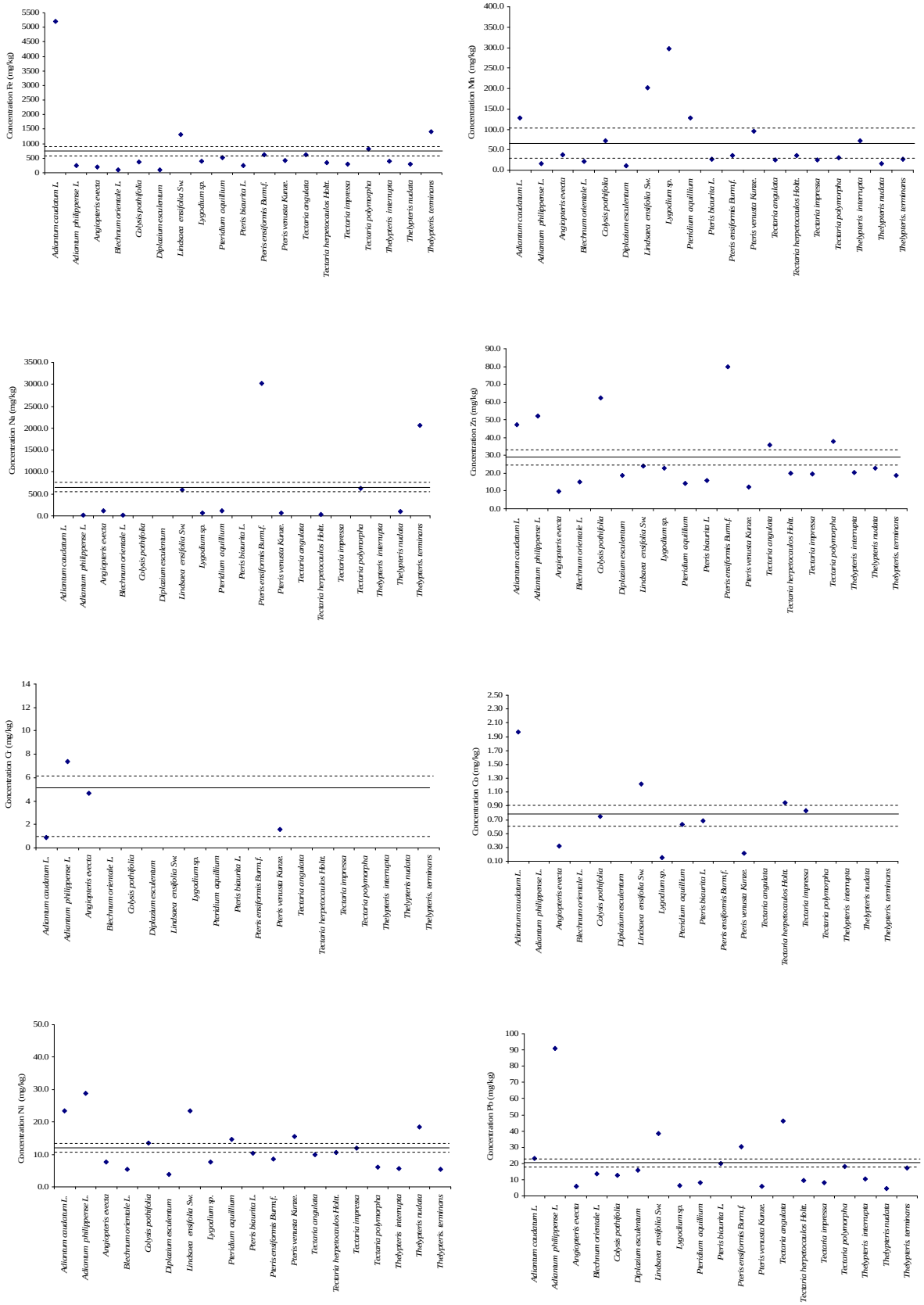

Figure 3 Specific distribution profile of Na, Zn, Cr in leaves.Solid line indicated the mean value of all species and dotted line showed the significant range with a critical value of α = 0.01 %.

The fern species showed highest N and Ca concentration is *Colysis pothifolia* while *Diplazium esculentum* showed significantly higher P concentration in leaves. The species *Adiantum philippense L.* showed significantly higher N and P concentration in the next, but it was highest K, Cu, Cr, Ni and Pb concentration in leaves. *Pteris ensiformis* Burm.f. showed highest concentrations of Mg, Na and Zn. Mn in leaves showed significantly higher concentrations such species as *Lygodium* sp., also Cd are less concentration in leaves of fern that was only found in *Lygodium* sp. and *Pteris venusta* Kunze. For the fern species showed highest Fe and Co concentration in leaves was *Adiantum caudatum* L. 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. The *Pteris cretica*, *P. longifolia*, along with *P. vittata*, *Pityrogramma calomelanos* would be considered arsenic hyperaccumulators. These species have previously been recorded as hyper-accumulators 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).The specific distribution profile of Cd showed no significantly concentrations (Table 1). Cd are less concentration in leaves of fern that was only found in *Lygodium* sp. and *Pteris venusta* Kunze. amount to 0.296 mg/kg and 0.172 mg/kg respectively.

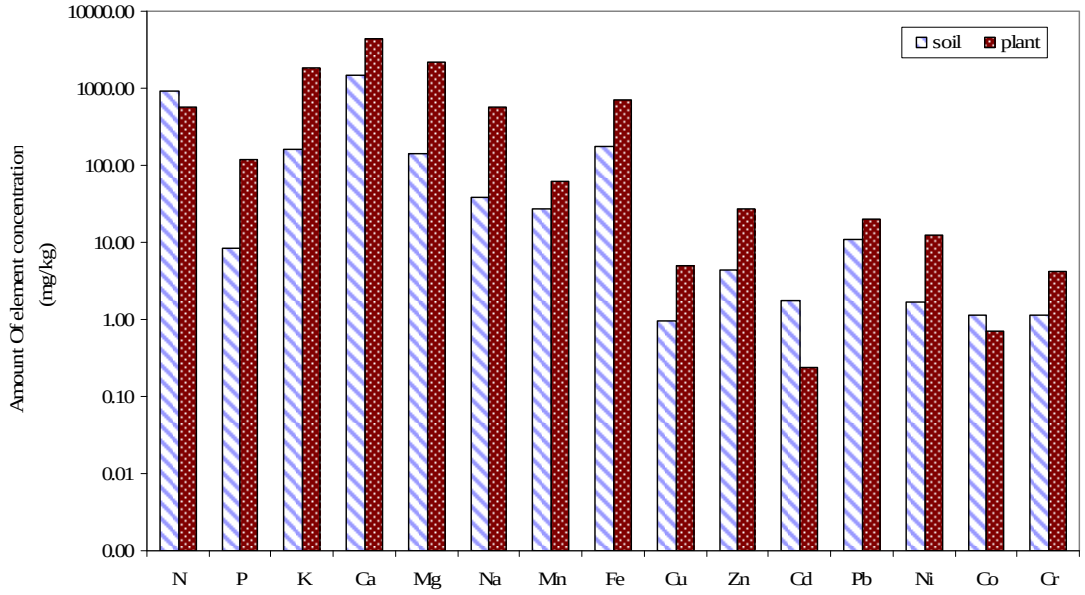

Figure 4 Comparison of elements concentration in soil and leaves

B. Comparison of elements concentration in soil and plant leaves

Each fern species have different level of element concentrations, depend on area base, soil fertility and forest type. N, P, K Ca and Mg are essential macro elements of plant nutrients. Mn, Fe, Cu, Zn, Ni are micro elements. The element Ca showed the highest concentration in plant and soil.The element concentrations in the leaves decreased in the following sequence :

Ca > Mg > K> Fe > Na >N > P > Mn > Zn > Pb > Ni > Cu > Cr > Co > Cd while The element concentrations in the soil decreased in the following sequence : Ca > N > Fe > K> Mg > Na > Mn > Pb > Zn > P > Cd > Ni > Cr > Co > Cu. It was observed that the ferns had higher potentials of absorbing the element than in the soil except N, Cd and Co (Figure 4)**.**

The absorption coefficients of different fern species in their leaves and soil are 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).

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

Analytical results are summarized and absorption coefficient were drawn. From 19 terrestrial fern species, some fern species had high element absorption due to the ecological effect**.** The element Ca showed the highest concentration in plant and soil. The result was observed that the ferns had higher potentials of absorbing the element than in the soil except N, Cd and Co. For a plant to hyperaccumulate a metal, level in fronds must be higher than in the soils (Meharg, 2002). *Colysis pothifolia* and *Diplazium esculentum* showed highest absorption coefficients of N and P respectively. The fern species showed highest absorption coefficients of Mg, Na and Zn was *Pteris ensiformis* Burm.f. For the *Adiantum* sp., *Adiantum philippense* L. showed highest absorption coefficients of K while *Adiantum caudatum* L. showed highest absorption coefficients of Co and Fe. Moreover, the highest absorption coefficients of Mn was found in *Lygodium* sp.

C. Soil characteristics each fern species

The soil characteristics of Phu Soi Dao National Park showed that : the soil texture were sandy loam and sandy clay loam. All soils contain acidic values in the ranged of 4.78 – 6.20 and 17.07 % of soil water content. Organic matter content are in the ranged of 0.89 – 10.70%. The cation exchange capacity is in the ranged of 4.31 – 11.91 me/100g. *Blechnum orientale* L. was found the highest organic matter content which correlated to the N concentrations in their soil.

The fern species that high absorption metal was *Adiantum caudatum* L., *Pteris biaurita* L. and *Lygodium* sp. Their soil charictics were sandy loam and sandy clay loam in texture, moderately acid of soil pH, organic matter and CEC was low to moderately low**.** Available phosphorus was low. It is well documented in literature, that plant availability of heavy metals depends on soil conditions such as soil type, adsorption and buffer capacity of the soil, concentration of clay and organic matter, soil pH and redox potential as well as concentration and forms of heavy metal and the origin of heavy metal contamination.(Kurz, Schulz and Römheld, 1999). In additional, Lasat *et al*.(1996) and McGrath *et al.* (1997) reported that the uptake of heavy metal by hyperaccumulator plants not only depens on the plant mechanism, but on the easily accessible pool in the soil. In this studied, the result showed that the element concentration found the soil samples were not significantly correlated to the concentrations in the fern samples. According to Schilling and Lehman (2002) that found metal concentrations in the Massanutten soil samples were not significantly correlated to the concentrations in the moss samples collected from the same location. The result observed that the high absorption's fern mostly has

the similar root system likes rhizome short, creeping to erect. Additionally, heavy metal concentration differs between plant species depending on morphology of shoots and root (Kurz, Schulz and Römheld, 1999).The pH level has effect to 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 explained 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.

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