The Identification of Metallophytes in the Fe and Cu Enriched Environments of Brookes Point, Palawan and Mankayan, Benguet and their Implications to Phytoremediation

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ABSTRACT

It is important to consider the potential of plants in the remediation or rehabilitation of areas affected by mining as well as their capability of absorbing metals in anomalous amounts which could be an alternative to traditional mining. Phytoremediation is an innovative way of addressing the environmental impacts of mining. Studies on metallophytes have identified some plant species that thrive in nickeliferous laterites and in cupriferous soils. It has been of interest as to how these plants accumulate the heavy metals the soil contains, and specifically how much of the available metals are being taken up by these plants. In Brookes Point, Palawan, some of the identified plants that thrive in nickeliferous laterites, were *Sapotaceae planchonella, Apocynaceae alstonia macropylla and Cunoniaceae weinmannia sp.* Results showed similar responses among the plant species in terms of the total Fe content in their leaves, stems and roots. The positive relationship between the soil and plant components was observed manifesting the characteristics of indicator plants for Fe. *Apocynaceae alstonia macrophylla* specifically had high Fe contents in the root system making it also an accumulator and phytostabilizer for Fe. In Mankayan, Benguet, four native fern species that can tolerate the Cu enriched soils were identified and analyzed for Cu using the root-stem-leaf components. These species were *Pteridium aquilinum*, *Dicranopteris linearis*, *Pteris sp.* and *Nephrolepis hirsutula*. The results showed *Nephrolepis hirsutala* and *Pteris sp.* to be the best Cu-tolerant species. These plants were not only considered as indicator plants for Cu manifested by the positive correlation between soil and plant components but also as accumulators and phytostabilizers for Cu due to the high Cu content in their roots. These are characteristics of metallophytes and thus such plants may be used for phytoremediation.

Keywords: Metallophytes, phytoremediation, Brookes Point, Mankayan

INTRODUCTION

Metallophytes are plants that can grow in and are tolerant to soils with elevated levels of metals and metalloids (Pyatt, 2001; Whiting, Broadly & White, 2002; Behmer et al, 2005). Studies have shown that these metallophytes have great potential in mineral and metal exploration and in phytoremediation and phytomining (Brooks, 1972; Rose, Hawkes & Webb, 1979; Bradshaw & Chadwick, 1980; Brooks, 1983; Anderson et al, 1999b; Baker, 2002; Lasat, 2002; Brooks & Robinson, 2004; Nicks & Chambers, 2004;). The identification and utilization of these plant species are being widely explored nowadays for phytoremediation which is a natural way of detoxifying contaminated soils (McGarth, 2004; Meharg, 2002a; Meharg, 2002b; Brooks, Chiarucci & Jaffe, 2004). Phytoremediation is also considered an innovative way in addressing the impacts of mining here in the Philippines (Cadiz, Cadiz & Vidal, 2005; Cadiz et al, 2006; Raymundo, 2009; Doronila, 2009). Recent works on the potential application of phytoremediation and the use of mycorrhizal plants to mine rehabilitation were done at Magpog, Marinduque; Toledo, Cebu; Antamok, Benguet; Paracale, Camarines Sur and Mankayan, Benguet (Aggangan et al, 2006; Mandoza, Ocampo & Aggangan, 2006; Aggangan & Segismundo, 2006; Manalo, Pampilona & Aggangan, 2007; Raymundo, 2009; Cadiz et al, 2009). Metallophytes have also been studied for phytomining which is the mining of metals using plants specifically the hyperaccumulators that can accumulate more than 0.1% of the metals in the leaves and stem irrespective of the metal concentration in the soil (Harper et al, 2002; USEPA, 1999; Anderson et al, 1999; Yang, Peng & Jiang, 2005). Understanding the metal accumulation property of the plants through the metal contents of the different plant components is important. Studies have shown that metal ions from the soil are absorbed by the root system and are transported to the stem tissues through the xylem components of the plant prior to their sequestration by the leaf system (Harper et al, 2002; Clemens, Palmgren & Kramer, 2002; Reichman, 2002; Hall, 2002; Boucher et al, 2004; Stoltz and Greger, 2001).

Two areas considered in this study, Brookes Point in Southern Palawan and Mankayan in Benguet. These areas are characteristically different specifically with the underlying geology and the associated type of soil formation. With vegetation largely influenced by soil type, it was inferred that unique plant species occur in each area.

Southern Palawan is underlain by ultramafic rocks predominantly serpentinized peridotites (Claveria & Fischer, 1991; Holloway, 1981; Mitchel et al, 1985). The weathering of these ultramafic rocks is referred to as laterization. In the process of laterization, continuous water percolation, otherwise known as hydrolysis, has led to the dissolution of soluble elements including the major nutrients essential for plant grown and the apparent enrichment of heavy metals such as iron, nickel and cobalt in the soil (Claveria & Adriano, 2008; Thorp & Baldwin, 1940; Gleason, Butt & Elias, 2003; Golightly, 1981; Santos-Ynigo & Esguerra, 1961). The laterites at Brookes Point are known for their anomalously high Fe contents (Fernandez, 1976; Claveria & Adriano, 2008). The loss of the major plant nutrients during laterite formation has initially led to the belief that this type of soil would have sparse vegetation. Studies however have shown that there were specific species distributions of vegetation in the laterites (Proctor, Bruijnzeel & Baker, 1999). These plants have thrived despite the relatively high concentrations of iron, which might actually be toxic to plants (Harper et al, 2002). It was then of interest to know the amount of iron accumulated by these plants. This study determined the amount of Fe accumulated by the plants from the soil and whether they accumulated enough Fe to be utilized for phytoremediation.

The Lepanto copper-gold deposit in Mankayan, Benguet is an epithermal high sulfidation vein-replacement type of mineralization (Hedenquist, Arribas & Reynolds, 1998; Disini, Robertson & Claveria, 1998; Claveria & Hedenquist, 1994). The principal ore minerals are enargite and luzonite ($Cu_3(As,Sb)S_4$), with significant presence of tennantite (-tetrahedrite) ($Cu_{12}(As,Sb)_4S_{13}$), base metal sulfides, electrum and tellurides (Claveria & Hedenquist, 1994). The copper ores are arsenic-rich and the processing of these types of ores would have an environmental impact due to arsenic toxicity (Lasat, 2002; Meharg, 2002a). Similarly the soil cover formed after the weathering of the copper ore at depth was known to have substantial copper (and arsenic) concentrations which are toxic to plants. Several fern species however are known to grow in these types of soil (Nishizono, Suzuki & Ishii, 1987;

Kertulis-Tartar et al, 2006; Meharg, 2002b). Understanding the accumulations and storage potentials of ferns for metal ions was important and relevant in appreciating metal nutrition applications and metal detoxification in soils (Cobbett, 2003). It was essential to study the concentrations of copper in the soil and correlate these with the Cu concentrations in some native fern species found in the area. The study determined how much of the copper quantified in the soil samples have been absorbed and accumulated by the fern species over time, and whether the fern species have accumulate enough of the metals to be utilized for phytoremediation.

MATERIALS AND METHODOLOGY

Field mapping and sampling were done at predetermined sites at Brookes Point, Palawan and at Mankayan, Benguet. Paired sampling was done on plants and soil in the selected sites to allow the study to be more determinate of the chemical relationships of the plants and the soil and to establish a correlation between their metal concentrations. The choice of what plant to sample and collect was dependent on its relative abundance in the area and its occurrence as pioneering plants. After sampling, the plant was washed with deionized water to remove the soil attached to the different plant components so as to avoid contamination during the chemical analyses. In the identification of the plants, proper documentation as to their habitat and physical characteristics was done in the field. Representative plant samples were preserved following certain protocols such as the plant press method (e.g. Middleton, 1976). It was intended that the various plant specimens be stored in a herbarium for future references. The identification and authentication of the different plant species were done by the Institute of Biology at the University of the Philippines, Diliman. The plant samples were separated into roots, stem and leaves and were oven dried to about 60^{o}C (Hobbs & Streit, 1986).

Standard protocols for plant digestion considered in this study were in reference to the Association of Official Analytical Chemist International (AOAC) methods (AOAC methods 922.02 and 975.03) which were deemed applicable to plant metal analyses. These methods considered dry ashing the plant samples using HNO_3 digestion (Demir, Esen & Gucer, 1990; Meharg, 2002b). Each compartment was ashed to about 500^{o}C in a muffle furnace for about 5-8 hours (Yates, Brooks & Boswell, 1974). About 0.1g of the plant ash was acid digested with 1.0ml concentrated HNO_3. The digestion was in preparation for Atomic Absorption Spectroscopy (AAS) analyses.

The soil samples were air dried. Similar to the sample preparation for plants, about 0.1g of soil was acid digested with concentrated HNO_3, in preparation for AAS analysis. The Environmental Protection Agency (EPA) standard method (EPA method 3050B) for soil digestion was utilized, which involve HNO_3 and H_2O_2 as acid metal extractors.

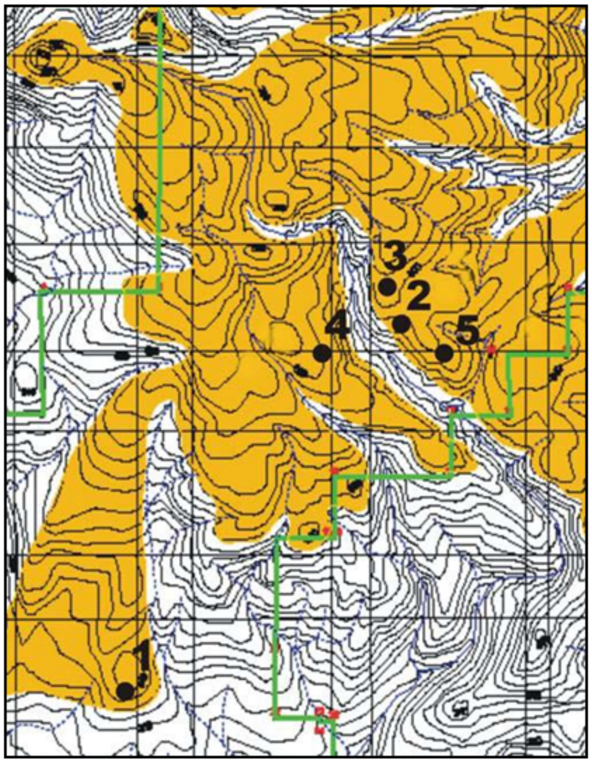
In the metal concentration analyses, the AVANTA GBC 932 AA Flame Atomic Absorption Spectrophotometer was used. The metal concentrations were determined using calibration curves derived from the analyses of appropriate standard solutions (Cui, Zhou & Chao, 2007). To check the validity and accuracy of the analyses, 3 technical replicates were done for all the samples analyzed.

RESULTS AND DISCUSSIONS

Brookes Point, Palawan

A total of 14 plant species have been acquired for the study and were taken at different sampling sites (Fig. 1). Out of these species, only 3 have been chosen because they had good representations of their leaf, stem, and root components. The selected plants were *Sapotaceae planchonella*, *Apocynaceae alstonia macrophylla,* and *Cunoniaceae weinmannia* and they were analyzed for Fe in their various components (see Table 1 and Fig. 2).

Laterite samples taken from 3 selected sites (e.g. Sites 1, 4 & 5) have high concentrations of total Fe that range from about 380,000 – 740,000 mg/L or ppm (Table 1). These values indicated that Fe was a major heavy metal that concentrated in the lateritic soils at the study area. For normal soils, trace metals including Fe do not exceed 100 ppm in amount (USEPA, 2006). The formation of Fe in laterites is a natural weathering process in ultramafic rocks. With



Sampling Site	Species Name
1	*Sapotaceae planchonella*
2	*Rubiaceae rothmannia*
	Rubiaceae wendlandia sp.
3	*Apocynaceae alstonia macrophylla*
	Moraceaef ficus sp.
	Rosaceae rhaphiolepis sp.
4	*Apocynaceae alstonia macrophylla*
	Rubiaceae wendlandia sp.
	Sapotaceae planchonella
	Casuarina sp. equisetifolia
	Sympolaceae symplocos polyandra
5	*Cunoniaceae weinmannia sp.*
	Dicranopteris linearis
No particular site	*Melastoma malabathricum*
	Spathoglothis kimballiana var. angustibolia
	Loganiaceae geniostoma rupestre Forst. (form prev. known as *G. pulgarense Elmer*)
	Nephentes alata

Figure 1. Map showing the location of the sampling sites. The yellow outline in the map indicates the extent of laterite occurrence. The table at the right is the list of identified plants acquired for the study.

the proper conditions of climate and topography, residual Fe would likely be enriched at the upper horizons of the laterite profile (Gleason, Butt & Elias, 2003; Golightly, 1981; Claveria & Adriano, 2008). The different physical characteristics of the site influenced the development of laterite as well as the concentration of Fe. It was noted that sites which are relatively flat and have good drainage system have mature or well-developed laterite and have higher Fe concentrations (Claveria & Adriano, 2008).

Table 1 shows the total Fe present in components of each plant. Higher total Fe concentrations were noted in the different components of *Cunoniaceae weinmannia* as compared to *Sapotaceae planchonella* and *Apocynaceae alstonia macrophylla*. *Cunoniaceae Weinmannia* had Fe values in its stems and leaves 700% higher than the other 2 plant species. Almost similar Fe concentrations in the leaves and stems were noted between *Sapotaceae planchonella* and *Apocynaceae alstonia macrophylla* ranging from 21,000 – 24,500 ppm. The total Fe percentages in the roots differ among the 3 plants. The highest Fe concentration is in the roots of *Cunoniaceae weinmannia*. (457,316 ppm) and the lowest is *Apocynaceae alstonia macrophylla* (389,065 ppm).

The results of the chemical analyses indicated that each plant species has its own individual nutritive requirements that somewhat differ from other species. It is believed that an excess of a particular element above a critical level in the nutrient solution will impair the health of the plant or even kill it (Rose, Hawkes & Webb, 1979). The Fe concentrations however were very high for all the plant species indicating possibilities of biogeochemical anomalies in the area. Though anomalies in Fe concentration are present in the plant samples, it would not follow the notion of eventual death of the plants. From field observations the study area even exhibited good vegetation (Claveria & Adriano, 2008).

It was noted that the plant species taken at Sites 1, 4 and 5 had varying responses to the concentrations of

Table 1. Total Iron analysis of the lateritic soil and the components of the different plant species. Data represents means +/- standard deviation (x +/- s.d.) (values x 10^3).

Sample Site	Soil Fe (ppm)	Plant Sample	Component	Plant Fe (ppm)
1	553.83 ±54.87	*Sapotac. planchonella*	Roots	426.53 ±15.72
			Stems	22.43 ±1.59
			Leaves	24.34 ±3.70
4	382.87 ±4.45	*Aposynac. alstonia macrophylla*	Roots	389.06 ±50.79
			Stems	21.06 ±3.10
			Leaves	21.6 ±3.40
5	737.55 ±32.02	*Cunoniac. winmannia*	Roots	457.32 ±156.16
			Stems	194.40 ±34.51
			Leaves	184.94 ±14.15

total Fe in the soil. A relationship existed where in the Fe concentrations in the soil were indicated or manifested in the metallophytes that have proportional amounts of Fe in their components. In general, the observed correlation of total Fe in the soil and plant components manifested indicator responses of metallophytes. These are indicator plants that can grow in specific metal enriched sites because of their metal tolerance which could be toxic to normal plants (USEPA, 1999; Baker and Whiting, 2002).

It was also noted that the concentrations of total Fe were highest in the roots for all plant samples and lowest in the leaves. These plant samples are classified as xerophytes, meaning shallow-rooted, yet they exhibited high amounts of total Fe in their roots. This could be attributed to the trace metal tolerance and stability of one plant organ under the function of time and location of accumulation. It was apparent that with the high total Fe concentrations present in the soil, the roots of the plant could make the Fe bio-available for absorption. Phytostabilization is related to the absorption and accumulation of trace metals within the root zones preventing or limiting the uptake of the metals into the plant shoots. This process also binds the substrate to the roots thus reducing the movement of metals in the soil (Robinson et al, 2003). Based on the Fe concentration values, it was apparent that the roots of *Apocynaceae alstonia macrophylla* have higher Fe content (about 1.6%) than the soil from where it was taken (e.g. Site 4). It could be considered that *Apocynaceae alstonia macrophylla* is an Fe accumulator and as a phytostabilizer as compared to the other 2 plant species.

Mankayan, Benguet

The locations of the 3 sampling sites (e.g. Sites A, B, C) were predetermined with consideration to increasing distances from the site of an exposed Cu ore body (e.g. Site 1) (Fig. 3). Sampling Site A was nearest to the exposed ore body while Site C was the farthest. In each site both soil and plant samples were taken. A sample of the exposed Cu ore was taken at Site 1 and the chemical analysis gave an expected high concentration of Cu at 117,000 ppm. The Cu values of the soil samples taken at Sites A to C ranged from 42-254 ppm (Table 2). In understanding the natural dispersion of Cu, the Cu values in each soil and plant samples from the different sites were compared with each other across sampling distances from Site 1. Interesting to note is the decreasing dispersion trend of Cu concentrations in soils from Site 1 to Site C. Similar observations were made using stream sediments taken at sampling Sites 2 and 3 (see Fig. 3). Taken along drainage systems that traverse the exposed ore body, Site 2 which was in a first order stream and located near the ore body gave Cu values of about 99 ppm while Site 3 which was in a second order stream and located much farther from the ore body gave lower Cu values of about 54 ppm.

The observed dispersion patterns of the metal concentrations in the soil with respect to distance from Site 1 were expected. As one moves away from a known Cu source, the concentration is expected to decrease. Similar observations were made in the works of Claveria (2000), Silberman & Berger (1985) and Sevillano & Fernando (1981). The natural dispersion of heavy metals are affected

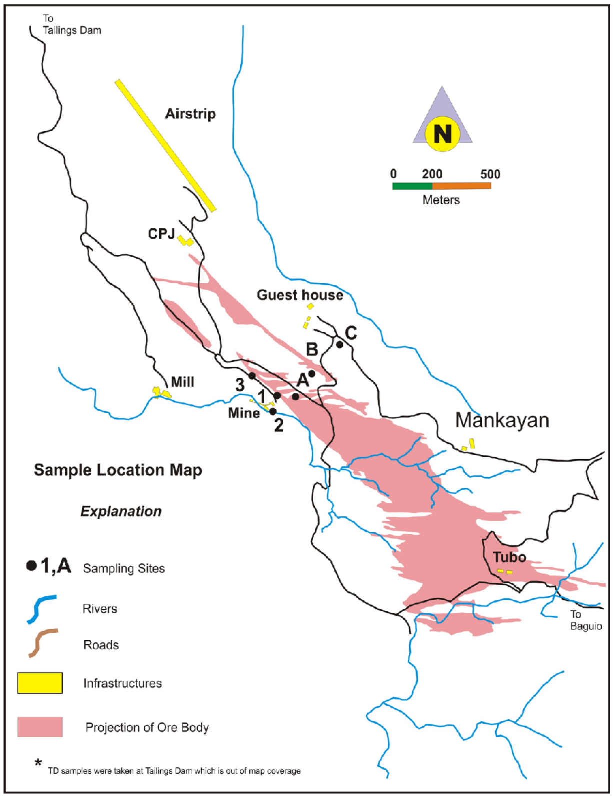
Figure 2. Photographs of some metallophytes found in nickeliferous laterite. a) *Sapotac. planchonella* found in Site 1 b) *Apocynac. alstonia macrophylla* found in Site 4 c) *Cunoniac. weinmannia* and *Apocynac. alstonia macrophylla* found in Site 5.

by the composition of the parent rock, which is the source of metals and various soil parameters such as pH, redox potential, ionic strength, competing ions and metal bioavailability (Singh & Steines, 1994; Dinelli & Tateo, 2001).

Across the different sampling sites, 4 fern species were identified. They were *Pteris sp., Nephrolepis hirsutula*, *Dicranopteris linearis* and *Pteridium aquilinum* (Fig. 4). In comparing the different Cu concentrations between plant and soil samples, it was observed that a positive relationship existed between the Cu concentrations in the plant to the Cu concentrations in the soil the ferns thrive in (see Table 1). In the same manner the lower Cu concentrations identified in the soil have caused the low to undetectable amounts of Cu in the plant samples. Such relationship manifested ferns to have characteristics of indicator plants (Baker and Whiting, 2002).

Relatively high Cu concentrations were observed in the root components of the different fern species while low to not detectable concentrations of copper are observed in the stem and leaf components. Among the 4 fern species spread across sites, only *N. hirsutula* from Site A was found to have detectable copper concentrations for all three plant components (e.g. roots, stems and leaves). It was noted that a decreasing trend of Cu concentrations from the roots, to the stems and to the leaves existed. Also the *N. hirsutula* species that was collected at Site A had a different set of concentrations in the different components from the same species collected at Site C. The *N. hirsutula* taken at Site C had a lower Cu concentration in the root component and not detectable (ND) Cu concentrations in the stem and leaf components. Aside from *N. hirsutula,* a comparison of similar fern species across different sites was made using *Pteris sp*. The *Pteris sp.* that was collected at Site A had high Cu concentrations in the roots and stems (488.18 ppm and 371.09 ppm respectively) and not detectable (ND) Cu concentration in the leaves. The other *Pteris sp.* that was collected at Site B showed that only the root component had a positive absorbance indicated by Cu concentrations of about 105.58 ppm. The Cu concentrations in the stem and leaf components were found to be not detectable. *D. linearis* collected at Site B had about 27.43 ppm Cu concentration in the root component and not detectable Cu concentrations in the leaf and stem components. *P. aquilinum* collected at Site C had Cu concentrations in the root and leaf components at about 53.09 ppm and 18.99 ppm respectively.

The fern species that were analyzed generally exhibited the highest Cu concentration in the root component. This was similar to the results of several studies exploring metal accumulation in different plant species (Cao et al, 2006; Bathia, Kachenko & Singh, 2007). This phenomenon indicated a low mobility of Cu from the roots to the other components. It manifested the immobilization of



Sampling Site	Species Name
A	*Pteris sp.*
	Nephrolepis hirsutula
B	*Pteris sp.*
	Dicranopteris linearis
C	*Pteridium aquilinum*
	Nephrolepis hirsutula

Figure 3. Sample location map showing the surface projection of the Lepanto main ore body at Mankayan and the different sampling Sites. Sites A, B and C are the sampling Sites where the coupled soil and fern samples were taken. Sites 2 and 3 are the sites where stream sediments were taken. Site 1 is where the exposed ore body is located. The table at the right is the list of fern species that were taken from Sites A, B and C and were used in this study.

Cu once it has been absorbed by the roots. This limited mobility and translocation in the roots was further explored through cellular analyses on the movement of the metals. It was noted that once the plant absorbed the Cu from the soil, the sequestered metal remains just in the roots, enabling the entire plant to grow and mature without its health being affected. These were considered important characteristics of plants to determine metal tolerance (Bathia, Kachenko & Singh, 2007). Ferns are considered good metal-tolerant plants as they sequester and accumulate Cu into one of their compartments, without allowing Cu and associated toxic metals to spread into the other components and inhibit the growth of the plant.

Relatively high Cu concentrations found in the other components and not only in the roots indicated that the absorption, translocation, transport and storage processes of heavy metals vary among different fern species. In general, various plants are noted to accumulate metals differently. The accumulation processes involved transport and chelating mechanisms that controlled the rate of uptake and storage (Clemens, Palmgren & Kramer, 2002).

The availability of the Cu metal in the soil played a huge factor on the Cu concentrations in the different fern species. *Pteris sp.* and *N. hirsutula* have relatively high concentrations of Cu compared to the rest of the plants samples, and this was due to the high Cu concentration of the soil they thrive in. In the same manner, the lower Cu concentrations identified in the soil from Sites B and C have caused the low to undetectable amounts of Cu in the plants samples collected from these sites. The *Pteris sp.* and *D. linearis* collected from Site B, along with *P. aquilinum* and *N. hirsutula* collected from Site C, however exhibited considerable Cu concentrations in the root components. Such concentrations are more likely related to the interaction between the soil and roots (Baker and Whiting, 2002; Robinson et al, 2003).

Table 2. Analytical results for Cu concentrations in soils and in the different plant components from samples taken at Sites A, B and C. Data represents means ± standard deviation (x ± s.d.). ND: not detected

Sample Site	Soil Cu (ppm)	Plant Sample	Component	Plant Cu (ppm)
A	254 ±46.04	*Pteris sp.*	Roots	488 ±172.35
			Stems	371 ±297
			Leaves	ND
		Nephrolepis hirsutula	Roots	73.2 ±15.71
			Stems	35.5 ±20.95
			Leaves	23.9 ±8.03
B	80.0 ±13.80	*Pteris sp.*	Roots	106 ±34.98
			Stems	ND
			Leaves	ND
		Dicranopteris linearis	Roots	27.4 ±4.75
			Stems	ND
			Leaves	ND
C	42.0 ±6.47	*Pteridium aquilinum*	Roots	53.1 ±3.92
			Stems	ND
			Leaves	19.0
		Nephrolepis hirsutula	Roots	11.8 ±7.46
			Stems	ND
			Leaves	ND

CONCLUSIONS AND RECOMMENDATIONS

The results of the study showed positive responses of *Sapotacaea planchonella, Aposynaceae alstonia macrophylla* and *Cunoniaceae weinmannia* in terms of absorbed total Fe content in their components with Fe concentrations in the soil at Brookes Point, Palawan. Such responses characterized these species as indicator plants. The root component of *Aposynaceae alstonia macropylla* had higher Fe content than the soil classifying it as a phytostabilizer, thus it could be a good species for phytoremediation.

Similarly, it was noted that the amount of Cu in the soil at Mankayan, Benguet corresponded well to the amount of Cu that was found in the fern samples that thrive in them. These ferns are characterized as indicator plants for Cu enriched soils. The results also indicated that *Pteris sp.* could be considered to have Cu accumulating characteristics especially in its root component, thus making it a good species for phytoremediation.

It is recommended that a study on the relationship between the availability of Fe and Cu in the soil and the uptake of the plants be done. Also a correlation study with other available heavy metals such as Ni, Co and As in the plants and soil should be done to understand better their chemistry and dispersion.

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Figure 4. Photographs of selected fern species found in the study area. Presented horizontally a) *Nephrolepis hirsutula,* b) *Dicranopteris linearis*, c) *Pteridium aquilinum*, d) *Pteris sp.*

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