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The identification of heavy metal accumulator ferns in abandoned mines in the Philippines with applications to mine rehabilitation and metal recovery

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Abstract

This paper focuses on the identification of some plant accumulators of heavy metals that can facilitate mine remediation and rehabilitation in the Philippines and metal recovery or phytomining. Most of these hyperaccumulators are ferns that thrive very well in different terrains and of particular interest are *Pityrogramma calomelanos*, *Pteris vittata*, and *Pteris melanocaulon* that are abundant in abandoned Cu−Au mining areas. The amounts of Cu and As in the soil and in the aboveground (AG) and belowground (BG) components of the accumulator ferns were determined and the Bioaccumulation Factor (BF) and the Translocation Factor (TF) were derived. Efforts to propagate the accumulator ferns identified from spores were successful, thus providing the opportunity of using them for various experiments on mine rehabilitation and metal recovery. The results of these experiments indicated that these hyperaccumulator ferns have the greatest potential for the remediation of metal contaminated soils, the rehabilitation of abandoned mines, and phytomining.

Keywords: hyperaccumulator ferns, mine rehabilitation, phytoremediation, phytomining

1. Introduction

Mining associated with the exploitation of metals such as cobalt, chromium, copper, iron, and nickel has been a global industry throughout the centuries. In the Philippines, the mining industry has greatly contributed to the socio-economic development of the country since the 1600s. With the history of progressive mining in the Philippines, which started even before World War II, comes the inevitability of a slow down with the expected depletion of metal resources. There have already been some reports of abandoned mines in various provinces such as those in the Cordillera and Mindanao and Bicol regions. Despite monetary gains, mining activities remain controversial due to their highly extractive nature. Mining sites tend to become contaminated areas of various metals, some of which could be toxic. The mined-out areas are left unviable for any use, because they are nutrient-deficient and erosion-prone with little to no vegetation. Current clean-up technologies via physical and chemical remediation methods remain expensive, especially for developing countries. The search for Green Technologies in mining has been a challenge, particularly within the perspective of what Responsible Mining is. It is inevitable that the apparent natural state of an ecosystem is disturbed during the various stages of mine development. Appreciating the ecological structure of an area could provide answers; one of which is the identification of an indigenous plant species that can be used for remediation and

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rehabilitation. Studies in phytoremediation have on various occasions provided opportunities to discover new plant species that can accumulate metals into their biological tissues. Interestingly, these plant species thrive in specific metalliferous terrains and are not commonly observed to occur elsewhere.

Understanding the accumulation potential of these plants is of great importance and from this understanding a certain plant species could be characterized and identified as a hyperaccumulator. A hyperaccumulator is 100x more capable of absorbing metals into its system than the metal content in the soil [1]. Hyperaccumulators are normally used in studies related to metal recoveries with the underlying concept of removing metals from contaminated soils with the use of the natural or induced metal tolerance and accumulation capacities of some plant species. The recovery of metals from contaminated soils and water is the goal of phytoremediation [2,3]. The use of plants to extract metals directly from the soil is called phytoextraction, which is the basis of the concept of phytomining, where plants are harvested and the metal contents extracted and sold. The utilization of hyperaccumulators will not only be beneficial for mine restoration, but also for the livelihood of communities around the mining area.

The Philippines is a mega-center for biodiversity and the challenge faced here is the identification of plants capable of copper (Cu) and arsenic (As) hyperaccumulation. These plant hyperaccumulators can be used in the rehabilitation of abandoned mining areas and consequently in the value adding (phyto) extraction of metals. This initiative is an important step in the ecological restoration of a previously mined area and could eventually lead to various stages of habitat successions [4]. Ferns are of particular interest because of their high adaptability to different kinds of terrains and to extreme environmental limiting factors. There are very few studies about ferns in the Philippines, and especially those of the Pteridaceae family which are known to be drawn towards hyperaccumulation. This study seeks to determine the metal accumulation capabilities of ferns. The information gathered could lead to the identification of hyperaccumulator ferns that are suitable for mass cultivation, phytoremediation and phytomining. They can provide additional economic benefits while remediating the soil for future agricultural use [5].

The objectives of this study were a) to identify the presence of indigenous ferns (as metallophytes and hyperaccumulators) found in selected study areas through field surveys, b) to determine the uptake capability of the selected ferns, and c) to recover Cu from the identified hyperaccumulators through metallurgical/chemical processes. The significance of the study is the feasibility of on-field applications of hyperaccumulator ferns in post-mining rehabilitation and the innovation of extracting Cu from a Cu accumulator and in turn the feasibility of phytomining in general. These applications were put forward to achieve certain benefits from what supposedly are useless contaminated areas such as abandoned mines. In order to appreciate the uptake and heavy metal accumulation of the identified indigenous ferns and consequently metal recovery, discussions on the different possible uptake mechanisms of the plant species are provided based on previous studies that were made on the selected ferns.

2. Materials and methods

Field surveys were conducted in some of the metal-rich areas in the Philippines covering the islands of Luzon, Visayas and Mindanao (Fig. 1). These areas were previously open pit mines and mine tailing dump sites which are likely to be enriched with heavy metals. It is of interest to identify plants that can accumulate heavy metals from the soil, and specifically to know how much of the available metals are being taken up by these plants. The selection of specific plants, particularly ferns, was based on a) their occurrence, being more abundant than other plant species, b) their presence, being the first and only plants that grow and thrive in the area and c) their tolerance, being able to grow in soils notably enriched or contaminated with heavy metals. The field surveys have evaluated the natural environment of the areas where the plants grow and propagate. Metallophytes were known to thrive in nickeliferous lateritic terrains. The 2 nickeliferous laterite deposits considered in this study were in the provinces of Zambales (Acoje) and Palawan (Brookes Point). In general, the 2 deposits are underlain by ultramafic rocks as part of some ophiolitic complexes [6,7]. The in-situ weathering of these ultramafic rocks has produced nickeliferous laterite profiles with varying thicknesses of the limonite and saphrolite horizons [8]. Laterite development was noted to thicken in plateau areas with approximately 500–1000 m elevations. As part of the limonite zone, the red to dark red near surface soils are enriched with iron oxides. Metallophytes and hyperaccumulators were also identified as growing abundantly in abandoned Cu–Au mines as well as in small scale mining areas such as those in

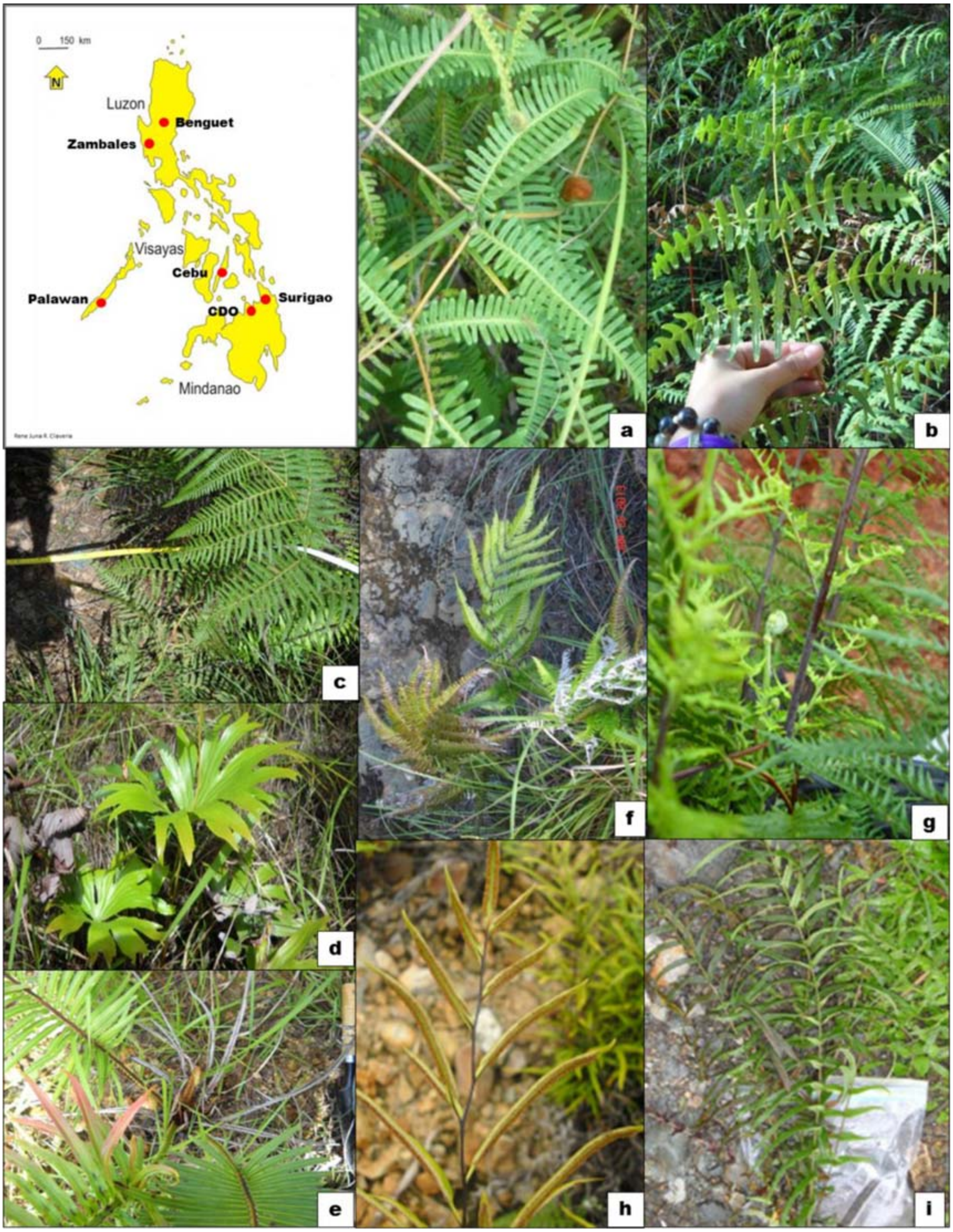

Fig. 1. Photographs of the common ferns found abundant in some Fe−Ni mines as well as Cu−Au mines in the Philippines. a) D. linearis, b) H. incisa, c) Pteridium sp., d) D. conjugate, e) B. orientale, f) Pteris sp., g) P. calomelanos, h) P. melanocaulon, i) P. vittata. Inset is a map of the Philippines showing the different sampling sites.

the provinces of Benguet, Cebu, and Surigao. In general, the Cu−Au mines in the 3 provinces are hydrothermal Cu−Au deposits occurring as Cu−Au porphyries and epithermal deposits [9]. The extraction processes are by open pit and underground mining and in some areas small scale or artisanal mining is being performed. The soils that are developed in these areas are products of the weathering of hydrothermally altered rocks which are significantly enriched with heavy metals. Soil development in these areas is also related to the weathering of old tailing ponds which are also enriched with heavy metals. It is also noted that these areas receive sufficient precipitation throughout the year making them conducive for plant growth. It was of interest to know how much heavy metals the plants and particularly the ferns could accumulate in their anatomical structures in consideration of the below and above ground components.

Plant and soil samples were collected. The plant samples gathered consisted of ferns. In total, about 14 different fern species were identified in the nickeliferous laterite and hydrothermal Cu–Au deposits. The entire fern with fronds and roots is taken as a sample. The aboveground (stem and leaves) and belowground (roots) components of the plants were taken with considerable care to avoid breakage of the different parts. In the selected sampling sites, the occurrences of the ferns vary. There are areas where a number of different ferns thrive together with some ferns being more abundant than others and there are also some areas where only 1 to 2 types of fern thrive either in abundance or are few in number. In areas where there is a relative abundance of specific ferns, triplicate sampling was performed and in areas where relatively few ferns occur, composite sampling was considered. The presence and relative abundance of the ferns in the selected study areas are significant indicators of the ability of the ferns to thrive in areas enriched with heavy metals. The corresponding soils where the plants have grown were taken with significant consideration of the depth to which the root system of the plant has extended. Similarly, composite soil samples of mixed rhizosphere and non-rhizosphere were taken in some areas where few fern species thrive. The collected plants were submitted for identification at the Institute of Biology, the University of the Philippines (Diliman) and the Philippine National Herbarium. The different plant species were identified accordingly. After following proper protocols, such as those of the Association of Analytical Chemists (AOAC) on sample preparation, the plant and soil samples were analyzed for metal and metalloid contents [10,11].

In the sample preparation of the plant samples, prior to chemical analyses, the samples were washed with tap water to remove any soil and dust that could be attached in the stem, leaf and root components of the plant and which may contain metals and metalloids. After washing, the plants were rinsed with deionized water and were oven dried to about 60–70 °C [11]. In order to know the elemental concentrations in the different parts of the plant, the fern samples were separated into root/rhizome, stipe, and lamina components. Some of the composite samples were prepared as whole plant samples and were not separated into above and belowground components. Such preparation was carried out in order to provide the study with an initial impression of the bioaccumulation factor of the ferns. During the preparation for analysis, the plant samples were acid digested with concentrated nitric acid [12]. The soil samples were air dried. When determining the element concentrations of the soil, the samples were prepared by acid digestion using concentrated nitric acid [12]. The elemental contents of the plant (whole plant, aboveground and belowground components) and soil samples were analyzed using the Avanta GBC Atomic Absorption Spectrophotometer utilizing various lamps. The metals analyzed included Cr, Co, Cu, Fe, and Ni. For As measurements an atomic absorption spectrophotometry with hydride generation (HG-AAS) analysis was used. The standard solutions were prepared with distilled water [12,13,14]. With the determined element concentrations in the plant and soil samples, the Bioaccumulation Factor (BF) and the Translocation Factor (TF) were calculated using the ratio between plant and soil (BF = Plant/Soil) and the ratio between aboveground and belowground components (TF = aboveground (AG)/belowground (BG)) [11,15].

In the post-mining rehabilitation experiments, the selected sites of the plots at Kias (Benguet) took into consideration the development of at least a few centimeters of soil cover found above the tailings. The presence of a soil cover above mine tailings was needed as ground preparation to enable the plants initial growth and survival. Such preparation included the use of uncontaminated soils taken within the vicinities of the mine tailings and the addition of soil amendments for plant growth. Two experimental plots with dimensions of approximately 3 m in length and approximately 1 m in width were prepared. In Surigao, the selected site for rehabilitation was Suyoc North which was formerly an open pit Cu mine. The area is about ¼ hectares of relatively flat terrain with no vegetation. The soil cover is either relatively thin or non-existent. Ground preparation was done specifically in areas where the ferns were to be planted. The preparations included the use of uncontaminated soils with soil amendments, some of which were used during the early propagation of the ferns in nurseries.

In the phytoextraction experiments, the hyperaccumulator ferns that exhibit efficient uptake of metals/metalloids, with the aboveground components having higher values than the belowground components, thus a TF ratio that is > 1, were candidates for the possible extraction of metals/metalloids directly from the plant. The higher TF values manifest the unique ability of the ferns to store heavy metals in the fronds and are not affected by phytotoxicity [1]. These are exemplified by hyperaccumulator ferns such as *Pityrogramma calomelanos, Pteris vittata* and *Pteris melanocaulon*. [16] indicated that the BG components of *P. melanocaulon* were relatively high in Cu with average values of about 4590 ± 386 mg kg^{-1}. In the same study, the authors indicated that the uptake of Cu by the fern in the roots was partly due to the high amounts of Cu in the soils in the mine area which had an average of about 1137.56 ± 297.48 mg kg^{-1} and that it was higher than the crustal average of Cu which is 58 mg kg^{-1}. The high content of Cu in the soils would have a major influence on the uptake of higher amounts of Cu by the plant [15]. From this information an experimental study on extracting metals from roots was performed using *P. melanocaulon*. Plant samples of *P. melanocaulon* were collected in an abandoned Cu–Au mine in Surigao where, apparently, the ferns thrive abundantly. The extraction process taken into consideration was electrowinning with the goal of separating Cu (as cations) from a solution that was laden with Cu and came from the dissolution of the root component of *P. melanocaulon*. The study placed emphasis on the extraction of Cu in the solution and did not considered the recovery of other metals which could be associated with Cu, such as As. In the experiment, the variable parameters analyzed included the digesting solutions which were nitric acid and *aqua regia*. The preparation of the solutions followed the protocols provided by [17]. The other variable parameters were voltage (1.5, 3 and 6 V) and time (30, 60 and 90 min). Changes in pH and temperature which may have an effect on metal recovery [18] were also taken into consideration. In addition, the types of electrodes used as well as the amount of agitation applied in the experiment were considered. Agitation allows metal ions to circulate in the solution and may assist in the deposition of the metals onto the cathodes [19]. Electrowinning setups with aluminum electrodes were made for each solution with 3 setups manifesting the tests on 3 different voltages and for each voltage the tests are on 3 different time frames. Atomic Absorption Spectrophotometry (AAS) was used to analyze the Cu concentration before and after electrowinning. The goal of the experiment was to determine the most efficient combination (solution, voltage and time) in extracting the highest % of Cu and thus the highest % of metal recovery. The limiting factor in the extraction process is the concentration of the metal in a solution which at high concentrations, the recovery of the metal would be high as well.

3. Results and discussions

3.1. Field surveys

3.1.1. Accumulator ferns in nickeliferous laterite mines

The analyses of the lateritic soils from the study sites confirmed the relatively high concentrations of Fe and Ni (Table 1). In the study sites, 3 metal tolerant ferns were found to thrive very well; these being *Pteridium aquilinum* and *Sphenomeris retusa* in the Zambales area and *Dicranopteris linearis* in Palawan. In all 3 fern samples, the amount of Fe absorbed by the plants was relatively high, at about 11 492.98 mg kg^{-1} for *P. aguilinum*, 4419.89 mg kg^{-1} for *S. retusa* and about 3043.62 mg kg^{-1} for *D. linearis*, as compared to other metals such as Ni where the values range from 7.79 to about 107.09 mg kg^{-1}. This was expected because of the very high concentrations of Fe in the soil which were approximately $52\,061.41 \pm 40\,836.92$ mg kg^{-1} on average in the Zambales (Acoje) study site to a high of 102 803.8 mg kg^{-1} in the Palawan (Brookes Point) study site. The consequential increase of Fe in plants due to the Fe increase in soil classifies them as indicator ferns for Fe.

The BF values of the different metals (e.g. Co, Cr and Ni) among the 3 fern species were < 1.0, which indicates relatively the inefficient uptake potential of the plants. This property may indicate the exclusionary mechanism of the plants to absorb just enough metals to avoid phytotoxicity, which is also the tolerant characteristics of other metallophytes to thrive well in soils with high metal contents [20]. The Cr TF values between the 3 ferns, however, were about ≥ 1.0 which shows the efficient transfer of Cr from roots to fronds. Such distribution is indicative of the capability of the ferns to tolerate absorbed Cr. These observations are of significant interest and it is highly recommended that further studies be made to confirm the Cr hyperaccumulation potentials for the plants.

3.1.2. Accumulator ferns in Cu–Au mines

The Cu–Au mines used as study sites were: a) the Lepanto Mine (in Mankayan, Benguet), b) the Acupan Mine (in Itogon, Benguet), c) Camp 6 (in Tuba, Benguet), d) the Philex Mine (in Tuba, Benguet), e) the Carmen Mine (in Toledo, Cebu), f) Manila Mining (in Placer, Surigao), g) the Silangan Mine (in

Table 1. Cu and As content of different fern species taken from nickeliferous laterite and Cu–Au mines in different localities in the Philippines. Indicated are the elemental contents of the soil as well as the belowground (BG) and aboveground (AG) components of the plant. Based on the elemental contents of the soil as well as the BG and AG of the plant, the bioaccumulation factor (BF) and the translocation factor (TF) were computed. Highlighted values are >1.00. The samples with computed averages are samples taken as triplicates and those without computed averages are samples taken as composite.

Site	1Plant	Element	Soil (mg kg^{-1})	BG (mg kg^{-1})	AG (mg kg^{-1})	BF	TF
Acoje	*Pteridium Aquilinum*	Co	410.77 ± 24.25	63.04		0.15	
Zambales		Cr	642.81 ± 258.62	62.45	95.62	0.25	**1.53**
Luzon (Nickeliferous		Fe	52 061.41 ± 40 836.98	9358.29	2134.69	0.22	0.23
Laterite Mine)		Ni	2904.13 ± 317.73	97.44	9.57	0.04	0.10
	Sphenomeris retusa	Co	410.77 ± 24.25	19.61		0.05	
		Cr	642.81 ± 258.62	47.70	45.49	0.14	0.95
		Fe	52 061.41 ± 40 836.98	3861.94	557.95	0.08	0.14
		Ni	2904.13 ± 317.73	36.16		0.01	
Brookes Point Palawan	*Dicranopteris linearis*	Cr	249.83	34.77	37.64	0.29	**1.08**
Luzon (Nickeliferous		Fe	102803.8	2974.80	68.82	0.03	0.02
Laterite Mine)		Ni	4610.67	7.79		0.001	
Camp 6	*Pteris vittata*	Cu	55.30	317.00	82.40	**7.22**	0.26
Benguet		As	13.70	53.70	175.00	**16.69**	**3.26**
Luzon (Cu–Au Small	*Pityrogramma*	Cu	55.30	816.00	51.70	**15.69**	0.06
Scale Mine)	*calomelanos*	As	13.70	15.50	106.00	**8.87**	**6.84**
Philex Mines	*Dipteris conjugata*	Cu	56.30	23.00	6.70	0.53	0.29
Benguet	*Pteridium* sp.	Cu	31.50	4.65	3.99	0.27	0.86
Luzon (Cu–Au Mine)	*Blechnum orientale*	Cu	73.80	10.80	10.50	0.29	0.97
Lepanto Mines	*Nephrolepis hirsutula*	Cu	697.23	76.52		0.11	
Benguet		As	1711.55	2.47		0.001	
Luzon (Cu–Au Mine)	*Dicranopteris linearis*	Cu	670.28	31.25		0.05	
		As	27.59	7.19		0.26	
	Histiopteris incisa	Cu	697.23	151.66		0.22	
		As	1711.55	2.47		0.00	
	Pteris sp.	Cu	1766.66 ± 32.14	2093.33 ± 97.12	17.00 ± 0.00	**1.19**	0.01
		As	1243.33 ± 132.03	179.33 ± 11.37	450.66 ± 20.55	0.51	**2.51**
	Pityrogramma	Cu	1265.66 ± 16.80	229.66 ± 11.84	41.00 ± 2.65	0.21	0.18
	calomelanos	As	2072.66 ± 105.11	473.33 ± 7.02	1227.33 ± 30.56	0.82	**2.59**
	Pteris vittata	Cu	351.00 ± 1.00	89.00 ± 7.21	17.60 ± 0.57	0.30	0.20
		As	645.00 ± 32.45	246.33 ± 8.32	722.33 ± 150.99	**1.50**	**2.93**
Acupan	*Pteris vittata*	Cu	106.33 ± 2.00	51.06 ± 0.50	9.92 ± 0.26	0.57	0.19
Benguet		As	25.80 ± 1.61	4.08 ± 0.07	80.10 ± 2.71	**3.26**	**19.63**
Luzon (Cu–Au Small	*Pityrogramma*	Cu	337.66 ± 2.30	404.66 ± 6.50	30.43 ± 0.21	**1.29**	0.08
Scale Mine)	*calomelanos*	As	33.83 ± 1.40	12.66 ± 0.31	113.00 ± 5.29	**3.71**	**8.93**
Carmen Mine	*Pityrogramma*	Cu	494.67 ± 1.15	1407.00 ± 0.00	117.33 ± 1.53	**3.08**	0.08
Cebu	*calomelanos*	As	4.01 ± 0.13	5.16 ± 0.09	86.90 ± 4.98	**22.96**	**16.84**
Visayas (Cu Mine)	*Pteris melanocaulon*	Cu	1538.33 ± 6.66	4623.33 ± 77.16	252.00 ± 0.00	**3.17**	0.05
		As	4.88 ± 0.30	1.83 ± 0.12	5.96 ± 0.31	**1.60**	**3.26**
	Blechnum orientale	Cu	255.33 ± 2.51	635.33 ± 0.58	82.17 ± 0.86	**2.81**	0.13
		As	3.68 ± 0.14	0.80 ± 0.07	0.30 ± 0.05	0.30	0.38
Silangan Mine	*Dicranopteris linearis*	Cu	151.80	49.30	5.00	0.36	0.10
Surigao		As	126.70	13.20	2.80	0.13	0.21
Mindanao (Cu–Au	*Blechnum orientale*	Cu	100.30	46.30	6.70	0.53	0.14
Mine)		As	131.50	21.20	0.50	0.17	0.02
	Cyathea sp. *(fern*	Cu	129.80	60.90	5.50	0.51	0.09
	tree)	As	161.10	4.10	2.50	0.04	0.61
Tompagon Misamis Oriental Mindanao (Au Small Scale Mine)	*Sphaerostephanos* sp.	Cu	44.60	43.80	5.90	**1.11**	0.13
Manila Mining	*Nephrolepis hirsutula*	Cu	181.50	86.20	12.20	0.54	0.14
Surigao		As	35.70	1.30	0.40	0.05	0.31
Mindanao (Cu–Au	*Pteris vittata*	Cu	134.00 ± 5.29	53.33 ± 3.21	8.13 ± 0.11	0.46	0.15
Mine)		As	64.66 ± 4.51	61.33 ± 3.78	388.00 ± 9.54	**6.95**	**6.33**
	Pityrogramma	Cu	134.00 ± 5.29	1136.66 ± 35.11	12.00 ± 0.00	**8.57**	0.01
	calomelanos	As	64.66 ± 4.51	14.00 ± 0.00	244.66 ± 21.73	**4.00**	**17.48**
	Pteris melanocaulon	Cu	184.33 ± 15.17	2190.00 ± 553.26	114.66 ± 42.01	**12.50**	0.05
		As	16.66 ± 1.15	8.56 ± 3.23	20.00 ± 17.32	**1.71**	**2.34**

Silangan, Surigao) and h) Tompagon (in Cagayan de Oro, Misamis Oriental). Various kinds of accumulator ferns were observed to thrive very well in a number of Cu–Au mines, these being: a) *Nephrolepis hirsutula* (Lepanto Mine and Manila Mining), b) *Pteris* sp. (Lepanto Mine), c) *Dicranopteris linearis* (Lepanto Mine and Silangan Mine), d) *Histiopteris incisa* (Lepanto Mine and Philex Mine), e) *Dipteris conjugata* (Philex Mine), f) *Pteridium* sp. (Philex Mine), g) *Blechnum orientale* (Philex Mine, Carmen Mine and Silangan Mine), h) *Cyathea* sp. (Silangan Mine), i) *Sphaerostephanos* sp. (Tumpagon), j) *Pityrogramma calomelanos* (Lepanto mine, Acupan, Camp 6, Carmen Mine and Manila Mining), k) *Pteris vittata* (Lepanto Mine, Acupan, Camp 6, Carmen Mine and Manila Mining) and l) *Pteris melanocaulon* (Carmen Mine and Manila Mining).

The results of analyses on the concentrations of Cu and As in the soils as well as the aboveground (shoots) and belowground (roots) components of the plants are shown in Table 1. It is noted that most of the ferns have BF values of < 1.0 indicating that these plants do not possess mechanisms for Cu and As absorption in any of their anatomical structures. It also shows that these ferns are tolerant to higher amounts of Cu and As in the soils that they thrive in. Instead of absorbing Cu and As into their system, they have the mechanism of excluding metals/metalloids in their uptake of micronutrients for plant growth [21].

There are, however, ferns that have BF values of > 1.0 indicating that the elemental concentrations in the plant are higher than that of the soil they thrive in. These ferns, which are commonly found in most of the study sites, are *P. vittata, P. calomenlanos* and *P. melanocaulon*. The range of Cu BF values of each species varies from: a) *P. vittata* (0.33–7.22); b) *P. calomelanos* (0.21–15.69) and c) *P. melanocaulon* (3.17–12.50). Other ferns that exhibited Cu BF values of > 1.0 are *Pteris* sp. (1.19), *B. orientale* (2.81) and *Sphaerostephanos* sp. (1.11). The range of As BF values also varies: a) *P. vittata* (1.50–16.69); b) *P. calomelanos* (0.82–22.96) and c) *P. melanocaulon* (1.60–1.71). With BF values of Cu and As > 1.0 these are classified as phytostabilizers of Cu and As and this capability enables them to thrive well in Cu and As enriched soils as metallophytes [16,22]. Studies have shown that *P. calomelanos,* which is a hyperaccumulator of As, Cd, and Zn, is a likely contender for Cu phytostabilization because of the dominant Cu accumulation in the roots and rhizome and hardly any translocation in the shoots [23].

There are ferns that exhibited TF values that are > 1.0. These ferns have the capability of transporting metals/metalloids from the roots to the shoots (stem and leaves). It is interesting to observe that there were no ferns from those identified that thrive in Cu and As enriched soils that exhibited Cu TF values of > 1.0. This illustrates that ferns had mechanisms that minimize the movement of Cu into the stem and leaves to avoid possible toxicity, if the uptake were in excess of what is needed for plant growth [24]. The phytotoxicity of Cu might have been reduced due to the presence of As, which some ferns tend to accumulate as part of their biological adaptation to Cu–As enriched soils [25]. The ferns with As TF values of > 1.0 are *P. vittata* (2.93–19.63); *P. calomelanos* (2.59–17.48) and *P. melanocaulon* (2.34–3.26). Another fern that has a high As TF value is *Pteris* sp (2.51). The capability of these ferns to transport As from the roots to the stem and leaves without experiencing possible phytotoxicity characterizes them as excellent extractors and therefore hyperaccumulators [26–28].

From the 2 different study sites, nickeliferous laterite and Cu–Au mines, specific ferns were identified and noted to thrive very well. These ferns are not only tolerant to the heavy metal enriched environments where they grow, but are also capable of accumulating considerable amounts of heavy metals into their anatomical systems, particularly the roots and fronds. In the nickeliferous laterite mines, *P. aguilinun*, *S. retusa* and *D. linearis* are the most dominant ferns as they are tolerant of, though not accumulators of, Fe, Co and Ni with BF values of < 1.00. However, initial observations from this study indicate that they could possibly be accumulators of Cr (TF values > 1.00), though this requires further studies and confirmation. The occurrence of these ferns is relatively low to non-existent in the Cu–Au mines. The ferns that are prominent in these areas are: *P. calomelanos*, *P. vittata*, *P. melanocaulon*, *B. orientale*, *N. hirsutula*, *H. incisa, D. conjugate*, *Pteris* sp. and *Cyatea sp*. Some of these ferns, such as *P. calomelanos*, *P. vittata* and *P. melanocaulon,* are identified as Cu and As hyperaccumulatros with TF values of >1.00 confirming previous studies on the ferns as Cu and As accumulators [15,16,29]. The other ferns appear tolerant to high amounts of Cu and As in the soils.

3.2. Post-mining rehabilitation

The concept behind rehabilitation using phytoremediation techniques is to enhance vegetation in areas normally not inhabited due to the high content of metals/metalloids which may not be conducive for plant growth [30]. Metallophytes have adapted to such conditions and their hyperaccumulation properties, in particular, could be the best option to use in mine rehabilitation. The use of hyperaccumulators to clean-up contaminated areas may take a very long period of time, but the reduction of toxicity in the soil is expected and therefore there is the possibility of other plants that are not metallophytes thriving and propagating. With the identification of hyperaccumulator ferns, particularly for Cu and As, experimental rehabilitation studies were performed in 2 selected sites, and these were Kias, Benguet and Suyoc North, Surigao. Kias is a Cu–Au ore processing plant used by small-scale miners. Small ponds were constructed to accommodate the tailings/waste from the processing of ores. The Cu and As values of the tailings vary from 1990.0 to 6112.0 mg kg^{-1}Cu and 63.1–130.0 mg kg^{-1} As. Suyoc North is an abandoned open pit Cu mine. The Cu and As values of the soil vary from 98.7 to 3390.0 mg kg^{-1} Cu and 16.8–100.0 mg kg^{-1} As. In both of the selected sites, very minimal vegetation is observed.

In Kias, *P. vittata* and *P. calomelanos* were propagated in a nursery following the protocol forwarded by [31]. Upon maturity, they were transferred to the experimental plots within the tailings pond. The growth of the plants was monitored for about 2–3 months during which time soil samples were analyzed for Cu content. The Cu values ranged from 3829.69 to about 4165.33 mg kg^{-1}. The ferns thrived very well accumulating Cu values at a range of about 43.0–294.0 mg kg^{-1} for *P. vittata*, and a range of 63.0–91.33 mg kg^{-1} for *P. calomelanos*. Building an ecosystem after 6 months promoted plant growth where other metallophytes apparently grew very well with the ferns. These plants were *Alternanthera sessilis*, *Solanum nigrum* and *Chenopodium ambrosioides*. Their growth extended to other parts of the pond, hence vegetation prospered. The Cu contents of the different metallophytes were 6598.0 mg kg^{-1} (*A. sessilis*), 2071.0 mg kg^{-1} (*S. nigrum*) and 1046.0 mg kg-1 (*C. ambrosioides*). The analyses of the different plants indicated that some have the potential capability of Cu accumulation and *A. sessilis* is of particular interest as it has a Cu BF value of 1.62. Understanding the efficiency of uptake of the plants may require further studies on the bioavailability of the metals/metalloids which are influenced by factors such as pH, organic matter, and NPK content of the soil.

At Suyoc North, *P. calomelanos* was used for the experimental rehabilitation of the abandoned open-pit Cu mine. *P. calomelanos* is an indigenous fern and naturally growing and abundant in the study site. After propagating *P. calomelanos* and cultivating it to maturity, it was transferred and planted at Suyoc North (Fig. 2). Immediate care was provided, e.g. in the form of watering and inputs of macronutrients. Sustaining the care process, however, was a challenge which resulted in an approximately 50% mortality rate. After 6 months, the soil and the ferns that survived and grew very well and were analyzed for Cu content. The original soil contained about 372 mg kg^{-1} Cu and after the experimental rehabilitation the Cu content of the soil was reduced to 263.67 mg kg^{-1}. The Cu content of the *P. calomelanos* was 689.67 mg kg^{-1} for the belowground components and about 112.67 mg kg^{-1} Cu for the aboveground components. Much of the absorbed Cu from the soil was apparently stored in the root system of the plant with minimal uptake to the stem and leaves. Further field studies on the survival of cultivated, supposedly indigenous ferns in any rehabilitation project should be pursued. Sustained care for the growth of the ferns during the rehabilitation followed an initial care program and in the process identified problems leading to the relatively high mortality rate of the ferns. One of the problems encountered was the handling of the ferns from the nursery to the rehabilitation sites which apparently was stressful for the plants. High mortality was observed when the roots of the plants were agitated during transplanting and therefore they should be handled with care as this is critical to the survival of the plants. Another problem was the time of planting, which was not during the seasonal rainfall and thus the soils were relatively dry and this might have affected the bioavailability of nutrients and led to the eventual death of some plants. It is important that the care of the plants be sustained by regularly providing water, since high rates of transpiration are expected due to the direct exposure of the plants to sunlight. Similar problems were encountered elsewhere such as the phytoremediation of As contaminated soils in Florida [32], China [33] and Australia [34]. The field trials used *P. vittata* and yielded a positive result, indicating the feasibility of more large-scale environmental clean-up projects concerning As contaminated soils. However, a number of problems were encountered: 1) the availability of substantial number of mother plants, 2) uniformity in age, 3) high transportation costs and improper handling of the plants, and 4) high stress and mortality during transport and transplanting.

3.3. Phytomining

Electrowinning is a relatively old metallurgical/chemical process of extracting metals from a solution. It uses an insoluble anode and cathode in an electrolyte cell (Fig. 3). With the use of electrical current and the reaction of metal ions, the win

Fig. 2. Photographs of post-mining rehabilitation activities. a) nursery at Xavier University where most of the ferns were propagated, b) the experimental rehabilitation site at Suyoc North (Surigao) which was completely barren of vegetation, c) rehabilitation experiment using P. calomelanos at Suyoc North.

metals of high purity are deposited on the cathode [35]. This method is also used by mining companies for extracting valuable minerals from Cu–Au ores. The use of electrowinning in bioremediation studies was initially performed by [36] in the removal of various metals from acid mine drainage. In the electrowinning experiments with the use of the nitric acid digestion method, the recovery of Cu from a solution during electrowinning was relatively high, ranging from 72 to 99%, with most of the samples having Cu recovery of > 90% (Table 2a). The Cu recovery using the *aqua regia* digestion method ranged from 42 to 96% with most of the samples having a recovery of > 80% (Table 2b). It appears that the nitric acid digestion method was more effective than *aqua regia* in recovering Cu from *P. melanocaulon*. Notable decreases in the Cu content of both solutions were observed. The recovery of Cu is already efficient at 30 min and at 1.5 V. The results of the experiments also indicated that there were no significant differences in the recovery of Cu across different times (e.g. 30, 60 and 90 min). In evaluating the effects of pH, it was noted in both solutions that there was high Cu recovery with low pH. It was also noted that there was an increase in the pH of the solution after electrowinning. The increase of the temperature of both solutions after electrowinning appears to be related to the increase of voltage which apparently increased Cu recovery. The increases in temperature are likely to put the metal ions into a more mobile state in the solution and thus increase the plating efficiency. The assessment of the overall efficiency of electrowinning shows that there is relatively high Cu recovery at low voltage as well as in a short period of time and this indicates the practical application of having an

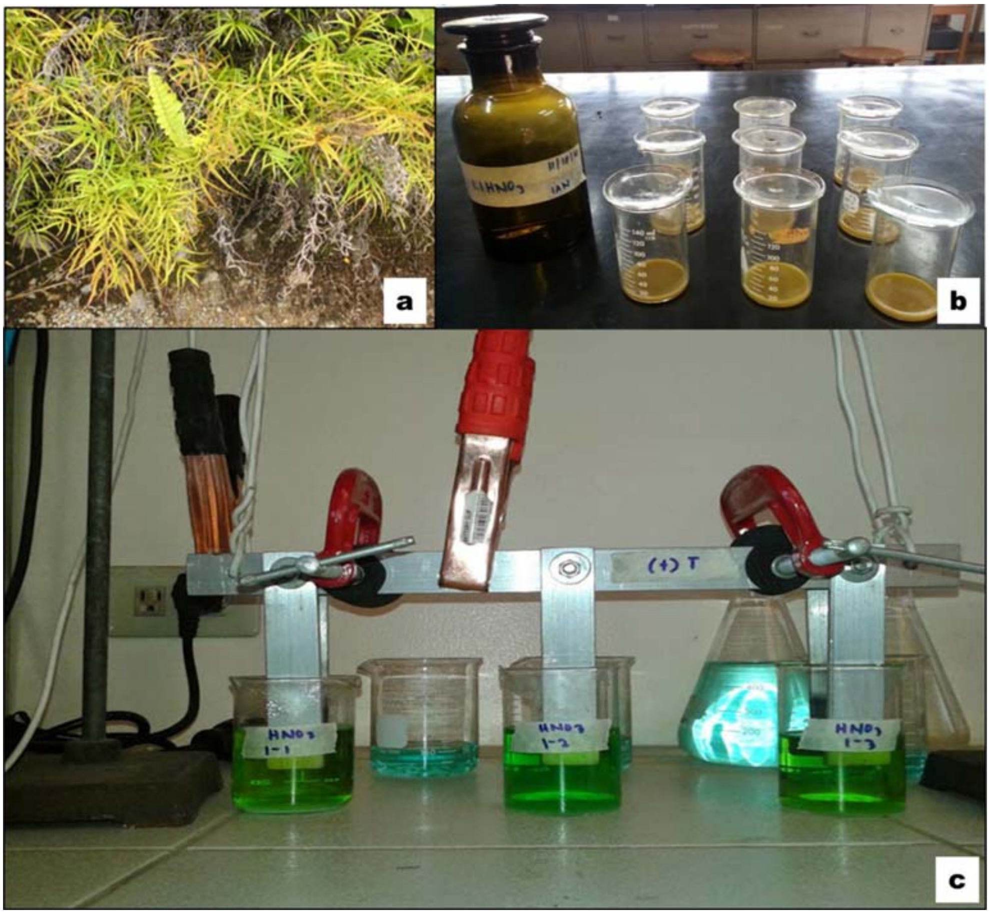

Fig. 3. Photographs on phytoextraction setups. a) P. melanocaulon was used as plant samples in the phytoextraction experiments, b) digested root components of P. melanocaulon in nitric acid. c) Electrowinning setup with 3 voltage variables considered. The greenish and bluish solutions are the results of tests using concentrated and diluted nitric acids.

electrowinning setup which leads to appreciable amounts of time and energy being saved in the recovery of Cu from hyperaccumulator plants. Due to the difficulty of using strong acids, such as nitric acid and *aqua regia*, the alternative of dry ashing the plant samples is recommended and after this there

Table 2b. Average concentrations (mg kg^{-1}) of Cu in the roots of P.melanocaulon digested with aqua regia before and after electrowinning. Percentages of Cu recovery are provided at different times and voltages.

Voltage	30 min			60 min			90 min		
	Before (mg kg^{-1})	After (mg kg^{-1})	%	Before (mg kg^{-1})	After (mg kg^{-1})	%	Before (mg kg^{-1})	After (mg kg^{-1})	%
1.5	8053 ± 2073	715 ± 489	91	8813 ± 874	667 ± 402	92	8610 ± 452	340 ± 207	96
3	6899 ± 832	3979 ± 3630	42	7374 ± 2773	728 ± 237	90	4665 ± 134	571 ± 564	88
6	5831 ± 383	689 ± 20	88	5735 ± 146	415 ± 132	93	5256 ± 705	658 ± 462	87

Table 2a. Average concentrations (mg kg^{-1}) of Cu in the roots of P.melanocaulon digested with nitric acid before and after electrowinning. Percentages of Cu recovery are provided at different times and voltages.

Voltage	30 min			60 min			90 min		
	Before (mg kg^{-1})	After (mg kg^{-1})	%	Before (mg kg^{-1})	After (mg kg^{-1})	%	Before (mg kg^{-1})	After (mg kg^{-1})	%
1.5	7409 ± 4013	2082 ± 930	72	4519 ± 1677	151 ± 25	96	7756 ± 923	119 ± 11	98
3	8291 ± 3086	114 ± 29	98	7675 ± 548	110 ± 14	98	7353 ± 1654	103 ± 25	98
6	10 337 ± 3363	178 ± 22	98	11 820 ± 3729	139 ± 29	98	10 923 ± 2180	86 ± 94	99

is the possibility of using weaker acids in the digestion processes.

4. Conclusions and recommendations

Indigenous ferns thrived very well in areas poorly inhabited by other plant species due to the enrichment of metals/metalloid in the soil. The identified ferns in this study are classified either as excluders or as extractors depending on their naturally/adaptive uptake mechanisms. The identification took into consideration the concentrations of heavy metals in the soil as well as in the AG and BG components of the plant. The concentration values were used to determine the BF and TF ratios. Ferns that are classified as excluders commonly have BF values of >1.00 and for the extractors the TF values are commonly >1.00. *P. vittata*, *P. calomelanos* and *P. melanocaulon have* TF values of As are >1.00 with *P. melanocaulon* also exhibiting BF values for Cu of >1.00. With BF and TF values of >1.00, these ferns are the best candidates for post mining rehabilitation and phytomining. In mine rehabilitation, these ferns can partly reduce the Cu and As content of the soil, paving the way for other plants, which are normally not tolerant of the contaminated soil, to survive and thrive very well, increasing the vegetative potential of the area. These hyperaccumulator ferns can also be harvested as raw materials for Cu recovery using electrowinning processes. It is highly recommended that further studies be carried out on the simultaneous or sequential recovery of associated heavy metals of Cu using electrowinning. This would be significant in providing assurances that the solutions used in the recovery processes are not released into the environment as contaminated wastewater. It is also recommended that application research be performed on the rehabilitation process to increase the survival of the ferns and on other recovery processes to efficiently extract the heavy metals from the plants.

Conflicts of interest

None declared.

Ethical statement

The authors state that the research was conducted according to ethical standards.

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