

Research Article

Copper and arsenic accumulation of *Pityrogramma calomelanos*, *Nephrolepis biserrata*, and *Cynodon dactylon* in Cu- and Au- mine tailings

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Received 4 February 2020, Accepted 3 March 2020

Abstract: Metallophytes are group of plants that can thrive on metal-rich substrate. These plants have potential in various green technologies. However, it is a must to first identify plants that can absorb heavy metals and tolerate the high concentration in their tissues. This study assessed the ability of plants thriving in a Cu-Au mined areas to uptake copper (Cu), and arsenic (As). The Cu and As content of the dried leaves, root tissues and soils were quantified using Atomic Absorption Spectrophotometer (AAS), and their bioaccumulation coefficient (BAC) were computed. Three species, *Pityrogramma calomelanos*, *Cynodon dactylon* and *Nephrolepis biserrata*, showed metal accumulation in the plant tissues. The three species have accumulation of Cu in the root and the estimated bioconcentration factor (BCF) is more than 1.0 which indicates the ability of these species to tolerate for said the metal hence is a good candidate for phytostabilization of polluted soils. Noteworthy was the accumulation of As in the shoot of the three species despite of the low soil As (<0.01 µg/g). *Nephrolepis biserrata* had the highest arsenic bioaccumulation factor of 30.91 followed by *Cynodon dactylon* (11.01) then *Pityrogramma calomelanos* (8.78) which make them potential species for clean-up of As through phytoextraction. Moreover, this study added *C. dactylon* as tolerant of arsenic in mined-out area in the Philippines.

Keywords: *cuprophytes, phytoextraction, pseudometallophytes, phytostabilization*

To cite this article: Ancheta, M.H., Quimado, M.O., Tiburan, C.L. Jr., Doronila, A. and Fernando, E.S. 2020. Copper and arsenic accumulation of *Pityrogramma calomelanos*, *Nephrolepis biserrata*, and *Cynodon dactylon* in Cu- and As- mine tailings. *J. Degrade. Min. Land Manage.* 7(3): 2201-2208, DOI: 10.15243/jdmlm. 2020.073.2201.

Introduction

Heavy metals are conventionally defined as elements with metallic properties and an atomic number > 20 are among the contaminants in the environment (Blaylock and Huang, 2000). The most common heavy metal contaminants are cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn) (Cho-Ruk et al., 2006). According to Pehlivan et al. (2009), metal pollution has harmful effects on biological

systems and does not undergo biodegradation. Recent concerns regarding environmental contamination have led to the development of appropriate technologies for metal cleaning in soil, water, and wastewater. At present, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil using plants. This technology is environment-friendly and potentially cost effective

(Erakhrumen and Agbontalor, 2007). For the restoration of deteriorated and metal-contaminated soils, the discovery and identification of plants that have the capacity to withstand and/or accumulate heavy metals are important.

Metallophytes or plants that can survive and grow on metal-rich substrates are generally classified into two, true metallophytes and pseudo-metallophytes. True metallophytes or plants that are native on natural metalliferous soils are divided into obligate and facultative. Obligate metallophytes cannot live outside their natural geographical distribution while facultative can grow outside their natural distribution range (Baker et al., 2010). In general, pseudo-metallophytes are species that are not native on natural metalliferous soils and can thrive on both metalliferous and normal soils (Dechamps et al., 2011). Further, various groupings are made depending on the metal that the metallophytes absorb. Cobaltophytes accumulates cobalt while cuprophytes absorb copper (Brooks et al., 1980).

Copper and arsenic are two of the common problem metals in metal contaminated soils. Discovery of copper hyperaccumulators is very important for phytoremediation of copper-contaminated soil. According to Kramer (2010), about 35 species belonging to 15 families accumulate copper. Copper tolerance and accumulation have been studied in *Haumaniastrum katangense*, a cuprophyte from Katanga (DR Congo), previously described as a copper hyperaccumulator (Chipeng et al., 2009). Another copper accumulator based on the study of Faucon et al. (2012) is *Crepidorhopalon tenuis* (pseudometallophytes of Katanga), the accumulation of copper ranges from 80-1400 mg/kg in shoots and 61-1105 mg/kg in roots, respectively. Wang et al. (2004) studied the copper accumulation in hyperaccumulator *Commelina communis* and its responses of antioxidative enzymes, including superoxide dismutase, guaiacol peroxidase, and ascorbate peroxidase. Holyoak and Lockhart (2011) studied the copper tolerant species of bryophytes in a copper mine spoils in Ireland. Some of the described are obligate Cu bryophytes; *Cephaloziella massalongi*, *Cephaloziella nicholsonii*, *Ditrichum cornubicum* and *Scopelophila cataractae*.

The most popular and very well studied ferns that accumulate copper are *Pteris vittata* (Chen et al., 2003; Silva Gonzaga et al., 2006; Zhang et al., 2004; Zheng et al., 2008) and the widespread *Pityrogramma calomelanos* (Francesconi et al., 2002). The accumulation capability of *Pityrogramma calomelanos* is 1,505 µg/g in the root part and 151 µg/g in the shoot part (De la Torre et al., 2015). As for the study conducted by Dahilan

and Dalagan (2017) they reported that the uptake of Cu in the belowground tissue of *Pityrogramma calomelanos* ranges from 262 µg/g to 6,883.32 µg/g. Another species of fern found out to accumulate copper is *Pteris melanocaulon* that have been studied by De la Torre et al. (2015). This species can accumulate high concentrations of copper in the root but in contrast to *Pteris vittata* and *Pteris melanocaulon* there is a shortage of documentation on the metal accumulation potential of this species. *Cynodon dactylon* is another species that has been reported to tolerate high amount of copper. Shu et al. (2002) revealed that *C. dactylon* collected from mine tailings have evolved ecotypes tolerant to Cu, Pb, and Zn and is therefore potential for re-vegetation of wastelands contaminated with these three metals. On the other hand, *C. dactylon* collected from polluted sites along the urban stream sediments of Nakivubo drainage ecosystem in Kampala, Uganda also accumulates Cu in the shoot (Sekabira et al., 2011).

Arsenic is a nonessential element for plants, and inorganic arsenic species are generally highly phytotoxic. Arsenic hyperaccumulation has been described for a number of *Pteris* species most notably *Pteris vittata* with up to 22,630 µg/g of As concentration in the shoot parts as studied by Ma et al. (2001) and Wang et al. (2002) and other ferns such as *Pityrogramma calomelanos* with up to 8,350 µg/g of As (Francesconi et al., 2002). *Pteris vittata* has been shown to be capable of taking up both inorganic and organic arsenic species, including arsenate, arsenite, and monomethylarsonic acid, concentrating up to 93% of the arsenic in the fronds (Ma et al., 2001; Kertulis et al., 2006). Their research on arsenic hyperaccumulation by *Pteris vittata* showed that arsenic exists in the plant mostly as inorganic species, and up to 94% of the arsenic in the fronds is present as arsenite. Similarly, in a study involving *Pityrogramma calomelanos*, most of the arsenic found in its fronds was arsenite. Chen et al. (2003) studied *Pteris vittata* in control pot trials with normal unpolluted soil containing 9 mg/kg of arsenic and found out that the bioaccumulation coefficient of the above ground parts and rhizoids of *Pteris vittata* were as high as 71 µg/g and 80 µg/g. Their study also revealed that *Pteris vittata* can grow rapidly with great biomass, wide distribution and easy adaptation to different environmental condition and therefore has a great potential in future remediation of arsenic contaminations.

This paper aimed to a) determine the heavy metal accumulation of plants growing in a copper and gold-mined area, and b) estimate the potential of the identified plants in phytoremediation of copper and arsenic metals. This research identified

plants that are capable of accumulating copper and arsenic in a mined-out area located in Nueva Vizcaya, Philippines that can be used in the phytostabilization and phytoextraction of heavy metals.

Materials and Methods

Site description

The study area is in a Cu-Au mine site in Didipio, Kasibu, Nueva Vizcaya. Three sampling sites within the mining tenement were chosen based on high category of mining activities, namely, mine tailing storage facility, mined pit and crusher. The plant assessment was focused on the three main sites where the mining activities are frequent, and the concentrations of heavy metals are high. For mined pit, three sub-sampling sites were surveyed namely bottom, mid and top.

Plant and soil sampling

The sampling method employed was opportunistic sampling (Brower et al., 1998). All plant species growing in the crusher area, mining pit, and the mine tailings were collected. Soil debris attached to each individual plant roots were removed manually and added to the composite soil samples. Herbarium voucher specimens were collected and deposited at the Forestry Herbarium (LBC), UPLB Museum of Natural History. For each plant sample, a representative soil sample was also taken from 0-25 cm depth of the rhizosphere. Weight of each sample was about 250 g, sufficient to yield 30-50g of representative sample powder used for the heavy metal analyses. The samples were excavated with a spade. The rhizosphere samples were mixed to form a composite. The soil samples were air-dried for 5-7 days. Residues including tiny roots of plants were removed before the soils were ground and sieved.

Plant and soil analyses

The collected plant samples were thoroughly washed with running water. After washing, the shoots of each plant were separated from their roots and were put in separate brown bags and oven dried at 60°C for three days or until the weight of the samples were already constant. The grinding of each sample using Wiley (Thomas Model 4) followed and preliminary screening for heavy metals was done using XRF (Thermo Scientific NDTTr-XL3-97002). All plant species that accumulated >100 µg/g of heavy metals based on XRF results including its representative rhizospheric soil samples were taken to the OSTREA Laboratories for AAS analysis.

Estimation of bioaccumulation factors

The analysis of the data includes the determination of the bioaccumulation factor (BAC) and bioconcentration factor (BCF) which are essentially the parameters that will serve as a key measure in determining the phytoremediation potential of a plant (Pachura et al., 2015).

Results and Discussion

Vegetation analysis

A total of ten species composed of 19 individuals belonging to five families were recorded from the three sampling areas. The most abundant species collected belong to Asteraceae with four species followed by Poaceae (2), Amaranthaceae (1), Pteridaceae (1), and Nephrolepidaceae (1). Most plants of the fern families (e.g. Pteridaceae, Nephrolepidaceae) thrive well in metal enriched soils such as those found within the mining area. Two fern species found abundantly growing in the mining area are *Pityrogramma calomelanos* and *Nephrolepis biserrata* (Figure 1). The former is present on all sampling areas while the latter is present only on the mine pit and crusher. Table 1 lists all the species of plants found growing in the sites of collection. The TSF has the most number of species of plants and *Cynodon dactylon* (Figure 1) was only collected from this site. *Celosia argentea* were observed to have purplish leaves while *Mikania cordata* and *Blumea lacineata* have purplish leaves and stems compared to the same species growing outside the collection sites. The purplish color is good morphological indicator for the three species growing on copper-rich soil. It is possible that the three species are producing a new ecotype. Shu et al. (2002) reported Cu tolerant ecotypes of *C. dactylon*, however, there is no report about different ecotypes from a plant species with different tolerance to As. The identification of tolerant ecotypes among metallophytes and the understanding of physiological mechanisms of tolerance is a key research to be able to implement phytoremediation (Verbruggen et al., 2009).

Soil analysis

The physico-chemical characteristics of the soil displayed that the soil pH is near neutral ranging from 6.31 to 7.3 for all sampling points (Table 2). As for the organic matter, all the sampling sites have 0.30% w/w and this can be described as low based on the descriptive scale provided by Nguyen et al. (1987). This can be attributed to the less vegetation and the excavation of the topsoil. The amount of calcium and magnesium are very low, but calcium is 5-10 times higher than magnesium.

Table 1. List of plants growing in the sampling areas in Didipio, Kasibu, Nueva Vizcaya.

Species	Location			Remarks
	Site 1 (TSF)	Site 2 (MP)	Site 3 (CRU)	
<i>Celosia argentea</i> L.	/	/	/	Pinkish to Reddish leaf color
<i>Imperata cylindrica</i> (L.) Raeusch.	/	/	/	
<i>Pityrogramma calomelanos</i> (L.) Link	/	/	/	
<i>Cynodon dactylon</i> (L.) Pers.	/	X	X	
<i>Crassocephalum crepidioides</i> (Benth.) S.Moore	/	X	/	
<i>Neprolepis biserrata</i> (Sw.) Schott	X	/	/	
<i>Mikania cordata</i> (Burm.f.) B.L.Rob.	/	X	/	Purplish leaf and stem color
<i>Desmodium sequax</i> Wall.	X	/	X	
<i>Blumea lacineata</i> (Wall. ex Roxb.) DC.	/	X	X	Purplish leaf and stem color
<i>Conyza sumatrensis</i> (S.F.Blake) Pruski & G.Sancho	/	X	X	
Total	8	5	6	

*/-present X-absent *TSF-Tailing Storage Facility *CRU-Crusher * MP- Mined Pit.

The soil physico-chemical characteristics of the soils in the sampling areas are unfavorable for the growth of ordinary crops resulting to very low number of species of plants in the collection sites. However, these parameters could have been conducive for the growth of *Pityrogramma calomelanos* as manifested by its widespread growth in the area.

Table 2. Physico-chemical characteristics of the rhizospheric soil in the three sampling areas.

Soil Parameter	Location		
	TSF	CRU	MP
pH	6.31	6.9	7.3
Organic matter	0.30	0.30	0.30
Electrical conductivity (mS/cm)	948	318	361
Cation exchange capacity	11.19	10.86	10.77
Soluble Calcium (Ca)	9.98	10.26	11.38
Soluble Magnesium (Mg)	1.51	2.18	0.50
Bulk Density	1.48	1.58	2.23

TSF-Tailings Storage Facility, MP-Mined Pit, CRU-Crusher.

The Cu values in the rhizospheric soil were high which is expected in a Cu-Au rich mining site. The Cu content in the soil ranged from 1,909 to 6,729 ug/g (Table 3). The Cu content of the soils vary with some areas having higher Cu contents than the others and this could be attributed to the weathering of unmineralized rocks and those with

Cu bearing ore minerals (Chen et al., 2013). On the other hand, the arsenic rhizospheric content ranged from <0.01 µg/g to 9.39 µg/g.

Heavy metal contents of plant tissues

Copper

Pityrogramma calomelanos collected from the crusher area exhibited the highest content in the root system (5,924 µg/g) among the three plant species. The other two species analyzed were also found out to accumulate higher concentration in the root than in the shoot. *Neprolepis biserrata* and *Cynodon dactylon* accumulated 5,756 µg/g and 2,250 µg/g respectively. Similar observations were noted in a study by Dahilan and Dalagan (2017) in which *Pityrogramma calomelanos* also had higher accumulation in the root than in the shoot tissue. Their study showed that the accumulation of copper in the root is 262 to 6,883 mg/kg. In the study of Shu et al. (2002), *Cynodon dactylon* was also described as a metal tolerant plant. They also found out that *Cynodon dactylon* and *Paspalum distichum* have been able to withstand higher concentrations of metals than the control populations, and might have evolved co-tolerance to Pb, Zn and Cu. The calculated bioconcentration factor of *Pityrogramma calomelanos* is 2.32 (Table 3). The high BCF of 2.32 indicated the capability of the fern to accumulate Cu from the soil into its biomass particularly the root components and would suggest the potential of the fern for rhizofiltration and phytostabilization (Niazi et al., 2012). *Neprolepis biserrata* and *Cynodon dactylon* also exhibited bioconcentration factor of more than 1 which means they are possible species

for phytostabilization of Cu (Table 4) and conferring to the study of Shu et al. (2002). It is also commonly known that *C. dactylon* can tolerate adverse edaphic conditions, including metal toxicity is widespread in temperate and tropical areas and also thrives on toxic mine wastes (Bradshaw and Chadwick, 1980).

Arsenic

The study of Matschullat (2000) stated that the normal concentration of arsenic that a plant can tolerate in uncontaminated soil is up to 6 mg/kg and the highly toxic is 1500 mg/kg and can reach up to 30,000 µg/g in a contaminated soil (Vaughan, 1993). Arsenic is commonly associated with ores such as copper, lead and gold and can be released during mining and smelting processes (Rathinasabapathi et al., 2006; Adriano, 2001). The results of the soil analysis in this study showed that the amount of arsenic present in the rhizospheric soil was low (<0.01 mg/kg). However, *Pityrogramma calomelanos* thriving in the mining pit was found to accumulate 280.18 mg/kg of As in the aboveground tissues and 161.82 mg/kg in the

root part. The level of accumulation in the plant tissues is hundred times the amount of As in soil which indicates that the species is an accumulator (Table 3). This was similar to the study of Ma et al. (2001) wherein they studied the ability of the fern *Pteris vittata*, that can accumulate as much as 1,500 µg/g arsenic into its fronds in a short time. Gonzaga et al. (2006) and Francesconi et al. (2002) reported that aside from *Pteris vittata*, *Pityrogramma calomelanos* was found to exhibit the same hyperaccumulating characteristics. Francesconi et al. (2002) studied the fern *Pityrogramma calomelanos*, an As hyperaccumulator and accumulates mostly in the fronds with up to 8,350 µg/g while the rhizoids contain the lowest concentrations of arsenic with a range of 88-310 µg/g.

In this study, *Pityrogramma calomelanos* also accumulated higher concentration of arsenic in the leaves (44-280 µg/g) and (<0.01-161 µg/g) in the roots, but the values did not meet the hyperaccumulation requirement of 1,000 µg/g for arsenic in the shoot part.

Table 3. Copper and arsenic concentrations in tissues and rhizospheric soils of *Pityrogramma calomelanos*, *Nephrolepis biserrata*, and *Cynodon dactylon* collected from the different sampling sites.

Species/ Site	Plant Tissue	Cu (mg/kg)	As (mg/kg)
<i>Pityrogramma calomelanos</i> / CRU	Leaves	174.43	44.73
	Roots	5924.14	<0.01
	Soil	2544	9.39
<i>Pityrogramma calomelanos</i> / TSF	Leaves	181.31	50.52
	Roots	1694.46	<0.01
	Soil	6729.35	5.79
<i>Pityrogramma calomelanos</i> / MP	Leaves	34.67	280.18
	Roots	578.08	161.82
	Soil	1446.23	<0.01
<i>Nephrolepis biserrata</i> / CRU	Leaves	191.41	153.96
	Roots	5756.07	<0.01
	Soil	5947.31	4.98
<i>Cynodon dactylon</i> / TSF	Leaves	20.65	47.34
	Roots	2250.32	<0.01
	Soil	1909.1	4.38

TSF-Tailings Storage Facility, MP-Mined Pit, CRU- Crusher.

Aside from the *Pityrogramma calomelanos*, *Nephrolepis biserrata* was also found to accumulate arsenic in the leaves which is 153.96 mg/kg (Table 2). *Cynodon dactylon* also accumulated more arsenic in the shoot (47 µg/g) than in the root (<0.01 µg/g) but not categorized as hyperaccumulator because the values did not likewise meet the hyperaccumulation level requirement.

Pityrogramma calomelanos, *Nephrolepis biserrata*, and *Cynodon dactylon* have a

bioaccumulation factor of greater than 1 which indicates that the species is an accumulator of arsenic. *Nephrolepis biserrata* was found to have the greatest bioaccumulation factor of 30.91 among the two species followed by *Cynodon dactylon* (Table 3).

Wang (2012) stated that the greater the BAC, the stronger the accumulation of the heavy metals in the plant, and greater BAC of more than 1 indicates the ability of the species in phytoextraction of arsenic. The high BAC of the

three species suggests that they are potential species for phytoremediation of arsenic through phytoextraction.

Based on the results of accumulation of copper and arsenic by the three species, the mechanisms of phytoremediation to be employed are phytoextraction and phytostabilization. *Pityrogramma calomelanos*, *Nephrolepis biserrata* and *Cynodon dactylon* plants showed capacity of growth and high copper phytoaccumulation in the roots after growth in copper -gold mined sites. Thus, phytostabilization with these species with

high potential of copper accumulation is the most suitable and feasible solution for remediation of the copper polluted soils. The translocation of heavy metals from the roots to shoots is important for phytoremediation where the shoots are easily harvested. In the copper mining area, this practice can be very useful, because of the characteristics of these areas such as unstructured, wide and plan with low soil loss by erosion, the removal of the total plant can be made mechanically and cost-friendly to the environment (Karczewska et al., 2015).

Table 4. Plant–soil bioconcentration ratios of copper and arsenic in *Pityrogramma calomelanos*, *Nephrolepis biserrata*, and *Cynodon dactylon* collected in the sampling areas in Nueva Vizcaya.

Species/ Site	Cu		As	
	BCF	BAC	BCF	BAC
<i>Pityrogramma calomelanos</i> / CRU	2.32	0.06	0.001	4.76
<i>Pityrogramma calomelanos</i> / TSF	0.25	0.3	0.001	8.78
<i>Pityrogramma calomelanos</i> / MP	0.4	0.42	<LOD	<LOD
<i>Nephrolepis biserrata</i> /CRU	1.03	0.07	0.002	30.91
<i>Cynodon dactylon</i> /TSF	1.18	0.19	0.002	11.01



Figure 1. Species that showed copper and arsenic accumulation, A) *Pityrogramma calomelanos*, B) *Nephrolepis biserrata*, and C) *Cynodon dactylon* in the sampling areas.

Phytoextraction of copper can be enhanced through the use of high biomass and fast-growing plant, an alternative to hyperaccumulating plant (Terry and Banuelos, 2000; Raskin and Ensley, 2000). Although the copper in the root system in *Pityrogramma calomelanos* plant was high, phytoextraction can be more efficient when the entire plant is harvested, so it is suggested to study the phytoextraction ability of *Pityrogramma calomelanos*.

Conclusion

The study identified 10 species of pseudometallophytes that grow in copper mined areas. *Pityrogramma calomelanos*, *Nephrolepis biserrata* and *Cynodon dactylon* showed potential abilities for phytostabilization of copper on copper-contaminated soil. The same three species also

showed potential for phytoextraction of arsenic. Phytoextraction of arsenic should be accompanied by appropriate technology for prevention of release of the accumulated arsenic to the environment. Another application of the three species is on revegetation of copper mined areas which is a real challenge. The three species of pseudometallophytes that were observed to grow and reproduce on such area are good ground cover for the build-up of organic matter and for eventual plant succession.

Acknowledgements

Financial support for this research was provided by a grant from the Philippines Department of Science and Technology (DOST) - ASHTRDP. Sincere thanks to Oceana Gold Mining Inc. Didipio, Nueva Vizcaya for the accommodation, thesis grant, and logistics during the fieldwork.

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Title:

Copper and arsenic accumulation of Pityrogramma calomelanos, Nephrolepis biserrata, and Cynodon dactylon in Cu- And Au- mine tailings

Date:

2020-04-01

Citation:

Ancheta, M. H., Quimado, M. O., Tiburan, C. L., Doronila, A. & Fernando, E. S. (2020). Copper and arsenic accumulation of Pityrogramma calomelanos, Nephrolepis biserrata, and Cynodon dactylon in Cu- And Au- mine tailings. *Journal of Degraded and Mining Lands Management*, 7 (3), pp.2201-2208. <https://doi.org/10.15243/jdmlm.2020.073.2201>.

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