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The metallophytes in the ultramafic soil of mt. Kiamo in Malaybalay, Bukidnon, Philippines

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Abstract

Forests over ultramafic soils are considered rarest. Ultramafic soils contain high concentrations of metal elements. Species colonizing such ecosystem are termed metallophytes because these are able to accumulate heavy metals and grow normally without exhibiting phytotoxicity. In determining the metallophytes species, ten 20x20m sampling plots were established within the forest vegetation of Mt. Kiamo. All plants within the plots with ≥5 cm diameter at breast height (dbh) were identified, measured and recorded. Samples of roots and shoots of the most dominant species determined through quantitative analyses were collected and analysed to determine the hyperaccumulation potentials. Seven plant species with highest species importance value were identified consisting of *Madhuca sp.*, *Elaeocarpus merritii* Merr., *Falcatifolium gruezoi* de Laub., *Scaevola micrantha* C.Presl., *Calophyllum soulattrii* Burm.f., *Polyosma integrifolia* Blume, and *Agathis philippinensis* Warb. Majority of the species accumulated extremely high varying levels of Fe, Mn and Cr while only *S. micrantha* could not be classified as Mn-hyperaccumulator based on shoot-root quotient. Both *F. gruezoi* and *P. integrifolia* are Ni-hypertolerant while *F. gruezoi* and *A. philippinensis* are Cu-hyperaccumulator. With the hyperaccumulation and hypertolerance exhibited by these species, we suggest to tests these plants under controlled condition to further verify if such characteristic is inherent to the taxon.

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Introduction

The Philippine archipelago is blessed with various types of forest formations and among them is the forest over ultramafic soil which is considered as the rarest. This forest ecosystem usually possessed unique vegetation and/or species composition coupled with high rates of species endemicity. However, due to the rich mineral content, ultramafic soils are exploited to mining hence, among the extremely threatened ecosystems. Ultramafic soils contain high concentrations of heavy metal elements and are classified as metalliferous soils. In most cases, this soil classification has extremely poor conditions, however, many plant species had evolved to adapt to such extreme conditions and are called 'metallophytes'. Metallophytes growing naturally on metal-rich soils were able to accumulate heavy metals in their biomass with minimal or no adverse effect on physiological processes, or without exhibiting phytotoxicity (Baker and Brooks, 1989; Reeves and Baker, 2000). Some metallophytes can accumulate very high concentrations of metallic or metalloid elements in their aerial tissues to levels exceeding their normal physiological requirement (Reeves, 2003). These plants are known to be hyperaccumulators. Hyperaccumulating plant species represent perhaps the ultimate in plant tolerance to extremely hostile edaphic environments that would kill many other species (Bondada and Ma, 2003). Lambinon and Auquier (1964) differentiated the plant species colonizing metalliferous soils into metallophytes and pseudo-metallophytes. Metallophytes are species found only on metal-rich soils while the pseudo-metallophytes could be found on both contaminated and uncontaminated soils within the same region. According to Baker and Brooks (1989), metal hyperaccumulation is generally restricted to species growing at a given locality due to a great variation in physical, chemical and biological factors which exist among contaminated areas. Further, metallophyte taxa either grow exclusively on metalliferous soils or in distinct phytogeographic areas and have very restricted geographical distributions, thus, correspond to rare species (Reeves, 1992). Although they are widely distributed in different genera and families, hyperaccumulators represent low percentage on all angiosperm (Baker *et al.*, 1999). About 450 angiosperm species have been identified so far as heavy metal (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn,) hyperaccumulators, accounting for less than 0.2% of all known species (Rascio and Navari-Izzo, 2010). Much of these species are least explored in the Philippines. Thus, this study was conducted to identify other potential metallophytes species as an addition to the few-known hyperaccumulator species in the Philippines that could be of future use particularly in the remediation and rehabilitation of heavy metal-contaminated sites.

Materials and methods

Study site

The study was conducted in Mt. Kiamo, Kibalabag, Bukidnon located approximately 12.5 km away from Malaybalay City, Philippines with geographic coordinates of 8.25409 North and 125.15610 East (Fig. 1). The climatic type of Mt. Kiamo falls under Type IV or intermediate B type based on Corona system and is characterized by evenly distributed rainfall throughout the year, with no distinct dry season and maximum rain period observed is from October to February. Mount Kiamo form part of the ultramafic areas in northern Mindanao as identified by Fernando *et al.* (2008).

Because of continued forest destruction and conversion to agriculture, Mt. Kiamo is consisted of forested areas and a vast marginal land. The marginal land was apparently an abandoned kaingin area indicated by the presence of few standing charred snags which can be observed at elevations 1,193 to 1,300 masl. The forested landscape occurring at elevations 1,400 to 1,790 masl is oriented on North-East cardinal direction. A preliminary survey was conducted on the month of October 2014 to assess the general topographic conditions as well as the possible location of the sampling plots. Establishment of sampling plots and data collection were conducted from the months of November to

January 2015.

Sampling plots

Ten (10) 20 x 20m sampling plots were randomly established from elevations 1,543 to 1,783 masl to sample the vegetation at the lower, mid, and topmost elevation. The plots on the lower elevation were located at 1,543 to 1,590 masl; mid-elevations at 1,600 to 1,630 masl; and top elevations at 1,721 to 1,783masl. All tree species with ≥ 5 cm diameter at breast height (DBH) within the sampling plots were identified and recorded. Diameters of individual trees were determined using a tree caliper. Voucher specimens were collected for species that needs further verification. Observations on bark exudates, presence of buttresses and stilt roots as well as cauliflory and ramiflory were also noted to facilitate the identification of species.

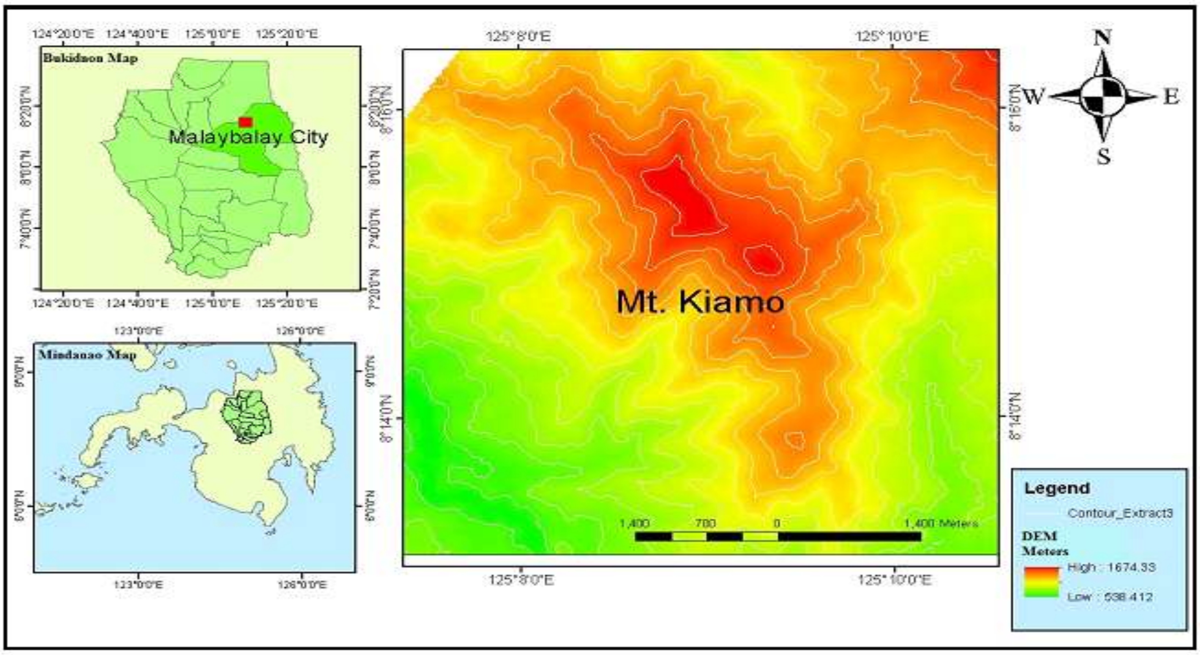

Fig. 1. Map of the study site.

Specimen identification

Identification of species was done with the aid of taxonomic keys and literatures from floras of Merrill, E.D. (1923-1926); Madulid, D. A. (1992); Rojo, J.P. (1999);Fernando *et al.* (2004) and (2008); Co *et al* (2006); Pancho & Gruezo (2006); LaFrankie (2010); van Balgooy (1997). The www.philippineplantlist.org was also used to compare voucher specimens to live photographed species while the updated botanical names were verified via www.ipni.org and www.theplantlist.org.

Quantitative analysis

The variables for measuring the magnitude of species, the following parameters were used: species density, dominance and frequency this will allow the determination of the overall importance of each species and the structure of the community (Curtis, 1959). Species with the highest importance values are considered as the most dominant species. The following equations were used:

$$\text{Dominance} = \frac{\text{Basal area of a species}}{\text{Area of the Plot}}$$

$$\text{Frequency} = \frac{\text{Number of plots where a species occur}}{\text{Total number of Plots}}$$

$$\text{Density} = \frac{\text{Total number of individual of a specie}}{\text{Total number of Plots}}$$

$$\text{Relative density of each species} = \frac{\text{Density of each species}}{\text{Density of all species}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Number of trees per species}}{\text{Total number of trees (n)}} \times 100$$

$$\text{Relative dominance} = \frac{\text{Basal area of each species}}{\text{Basal area of all species}} \times 100$$

$$\text{Importance value} = \text{relative density} + \text{relative dominance} + \text{relative frequency}$$

Soil sampling

A composite soil sample was collected from each plot to determine the physicochemical properties and heavy metal content. Sampling was done by collecting soil from the surface up to 30cm-depth on ten randomly distributed points. Collected soils were mixed thoroughly and a 2-kg representative sample was taken and placed on tight-sealed container for analyses.

Collection of plant samples

Samples of roots and shoots of the plant species with highest importance values (IV) based on quantitative analysis were collected on ten (10) randomly chosen individuals per species per plot. All roots and shoots samples were mixed separately and a composite sample on both roots and shoots was taken.

Analysis of plant and soil samples

Both soil and plant tissue samples were sent to the University of the Philippines Los Baños (UPLB) - National Biotech laboratory for analyses of heavy metal uptake. Prior to shipping, samples of plant tissues were grounded and oven-dried at 80°C for 5 days while the soil samples were air-dried, pulverized and screened using 2mm wire mesh. Both soil and plant samples were analyzed via X-ray Fluorescence (XRF) and atomic absorption spectrophotometry (AAS). Other variables such as OM and pH and texture were also determined at the Soil and Plant Tissue Laboratory of Central Mindanao University.

Results and discussion

Quantitative vegetation analysis

There were seven (7) species that obtained the highest importance value (IV) and are considered as the most dominant species in Mt. Kiamo (Table 1). These include the following: *Polyosma integrifolia* Blume (Escalloniaceae), *Calophyllum soulattri* Burm.f. (Clusiaceae), *Scaevola micrantha* C.Presl. (Goodeniaceae), *Falcatifolium gruezoi* de Laub. (Podocarpaceae), *Madhuca sp.* (Sapotaceae), *Elaeocarpus merrittii* Merr. (Elaeocarpaceae), and *Agathis philippinensis* Warb. (Araucariaceae). *Falcatifolium gruezoi* and *Agathis philippinensis* were the only gymnosperms among the group (Table 1).

Table 1. Species with highest importance value in Mt. Kiamo.

Species	Occurrence	Density	Dominance	Relative Dominance	Relative Frequency	Relative Density	SIV
Calophyllum soulattri Burm.f.	3	58	24.404	10.173	3.191	11.373	24.737
Madhuca sp.	3	32	20.584	8.581	3.191	6.275	18.047
Polyosma integrifolia Blume	2	14	29.361	12.239	2.128	2.745	17.112
Scaevola micrantha C.Presl.	3	38	12.328	5.139	3.191	7.451	15.781
Agathis philippinensis Warb.	3	12	24.038	10.020	3.191	2.353	15.565
Elaeocarpus merrittii Merr.	2	38	13.782	5.745	2.128	7.451	15.324
Falcatifolium gruezoi de Laub.	3	37	7.902	3.294	3.191	7.255	13.740
*Ardisia elliptica*Thunb.	2	25	11.673	4.866	2.128	4.902	11.896
Phyllanthus everettii C.B.Rob.	2	24	9.836	4.100	2.128	4.706	10.934
Podocarpus rumphii Blume	2	16	8.899	3.710	2.128	3.137	8.975
Kibatalia sp.	3	13	3.978	1.658	3.191	2.549	7.399
Garcinia sp.	2	15	5.086	2.120	2.128	2.941	7.189
Turpinia ovalifolia Elmer	3	6	5.704	2.378	3.191	1.176	6.746
Myristica sp.	2	8	5.202	2.168	2.128	1.569	5.865
Ascarina philippinensis C.B.Rob.	2	13	2.82	1.176	2.128	2.549	5.852
Eurya coriacea Merr.	3	7	1.62	0.675	3.191	1.373	5.239
Myrmeconauclea strigosa (Elmer)	2	9	2.741	1.143	2.128	1.765	5.035

Pipoly							
Syzygium sp.1	1	16	1.875	0.782	1.064	3.137	4.983
Symplocos ophirensis C.B.Clarke	2	7	3.215	1.340	2.128	1.373	4.840
Rauvolfia sumatrana Jack.	2	3	5.075	2.116	2.128	0.588	4.831
Phyllocladus hypophyllus Hook.f.	2	9	1.483	0.618	2.128	1.765	4.511
Dacrycarpus imbricatus(Blume) de Laub.	2	2	4.306	1.795	2.128	0.392	4.315
Osmoxylon simplicifolium (Elmer)Philipson	2	10	0.455	0.190	2.128	1.961	4.278
Weinmannia urdanetensis Elmer	1	11	2.238	0.933	1.064	2.157	4.154
Vaccinium sp.	2	4	0.692	0.288	2.128	0.784	3.200

These plant taxa colonizing Mt. Kiamo has similarity with that of Fernando *et al.* (2013) in Acoje mine of Zambales Mountains, Amoroso and Aspiras (2010) on Mt. Hamiguitan Range and Wildlife Sanctuary and Salas (unpublished) in Mt. Diwata of Surigao del Norte specifically on the genera *Calophyllum*, *Scaevola, Falcatifolium* and family *Podocarpaceae* while *Madhuca sp.* was also observed by Proctor (2003) in Mt. Bloomfield in Palawan. Further, *S. micrantha* is a typical species on ultramafic soils (Podzorski, 1985; Proctor, 2003; Fernando, 2008). Because of these similarities, it is concluded that these taxa are "edaphic endemics" (Bondada and Ma, 2003). These dominant species are the resistant plants that have adapted to noxious soil conditions (Baker *et al.*, 1988) and have evolved biological mechanisms to resist, tolerate, and thrive on the toxic metalliferous soils (Whiting *et al.* 2004).

Table 2. Heavy metal on the soil samples of Mt. Kiamo in µg/g dry weight.

Elevation	Iron(Fe)	Nickel (Ni)	Magnesium (Mg)	Chromium (Cr)
Top elevation	353,786.21	1,347.27	2,337.30	5,060.19
Mid elevation	585,155.08	2,616.54	17,461.53	5,041.82
Lower elevation	482,925.28	1,248.29	7,924.13	6,738.86

Soil sampling

The soil pH on the lower, mid and top elevation of Mt. Kiamo is 6.41, 5.89, and 5.18, respectively while the organic matter is 5.24, 5.38, and 3.86, respectively. These values are similar to other tropical serpentine regions according to Brooks (1987). Among the different heavy metals evaluated, only Iron (Fe), Nickel (Ni), Magnesium (Mg), and Chromium (Cr) were present in the samples with Fe as the most dominant (Table 2). Other heavy metal elements such as Molybdenum (Mo), Lead (Pb), Copper (Cu), Cobalt (Co), and Arsenic (As) were found to be below the detection limit.

Potential metallophytes and metal uptake

Threshold values were successively provided to define the heavy metal hyperaccumulation of species based on its specific phytotoxicity (Rascio and Navari-Izzo, 2010). The threshold metal content used to define a hyperaccumulator depends upon the particular metal accumulated (Bondada and Ma, 2003). For instance, proposed thresholds on a dry weight basis are 100µg/g for Cd, 1,000µg/g for Co, Cu, Ni, and Pb, and 10,000µg/g for Mn and Zn (Baker and Brooks,1989).

Recent definition provided by Rascio and Navari-Izzo (2010) defined hyperaccumulators as plants that, when growing on native soils, concentrate>10µg/g (1%) for Mn or Zn;>1µg/g (0.1%) for As, Co, Cr, Cu, Ni, Pb, Sb,Se or Tl; and >0.1µg/g (0.01%) for Cd in the aerial organs, without suffering phytotoxic damage. Further, Rotkittikhun *et al.* (2006) stressed that shoot-root quotient (SRQ) should be used to evaluate the ability of the plant species to accumulate heavy metals in their tissue. The SRQ could be obtained by simply dividing the accumulated metals of the shoots over the accumulated metals in the roots such that a species obtained an SRQ value of >1 is classified as a hyperaccumulator otherwise they are heavy metal excluder. The metal uptake and SRQ of the most dominant species is presented in Table 3.

Table 3. Metal uptake and shoot-root quotient of the seven (7) most dominant species in Mt. Kiamo in µg/g dry weight.

Species	Tissue	Fe	SRQ	Ni	Mg	SRQ	Cr	SRQ	Cu	SRQ	S
Scaevola micrantha	Shoots	1,172.93	0.236	bdl	316.02	0.471	89.32	0.199	43.91	0.239	bdl
	Roots	4,978.11		bdl	670.84		448.98		184.01		bdl
Elaeocarpus merrittii	Shoots	5,673.30	0.180	bdl	4,991.95	2.937	835.03	0.567	37.32	0.371	bdl
	Roots	31,573.01		bdl	1,699.57		1,472.66		100.55		bdl
Calophyllum soulatri	Shoots	1,143.97	0.394	bdl	3,029.82	2.169	68.11	0.386	33.86	0.403	bdl
	Roots	2,904.59		bdl	1,396.99		176.4		84.12		bdl
Polyosma integrifolia	Shoots	4,400.70	0.115	107.72	3,466.97	1.288	779.16	0.215	33.57	0.538	21,081.85
	Roots	38,215.68		bdl	2,690.85		3,631.09		62.34		bdl
Madhuca sp.	Shoots	1,867.99	0.240	bdl	676.1	4.338	133.36	0.253	Bdl		bdl
	Roots	7,775.43		bdl	155.84		527.37		57.57		bdl
Falcatifolium gruezoi	Shoots	4,826.26	0.118	bdl	3,436.36	2.718	326.15	0.038	70.36	1.668	bdl
	Roots	40,809.61		189.72	1,264.19		8,671.23		42.19		bdl
Agathis philippinensis	Shoots	1,774.93	0.859	bdl	836.71	2.538	200.36	0.835	48.51	1.164	bdl
	Roots	2,065.26		bdl	329.64		240.06		41.69		bdl

bdl = below detection limit

SRQ = Shoot-Root Quotient, obtained by dividing the amount of accumulated in shoot by the root.

Iron

Among the seven (7) most dominant species, *F. gruezoi* obtained the highest uptake of Fe in the roots followed by *P. integrifolia* with 40,808.61µg/g and 38,215.68µg/g, respectively. For the shoots, *E. merrittii* has the highest Fe accumulation with 5,673.30µg/g and was followed by *F. gruezoi* and *P. integrifolia* with 4,826.26µg/g and 4,400.70µg/g, respectively. The accumulated amounts of Fe showed beyond the usual amounts of plant uptake. In fact, these values are far greater compared to the uptake of *Planchonella sp.* and *Alstonia macrophylla* as reported by Claveria *et al.* (2010) which ranges only from 21,000 to 24,500µg/g.

Nickel

F. gruezoi and *P.integrifolia* were the only dominant species that showed Ni accumulation on the roots. *F. gruezoi* and *P. integrifolia* had accumulated 189.72µg/g and 107.72µg/g, respectively. Both species could not be considered as hyperaccumulator because the concentration falls below the threshold level recommended by Baker and Brooks (1989) whilst Rascio and Navari-Izzo (2010). Moreover, *S. micrantha*, the heavy-metal indicator however, did not show Ni accumulation contrary to the findings of Fernando *et al.* (2013) in Acoje mine of Zambales Mountains.

Magnesium

P. integrifolia had the highest concentration of Magnesium (Mg) in the roots with 2,690.85µg/g and is followed by *E. merrittii* with 1,699.57µg/g. However, the shoots of *E. merrittii* had the highest concentrations of 4,991.95µg/g while *P. integrifolia* had only 3,466.97µg/g. Among the dominant species only the *S. micrantha* obtained <1 SRQ.

Chromium

Three (3) species showed higher accumulation of Cr in their root tissue. Normal accumulation of Cr by plants is up to 1,000µg/g however, *F. gruezoi*, *P.*

integrifolia and *E. merrittii* had accumulated 8,671.15µg/g, 3,631.09µg/g and 1,472.66µg/g, respectively.

Copper and Sulphur

The observed copper and sulphur uptake of the dominant plant species is unusual because the amount of these heavy metals in the soil sample is below detection limit however laboratory analyses showed that only *Madhuca sp.* did not accumulate Cu in the shoots. On the other hand, *P. integrifolia* showed S accumulation at very high amounts of 21,081.85µg/g. Further, both the roots of *F. gruezoi* and *A. philippinensis* had lesser concentration of Cu compared to the shoots implying that these are potential Cu-hyperaccumulator species.

Conclusion

The soils of Mt. Kiamo is naturally-rich in heavy metals as manifested from both soil samples and plant tissues. The seven (7) most dominant species colonizing Mt. Kiamo exhibited the ability to accumulate varying levels of heavy metals in their system although adhering to SRQ criteria, some of these species cannot be classified as hyperaccumulators. However, on the basis of unusual accumulation of extremely high levels of heavy metals, this would mean therefore that some of these species are hypertolerant or excluder which is a necessary characteristic for these plants to survive without suffering toxicity. Therefore, it is recommended that these species be subjected to experimentation to verify if such trait would be exhibited under controlled conditions otherwise they should not be considered as potential species for phytoremediation.

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