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Assessment of Plant Diversity and Associated Mycorrhizal Fungi in the Mined-out Sites of Atlas Mines in Toledo City, Cebu for Bioremediation

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ABSTRACT

The diversity of plant and mycorrhizal fungi within the 35,000-ha of the Atlas Mines in Toledo, Cebu, was surveyed with the goal of rehabilitating their mined-out area through bioremediation (the use of dominant plants and mycorrhizal fungi present in area to contain/ reduce pollutants). From five one km transect lines in five sites, the survey indicated that the vegetation was classified as a disturbed grass-shrubland-savanna-agroforest plant community with tree plantations in rehabilitated sites. Plant composition comprised 69 species belonging to 66 genera and 35 families which include trees, shrubs, herbs, creepers, vines, agricultural or agroforest crops. Fruit bodies of ectomycorrhizal fungi (ECMF), namely: Pisolithus, Scleroderma, Thelephora and Boletellus were found under Acacia auriculiformis, A. mangium, Eucalyptus urophylla and E. camaldulensis. Pisolithus were the most dominant. For arbuscular mycorrhizal fungi (AMF), out of 50 plants collected, 10-100% roots of Lycopodium, Saccharum spontaneum, Nephrolepsis, Acacia mangium and Stachytarpheta jamaicensis were colonized by AMF. All roots of S. jamaicensis were colonized solely by Glomus sp. Pithecellobium dulce harboured the highest spore density (2,575 spores/plant/30g dry soil), consisting of Glomus (42%), Acaulospora (24%) and Entrophospora (37%). Muntingia calabura was the only plant associated with Glomus, Gigaspora, Scutellospora, Acaulospora and Entrophospora, though with low spore population. Glomus was the most prevalent among the AMF. The above AMF and ECMF can be mass-produced as biofertilizers for use in bioremediation of mined-out sites and other areas with similar conditions.

Key words: *arbuscular mycorrhiza, ectomycorrhiza, mined-out area, plant diversity*

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INTRODUCTION

Mining activities produce many waste products containing high concentrations of toxic heavy metals. Unlike organic pollutants, heavy metals cannot be degraded into harmless forms such as carbon dioxide, but persist indefinitely in the environment. These toxic heavy metals do not only affect public health, nor only pollute and damage our environment, but also cause (as a consequence) tremendous loses in the biodiversity of the area (*Raymundo 2005*). The wetland and marine ecosystems in particular sustain much damage from chemical wastes from mine tailings and mining activities like that of Calancan Bay in Marinduque, between Negros and Cebu.

The only approaches available for remediating heavy metal–polluted soils are to extract, immobilize or to convert the heavy metals into less bioavailable forms (*Raskin, Smith and Salt 1997*). Bioremediation and phytoremediation is the use of pollutant-accumulating plants/microbes to remove, transfer, stabilize, or destroy in soil, sediment, and groundwater, heavy metals from soil, or to reduce their bioavailability (*Khan 2006, Cadiz, De Guzman and Davies 1999*; *Cadiz and Principe 2005*). Phytoremediation, a strategy that uses plants, is affordable and easily implementable. These approaches may be applied *in situ* or *ex situ*, to soils, sludges, sediments, other solids, or groundwater.

Despite the presence of toxic concentrations of heavy metals in the environment (e.g., mine tailings), it is unlikely to find them bare of vegetation. While most plants will not survive in land laden with heavy metals like cadmium and mercury, some plants tolerate them and pave the way for succession to proceed. The evolution of heavy metal tolerant races within plant species is brought about by natural selection, which acts on available genetic variation, so that the few metal-tolerant individuals emerging from the 'normal populations' may serve as "founders" of the tolerant population. While not all species have the potential to evolve heavy metal tolerance, a vast number of potential colonizers are yet to be discovered. For the past decade, plant scientists continually search for species that could clean up contaminated areas (*Salt, Smith and Raskin 1998*).

Toledo City, Cebu in the Visayas group of islands, has about 35,000 ha of mined-out sites, which have to be rehabilitated the soonest. Some companies expressed their interest in utilizing these mined-out sites for establishing plantations of the nut producing plant *Jatropha* which will serve as feedstock for biodiesel production. In order to achieve such goal, an assessment of the mined-out sites in Toledo areas is needed to develop strategies for bioremediation. Nursery and field trials have shown that soil

microorganisms such as mycorrhizal fungi, can enhance plant growth and survival in acidic soils and can increase plant tolerance to heavy metals (*Aggangan 1996, Aggangan, Dell and Malajczuk 1996, 1997*; *Tordoff, Baker and Willis 2000*; *da Silva et al. 2005*; *Chen et al. 2005*). The interaction of plant roots with mycorrhiza is one of the key determinants of successful rehabilitation (*Prasetyo et al. 2010*).

In mine sites and tailings, nitrogen, phosphorus, and potassium are deficient and can be increased in plant intake by mycorrhizae (*Khan 2006*). Other essential nutrients such as calcium, magnesium, sulfur, iron, zinc, aluminum, and sodium have shown increases in plant intake with mycorrhizal fungi (*Gohre and Paskowski 2006*). Higher concentrations of heavy metals which can be harmful to plants (such as aluminum, arsenic, barium, boron, cadmium, copper, iron, lead, manganese, nickel, selenium, and zinc), can exist on mined sites and, at the same time, be filtered to tolerable amounts by the fungi for the plant (*Malajczuk et at. 1994*). Increasing plant hormones and acting as a barrier to plant pathogens are other benefits to the plant provided by mycorrhizae (*Khan 2006*; *Leung et al. 2007*). Mycorrhizae can also alleviate the stress of higher surface temperatures, acidity and drought in mined-out sites (*Salt, Smith and Raskin 1998*).

Ectomycorrhizal fungi (ECMF) and the endomycorrhizal fungi also known as arbuscular mycorrhizal fungi (AMF) are the most economically and important groups of fungi for agriculture and forestry applications. Ectomycorrhiza (Greek words: ektos, meaning outside; and mykós, meaning fungus; and riza, meaning roots; plural ectomycorrhizas or ectomycorrhizae) is a form of symbiotic relationship where the fungus or fungi covers the root tips, thus, they are sometimes called sheathing fungi of selected host plants (*Brundrett et al. 1996*). The fungus penetrates in between the epidermal of the root forming a Hartig Net. The Hartig Net is the site of nutrient exchange between the host plant and the fungus (*Brundrett et al. 1996*). ECMF host plants are pines, eucalypts, acacias, dipterocarps, alnus, casuarinas, etc. The ECMF forming symbionts belong to the Basidiomycetes. During the rainy season, Basidiomycetes produce fruit bodies in the form of mushrooms, puffballs or truffles which can be seen below or above the soil surface. Spores (extracted from the fruit bodies) and mycelia (produced in aseptic cultures) can be used as mycorrhizal inoculants.

An endomycorrhizal fungus forms hyphae that penetrate the cells of plant roots where they form balloon-like vesicles and branch out arbuscules dedicated as exchange site for minerals and carbohydrates (*Brundrett et al. 1996*) between the fungus and the host plant. These structures gave rise to the name arbuscular mycorrhizal fungi (AMF) formerly known as vesicular-arbuscular mycorrhizae (VAM) (*Brundrett et al. 1996*). AMF are associated with almost all (90%) plants.

Published information on field surveys of mycorrhizal status of plants growing on heavy metal contaminated or mined-out sites in the Philippines is wanting. Thus, the general objective of this survey was to assess plant and mycorrhiza diversity in the mined-out sites in Toledo City. The specific objectives were: to identify mycorrhizal fungi associated with plants growing in the abandoned mine sites in Atlas Mines, Toledo, Cebu, to quantify mycorrhizal population associated with selected plants species thriving in the mine site and to determine the most prevalent mycorrhizal fungi and plant species that harbored the highest mycorrhizal population that could possibly be utilized in a bioremediation strategy in the rehabilitation of the mined-out areas.

MATERIALS AND METHODS

Geographic Setting and Description of the Study Area

Atlas Consolidated Mining and Development Corporation (ACMDC) is located southeast of Toledo City in Barangay Don Andres Soriano, in the province of Cebu, Visayas group of islands. The study area is geographically situated in three barangays of Biga, Bagakay and Lu-ay (**Figure 1**). The sampling site is an open area with patches of disturbed communities of plants that cover the undulating terrain and hills of unearthed soil. Part of the sampling area was also close to a dump site, where local residents were resettled in Sitio Udon, Barangay Lu-ay. The dump site appeared fertile because of the agricultural crops being raised. A nursery of forest plants was also established in this site for the rehabilitation of the mined-out areas. The water system, which drains to the mining pit, and Malubog Lake, were located near the Carmen Primary Crusher and Biga Pit. A spring that serves as a source of potable water for the community is situated in the creek of Barangay Lu-ay.

The soil is extremely acidic (pH 2.55±0.05 in H_2O), with low organic matter (0.40±0.05%) and cation exchange capacity (7.70±0.30 meq/100 g soil) (**Table 1**). Cu levels in all sampling plots are high ranging from 154 – 638 mg kg^{-1}

Table 1. General soil characteristics in Atlas Consolidated Mining Corporation, Toledo City provided by D1 Oils Asia Pacific Inc.

Soil Characteristics	Value
pH	2.55 ± 0.05
OM (%)	0.40 ± 0.05
CEC (meq/100 g soil)	7.70 ± 0.30
Copper (mg kg^{-1})	31.61 ± 0.72
Cadmium (mg kg^{-1})	0.09 ± 0.01
Lead (mg kg^{-1})	198 ± 0.24
Zinc (mg kg^{-1})	Not Analyzed

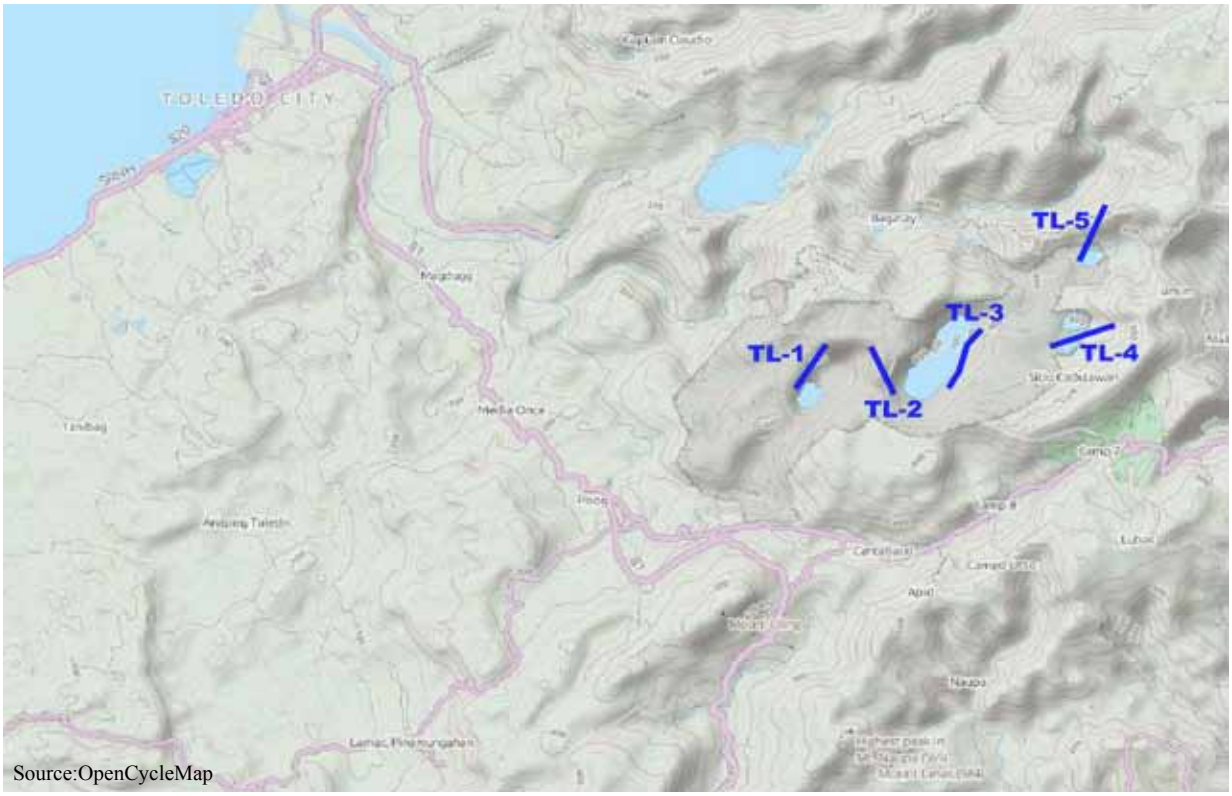

Figure 1. Map (with inset) showing the collection sites (TL1-TL5) within the mined-out areas of Atlas Consolidated Mining and Development Corporation, Toledo City, Cebu.

Table 2. Heavy metal content (mg kg^{-1}) of soils obtained from the different sampling sites in Atlas Consolidated Mining Corporation.

Sampling site	Copper	Cadmium	Lead	Zinc
S1 & S2	168.160 ± 0.410	0.066 ± 0.041	3.435 ± 0.000	11.880 ± 0.000
S3	154.009 ± 0.040	ND	0.57 ± 0.00	5.140 ± 1.010
S4 & S5	637.805	0.428	21.365	51.005

(ND = Not Detectable: Cd < 0.05)

dry soil (**Table 2**), which are beyond the maximum allowable limit for Cu (36 mg kg^{-1} soil) (**Table 3**). On the other hand, cadmium (Cd), lead (Pb), and zinc (Zn) levels are below the maximum allowable limit of 0.8, 85, and 140 mg kg^{-1} soil, respectively (**Table 3**).

Assessment of Plant Composition and Diversity

Establishment of Transect and Sampling Plots

Reconnaissance survey of the ACMDC area was done during the wet season using a 1:2000 scale map, showing extent of the mining operation and rehabilitation of the company. Five transect lines (TL1 to TL5) representing five sampling sites (S1 to S5) were established based on the location of the mining pit, dump site, water systems and vegetation around the different Barangays (**Figure 1**). The five sites were as follows: S1 in Carmen Primary Crusher in Barangay Biga, S2 near the Biga Repeater Tower, S3 in Biga Pit, S4 around Barangay Bagakay, and S5 in the nursery area of Sito Udon, Barangay Lu-ay and vicinity.

A transect approximately a 1.0 kilometer in length was stretched to cover the terrestrial vegetation in each sampling site. Five sampling plots measuring 10 m x 10 m each were systematically allocated alternately along each transect at an interval of 200 m.

Quadrat Sampling Technique and Plant Collection

Sampling procedure for plant diversity analysis followed the Quadrat Sampling Technique (*Pampolina et al. 2003*). Plants within quadrants were identified morphologically, taking note of their habit and habitat to determine their kind and type of vegetation, including abundance by counting the number of individuals for every species.

Representative samples of plants were collected for authentication and herbarium purposes. Plant materials were placed in a wooden presser and sprayed with 95% ethyl alcohol for preservation. All activities and biological samples

Table 3. Dutch standards for soil contamination assessment, in terms of total concentration of heavy metals in soils.

Element	Target value (mg kg^{-1} soil)	Intervention value* (mg kg^{-1} soil)
Arsenic	29	55
Barium	200	635
Cadmium	0.8	12
Chromium	100	380
Cobalt	20	240
Copper	36	190
Mercury	0.3	10
Lead	85	530
Molybdenum	10	200
Nickel	35	210
Zinc	140	720

*Intervention value. This indicates serious contamination of soils where remediation is necessary.

Notes:

(1) Intervention value. This indicates serious contamination of soils where remediation is necessary.

(2) For heavy metals, the target and intervention values are dependent on the clay/silt and organic matter content of the soils. Standard soil values must be modified by the formula:
Lb = Is [(A + B% clay/silt + C% organic matter)]/(A + 25 B + 10 C)
Where Lb = intervention values for a particular soil.
Is = Intervention values for a standard soil (10% organic matter and 25% clay)

were documented using digital and SLR cameras. Herbarium samples were oven-dried at 65°C for 24 hours. Unidentified plants were verified from taxonomic references and the Herbarium of the University of the Philippines Los Baños, College of Forestry and Natural Resources (UPLB-CFNR).

Parameters Measured

The ecological parameters to characterize and analyze species composition, biometrics, population density or abundance, plant diversity, and evenness were based from Shannon-Wiener Index (*Maguran 1988*), to determine the number of species or species richness in every site and know how evenly individuals are distributed among species. Tree height was estimated in meter while diameter at breast height (DBH) was measured in centimeter using a caliper. The DBH of standing individual tree is commonly used by Scientists globally though invariably accepted at 1.3 or 1.4 m aboveground (*Avery and Burkhart 1983*; *Husch et al. 2003*). The diversity values computed from proportion of population density provided values ranging from 1.00 to 4.00 while evenness was 0.00 to 1.00 and described as poor, moderate, and high. Below are the formulae used:

$$\text{Species Richness} = \frac{\text{Number of Species}}{\text{Sampling Plot}}$$

where each plot was measured 100 m^2

$$\text{Population Density} = \frac{\text{Number of Individual Species}}{100\ m^2}$$

$$\text{Relative Diversity} = \frac{\text{Density of a Species}}{\text{Total Density of all Species}}$$

$$\text{Shannon-Diversity Index (H')} = -\Sigma\, pi \ln (pi)$$

where: *pi* refers to the proportion of the population density of a species relative population density value of all species while ln the logarithm value

$$\text{Evenness Index (E)} = H'/\text{In (Species Richness)}$$

Assessment of Plant-Mycorrhizal Fungi Diversity

Sampling area and sample collection

Sample collection was done during the wet season, same time as the survey for plant diversity assessment in the area. The same imaginary transect (1 km) that bisected each sites were used (**Figure 1**). Three established sampling plots (10 m x 10 m each) that were alternately established in cardinal directions for plants were used in every transect. Inside each plot were three smaller subplots (5 m x 5 m each) where wildlings (30-60 cm height) were randomly collected, including fine roots and rhizosphere soil (0 – 20 cm depth). A total of 6 to 19 samples (6 in S1, 11 in S2, 13 in S3, 14 in S4 and 19 in S5) were obtained in each transect or a total of 63 samples. Plant sample size differed because plants growing in the 5 m x 5 m sampling area were few. Data were gathered in three replicates per sample.

Identification of host plants

Plant samples were excavated using a shovel and hand trowel. Collected plant samples were identified morphologically after collection with assistance from local community and using reference materials (*Fernando and Castillo 2003*). Herbarium specimens were brought to UPLB for the verification of their identity by comparing them with herbarium specimens at the CFNR, UPLB.

Collection and identification of ectomycorrhizal fungi

Fruit bodies of ectomycorrhizal fungi (ECMF) were collected from the established three 5m x 5m areas. These were measured, identified and photographed immediately and dried overnight using a portable drier (45°C) at the field station. Samples were kept for microscopic characterization and as herbarium collection. Collected fruit bodies of ECMFungi were described and identified immediately by comparing them to reference books (*Schenck and Perez 1990*; *Brundrett et al. 1996*).

Assessment of root colonization by arbuscular mycorrhizal fungi

Usually, an AMF colonized root cannot be easily distinguished from a non-mycorrhizal root without the aid of a microscope and special dying techniques. Attached fine roots (diameter <0.5 mm) of the collected plant samples (wildlings or any plant below 1.5 m height) were washed in running water, detached from the main root system and fixed in 50% alcohol for estimation of root infection. The fixed root segments were washed in tap water several times to remove the alcohol. They were then cleared with 10% (w/v) KOH solution at 90 °C and stained with 0.05% trypan blue in lactoglycerol (*Phillips and Hayman 1970*). Samples containing old and/or heavily pigmented roots required several changes of KOH solution and longer clearing time for adequate clearing. Stained roots were viewed under a stereomicroscope and mycorrhizal and non-mycorrhizal infected roots were counted using the grid-line intersect method (*Giovannetti and Mosse 1980*). The presence of attached hyphae, vesicles or arbuscules inside the roots was scored as mycorrhiza infected root. Percentage of colonization is calculated as the number of infected root segments over the total number observed multiplied by 100 *(Giovannetti and Mosse 1980*).

Isolation and identification of AM fungi

Rhizosphere soils (0-20 cm depth) were collected using a hand trowel. About one kg of rhizosphere soils packed in a plastic bag comprised one sample. Samples were brought to the Mycorrhiza Laboratory, BIOTECH, UPLB. The samples were first passed through a 2 mm sieve from which a 100 g sample was oven dried set at 70 °C and oven dry weight was measured after three days. This was done to expressed the AMF spore count per dry soil.

AMF spores were isolated from 30 g of air-dried rhizosphere soil following the wet sieving and decanting technique (*Gerdemann and Nicolson 1963*). The AMF were identified according to spore size, color, surface characters and wall layers (*Schenck and Perez 1990*) and using standard monographs (*Morton and Benny 1990*; *Hall 1984*; *Schenck and Perez 1990*). Most of the spores were identified up to genus level. Spores of AMF were inoculated into Bahia (*Paspalum notatum*) grass and maintained at the BIOTECH screen house for production of adequate samples for further identification up to the species level.

Assessment of AMF density

The density or population and percent root colonization of AM fungi were assessed based on density index of *Maguran* (*1988*) and *Giovannetti and Mosse* (*1980*) respectively. The following formulae were used:

$$\text{AMF Density} = \frac{\text{No. of isolated spores}}{\text{30 g dry soil}}$$

$$\text{Root colonization (\%)} = \frac{\text{Number of roots with mycorrhizal fungi}}{\text{Total number of roots observed}} \times 100$$

RESULTS

Plant Diversity

Plant community structure and taxonomic composition

The type of vegetation represented within the sampling area was generally classified as a disturbed grass-shrubland plant community with patches of savanna and agroforest ecosystems with tree plantations in rehabilitated sites. The terrestrial vegetation exhibited some early stage of plant succession exemplified by vast open areas covered by different species of shrubs and ferns with spots of pioneer trees that serve as shade crops to undergrowth (**Table 4**). Fertile sites were planted with agricultural crops interspersed with forest tree species. Remnants of mining activities including ground excavation, a network of road systems, lakes in mining pits, and hilly dump sites which provided a landscape where green vegetation contrasted with brownish soil. Some of the dominant and heavy metal tolerant vegetation types observed from the different sampling sites were *A. auriculiformis*, *S. spontaneum*, *S. jamaicensis*, and *Lycopodium* (**Figure 2**).

Structurally, the horizontal profile of the plant community occupied approximately 40-60% of the area, with the remaining percentage covered by exposed excavated soil. The vertical horizon of vegetation showed the dominance of undergrowth less than a meter in height and shrubs of 1-2 m tall. Trees reached a height of 3 - 6 m, with a DBH of about 15-35 cm.

The plant composition comprised of 69 species belonging to 66 genera, 35 families, and 23 orders (**Table 4**). Plants were categorized based on habit, as trees, shrubs, herbs, creepers, ferns, vines; and agricultural crops, or agroforestry crops. About 38% of the vegetation comprised of trees, including reforestation, agroforest and pioneer species. The intermediate layer consisted of 18% represented by saplings and small trees. The remaining proportion of plant composition were agricultural crops classified as under-growths like shrub, herb, creeper, together with wildlings which were important for mycorrhizal analysis.

Ecological values

The number of species from various sampling sites ranged from 15- 41 different kinds (**Table 5**). The sampling site near the pit close to the Biga Repeater (S2) and the

Table 4. List of plant taxa and their respective habit from different sampling sites in Atlas Consolidated Mining and Development Corporation in Barangay Don Andres Soriano, Toledo City.

Common Name	Scientific Name	Family Name	Order	Habit
Limuran	*Calamus ornatus* Blume	Arecaceae	Arecales	Climbing palm
Panibat	*Bidens pilosa* L.	Asteraceae	Asterales	Shrub
Yautia	*Xanthosoma violaceum* Schott	Araceae	Alismatales	Agricultural crop
Kamoting baging	*Ipomea batatas* (L.) Lamk.	Convulvolaceae	Solanales	Agricultural crop
Ampalaya	*Momordica charantia* L.	Cucurbitaceae	Cucurbitales	Agricultural crop
Cassava	*Manihot esculenta* Crantz	Euphorbiaceae	Malpighiales	Agricultural crop
Latundan	*Musa x paradisiaca* L.	Musaceae	Zingiberales	Agricultural crop
Sugarcane	*Saccharum officinarum* L.	Poaceae	Poales	Agricultural crop
Corn	*Zea mays* L.	Poaceae	Poales	Agricultural crop
Palay	*Oryza sativa* L.	Poaceae	Poales	Agricultural crop
Tanglad	*Andropogon citratus* DC	Poaceae	Poales	Agricultural crop
Mango	*Mangifera indica* L.	Anacardiaceae	Sapindales	Agroforest
Ipil-ipil	*Leucaena leucocephala* (Lam.) de Wit	Fabaceae	Oxidales	Agroforest
Kaliskis dalag	*Desmodium trifflorum*	Fabaceae	Oxidales	Creeping shrub
Pole	*Vigna unguiculata* Linn. Sp. Sesquipedalis (Verde)	Fabaceae	Oxidales	Agricultural crop
Maning aso	*Senna occidentalis* (L.) Link.	Fabaceae	Oxidales	Creeping shrub
Akle	*Albizia acle* (Blanco) Merr	Fabaceae	Oxidales	Tree
Santol	*Sandoricum koetjape* (Burm. f.) Merr.	Meliaceae	Sapindales	Agroforest
Nangka	*Artocarpus heterophyllus* Lam.	Moraceae	Rosales	Agroforest
Bayabas	*Psidium guajava* L.	Myrtaceae	Myrtales	Agroforest
Duhat	*Syzygium cumini* (L.) Skeels	Myrtaceae	Myrtales	Agroforest
Kalobkob	*Syzygium calubcob* (C.B. Rob) Merr	Myrtaceae	Myrtales	Tree
Lycopodium	*Lycopodiella cernua* L. Pic. Serm.	Lycopodiaceae	Lycopodales	Creeper
Kilob	*Dicranopteris linearis* (Burm.f) Underw.	Gleicheniaceae	Gleicheniales	Fern
Kabkab	*Drynaria quercifolia* (L.) J. Sm.	Polypodiaceae	Polypodiales	Fern
Mutha	*Cyperus rotundus* L.	Cyperaceae	Poales	Herb
Paragis	*Eleusine indica* L.	Poaceae	Poales	Herb
Cogon	*Imperata cylindrica* (L.) Beauv.	Poaceae	Poales	Herb
Talahib	*Saccharum spontaneum* L	Poaceae	Poales	Herb
Gabi	*Colocasia esculentum* (L.) Schott	Araceae	Alimatales	Herb
Tagulinao	*Vernonia ceneria* L.	Asteraceae	Asterales	Herb
Tari-tari	*Commelina benghalenis* L.	Commelinaceae	Commelinales	Herb
Tawa-tawa	*Euphorbia hirta* (L.) Millsp.	Euphorbiaceae	Malpighiales	Herb
Tagbak	*Alpinia elegans* (Presl.) K. Schum.	Zingeberaceae	Zingiberales	Herb
Anabiong	Trema orientalis (L.) Blume	Cannabaceae	Rosales	Pioneer tree
Balanti	*Homolanthus populneus* (Gelsel.) var. populneus Pax	Euphorbiaceae	Malpighiales	Pioneer tree
Binunga	*Macaranga tanarius* (L.) Muell.-Arg.	Euphorbiaceae	Malpighiales	Pioneer tree
Marasili	*Chromolaena odorata* (L.) R.M. King & H. Rob	Asteraceae	Asterales	Shrub
Suab kabayo	*Hyptis soaveolens* (L.) Polt	Asteraceae	Asterales	Shrub
Wild sunflower	*Tithonia diversifolia* Asa Gray	Asteraceae	Asterales	Shrub
Tridax	*Tridax procumbense* L.	Asteraceae	Asterales	Shrub
Flemengia	*Flemengia macrophylla* (Willd.) Merr.	Fabaceae	Oxalidales	Shrub
Saluyot	*Corchorus acutangulus* Lam.	Malvaceae	Malvales	Shrub
Malatungaw	*Melastoma malabathricum* L.	Melastomataceae	Myrtales	Shrub
Coronitas	*Lantana camara* L.	Verbenaceae	Lamiales	Shrub
Kandikandilaan	*Stachytarpheta jamaicencis* (L) Vahl.	Verbenaceae	Lamiales	Shrub
Datiles	*Muntingia calabura* L.	Malvaceae	Malvales	Small tree
Dalunot	*Pipturus arborescens* (Link) C.B. Rob.	Urticaceae	Rosales	Small tree
Agoho	*Casuarina equisetifolia* Forst	Casuarinaceae	Fagales	Tree
Japanese acacia	*Acacia auriculiformis* A. Cunn.ex Benth.	Fabaceae	Oxalidales	Tree
Akleng parang	*Albizia procera* (Roxb.) Benth	Fabaceae	Oxalidales	Tree
Fire tree	*Delonix regia* (Boj. ex Hook.) Raf.	Fabaceae	Oxalidales	Tree
Pineapple	*Ananas comosus* L.	Crassulaceae	Poales	Agricultural crop
Silver fern	*Pityrogramma calomelanos* (L.) Link	Adiantaceae	Polypodiales	Fern
Kamachile	*Pithecellobium dulce* (Roxb) Benth.	Fabaceae	Oxalidales	Tree
Smooth Narra	*Pterocarpus indicus* Willd. forma indicus	Fabaceae	Oxalidales	Tree
Yemane	*Gmelina arborea* Roxb.	Lamiaceae	Lamiales	Tree
Mahogany	*Swietenia macrophylla* King	Meliaceae	Sapindales	Tree
Tibig	*Ficus nota* (Blanco) Merr.	Moraceae	Rosales	Tree
Pakiling	*Ficus odorata* (Blanco) Merr	Moraceae	Rosales	Tree
Hauili	*Ficus septica* Burm. f.	Moraceae	Rosales	Tree
Red gum	*Eucalyptus camaldulensis* Dehnh.	Myrtaceae	Myrtales	Tree
Wisak	*Neonauclea media* (Havil.) Merr.	Rubiaceae	Gentianales	Tree
Uoko	*Mikania cordata* (Burm. f.) B.L. Rob.	Asteraceae	Asterales	Vine
Kudzu	*Calopogonium mucunoides* Desv.	Fabaceae	Oxalidales	Vine
Dilang butiki	*Centrosema pubescens* Benth.	Fabaceae	Oxalidales	Vine
Giant mimosa	*Minosa pudica* L.	Fabaceae	Oxalidales	Vine
Silong pugo	*Pericampylus glaucus* (Lam.) Merr.	Menispermaceae	Ranuculales	Vine
Nito	*Lygodium flexuosum* (L.) Sw.Schizaeales	Lygodiaceae	Schizaeales	Vine
Patogo	*Blechnum egregium* Copel.	Blechnaceae	Cyathales	Fern

Table 4. List of plant taxa and their respective habit from different sampling sites in Atlas Consolidated Mining and Development Corporation in Barangay Don Andres Soriano, Toledo City. (cont...)

Common name	Scientific name	Family name	Order	Habit
Pakong kalabaw	*Nephrolepis bisserata* Schott	Nephrolepidaceae	Polypodiales	Fern

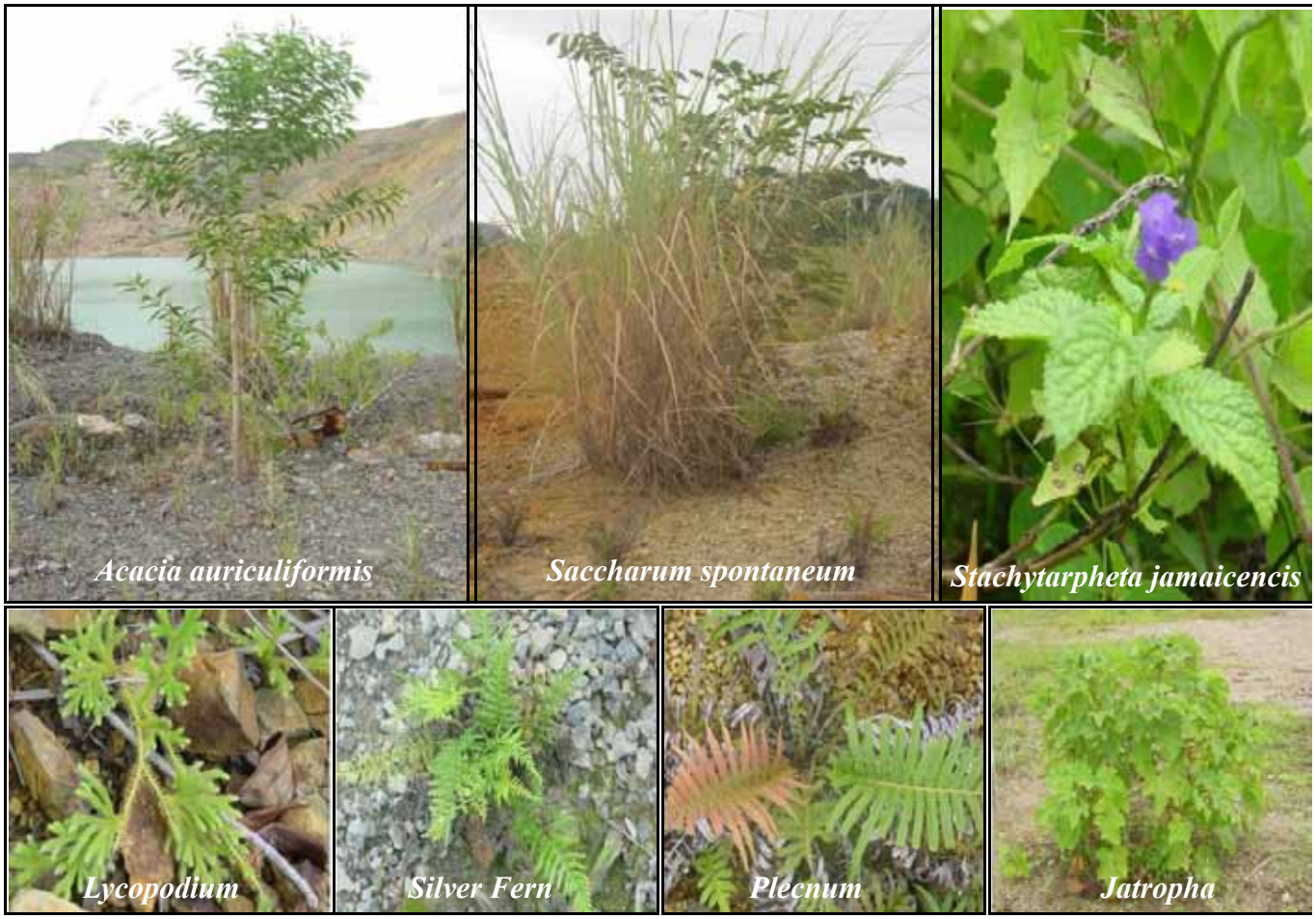

Figure 2. Example of heavy metal tolerant plants in the mined-out sites of Atlas Consolidated Mining and Development Corporation, Toledo City, Cebu.

Table 5. Species richness, density value, diversity indices, and dominant species from different sampling stations in the Atlas Mining site in Barangay Don Andres Soriano, Toledo City.

Transect/ Sampling Site (S)	Site Description	Species Richness	Population Density (per 100 m^2)	Diversity Index (H')	Evenness Index (e)	Dominant Species
S1	Carmen Primary Crusher, Brgy Biga	31	40	3.0998	0.9027	Kudzu, Talahib, Corn, Silver fern, Uoko, Japanese acacia
S2	Biga Repeater Tower	41	79	3.0080	0.8100	Talahib, Malatungaw, Kaliskis dalag, Cogon, Kilob, Camote, Silver fern
S3	Biga Pit	15	34	2.0364	0.7520	Silver fern, Blechnum, Malatungaw, Lamon babae
S4	Bgy Bagakay	16	49	1.8049	0.6510	Silver fern, Malatungaw, Cogon
S5	Nursery area Bgy Lu-ay, Sitio Udon	40	84	3.1227	0.8465	Kudzu, Silver fern, Ampalaya, Kamote, Japanese acacia, Hagonoy

Note: Category of Diversity index: Very high = 3.500-4.00; High = 3.00-3.499; Moderate = 2.500-2.999; Low = 2.000-2.499; Very Low = 1.000-1.999.

nursery in Sitio Udon, Baranga Luay (S5) gave the highest species richness (41 and 40, respectively). Highest plant density per 100 m^2 was observed in S5 with 84 individuals while the lowest was observed in S3 with only 34. The diversity indices were high in sampling plots S1, S2 and S3 with values ranging from 2.01 to 3.12. These sites have plant species that were evenly distributed with values ranging from 0.81 to 0.90. By contrast, values obtained from sampling plots S3 and S4 were all low in diversity (1.8 and 2.04, respectively) and evenness (0.75 and 0.65, respectively).

Mycorrhizal Fungal Diversity

Ectomycorrhizal fungi

Fruit bodies of ECM fungi were observed under *Acacia mangium*, *A. auriculiformis* and *Eucalyptus camaldulensis* and *E. deglupta* trees (**Figure 3**). ECMF belong to the genera *Scleroderma*, *Thelephora*, *Boletellus* and *Pisolithus* (**Figures 3b-3i**). *Pisolithus* was the most prevalent (**Figure 3i**), being found in almost all trees in the five to ten-year old plantations. *Scleroderma* was next to *Pisolithus* in abundance while the *Boletellus* was rare. Fruit bodies of *Pisolithus* differred in terms of outside appearance. Some of the *Pisolithus* fruit bodies were square in shape (**Figure 3f**) or rounded top (**Figure 3g**), some are conical and some are thin slender tube (**Figure 3h**). The conical shaped fruit bodies were the most prevalent (**Figure 3i**). It was not determined if the *Pisolithus* observed were of the same strain.

Root Colonization by mycorrhizal fungi

Freshly collected root tips of *A. mangium*, A. *auriculiformis*, *E. camaldulensis* and *E. deglupta* were heavily colonized with *Pisolithus* and *Scleroderma*. *Pisolithus* colonized root tips were golden yellow to dark brown in color while those colonized with *Scleroderma* were white. On the other hand, most of the other plants (not mentioned above) collected did not show any root colonization by AMFungi (**Table 6**).

For root colonization by AMF, out of the 63 collected plant samples, only five [(i.e. clubmoss, talahib (*Saccharum spontaneum*), *Nephrolepis*, *A. mangium* and kandikandilahan (*Staphytarpheta jamaicensis*)] collected from two sites (three plants in S2 and 2 in S4) showed colonization by AMF, with infection ranging from 10% to 100% (**Table 6**). All roots of *S. jamaicensis* were heavily colonized (100%) with mycelia, vesicles and arbuscules of AM fungi (**Figure 4**). The attached spores were observed only in the roots of *S. spontaneum* in S2, datiles (*Muntingia calabura*), tuhogtuhog and anabiong (*Trema orientalis*) in S5 (**Table 6**). Most of the collected plant samples were not colonized by AMF and there were very rare plants with spores inside the stained roots.

Occurrence and abundance of AM fungi

Among the five sites surveyed, S3 gave the highest (258 spores/plant/30g dry soil) spore count (irrespective

Figure 3. Fruit bodies of ectomycorrhizal fungi associated with *Acacia mangium*, *A. auriculiformis* and *Eucalyptus camaldulensis* trees growing in the mined-out sites of Atlas Consolidated Mining and Development Corporation, Toledo City, Cebu. *A. mangium* tree (a) associated with different ECM fungi. Fruit bodies are: *Scleroderma* (b and c), *Thelephora* (d), *Boletellus* (e), *Pisolithus* (f-i). Spores or vegetative mycelia of these ECM fungi can be used to inoculate plants for bioremediation purposes.

Table 6. Percentage of AMF root colonization and spore count inside the roots from five sampling sites in Atlas Mining, Toledo City, Cebu.

Sampling site (S)/ No. of plant samples	Plants evaluated	Mycorrhizal plants and percent root colonization	Spore count in stained roots
S1/6	Corn (2), Tagunilaw, Hauili, Silver fern, gabi	No mycorrhizal root colonization	No spores observed
S2/11	Silver fern, Malatungaw, *Acacia auriculiformis*, *Plecnum*, *Lycopodium*, Talahib, Kalubkob, Limuran, Kaliskis dalag , *Nephrolepsis*, Camote	Talahib (20%) *Lycopodium* (21%) *Nephrolepsis* (29%)	No spores observed
S3/13	Malatungaw, *Acacia auriculiformis*, Blechnum (3), Kamachile, Mahogany, *Eucalyptus*, *Nephrolepis* Narra, Agoho, Kaliskis dalag Tanglad, *Lycopodium*,	No mycorrhizal infection	No spores observed
S4/14	Malatungaw, *Acacia auriculiformis* (2), *Plecnum* (3), Kandikandilaan, Cogon (3), Hauili, Wisak, Maning aso, Kudzu	Kandikandilaan (100%) *A.auriculiformis* (10%)	No spores observed
S5/19	Tuhogtuhog, Flemingia, Malunggay, Pole sitaw, pine-apple, Bagras, Sapinit, *Nephrolepis*, ampalaya, akle, *Acacia auriculiformis*, Datiles, Anabiong, Cassava, Hagonoy, Rice, Camote, Corn, Yautia	No mycorrhizal infection	Tuhogtuhog (3 spores in 2 g root samples)

S1 = Carmen Primary Crusher, Bgy Biga, S2 = Biga Repeater Tower, S3 = Biga Pit, S4 = Bgy Bagakay, S5 = Nursery area Bgy Lu-ay, Sitio Udon

of plant species) followed by that in S5 (98±127), S2 (78±112) and S1 (29±32) (**Table 6**). *Glomus*, *Acaulospora* and *Entrophospora* species were present in all sites. *Gigaspora* species were observed only in S1, S4 and S5 while *Scutellospora* species were found only in S5. Except in S3 where spore density of *Entrophospora* was highest (155 spores/plant), spore density of Glomus species in S1 (11±13), S2 (28±33), S4 (42±106) and S5 42±88) were higher than the other AM fungi.

Pithecellobium dulce harboured the greatest number (2,575 spores/plant/30g soil) of mycorrhizal spores of *Glomus* (739 spores/30 g dry rhizosphere soil), *Acaulospora* (410 spores/30 g dry rhizosphere soil) and *Entrophospora* (1,426 spores/30g dry soil) (**Table 7a**). *Pithecellobium dulce* was growing in S3. *Flemingia* (S5) and *S. jamaicensis* (S4) were next to P. dulce, with spore counts of 450 and 401 spores/30g dry soil/plant, followed by malunggay (*Moringa oleifera*) (394 spores/plant) growing in S5. Datiles (*Muntingia calabura*) growing in S5 was the only plant that was associated with *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora* (**Table 7b**).

AM spores were identified to belong under the genera *Glomus* (**Figure 5**), *Gigaspora* (**Figure 6a-c**), *Scutellospora* (**Figure 6d**), *Acaulospora* (**Figure 7**) and *Entrophospora* (**Figure 8**). Plants growing in S3 has the highest spore density (258±699 spores/30 g dry soil/plant) and the lowest (29±32 spores/30 g dry soil/plant) was in S1 (**Table 6**). *Glomus* was observed in all sampling sites (**Table 6**) and it is the most dominant genera (216 spores/plant/30 g dry soil) followed by *Entrophospora* (189 spores/plant/30 g soil) and *Acaulospora* (124 spores/plant/30 g dry soil), *Gigaspora* (15 spores/plant/30 g soil) and spores of *Scutellospora* were very rare (0.11) (**Table 7b**). *Glomus* are known to have funnel shaped subtending hyphae (**Figure 5**), bulbous base for the *Gigaspora* (**Figure 6**), distinct spore ornamentations for the *Acaulospora* (**Figure 7**) and protrusions for the *Entrophospora* (**Figure 8**). *Scutellospora* also has a bulbous base with germination shield (**Figure 6d**), thus, it differs with that of *Gigaspora*.

DISCUSSION

Disturbed ecosystems such as mine waste dumpsite, mined-out areas or mine tailings normally support less plant and mycorrhiza species as compared with undisturbed sites fully covered with green plants. These disturbed sites are mostly hostile environments for plant growth due to the presence of many limiting factors, particularly residual high levels of heavy metals, nutrient deficiencies, unfavorable microclimate, and poor substrate structure (*Tordoff, Baker and Willis 2000*). These features result in many heavy metal contaminated areas being largely devoid of any natural vegetation similar to that observed in the Atlas Mines mined-out areas, even many years after abandonment. It is most desirable to find native plants that may already be adapted to local soil and climatic conditions.

Mycorrhizal associations are ubiquitous in natural ecosystems acting as biofertilizers, bioprotectants, and biodegraders (*Khan 2006*), among which AMF is the most widely distributed type. The wide distribution of AMF on heavy metal contaminated sites has been shown to be due to general adaptation and tolerance of these symbionts to heavy metals (*Aggangan, Dell and Malajczuk 1997*) and heavy

Table 7a. AMF spore density (spore count per 30 gram of dry soil) of mycorrhizal fungi associated with plants growing in five sampling sites in mined-out area in Atlas Consolidated Mining and Development Corporation, Toledo, Cebu. n = 3.

Sampling Plot/ No.of plant samples	Plants evaluated	Glomus	Gigaspora	Scutellospora	Acaulospora	Entrophospora	Spore density (Total spore count)
S1/6	*T*agunilaw	4	0	0	2	0	6
	Gabi	3	0	0	9	2	14
	Hauili	4	4	0	2	0	10
	Silver fern	3	0	0	0	0	3
	Corn (healthy)	37	38	0	2	0	77
	Corn (Sick)	18	35	0	7	2	62
	Total	69	77	0	22	4	172
	Average	11.50	12.83	0	3.67	0.67	28.67
	SE	13.78	18.42	0	3.50	1.03	32.20
S2/11	*A. auriculiformis*	22	0	0	0	0	22
	Camote	41	0	0	0	0	41
	Talahib	24	0	0	0	87	111
	Fern	1	0	0	6	2	9
	Silver Fern	8	0	0	3	0	11
	Lycopodium	31	0	0	5	34	70
	Lycopodium	14	0	0	11	32	57
	Kalubkob	119	0	0	0	0	119
	Limuran	0	0	0	0	0	0
	Malatungaw	13	0	0	4	2	19
	Neprolepsis	38	0	0	0	0	38
	Total	311	0	0	29	157	497
	Average	28.27	0	0	2.64	14.27	45.18
	SE	33.08	0	0	3.61	27.42	40.43
S3/13	*Neprolepsis*	8	0	0	0	0	8
	Eucalyptus	0	0	0	0	27	27
	Narra	92	0	0	17	20	129
	Agoho	68	0	0	0	0	68
	Mahogany	36	0	0	0	190	226
	Oliva fern	175	0	0	0	0	175
	Oliva fern	0	0	0	0	0	0
	Camachile	739	0	0	410	1426	2575
	Lycopodium	5	0	0	21	7	33
	Malatungaw	24	0	0	0	0	24
	Oliva fern	22	0	0	0	0	22
	Kaliskis dalag	7	0	0	0	0	7
	Tanglad	25	0	0	0	36	61
	Total	1201	0	0	448	1706	3355
	Average	92.38	0	0	40.73	155.09	258.08
	SE	200.42	0	0	113.06	392.45	699.67
S4/14	Kandikandilaan	401	0	0	0	0	401
	Cogon	1	0	0	7	14	22
	Cogon	0	0	0	50	51	101
	Cogon	4	2	0	6	4	16
	Wisak	2	0	0	0	0	2
	Hauili	55	0	0	0	0	55
	A. auriculiformis	0	0	0	213	0	213
	Silver Fern	5	0	0	1	0	6
	Silver Fern	14	0	0	0	0	14
	Silver Fern	1	0	0	0	2	3
	Malatungaw	0	0	0	135	0	135
	Maning aso	80	0	0	16	0	96
	Unidentified	9	0	0	0	6	15
	Unidentified	10	0	0	4	4	18
	Total	582	2	0	432	81	1097
	Average	41.57	0.14	0	30.86	5.79	78.36
	SE	106.14	0.53	0	63.92	13.59	111.77

Table 7a. AMF spore density (spore count per 30 gram of dry soil) of mycorrhizal fungi associated with plants growing in five sampling sites in mined-out area in Atlas Consolidated Mining and Development Corporation, Toledo, Cebu, n = 3. (cont...)

Sampling Plot/No.of plant samples	Plants evaluated	Glomus	Gigaspora	Scutellospora	Acaulospora	Entrophospora	Spore density (Total spore count)
S5/19	Tuhogtuhog	29	0	0	0	0	29
	Flemengia	0	0	0	428	22	450
	Malunggay	394	0	0	0	0	394
	Pole Sitaw	95	1	0	0	0	96
	Pineapple	0	0	0	86	0	86
	Bagras	60	0	0	0	0	60
	Sapinit	37	0	0	55	16	108
	Datiles	17	25	2	164	16	224
	Anabiong	25	0	0	8	0	33
	Neprolepsis	9	0	0	7	8	24
	Rice	45	0	0	13	3	61
	Ampalaya	48	0	0	44	54	146
	Hagonoy	5	0	0	0	0	5
	Akle	0	0	0	0	0	0
	Auri	26	0	0	0	8	34
	Cassava	4	0	0	61	0	65
	Corn	4	3	0	0	0	7
	Camote	0	3	0	16	13	32
	Yautia gabi	6	0	0	0	0	6
	Total	804	32	2	882	140	1860
	Average	42.32	1.68	0.11	46.42	7.37	97.89
	SE	88.86	5.73	0.46	101.59	13.31	127.31

S1 = Carmen Primary Crusher, Bgy Biga, S2 = Biga Repeater Tower, S3 = Biga Pit, S4 = Bgy Bagakay, S5 = Nursery area Bgy Lu-ay, Sitio Udon

Table 7b. Summary of AMF spore density (spore count per 30 g dry rhizosphere soil) under each genera irrespective of plants growing in five sampling sites in mined-out site in Atlas Consolidated Mining and Development Corporation, Toledo, Cebu.

Sampling site	Glomus	Gigaspora	Scutellospora	Acaulospora	Entrophospora	Spore density (Total spore count)
S1	11.50±13.78	12.83±18.42	0.00±0.00	3.67±3.5	0.67±1.03	28.67±32.2
S2	28.27±33.08	0.00±0.00	0.00±0.00	2.64±3.61	14.27±27.42	45.18±40.43
S3	92.38±200.42	0.00±0.00	0.00±0.00	40.73±113.06	155.09±392.45	258.08±699.67
S4	41.57±106.14	0.14±0.53	0.00±0.00	30.86±63.92	5.79±13.59	78.36±111.77
S5	42.32±88.86	1.68±5.73	0.11±0.46	46.42±101.59	7.37±13.31	97.89±127.31
Total	216.04	14.65	0.11	124.32	189.19	508.18±202.28
Percentage relative to the total population	42.52	2.88	0	24.46	37.23	

S1 = Carmen Primary Crusher, Bgy Biga, S2 = Biga Repeater Tower, S3 = Biga Pit, S4 = Bgy Bagakay, S5 = Nursery area Bgy Lu-ay, Sitio Udon

metal tolerant fungal strains have been isolated (*Mendoza, Ocampo and Aggangan 2006*; *Aggangan and Segismundo 2006*). Numerous studies have indicated that mycorrhizal colonization may increase plant tolerance to heavy metal contamination (*Leyval, Turnau and Haselwandter 1997*). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity (*Van der Heijden et al. 1998*; *Genny, Hartley and Alexander 2001*).

The 3,500 ha surveyed mined-out area of ACMDC is supporting vines, shrubs, trees, pioneer species, herbs and even agricultural crops which may be due to bioremediation. The presence of diverse plant composition and successional stages are indications that bioremediation can address rehabilitation of mined-out areas of the ACMDC. Microscopic examination of plant roots and soil suggest that microbial interactions could be behind plant survival in Cu-rich ecosystem. The persistence and dominance of silver ferns (*Pitygramma calomelanos*), malatungaw (*Melastoma malabatricum*) and *A. auriculiformis* in all transects or sampling sites are indications of their potential to rehabilitate marginal sites as in ACMDC. An additional factor that can be considered is the presence of mycorrhizal species found established in many of the plants existing in the area.

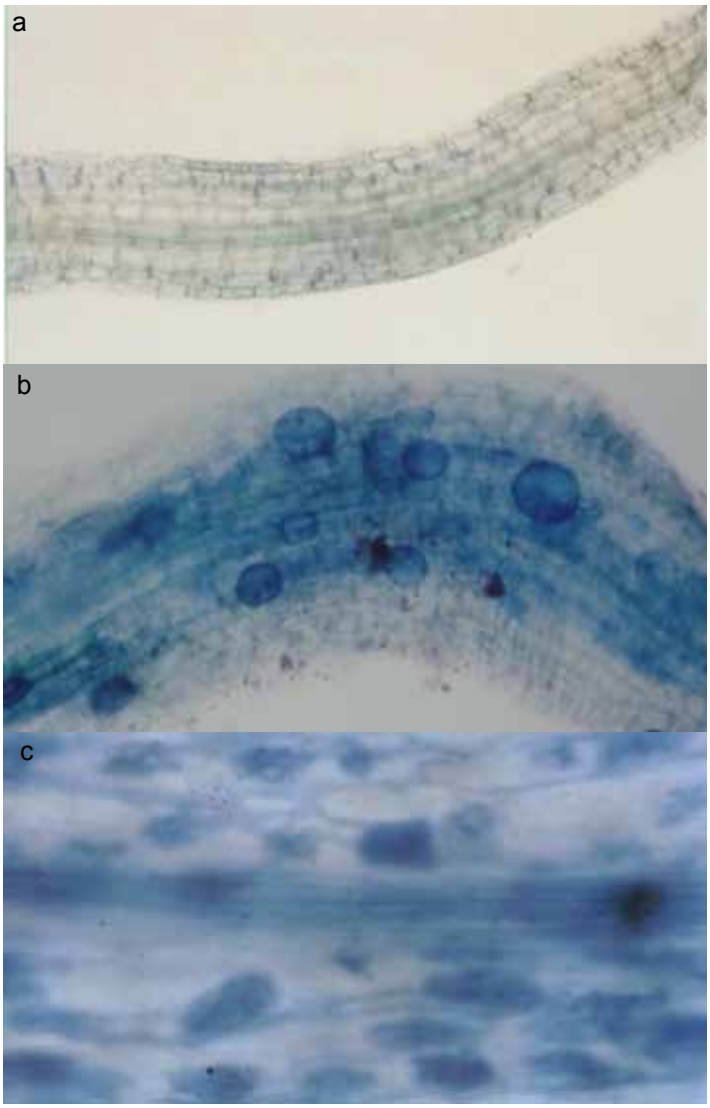

Figure 4. Non-mycorrhizal (a) and AM fungi infected roots showing vesicles, the food storage organ of the fungus (b) and arbuscules which is the site of nutrient exchanges between the plant and the fungus (c).

Other beneficial microorganisms can be explored that could contribute to the success of bioremediation in mine tailings areas similar to that of ACMDC in Toledo, Cebu.

ECMF were present in the rehabilitated area in the collection sites. Fruit bodies of *Pisolithus*, *Scleroderma*, *Thelephora* and *Bolettellus* were found under *A. auriculiformis*, *A. mangium*, *Eucalyptus urophylla* and *E. camaldulensis*. Accordingly, these acacias and eucalypts planted by the Atlas Commission Reforestation program have used Mycogroe tables produced at BIOTECH, UPLB. Mycogroe tablets contain mix spores of *Pisolithus* and *Scleroderma*. This could be the reason of the dominance of *Pisolithus* among ECMF present in the collection sites followed by the *Sclerodermas*. *Pisolithus* was found to be an acid and heavy metal tolerant ECM fungus (A*ggangan, Dell and Malajczuk 1996*, *1997*; *Aggangan and Aggangan 2005*, *2012*). The occurrence of *Bolettellus* and *Thelephora* species

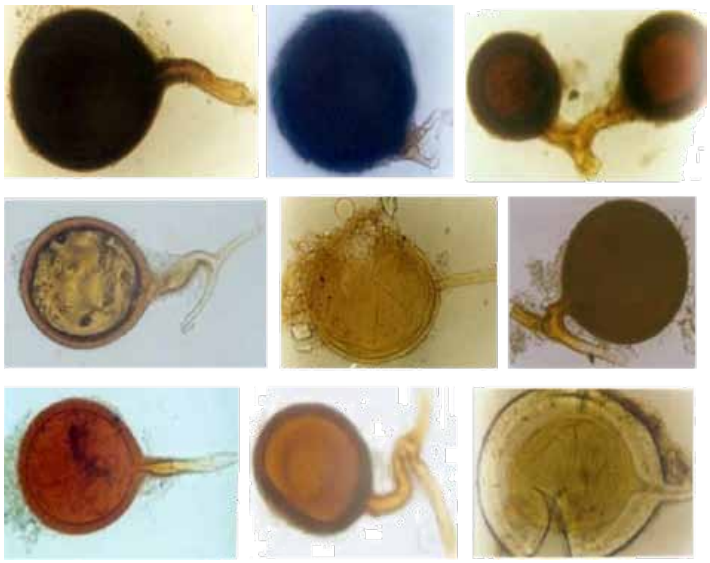

Figure 5. Glomus as the most prevalent and diversified VAM fungi in abandoned mine sites in Atlas Consolidated Mining and Development Corporation, Toledo City, Cebu.

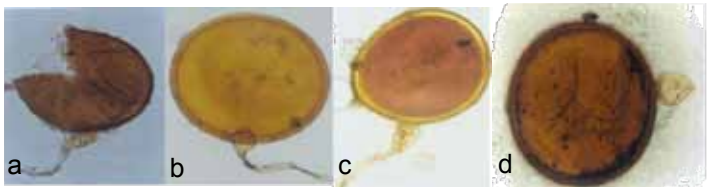

Figure 6. Spores of mycorrhizal fungi belonging to the genus *Gigaspora* (a-c) showing bulbous subtending hyphae (distinct characteristic of *Gigaspora* species) and germination shield (with asterisk *) of *Scutellospora* (d).

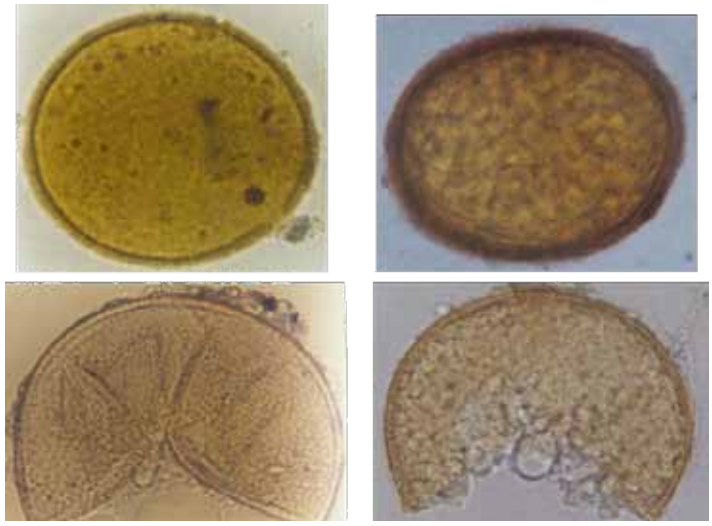

Figure 7. Spores of mycorrhizal fungi belonging to the genus *Acaulospora* showing different spore wall ornamentations (distinct characteristic of *Acaulospora* species).

may have come from adjacent tree species associated with ECMF or it could possibly be that the spores of these fungi which have been dormant for a long time became active when the area was planted with ECMF plant hosts (*Malajzcuk et al. 1994*).

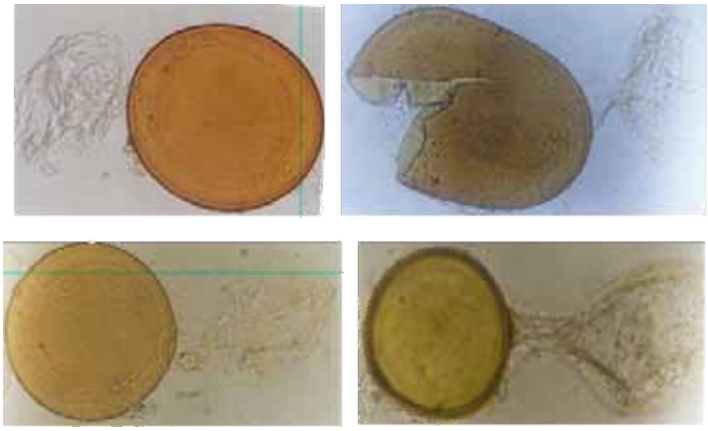

Figure 8. Spores of mycorrhizal fungi belonging to the genus *Entrophospora.*

Atlas mined-out sites also support a diverse species of AMF. The sequence of dominance was as follows: *Glomus* (42%) >*Entrophosphora* (37%) >*Acaulospora* (24%,) *Gigaspora* > (3%) and the rarest was *Scutellospora* (0.11 spore count/30 g dry soil). *Glomus* was the dominant AMF indigenous in the mine site of Atlas Mines, Toledo City, Cebu similar to those reported by *Muyzer, De Waal and Uittenlinden* (*1993*), *Aggangan et al.* (*2004*), *Li et al.* (*2010*). *Da Silva et al* (*2005*) reported more mycorrhizal genera (*Glomus*, *Acaulospora*, *Archaeospora*, *Entrophospora*, *Gigaspora*, *Paraglomus* and *Scutellospora*) from six mine sites in the Mineração Caraiba, Bahia State, Northeastern Brazil.

The kamachile tree growing in S3 harboured the greatest number of mycorrhizal spores (2,575 spores/plant). Out of the 2,575 spore density in this tree, 55% belong to the genus *Entrophospora* followed by *Glomus* (29%) and *Acaulospora* (16%). On the other hand, datiles, a pioneer species, was the only plant that was associated with *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora*.

The effectiveness of an *Entrophospora* species (coded as Paracale isolate) collected in a Cu-mine tailings in Paracale, Camarines Norte improved growth of various forest crops. Examples were done by *Aggangan and Segismundo* (*2006*) and *Aggangan and Aggangan* (*2012*). *Aggangan and Segismundo* (*2006*) inoculated *A. aulacocarpa*, *A. mangium* and *Swietenia macrophylla* seedlings with Mykovam® (containing a mixture of two species of *Glomus* and *Gigaspora margarita*) and mycorrhizal fungi indigenous (coded as Paracale isolates) in an abandoned mine site in Paracale, Camarines Norte. They found that growth was better in plants inoculated with Paracale isolates than those inoculated with the commercial mycorrhizal inoculant Mykovam®. *Aggangan and Aggangan* (*2012*) inoculated three-week old *A. aulacocarpa* seedlings with ECMF *Pisolithus*, *Scleroderma* and *Astreus* grown in Cu mine tailing soil collected from Barangay Capayang, Mogpog, Marinduque. The four months growth under screenhouse conditions showed that plants inoculated with mycorrhizal fungi collected in heavy metal-contaminated sites promoted better growth and heavy metal tolerance than those isolates collected in uncontaminated sites. Survival rate was 100% in the mycorrhizal-inoculated plants as compared with 50% in the uninoculated ones. In another experiment, *Mendoza, Ocampo and Aggangan* (*2006*) inoculated *Jatropha* seedlings with a species of *Gigaspora* and *Glomus* (not collected in mine sites) and planted in soils collected in abandoned mine sites in Antamok and Lepanto, Benguet; Paracale, Camarines Norte; Toledo, Cebu and in commercial garden soil representing a fertile soil as the control. As expected, plant growth in garden soil was the best. However, *Jatropha* seedlings were able to survive in some mine soils, but not in soils from Benguet such as TP2, TP3B and TP4 (codes given by the Benguet Mines Corporation). Plants planted in TP3B from Mankayan, Benguet and from Toledo, Cebu died after a week in the screen house. Moreover, plants planted in TP2 also from Mankayan, Benguet, died after two weeks. *Mendoza, Ocampo and Aggangan* (*2006*) attributed the poor survival to the low pH (2 to 2.4 in H_2O). These results imply careful selection which among the indigenous mine site mycorrhizal fungi that need to be continuously mass-produced for the establishment of large-scale forest plantations.

In this study, selection of AMF adapted for the different sites in relation to soil Cu content would be follows: *Glomus* plus *Acaulospora* for both S4 and S5 (with 638 mg Cu kg soil^{-1}), with spore counts of 41-42 and 31-46 spores per 30 g soil, *Glomus* plus *Entrophospora* for S3 (with 154 mg Cu kg soil^{-1}) and *Glomus* for S1 and S2 (with 168 mg Cu kg soil^{-1}). Except for S3 where *Entrophospora* was the most prevalent, *Glomus* could be a general inoculant for S1 to S5. However, the effectiveness of the different species under each genera in promoting growth and survival in mine-out sites should be studied before a cocktail of mycorrhizal fungi be formulated and be used as inoculant for planting mined-out sites similar to that in the Toledo City, Cebu.

AMF differ in their capacities and heavy metal tolerance. Some are better at imparting drought resistance while others may be more effective in protecting against pathogens or have tolerance to soil temperature extremes. Because of the wide variety of soil, climatic and biotic conditions characterizing natural or man-made environments, it is improbable that a single mycorrhizal fungus could benefit all host species and adapt to all conditions. The types and activities of mycorrhizal fungi associated with young plants may be quite different from those associated with mature plants. Thus, the mycorrhizal fungi reported in this paper which were collected from young wildlings, may differ from those living in the rhizosphere of mature trees.

The diversity of mycorrhizal fungi does not follow

patterns of plant diversity but the dominant type may regulate plant species diversity (*Van der Heijden et al. 1998*). For instance, coniferous forests of northern latitudes may have more than 1000 species of ECM fungi where only few ECM host plant species dominate, in contrast, there are fewer than 25 species of AMF in tropical deciduous forest in Mexico with 1000 AMF host plant species (*Amaranthus and Luoman 1997*). Below ground diversity of AMF is one of the major factors contributing to the maintenance of plant biodiversity and to the ecosystem functioning (*Van Der Heijden et al. 1998*). The lack of mycorrhizal fungi on plant root systems is one of the leading causes of poor plant establishment and growth in a variety of forest, restoration, agricultural, suburban and urban landscapes (*Tordoff, Baker and Willis 2000*).

To our knowledge, no information is so far available on the plant diversity and number or diversity of mycorrhizal fungi associated with plant communities sampled in the mined-out areas of Atlas Mines in Toledo City, Cebu. In spite of the limitations that only one sampling has been done, our work is an important contribution to the assessment of plant and mycorrhizal diversity in the study area.

A greater percentage of plant species, particularly shrubs commonly found in the area, are also common in many regions of the country. Graminae and Compositae were the most dominant plant colonizers similar to that reported in China (*Leung, Ye and Wong 2007*). Some species were indigenous, while others were introduced reforestation (e.g. acacias and eucalypts from Australia) and agricultural crops in the mine site being assessed. Based on the ecological status, none of the species was classified as threatened according to the Red List prepared by the International Union of Nature Conservation or IUCN (*Hilton-Taylor 2000*).

Most of the plants identified during the survey are associated with beneficial mycorrhizal fungi and could also be associated with other beneficial microorganisms. This was expected, considering the acidic and infertile soil condition of Cu-rich ecosystems. Soil microorganisms are important in the fixation and absorption of essential elements for growth, survival, and plant development. The presence of leguminous Fabaceae plants and nodulated roots are indications of rhizobial interaction that fix atmospheric nitrogen. The abundance of mycorrhizal fungi both ECMF and AMF in the *A. auriculiformis* and *E. camaldulensis* plantations in the rehabilitated areas of Atlas Mines suggests their potential in phosphorus uptake. The existence of different kinds of ferns and the presence of *Melastoma* species have been reported in many areas high in heavy metals where these are reportedly prolific. Plant succession and the corresponding microorganism interactions are vital information that should be considered in the rehabilitation of abandoned mining sites in ACMDC, Toledo City, Cebu in the Visayas group of islands.

The plant and mycorrhizal interaction currently investigated are essential to understand the ecological dynamics that will provide information for the management of the mine site regarding plant succession for the rehabilitation strategies that will be implemented during the decommissioning phase as required under the existing environmental law. It is therefore recommended that another similar study should be implemented to monitor the diversification of plant-mycorrhizal composition above and below ground ecosystems.

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