



## Determination of Soil Properties and Fungal Interrelations Influenced by Forest Restoration on a Contaminated Zinc Mining Area

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### Abstract

Forest restoration in a contaminated zinc mining area must take into account the environmental soil properties. This study aimed to analyze the soil properties from the three different stages of restored forests, at 3, 7 and 17, focusing on the impact on soil microorganisms and availability of fungal species. Three study sites were selected, all located in a zinc mining area in Mae Sot District, Tak Province, Thailand. The results showed that observed soil properties especially soil texture, moisture and nutrients differed between stand initiation and regrowth forests and affected the distribution of fungal species. Soils in older forest stands up to 17 years old showed higher sand content and reduced soil moisture, SOM, and certain nutrients. Forest restoration was found to favour development of a range of soil properties, some of which were suitable for fruiting in nine fungal species from the following families: *Agaricaceae*, *Psathyrellaceae*, *Cantharellaceae*, *Phallaceae*, *Coprinaceae*, *Sclerodermataceae*, *Lycoperdaceae*, *Marasmiaceae*, and *Clavariaceae*.

**Keywords:** Forest restoration; Zinc mining; Soil properties; Fungi

### Introduction

Forest ecosystems provide both direct and indirect benefits in terms of economic, social and environmental services. Forest ecosystems provide food sources and habitats for many species of flora and fauna [1-2] through ecological production including biogeochemical mechanisms regulated by microorganisms in a

soil ecological system. Soil microorganisms play an important role in nutrient cycling within forest ecosystems and are critical to forest ecosystem development and integrity [3]. Bacteria, actinomycetes, and fungi [4] are crucial to breakdown of organic matter. Among these, fungi play the most important role in soil nutrient cycling through microbial degradation of

organic matter [5]. Their role in biodegradation is most intensive in the rhizosphere where fungi are associated with plant roots [4]. Some, such as mycorrhiza, enjoy a symbiotic relationship with plant roots [6], exchanging nutrients from mineralization of organic matter with carbohydrates from plant photosynthesis. The exchanges may also be a non-symbiotic relationship (e.g. *Basidiomycota*, *Ascomycota*, and *Zygomycota*).

Mining disturbs soil cover and causes loss in topsoil fertility, while often contaminating soils with surface deposits of heavy metals. Contaminated wastes from mining process interrupt the functions of soil microbial activity [7], causing severe environmental damage and ecological degradation [8]. Surface cover influences available moisture for plants, so mining tends to accelerate soil degradation, especially causing reduction of capillary barriers in soil organic matter and reduction in fine soil particles. The layer of soil cover, typically mixed with foliage and litter, together with clay and mineral particles maintain the soil's water storage capacity- vital for plant growth [9]. Removal of topsoil as part of typical mining practices severely depletes soil nutrients such as nitrogen, carbon, phosphorus and sulfur [10]. Soil organic matter (SOM) provides energy for micro-organisms that unlock nutrient sources for all living organisms in the rhizosphere [11]. SOM and adequate water availability are fundamental prerequisites for effective forest restoration [8].

Restoration of forest ecosystems in mining areas requires healthy, diverse populations of soil microorganisms [12-13]. Many previous research studies have focused on the influence of soils on forest ecosystem restoration, but have neglected soil formation as a result of forest ecological production which contributes to restoration of forest ecosystem services [11]. This research was conducted to determine the influence of soil properties on fungal populations

and the contributions of these fungal species to restoration of forest ecosystems.

### Materials and methods

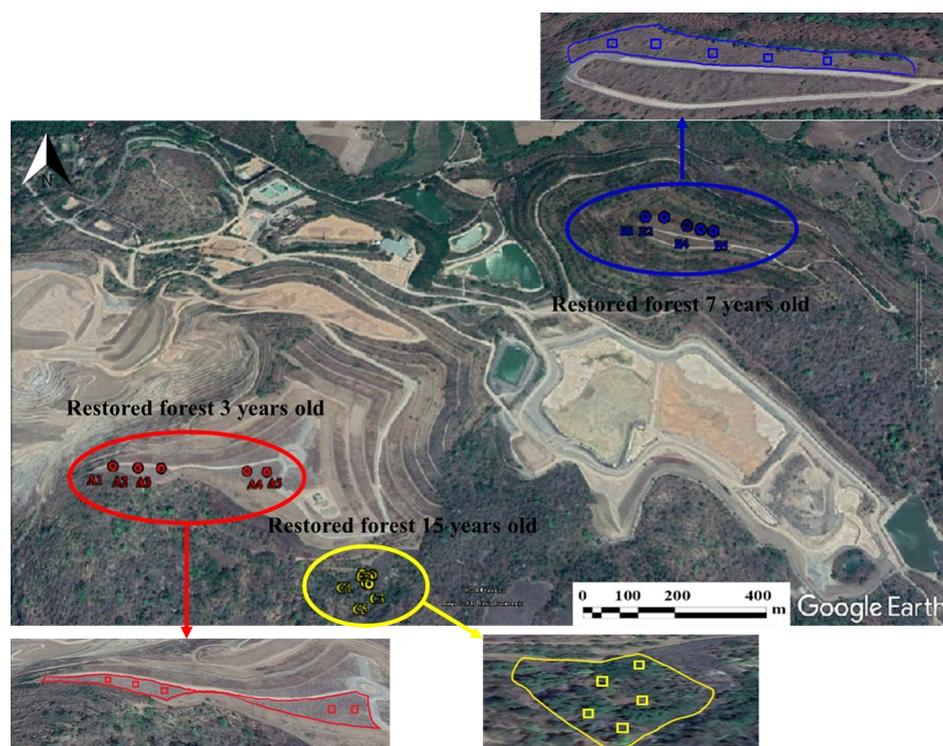
A mixed deciduous forest comprising native species was restored over a zinc mining area in Mae Sot District, Tak province, Thailand. The study sites were former landfill areas for the mine, that were later reclaimed for forest development during the mining closure phase mandated under the Enhancement and Conservation of National Environmental and Quality Act, B.E. 2535 (A.D. 1992). At a reclaimed cell of landfills for uneconomic zinc ores and mining wastes, a 30-cm topsoil layer was prepared for indigenous tree planting in 2000. Three different forest plantations were selected based on soil properties at different restored forest stages including stand initiation stage at 3 years old, young forest regrowth stages at 7 years old, and 17 years old (Figure 1). The sites were characterized by elevated slopes below 25 degrees, with narrow contour strip planting.

Field survey and the line transect method with five replicated plots of 10x10 m<sup>2</sup> were conducted and marked with stakes to count fungal species and to collect soil samples for the observation period. Fungal species identified in any plot from the three stages of restored forest were collected and recorded during the field study in June, September and October, 2017. Their scientific names were identified by morphological characterization using a guidebook, Mushroom Diversity in Thailand [14], and were confirmed by expert opinion using images showing characteristic identifying structures.

Soil samples from 15 replicated plots with three sampling replications were collected in June 2017, to represent soil properties of those forest stages. Composite sampling was used to produce replicate soil samples. The soil samples were collected at a depth of 2-15 cm using a

soil core sampler. Soil temperatures were measured and recorded at the field sites. Samples were stored in polyethylene bags and preserved at under 4 °C for later physical analysis (texture and bulk density), chemical (pH, cation exchange capacity (CEC), organic matters (OM), total carbon (TC), total nitrogen (TN), available phosphorus, zinc, cadmium,

and iron) and biological (fungi, actinomycete, and bacteria). Several standard analytical methods for soil analyses (Table 1) were employed at the laboratory of the Interdisciplinary Program in Environmental Science and at the Environmental Research Institute of Chulalongkorn University, Bangkok, Thailand.



**Figure 1** Study sample locations for restored forest stages aged 3, 7 and 17 years, using Google Earth 2016.

**Table 1** Analytical methods of soil properties

Parameter	Method	Reference
Soil texture (%)	Hydrometer method	[15]
Soil bulk density (%)	Core method	[16]
Soil moisture (%)	Dried at 105°C	[17]
Soil temperature	Thermometer	[16]
pH	pH meter	[16]
CEC (cmol kg <sup>-1</sup> )	Ammonium saturation method	[18]
Organic Matter, OM (%)	Walkley & Black, 1996	[19]
Total carbon, TC (%)	Walkley & Black, 1996	[19]
Total nitrogen, TN (%)	Kjeldahl	[20]
Available phosphorus (mg kg <sup>-1</sup> )	Bray 2 & Kurtz	[20]
Sulfur (mg kg <sup>-1</sup> )	Spectrophotometer	[20]
Iron, Zinc, and Cadmium(ppm)	Atomic Absorption Spectrophotometer (AAS)	[21]
Fungi, Actinomycete, and Bacteria (CFU g <sup>-1</sup> )	Plate count	[22]

## Results and discussion

Changes in soil physical and biological properties are complex and influenced by many factors such as weather, soil structure, vegetation cover, and soil microflora. In a natural forest ecosystem, a dynamic equilibrium emerges that results ecosystem productivity. In forests restored with native plant species, biomass production generally generates at very slow rate and regularly faces wild fires as a human disturbing activity. The fires left C:N ratios largely unaffected but accelerated soil erosion from elevated sloping study areas [23]. After 20 years of forest restoration, soil microbiological functions approached a new dynamic equilibrium.

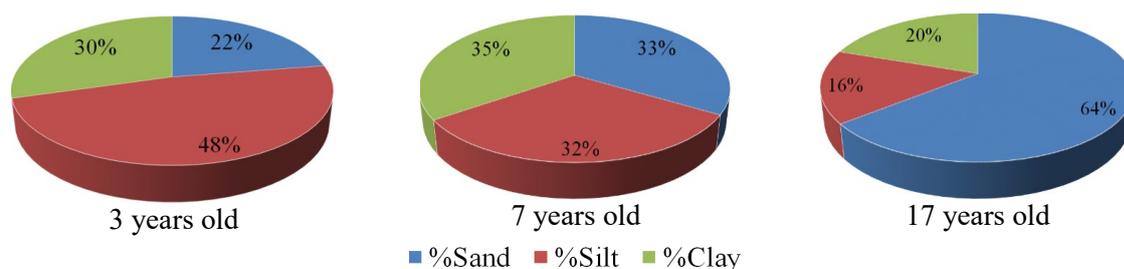
### 1) Soil properties influenced by forest development stages

Typical soil textures in Mae Sot District are mostly categorized as loam sands [24]. A study of soil physical properties for three stage of forest growth revealed that soil textures of 3-year old restored forest was typically categorized as clay loams, with sand, clay and silt present at  $22.4\pm 6.1\%$ ,  $29.5\pm 5.4\%$ , and  $48.1\pm 7.3\%$ , respectively. At 7 years, the soil structure was similar, with proportions of sand, clay and silt at  $32.1\pm 2.1\%$ ,  $32.7\pm 2.6\%$  and  $35.3\pm 3.0\%$ , respectively. However, at 17 years, the texture of restored forest soils had changed to a sandy clay loam comprising silt, clay and sand in proportions of  $15.9\pm 6.6\%$ ,  $19.8\pm 8.9\%$  sand  $64.4\pm 15.4\%$ , respectively (Figure 2).

As seen in Figure 2, the older restored forests showed a loss of clay and silt particles

while sand particles remained. This may be caused by water erosion from rainfall and surface runoff, exacerbated by rapid loss of organic matter that bind the soil and slows down water flow [25- 26]. At high elevations, fast growing trees would have the most detrimental consequences on soil formation [27]; native species are generally preferable for forest restoration. In younger restored forests, the canopy cover provides insufficient protection from rain impacts, resulting in severe soil erosion; the foliage and ground litter is also degraded rapidly in the tropics [2, 29]. However, this decomposition was insufficient to help build up sufficient reserves of SOM.

The change in soil texture also influenced SOM. Among soils in similar climatic zones, SOM in fine textured clay soils may be two to four times higher than SOM found in coarse textured sandy soils [28]. Clay particles and organic matter determine the soil's cation exchange capacity (CEC), which is critical to the supply of nutrients to plants. The results also showed reductions in CEC as the clay content declined. The results of this study indicate that very low levels of total N for all ages of restored forest, suggesting that the sampling sites had been disturbed by fire (Table 2). Forest fires burn away organic matter (OM), nutrients and soil microorganisms including actinomycetes, bacteria and fungi. From the results, it was found that available phosphorus levels in restored forest soils decreased with forest age ( $64.89\pm 26.44$ ,  $22.49\pm 6.02$ , and  $29.63\pm 13.29$  mg kg<sup>-1</sup>), respectively.



**Figure 2** Composition of soil texture; sand, silt, clay found in soil samples collected from the three restored forest areas.

**Table 2** Soil parameters measured according to the restored forest ages of 3, 7 and 17 years

Parameter	3 years	7 years	17 years
Depth (cm)	15	15	15
bulk density (g cm <sup>-2</sup> )	0.28±0.02	0.31±0.02	0.32±0.02
Soil moisture (%)	20.68±3.53	20.25±3.90	13.09±3.45
Temperature (°C)	26±0.23	25±0.23	24±0.23
pH	6.8±0.1	7.4±0.2	7.2±0.2
CEC (cmol kg <sup>-1</sup> )	20.4±3.79	22.44±0.91	9.81±7.33
OM (%)	2.70±0.11	2.32±0.10	1.69±0.06
TC (%)	1.57±0.06	1.35±0.06	0.98±0.03
TN (%)	0.12±0.05	0.10±0.02	0.11±0.04
Available P (mg kg <sup>-1</sup> )	64.89±26.44	22.49±6.02	29.63±13.29
Sulfur (mg kg <sup>-1</sup> )	10.16±4.51	12.26±3.16	7.62±2.15
Cadmium (ppm)	0.74±0.2	2.72±2.4	12.0±5.7
Zinc (ppm)	73.86±13.7	250.26±166.8	1,416.2±698.4
Iron (ppm)	21,120.8±1976.7	30,065.4±3.1	31,079.4±7216.2
Fungi (CFU g <sup>-1</sup> )	8.05x10 <sup>6</sup> ±6.66x10 <sup>6</sup>	8.66x10 <sup>8</sup> ±6.10x10 <sup>8</sup>	1.40x10 <sup>9</sup> ±8.25x10 <sup>8</sup>
Actinomycetes (CFU g <sup>-1</sup> )	1.67x10 <sup>5</sup> ±2.48x10 <sup>5</sup>	3.31x10 <sup>5</sup> ±2.48x10 <sup>5</sup>	4.71x10 <sup>5</sup> ±7.32x10 <sup>5</sup>
Bacteria (CFU g <sup>-1</sup> )	4.98 x10 <sup>5</sup> ±2.97x10 <sup>5</sup>	3.84x10 <sup>6</sup> ±4.16x10 <sup>6</sup>	2.81x10 <sup>6</sup> ±1.63x10 <sup>6</sup>

Changes in soil texture caused by water erosion is common in young restored deciduous forests [30]. Despite this, the loss of clay and silt particles did not change the soil's bulk density, which increased slightly from stand initiation to young forest regrowth stages (0.28 ±0.02 g cm<sup>-2</sup>, 0.31±0.02 g cm<sup>-2</sup>, and 0.32±0.02 g cm<sup>-2</sup>, respectively). Sandy soil particles from mining wastes were closely associated with heavy metal contamination. Soil concentrations of heavy metals, especially cadmium, iron and zinc were found to increase with age of the restored forest (Table 2).

The increase in cadmium, iron and zinc concentrations resulted from erosion of organic matter from the surface soil, particularly in the younger stages of regrowth. The study also found that populations of soil microorganisms including fungi, actinomycetes and bacteria, increased with the age of restored forest (Table 2), although the increase for bacterial populations were not statistically significantly.

Soil pH did not vary significantly with age, remaining predominantly neutral. However, soil moisture content also decreased with age of restoration to 20.67 ± 3.53%, 20.25 ± 3.90% and 13.09 ± 3.45%, respectively.

Additionally, organic matter and total organic carbon content for the 3-year-old restored forest were higher than for older restored forest, due to the impacts of human activities such as wood cutting and wild fires on organic matter and soil nutrients [29], as well as the nutrient cycling when SOM is decomposed by microorganisms to form organic, soluble carbon (dissolved organic carbon). Organic carbon dissolved in water plays an important role in soil processes and contributes to maintaining nutrient balance [31]. In tropical forests, rapid decomposition of organic matter results in depletion of the soil and loss of soil structure and fertility and water-holding capacity [32].

## 2) Effects of changing soil properties of restored forest on fungal species

The study investigated soil properties including pH, moisture, temperature, particle size distribution and soil bulk density in order to identify their effect on distribution of fungal species in each area of restored forest. The results indicated that fungal growth required neutral or slightly alkaline soils (pH  $6.70 \pm 0.1$  to  $7.4 \pm 0.2$ ), soil texture in the range of clay loam to sandy clay loam: clay ( $11.1 \pm 8.9$  to  $41.3 \pm 3.1\%$ ), silt ( $11.2 \pm 6.6$  to  $50.0 \pm 7.3\%$ ) and sand ( $17.7 \pm 6.1$  to  $73.9 \pm 15.4\%$ ), having  $0.59 \pm 0.09$  to

$3.83 \pm 0.99\%$  of organic matter and  $13.09 \pm 3.45$  to  $20.67 \pm 3.53\%$  of soil moisture. Also, macronutrients and microelements including TN ( $0.05 \pm 0.04$  to  $0.29 \pm 0.05\%$ ), OM ( $0.41 \pm 1.02$  to  $6.43 \pm 1.15\%$ ), TC ( $0.24 \pm 0.59$  to  $3.73 \pm 0.67\%$ ), and available P ( $11.82 \pm 13.29$  to  $123.28 \pm 26.44\%$ ), and Zn ( $0.155$  to  $1.567 \text{ mg kg}^{-1}$ ) were needed for growth and reproduction of fungal species identified in the three restored forest stages. The field survey results showed a range of fungal species in those three restored forest plots as shown in Figures 3-5.



a: *Leucocoprinus birnbaumii* (corda) Family: Agaricaceae  
 b: *Cantharellus cibarius* Family: Cantharellaceae  
 c: *Coprinus plicatilis*, *Agaricus plicatilis* Family: Psathyrellaceae  
 d: *Lycoperdon pretense* Family: Lycoperdaceae

**Figure 3** Four mushroom species found in the three years old restored forests.



e: *Coprinopsis lagopus* Family: Psathyrellaceae

**Figure 4** One mushroom species found in the seven years old restored forests.



f: *Dictyophora indusiate*

Family: Phallaceae

g: *Coprinus disseminates*

Family: Coprinaceae

h: *Scleroderma verrucosum* Pers

Family: Sclerodermataceae

i: *Scleroderma flavidum*

j: *Calvatia craniiformis*

Family: Lycoperdaceae

k: *Marasmius maximus*

Family: Marasmiaceae

**Figure 5** Six fungal species found in the 17-year old restored forests.

This study found 6 types fungal species in the restored 17-year old restored forest. The environmental conditions of their habitat were moderate pH with sandy clay loam soils. Soil moisture at 15 cm depth was between  $9.96 \pm 2.35$ - $16.90 \pm 1.92\%$ .

Soil organic matter levels we boosted by higher levels of soil moisture, which also stimulated higher biomass production, which in turn provided potential food for soil biota. Soil ecosystems require adequate aeration and moisture to favour soil microbial activities. Soil water in the three sites soil were equivalent to 60-percent of the water-filled pore space [33]. Soil moisture was predictably lowest in the more sandy, highly degraded soils in the older 17-year old restored forest area. However, the range of soil moisture of 10-20% provided optimal condition for fungal growth and reproduction. On the other hand, poorly aerated or saturated soils result in reductive conditions leading to a change in soil microorganisms. Without active microbial decomposition under aerobic conditions, organic matter remains intact in the soil. Due to these optimal soil conditions, the fruiting bodies of a range of fungi were found in all stages of restored forest. Soil sampled from the 17-year old forest contained SOM at levels of 1.50-3.16% with 0.87-2.19% carbon; soil temperature was 24-26 °C and soil moisture of 10-16%. A study by Gezer and Kaygusuz [34] found that *Agaricus langei*, a fungus species in the Agaricaceae family thrived optimally at pH 7.7-8.9, which was consistent with its presence in these study soils.

### Conclusions

The mixed deciduous forest was restored with native species on prepared topsoil covering an old landfill areas of a zinc mine in Mae Sot District, Tak province of Thailand in 2000 as mandated by law. The soil properties of different restored forest stages including stand

initiation stage at 3 years old, young forest regrowth stages at 7 and 17 years old were found differently at different development stages of restored forest. The proportion of sand increased with age of the restored forest, while the study plots showed minimum SOM and very low total C, reflecting soil disturbance, particularly due to forest fires. The optimal environment for young restored forest resulted in growth of nine fruiting fungal species. The restored forest stages showed differences in soil properties; further work is needed on soil processes, particularly for timescales beyond the scope of this study to cover monitoring of fully mature restored forests older than 20 years.

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