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Effects of Acid Soils on Plant Growth and Successful Revegetation in the Case of Mine Site

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<http://dx.doi.org/10.5772/intechopen.70928>

Abstract

Acid soils are caused by mining, potentially causing the death of plants. Although soil pH is one of the useful indicators to evaluate acid soil conditions for successful revegetation, the dissolution of harmful elements under acidic conditions should be considered in addition to the tolerance mechanism of plants in mines. Thus, this study aims to report the current situation of acid soils and plant growth in mine site and to elucidate the effects of acid soils on plant growth over time through field investigation and a vegetation test. The results showed that the dissolution of Al from acid soils which were attributed to the dissolution of sulfides influenced plant growth. Not only soil pH but also the assessment of the dissolution of sulfides over time is crucial for successful revegetation, suggesting that net acid producing potential (NAPP) and net acid generation (NAG) pH, which are used for evaluating the formation of acidic water, are useful to evaluate soil conditions for the revegetation. Furthermore, acid-tolerant plant survived under acidic conditions by increasing the resistance against acidic conditions with the plant growth. Such factors and the proper selection of plant species play an important role in achieving successful revegetation in mines.

Keywords: acid soils, plant growth, revegetation, mine site, acidic water, Al tolerance, sulfides

1. Introduction

Acid soils are formed with human activities, such as construction and mining, potentially causing the death of plants [1]. Plants wither due to not only low pH conditions in acid soils

but also dissolution of harmful elements, such as Al, Fe, and Mn, dissolving under acidic conditions. Although soil pH is one of the useful indicators to evaluate acid soil conditions for successful revegetation and/or farming, the dissolution of harmful elements under acidic conditions should be taken into account [2]. For example, Al, which constitutes approximately 7% of the Earth's mass, is easily released in water with the change of pH, thereby inhibiting plant growth, including root growth and its function [3]. Al generally existing as $\text{Al}(\text{OH})_3$, which is insoluble in soils, dissolves in water as Al^{3+} under acidic conditions ($\text{pH} < 4.5$) and is released as $\text{Al}(\text{OH})_4^-$ under alkaline conditions. The Al^{3+} easily reacts with phosphoric acid and then it causes phosphorus deficiency on plants with the formation of insoluble aluminum phosphate in soils [4]. Other harmful elements, such as Fe and Mn, also inhibit plant growth. Therefore, acid soils affect plant growth through indirect factors like dissolution of harmful elements, indicating that the understanding of the effect of acid soils on plant growth in terms of not only soil pH but also harmful elements is important for successful revegetation.

With respect to plant species, there are some plants that can survive in acid soils owing to tolerance characteristics, such as acid tolerance and Al tolerance. Acid-tolerant plants can survive under low soil pH conditions by setting up several tolerance mechanisms, such as the increase of soil pH around the root apices [5, 6]. The plants with Al tolerance are resistant to the effects of Al as described above. They are separated into Al-tolerant plant and Al-stimulated plant and additionally categorized as Al-excluders, Al root-accumulators, and Al-accumulators [7]. While *Camellia sinensis* localizes Al in the cell walls of epidermal cells of the leaves against Al toxicity [8], *Acacia mangium* (*A. mangium*) excludes Al from the root apices [9]. Furthermore, Saifuddin et al. found that the photosynthetic rates rose by increasing soil pH in *Leucaena leucocephala* after the pre-aluminum treatment [9, 10]. Thus, Al-tolerant mechanism of plants depends on the plant species, and the effects of acid soils on plant growth change according to the species. This indicates that tolerance characteristics of plants should be considered in regard to the effect of acid soils on plant growth in addition to soil pH.

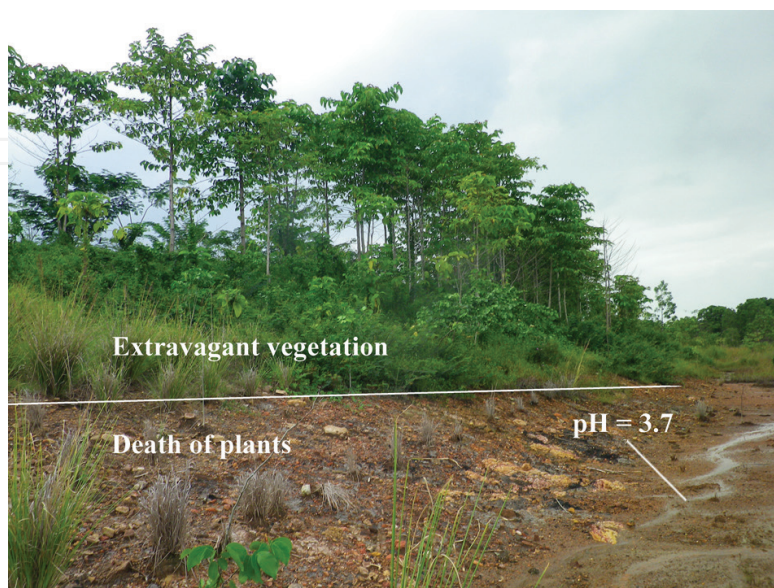


Figure 1. Death of plants under acidic condition in the waste dump in mine site.

The research on the effects of acid soils on plant growth has been performed in the world; however, it is still a serious problem, especially in mine site where revegetation is necessary for environmental reclamation as shown in **Figure 1**. The formation of acid soils resulted from construction and/or resource exploitation has been often highlighted as the cause of plant death. The information on the effects of acid soils on plant growth in mine site is crucial for successful revegetation. Therefore, this study aims to report the current situation of acid soils and plant growth in mine site and to elucidate the effects of acid soils on plant growth over time through field investigation and a vegetation test. On the basis of the results, the key to successful revegetation was discussed from the point of view of soil acidification and tolerance mechanism of plants.

2. Methods

2.1. Vegetation survey

Plant species were investigated at two points (namely, Point A and Point B) in the waste dump in a coal mine in Indonesia in conjunction with literature research on plant species focusing on fast-growing characteristics and acid tolerance in order to understand the effects of acid soils on plant growth. Point A is about 400 m away from Point B in the same waste dump. This waste dump was constructed more than a year ago by piling up waste rocks, followed by revegetation which is mandatory for environmental conservation in mines. The revegetation in the research area had been conducted in the two stages (primary and secondary revegetation) in terms of growth rate of plants based on the experiences of the revegetation. Fast-growing trees were planted in the first stage of the revegetation for 3 years to improve soil conditions by increasing organic matter, followed by planting local plants in the second stage. In this mine, the death of plants has been reported along with the formation of acidic water at Point A as shown in **Figure 1**; on the other hand, it has not been reported at Point B.

In addition, three samples of *Eleusine indica* (*E. indica*) and *Melaleuca leucadendra* (*M. leucadendra*) (*Melaleuca cajuputi*) which were observed at the both points were taken, and they were separated into leaves, stems, and roots. The samples were washed with deionized water using a sonication (UT-106H, SHARP) at room temperature to remove soil particles. Finally, they were dried at 60°C for 72 hours and pulverized using mortar and pestle by each part of the plants. 0.25 g of each part of the samples were digested by 5 mL of a mixture of 61% nitric acid (HNO₃) and 35% hydrochloric acid (HCl) at a ratio of 3:1 at 110°C in a DigiPREP Jr. (SCP Science, Quebec, Canada) until they were completely digested with reference to the method by Quadir et al. [11]. In the case that they were not dissolved in the mixture, 1 mL of the mixture was added and the dissolution process was repeated. After the dissolution process, the volume of the solution was adjusted to 20 mL by adding deionized water. The solutions were subjected to Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, VISTA-MPX ICP-OES [Seiko Inst., Japan]) after the filtration with 0.45 µm of membrane filter in order to quantify the content of Al, B, Fe, Mn, S, and Zn, which are thought to affect plant growth and be linked with the formation of acidic water, in each part of plant body. The results were calculated with mg

per dry unit weight (mg/g) and compared with the waste water quality, which was recorded at Point A and Point B, so as to understand the linkage between the formation of acidic water and the accumulation of the elements in the plants.

2.2. Soil analysis

Soils within the waste dump were sampled until 100 cm depth with a 8-cm diameter hand auger (DIK-100A-55) at Point A and Point B in order to understand the current soil conditions. Soil pH was measured at each depth using Soil Acid meter (SK-910A-D, Sato, and DM-13, Takemura Electric Works Ltd.). The samples were separated by 20-30 cm and supplied to X-ray diffraction (XRD) and X-ray fluorescence (XRF) analysis, paste pH test, acid base accounting (ABA) test, and net acid generation (NAG) test so as to investigate the cause of acidic water in this area [12, 13]. The XRD analysis was conducted using wide angle goniometer RINT 2100 XRD after the drying process at 50°C for 48 hours in a nitrogen atmosphere under the following conditions: radiation $\text{CuK}\alpha$, operating voltage 40 kV, current 26 mA, divergence slid 1°, anti-scatter 1°, receiving slit 0.3 mm, step scanning 0.050°, scan speed 2.000°/min, and scan range 2.000–65.000°. In paste pH test, the change of pH was reported as paste pH after 12 hours of the dissolution process at the 1:2 of mixing ratio of sample and deionized water ($\text{pH}_{1:2w}$). The ABA and NAG test were performed to evaluate the acid producing potential of the soils. Net acid producing potential (NAPP) was calculated with acid potential (AP) and acid neutralizing capacity (ANC) of the samples as an acid producing potential in addition to NAG pH [12, 13]. Soils with NAG pH < 4.5 and NAPP > 0 were considered the source of acidic water, which can produce acids [14]. Acid producing potential generally rises with the increase of NAPP and the decrease of NAG pH.

2.3. Vegetation test

The vegetation test was conducted with a focus on the effect of acidic conditions on plant growth over time based on the results of the vegetation survey. Simulated acid soil was prepared by mixing sand, clay, and pyrite. Pyrite was mixed in the simulated soils, which were prepared using sand and clay in conformity to the soil texture and the physical properties of the topsoil in the mine site, aiming at setting the sulfur content as from 0 to 30% by weight on the basis of the result of XRF analysis. Each sample was labeled as S0.0, S0.5, S1.5, S3.0, S5.0, S7.5, S10.0, S15.0, and S20.0. The prepared acid soils were evenly mixed in each flower pot at a constant rate to uniform physical conditions in each pot, and used for the vegetation test. In this study, *A. mangium* which inhabits tropical forests in Southeast Asian countries, including Indonesia, and has acid tolerance was used: the seeds were obtained in Japan. *A. mangium* occurs naturally in the humid tropical lowland and has been successfully applied in reclamation in post-mine lands for bauxite, copper, coal, and iron in the world. It grows in compacted soils, dry area, on the slopes of hills, and humid area owing to high adaptability. *A. mangium* is designated an Al-excluder plant as well as *M. leucadendra* (*M. cajuputi*) [7].

In order to elucidate the effects of acidic conditions, including low pH, Al, B, Fe, Mn, S, and Zn on plant growth, *A. mangium* was planted on the prepared acid soils in the phytotron glass room G-9 in Biotron Application Center, Kyushu University under the following conditions: at 30°C room temperature and 70% relative humidity assuming the local climate in the mine

site in Indonesia. The 9 flower pots with different content of pyrite were used for the vegetation test. In this test, five plants were planted in pots by the content of pyrite, and the height and the diameter were measured every week. About 500 mL of water was supplied to the pots every 3–4 days. The liquid fertilizer HYPONeX-R (N-P-K = 6-10-5) diluted to 1000 mg/L with water was, additionally, added to them once per week. The test was continued for 133 days until a clear distinction is observed.

To understand the effects of chemical and physical conditions of the prepared acid soils on plant growth, paste pH, NAG test, ABA test, Atterberg Limits test, and particle size distribution test were conducted according to the standard of AMIRA [13], ASTM D4318-05 [15], and ASTM D422-63 [16]. Moreover, the leachate from the bottom of the pots was taken every week to monitor the change of pH. At the end of the vegetation test, each part of the plants was digested by the acids in the same way as in Section 2.1 [11]. On the basis of the concentration of Al, B, Fe, Mn, S, and Zn in each part of the plants, the effect of the elements on plant growth over time was elucidated.

3. Results and discussion

3.1. Effects of acid soils on plant growth in mine site

Tables 1 and **2** summarize the main species of plants, which were planted in each stage of the revegetation in the mine and were characterized according to the literatures. The plants, which are acid tolerant, were planted in the first stage of the revegetation, and most of them were classified into fast-growing species. The plants generally planted for soil improvement, such as *Calliandra calothyrsus*, *Gliricidia sepium*, *Pterocarpus indicus*, and *Paraserianthes falcataria*, were planted in the first stage, aiming at improving soil conditions by increasing organic matter in the waste dump before planting local plants that are not acid tolerant in the second stage. Such fast-growing species shorten the time for revegetation and enable us to perform an earlier improvement of soil conditions in the waste dump. On the other hand, local plants which were planted in the second stage are utilized for industrial use as timber and ecosystem protection, such as *Intsia bijuga* and *Inocarpus fagifer*. The results indicated that plants were transplanted in the waste dump in the two stages for different purposes for successful revegetation in this mine.

The plants, which were observed in the waste dump at Point A and Point B, are summarized in **Table 3**. *Swietenia macrophylla*, *Mimosa pudica*, and cover crop (*Convolvulaceae*) plants, which are not acid tolerant, were not observed at Point A. Although *Intsia bijuga*, which is not acid tolerant, was observed at Point A, some of them withered. By contrast, *Paraserianthes falcataria*, and *M. leucadendra*, which are acid tolerant, were found at Point A and Point B, indicating that the plant growth in the waste dump may have been affected by acidic conditions. Additionally, the revegetation failed at Point A since *Swietenia macrophylla* which was planted in the second stage of the revegetation and the cover crop (*Convolvulaceae*) plants which are important for improving soil conditions withered.

| Name of plants | Fast growing | Acid tolerance | References |
|-----------------------------------|--------------|----------------|---|
| <i>Paraserianthes falcataria</i> | + | + | Evans and Szott [17] Krisnawati et al. [18] |
| <i>Pterocarpus indicus</i> | - | + | Evans and Szott [17] Thomson [19] |
| <i>Michelia champaca</i> | - | + | Orwa et al. [20] Fern [21] |
| <i>Gliricidia sepium</i> | + | + | Orwa et al. [20] Evans and Szott [17] |
| <i>Anthocephalus cadamba</i> | + | + | Irawan and Purwanto [22] Krisnawati et al. [18] |
| <i>Anthocephalus macrophyllus</i> | + | + | Irawan and Purwanto [22] Krisnawati et al. [18] |
| <i>Senna siamea</i> | - | + | Orwa et al. [20] |
| <i>Calliandra calothyrsus</i> | + | + | Orwa et al. [20] |
| <i>Melaleuca leucadendra</i> | + | + | Masitah et al. [23] Turnbull [24] Nakabayashi et al. [25] |
| <i>Nauclea orientalis</i> | - | - | Orwa et al. [20] |
| <i>Enterolobium cyclocarpum</i> | + | + | National Research Council [26] Evans and Szott [17] |

Table 1. Plant species in the first stage of the revegetation and the growth characteristics and the acid tolerance: + indicates that it has the characteristics; - indicates it does not.

| Name of plants | Fast growing | Acid tolerance | References |
|-------------------------------|--------------|----------------|--------------------------------|
| <i>Pterospermum javanicum</i> | - | - | |
| <i>Ficus benjamina</i> | ++ | - | Fern [21] |
| <i>Aquilaria spp.</i> | - | - | Soehartono and Newton [27] |
| <i>Inocarpus fagifer</i> | + | + | Pauku [28] |
| <i>Tectona grandis</i> | ++ | - | Orwa et al. [20] |
| <i>Hevea brasiliensis</i> | - | - | Orwa et al. [20] |
| <i>Pericopsis mooniana</i> | - | - | Ishiguri et al. [29] |
| <i>Swietenia macrophylla</i> | - | - | Krisnawati et al. [18] |
| <i>Shorea balangeran</i> | - | - | |
| <i>Intsia bijuga</i> | - | - | Thaman et al. [30] |
| <i>Schima wallichii</i> | - | - | Orwa et al. [20] |
| <i>Mimusops elengi</i> | - | - | Kadam [31] |
| <i>Fagraea fragrans</i> | - | - | Steinmetz [32] |
| <i>Samanea saman</i> | ++ | - | National Research Council [26] |

Table 2. Plant species in the second stage of the revegetation and the growth characteristics and the acid tolerance: ++ indicates that it has the characteristics; + indicates moderate; and - indicates that it does not.

| Name of plants | Point A | Point B | Acid tolerance |
|--------------------------------------|---------|---------|----------------|
| <i>Paraserianthes falcataria</i> | ○ | ○ | + |
| <i>Anthocephalus cadamba</i> | ○ | ○ | + |
| <i>Melaleuca leucadendra</i> | ○ | ○ | + |
| <i>Intsia bijuga</i> | ○ | ○ | - |
| <i>Eleusine indica</i> | ○ | ○ | + |
| <i>Swietenia macrophylla</i> | × | ○ | - |
| <i>Mimosa pudica</i> | × | ○ | - |
| Cover crop (<i>Convolvulaceae</i>) | × | ○ | - |

Table 3. Plant species, which were observed in the waste dump at Point A and Point B, and its acid tolerance: ○ indicates that it was observed; × indicates that it was not observed; + indicates acid tolerant; and - indicates that it is not acid tolerant.

In regard to waste water quality in the waste dump, electric conductivity (EC) and oxidation reduction potential (ORP) exhibited higher values at Point A (EC = 3.00 mS/cm, ORP = 588 mV) than at Point B (EC = 0.62 mS/cm, ORP = 568 mV). Moreover, a higher concentration of total Fe, SO_4^{2-} , and Al related to the formation of acidic water was recorded at Point A than at Point B, suggesting the formation of acidic water with the dissolution of sulfides at Point A based on the XRD results. This implies that acid soils can be formed with the intrusion of acidic water into the ground at Point A. While acid soils in Southeast Asian countries are often attributed to sulfuric sediments formed in mangrove, acidic water caused by the exposure of sulfides to oxygen and water with the excavation in mine site may have resulted in the formation of acid soils in this case [33]. The geochemical properties of the soils at Point A and Point B are summarized in **Table 4**. Paste pH showed similar values at each depth at the points. Furthermore, there was not a significant difference in soil pH showing 5.0–6.4 at each depth at the points. This was resulted from acid soils, which are common in the Southeast Asian countries. Meanwhile, there was a large difference in sulfur content, NAPP, and NAG pH between the points. Sulfur content showed larger values especially at 30–70 cm depth at Point A than at Point B. The results of XRD analysis suggested the presence of sulfides at the points, thus showing that the difference in the content of sulfides led to the difference in sulfur content at 30–70 cm depth between Point A and Point B. Since NAPP is calculated by subtracting ANC from AP, NAPP showed negative values at Point B where ANC showed positive values because of neutralization by carbonate and/or clay minerals. Besides, considering the similar values of paste pH and soil pH between Point A and Point B and complete dissolution of the samples with H_2O_2 in NAG test, the differences in NAG pH between the points were triggered by the abundance distribution of sulfides. The change of paste pH is not greatly affected by the dissolution of sulfides as soluble minerals mostly affect paste pH in a relative short time as well as soil pH. On the other hand, NAG pH significantly depends on the dissolution of sulfides owing to the complete dissolution of samples with H_2O_2 in NAG test. Therefore, NAG pH was lower at Point A than Point B since sulfides at Point A were sufficient to react with H_2O_2 . With respect to the formation of acidic water, the presence of sulfides leads to the continuous formation of acidic water for a long time,

| Point | Depth (cm) | Paste pH | S (%) | AP | ANC | NAPP (kg H ₂ SO ₄ /ton) | NAG pH |
|---------|------------|----------|-------|------|------|---|--------|
| Point A | 0–20 | 4.47 | 0.13 | 4.1 | 0.0 | 4.1 | 4.24 |
| | 30–50 | 4.72 | 0.75 | 23.1 | 0.0 | 23.1 | 3.76 |
| | 50–70 | 5.05 | 1.06 | 32.5 | 3.2 | 29.3 | 2.74 |
| | 70–100 | 4.41 | 0.52 | 16.0 | 0.0 | 16.0 | 3.22 |
| Point B | 0–20 | 4.74 | 0.09 | 2.9 | 43.9 | -41.0 | 4.39 |
| | 30–50 | 4.76 | 0.08 | 2.5 | 43.6 | -41.1 | 4.57 |
| | 50–70 | 4.57 | 0.11 | 3.4 | 43.4 | -39.9 | 4.30 |
| | 70–100 | 4.63 | 0.11 | 3.3 | 43.4 | -40.1 | 4.06 |

Table 4. Geochemical properties of the samples in the waste dump at Point A and Point B.

which is considered as a lag time due to the difference in the solubility of sulfur in various minerals, such as sulfates and sulfides [34]. The continuous dissolution of sulfides for a long time resulted in a high concentration of total Fe, Al, and SO₄²⁻ in the waste water at Point A. Additionally, NAG pH < 4.5 and NAPP > 0 at Point A indicated the source of acidic water. This suggested that the evaluation of soil pH combined with NAPP and NAG pH, which are used to predict the formation of acidic water, enables us to understand the formation of acidic water and acid soils over time. Hence, in this case, the formation of acidic conditions in soils triggered by acidic water for a long time with the continuous dissolution of sulfides caused plant death at Point A. It is necessary to consider a lag time of the dissolution of sulfur in addition to soil pH for successful revegetation.

Figures 2 and **3** show the concentration of Al, As, B, Fe, Mn, S, and Zn in each part of *E. indica* and *M. leucadendra*, which were sampled in the waste dump at Point A and Point B. In addition, the standard deviation of the results was summarized by the species as shown in **Figure 4**. In particular, Fe and Al were accumulated in the roots of the plants. S was accumulated in the roots and leaves of the plants, and the concentrations of the elements were higher at Point A than that at Point B. This was attributed to the biological action to accumulate the excess of the harmful elements for plant growth on the roots. As the accumulation of Al in the roots causes the death of plants by preventing the absorption of nutrients from the roots, the high concentration of Al caused the inhibition of the growth of *Intsia bijuga*, *Swietenia macrophylla*, *Mimosa pudica*, and cover crop (*Convolvulaceae*) at Point A [35]. Moreover, a high concentration of Fe and S, which were derived from the dissolution of sulfides such as FeS₂, suggested that the dissolution of Al under acidic conditions was caused by the formation of acidic water with the dissolution of sulfides. The higher concentration of Fe, S, and Al was, additionally, obtained in *M. leucadendra*, which is acid tolerant, than that in *E. indica*, indicating that the accumulation capacity of the elements in the plant body depends on the species. Compared to the standard deviation of B, Mn, and Zn with that of Al, Fe, and S, the standard deviation of B, Mn, and Zn was near zero as shown in **Figure 4**, suggesting that B, Mn, and Zn were ubiquitous in the plants. B is an essential element for the maintenance of cell wall and carbohydrate metabolism [36], and the atomic number of B is similar with that of C, which is utilized for organic substances through the formation of carbon

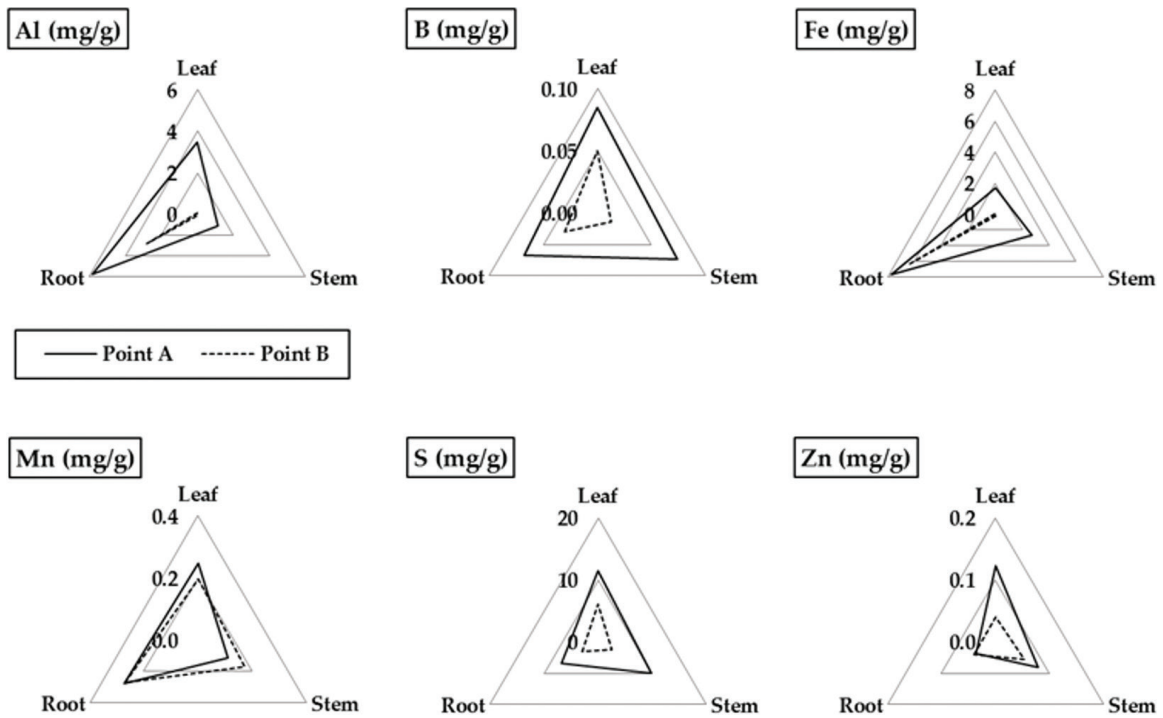


Figure 2. Concentration of Al, As, B, Fe, Mn, S, and Zn in each part of the plant body of *E. indica* at Point A and Point B.

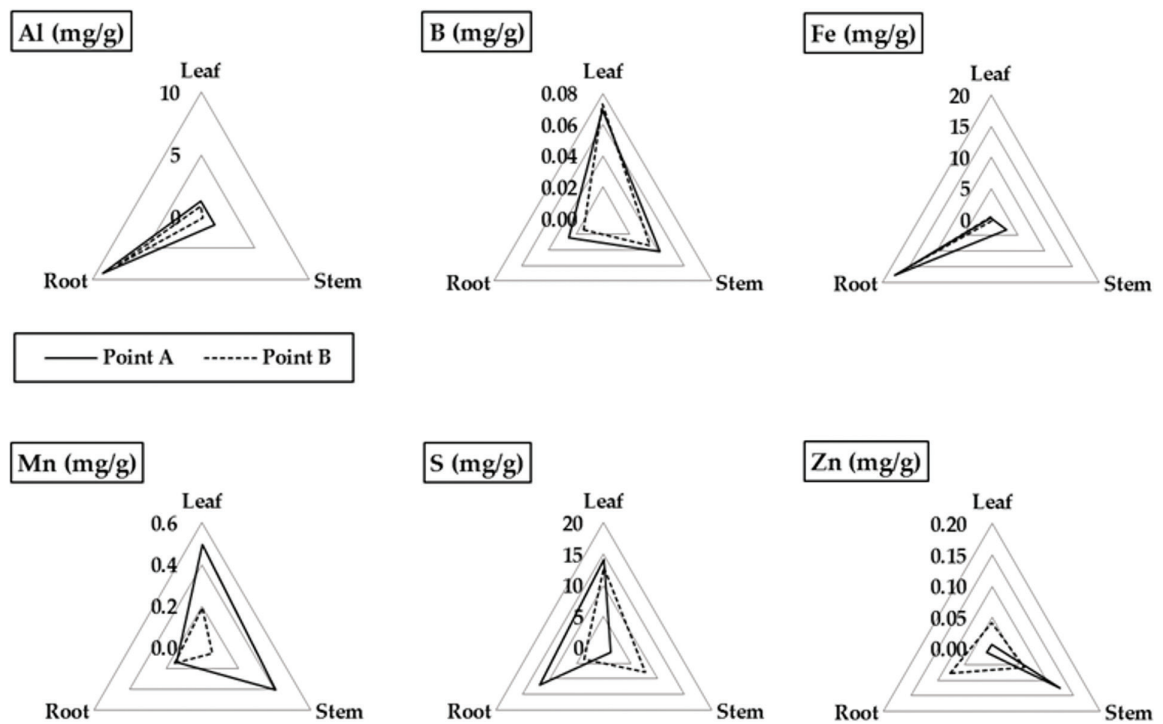


Figure 3. Concentration of Al, As, B, Fe, Mn, S, and Zn in each part of the plant body of *M. leucadendra* at Point A and Point B.

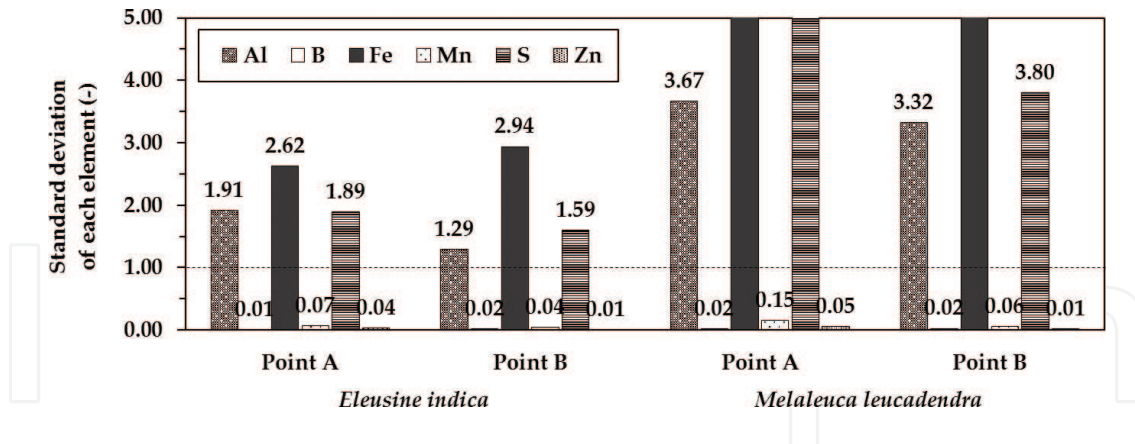


Figure 4. Standard deviation of the concentration of Al, As, B, Fe, Mn, S, and Zn in each part of the plant body: the distribution of the elements is homogeneous when standard deviations are nearly zero.

hydride in plant body. Thereby, B was widely distributed in the body of both plants in the similar way with C as a consequence of the transport mechanism of Mn to the leaves for photosynthesis [37, 38]. Zn is also one of the essential elements for plant growth as coenzyme to accelerate photosynthesis and DNA synthesis [39, 40]. Consequently, the dissolution of Al in acid soils triggered by the continuous formation of acidic water along with the dissolution of sulfides influenced the plant growth at Point A. For the presence of Al, the neutralization of soil conditions with limestones and/or chemicals is not always useful to improve soil conditions in acid soils because Al is released as $\text{Al}(\text{OH})_4^-$ under alkali conditions. The evaluation of soil conditions before revegetation and/or farming is more important than the treatment of such acidic conditions. Likewise, even if the plants which are planted in the first stage of revegetation are acid tolerant, it is still necessary to select a proper plant for successful revegetation from the point of view of Al tolerance of plants and the dissolution of Al with the formation of acidic water over time in this case.

3.2. Tolerance characteristics of plants to acid soils

Figure 5(a) and **(b)** shows the relationship between plasticity index (I_p) and liquid limit (W_L), which shows soil conditions under different water content and particle size distribution of the prepared acid soils, respectively. Soil classification is closely associated with physical characteristics of soils such as particle size distribution, which affects plant growth [41]. All of the prepared acid soils were categorized as high W_L silt, and there were not significant differences in the particle size distribution among the samples as shown in **Figure 5**. This indicated that the growth of plants was not affected by the physical characteristics of the soil samples during the vegetation test.

The geochemical properties of the soil samples are summarized in **Table 5**, and **Figure 6** describes the content of Al, Fe, and S in the prepared acid soils. Besides, **Figure 7** shows the changes of the height of seedlings and the diameter of stem of *A. mangium* during the vegetation test. In **Table 5**, NAG pH dropped between S0.0 and S0.5, showing that NAG pH was significantly affected by the content of sulfides such as pyrite. The content of Fe and S rose with the increase in the mixing ratio of pyrite in **Figure 6**, whereas that of Al decreased, attributing to the decrease in the mixing ratio of simulated soils containing Al. In **Figure 7**,

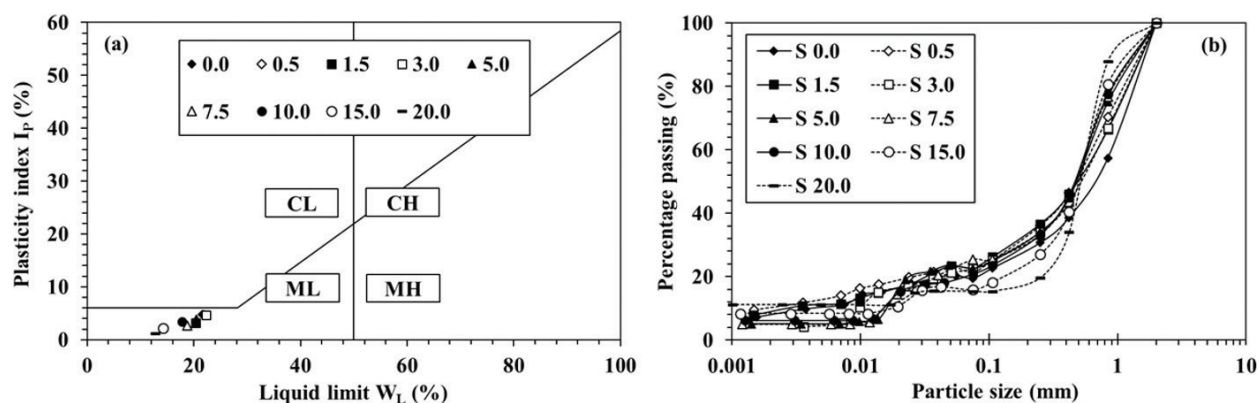


Figure 5. (a) Relationship between I_p and W_L of the prepared acid soils (C: clay; M: silt; H: high liquid limit; and L: low liquid limit). (b) Particle size distribution of the prepared acid soils.

the plants under the condition of S1.5–S20.0 withered after 56 days (8 weeks). By contrast, the plants under the condition of S0.0–S0.5 had grown after 56 days. For these results, the excess of sulfides in soils inhibited the growth of *A. mangium*, and the sulfur content for the limitation of the growth of *A. mangium* lied between 1.13 and 1.94% in this case. Furthermore, the plant growth declined with the increase in the mixing ratio of pyrite as indicated by the decrease of the height and the diameter as shown in **Figure 7**. The sulfur content at Point A showed 0.13–1.06%, especially 1.06% at 50–70 cm depth, as shown in **Table 4**, suggesting that the growth of plants which are subject to effects of acid soils compared to *A. mangium* can be inhibited under the soil conditions in the mine site.

In **Figure 8**, the changes of pH of the leachate from the bottom of the pots during the vegetation test are plotted. The pH of the leachate ranged from pH 2.0 to pH 4.0 in S1.5–S20.0 from the beginning of the experiment, resulting in the death of *A. mangium* contrary to the growth with constant pH 7.0 in S0.0. On the other hand, *A. mangium* in S0.5 had grown even if pH dropped

| Sample | Sulfur content (%) | NAPP (kg H ₂ SO ₄ /ton) | NAG pH |
|--------|--------------------|---|--------|
| S0.0 | 0.04 | –8.9 | 5.67 |
| S0.5 | 1.13 | 24.1 | 2.34 |
| S1.5 | 1.94 | 48.8 | 2.24 |
| S3.0 | 3.83 | 106.1 | 2.14 |
| S5.0 | 6.86 | 198.2 | 2.05 |
| S7.5 | 9.08 | 265.3 | 1.92 |
| S10.0 | 11.60 | 340.1 | 1.98 |
| S15.0 | 18.20 | 541.7 | 1.98 |
| S20.0 | 28.20 | 846.0 | 1.91 |

Table 5. Geochemical properties of the prepared acid soils: the mixing ratio of pyrite in the prepared soils is labeled as sample names, e.g. the mixing ratio of pyrite is 0.5% in S0.5.

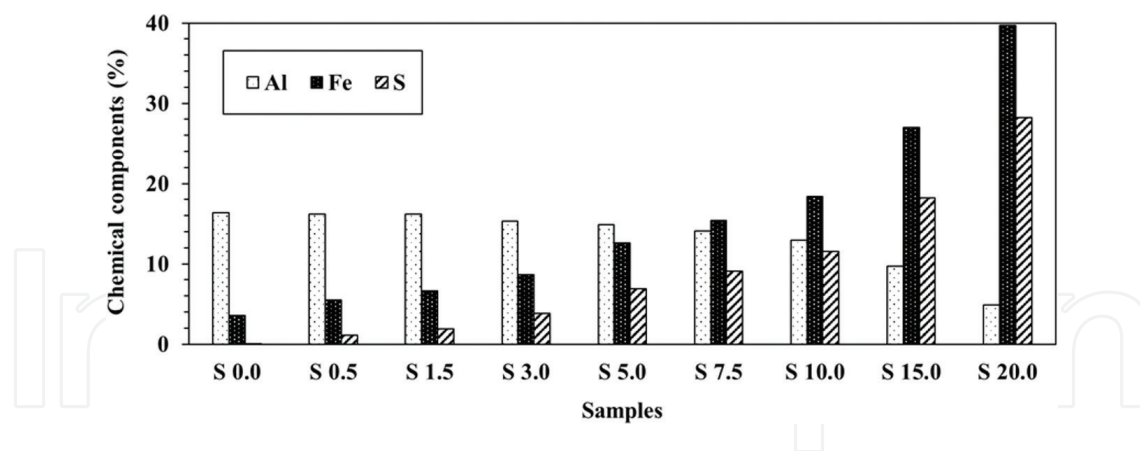


Figure 6. Content of Al, Fe, and S in the prepared acid soils with different contents of pyrite.

from pH 8.0 to ca. pH 2.0 after 70 days. It would appear that *A. mangium* can survive under acidic conditions by increasing the resistance against acidic conditions with the plant growth during 70 days [7]. The 8 cm height of seedlings were transplanted at the beginning of the experiment, and they died when they were exposed to pH 2.0 in S1.5–S20.0. However, *A. mangium* in S0.5 was exposed to pH 2.0 after 70 days when the height became 15 cm, leading to the existence of the seedlings due to the development of acid tolerance with the plant growth. Additionally, the sudden drop of the pH of the leachate may have resulted from the lag time of the dissolution of sulfides. Sulfides gradually dissolve in water over time, causing the formation of acidic water for a long time [34]. Thus, both formation of acidic water over time and the development of acid tolerance with the plant growth have to be taken into account for successful revegetation in the waste dump in mine site.

In Figure 9, the concentration of Al, As, B, Fe, Mn, S, and Zn in each part of *A. mangium* is summarized, and in Figure 10, the standard deviation of the results is described by the soil samples. Compared to the results in Figures 2 and 3 and the concentration of the elements in *A. mangium* in Figure 9, Fe and Al were equally accumulated in the roots and S was accumulated in the roots

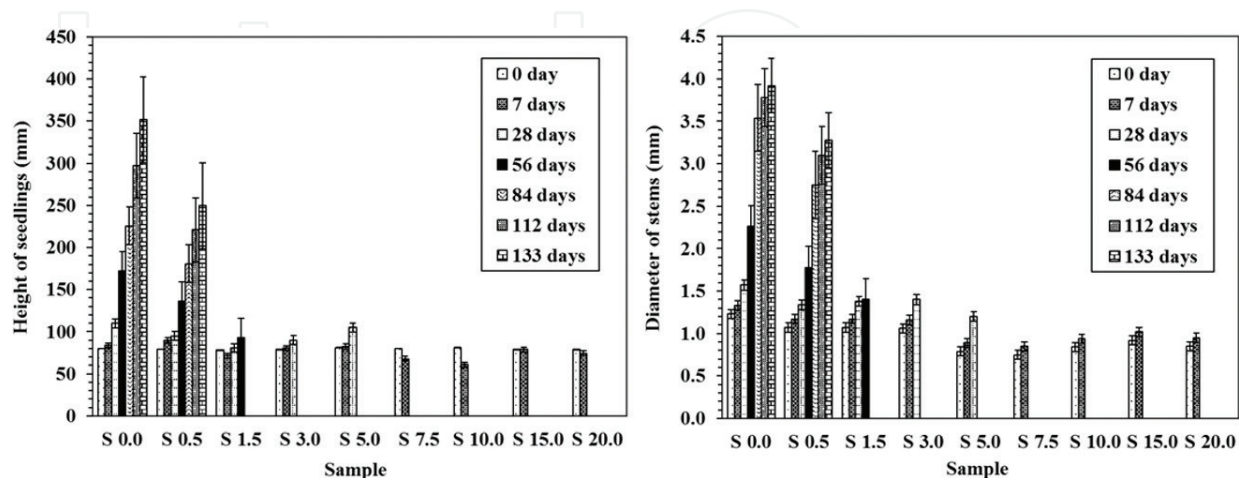


Figure 7. Change of the height and the stem diameter of *A. mangium* with different contents of pyrite in soils: the values were calculated based on the average of five samples by each content.

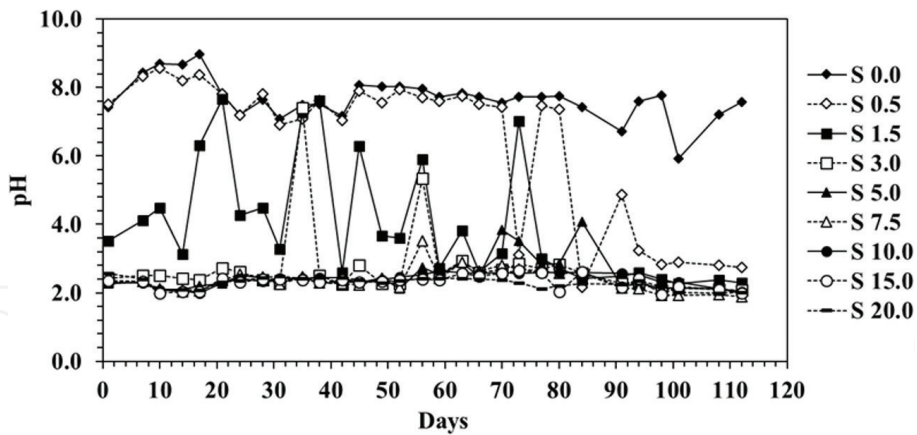


Figure 8. Change of pH of the leachate from the pots with different contents of pyrite in soils in the vegetation test.

and the leaves. Moreover, the accumulation of Al in the roots significantly rose with the increase in the mixing ratio of pyrite as shown in Figure 9. The high concentration of Al in the roots resulted in the death of *A. mangium* by preventing the absorption of nutrients from the roots [10]. In Figure 10, the standard deviation of Al, Fe, and S showed more than 0.5, whereas that of the other elements ranged from 0.01 to 0.1. The standard deviation of Al, Fe, and S, besides, rose with the increase in the mixing ratio of pyrite, revealing the accumulation of Al, Fe, and S in the plant body: the standard deviation of Al, Fe, and S was 2.98, 13.17, and 1.43 in S1.5, respectively. This was caused as a consequence of the biological action to accumulate the excess of the harmful elements for plant growth on the roots in the same case as in Section 3.1. The results in Section 3.1 also support that B, Mn, and Zn were distributed through the body of *A. mangium*. Furthermore, a larger amount of Fe, S, and Al was obtained in *M. leucadendra* compared to

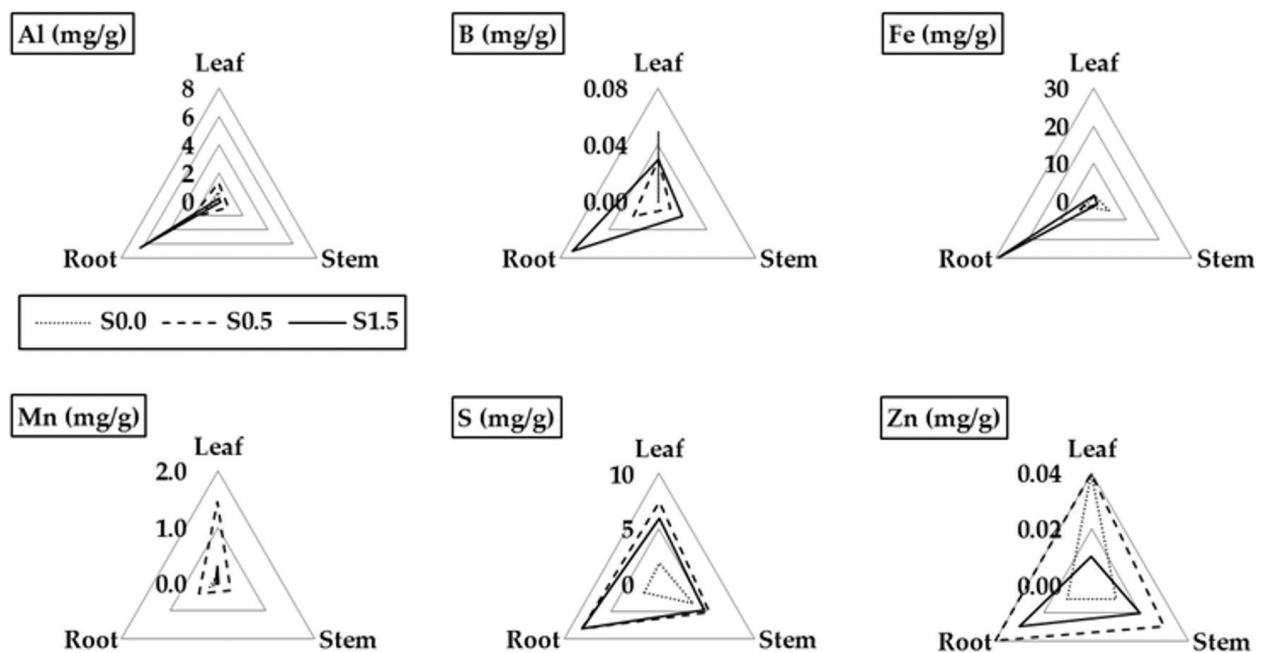


Figure 9. Concentration of Al, As, B, Fe, Mn, S, and Zn in each part of *A. mangium*.

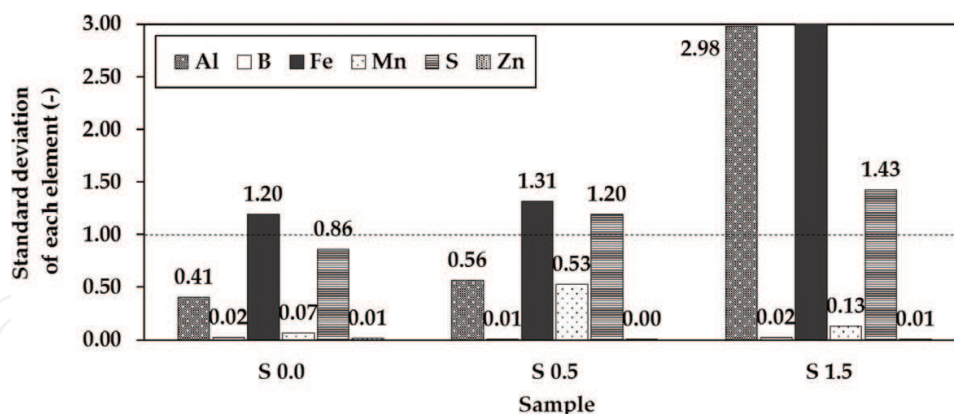


Figure 10. Standard deviation of the concentration of Al, As, B, Fe, Mn, S, and Zn in each part of *A. mangium*.

A. mangium although both *M. leucadendra* and *A. mangium* are acid tolerant. This indicated that the accumulation capacity of the elements in the plant body depends on the species.

Figure 11 demonstrates the growth of *A. mangium* in S0.0–S1.5, and Figure 12 shows the change of height and length of the seedlings and roots of *A. mangium*. The number of the leaves and the height of *A. mangium* obviously decreased with the increase in the mixing ratio of pyrite as shown in Figure 11. The length of the roots also decreased with the increase of the content of pyrite as shown in Figure 12, attributing to the inhibition of the root elongation in response to Al-stress [10, 42]. This result and the increase of the content of Al in the roots of *A. mangium* with the increase of mixing ratio of pyrite revealed that the accumulation of Al in *A. mangium* resulted in the death in S1.5 regardless of the similar content of Al in S0.0–S1.5 as shown in Figure 6. In short, the immobilization of Al in soils without absorption in the plant body due to neutral pH led to the growth of *A. mangium* in S0.0, and *A. mangium* survived in S0.5 by increasing the resistance against acidic conditions with the growth and without absorption of Al in the plant body at around pH 7.0 during 70 days even if pH dropped in



Figure 11. Growth of *A. mangium* at different mixing ratios of pyrite in the acid soil.

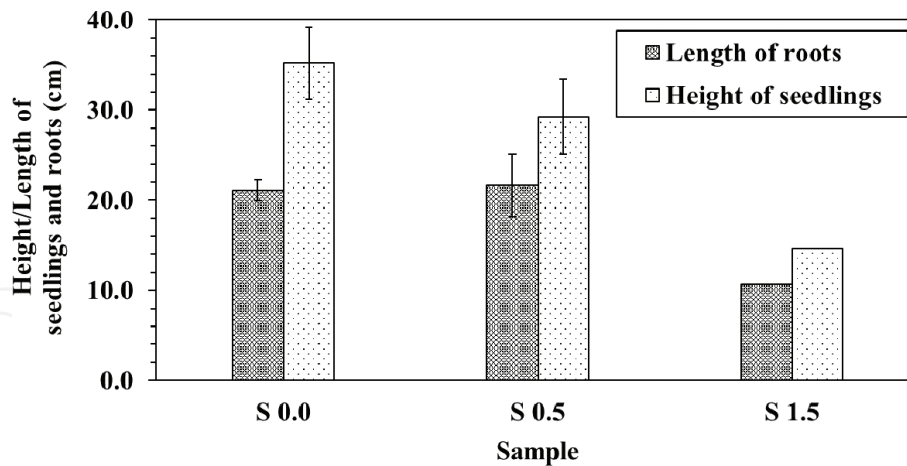


Figure 12. Change of the height of the seedlings and the length of the roots of *A. mangium* at the end of the vegetation test.

pH 2.0 after 70 days. In contrast, the accumulation of Al in the roots resulted in the inhibition of the root elongation and the death of *A. mangium* because of the large amount of dissolved Al and its accumulation in plants at pH 3.0 in S1.5. In S3.0–S20.0, *A. mangium* died resulting from the dissolution of Al and its accumulation in the roots with the continual production of H^+ at pH 2.0 from the beginning of the vegetation test. Therefore, the timing of the transplant of plants and acidification of soils over time should be taken into account for the revegetation.

Acid-tolerant plants can survive in acid soils by setting up several tolerance mechanisms, such as the increase of soil pH around the root apices [5, 6]. However, the results in this study suggest that Al tolerance of plants has to be considered in addition to acid tolerance in the case that the accumulation of Al in plants inhibits plant growth. As shown in Ref. [7], there are differences in the Al-tolerant mechanism, such as Al-excluder and Al-accumulator. In regard to the effects of Al accumulation in plant body on plant growth, the plant classified as Al-excluder seems to be a suitable plant for the first stage of the revegetation in which acidic conditions are formed. Likewise, the content of Al was 6.45 mg/g in the roots of *A. mangium* in the vegetation test and it was 9.05 mg/g in *M. leucadendra*, which survived in acid soils in this study, although both plants are similarly categorized as Al-excluder in Ref. [7]. This indicates that *M. leucadendra* is more suitable for the first stage of the revegetation than *A. mangium* from the point of view of the effects of Al. Thereby, the plant for revegetation should be carefully selected from various perspectives, such as acid tolerance and Al tolerance of plants, and soil conditions. The evaluation in terms of not only soil conditions but also plant species has to be highlighted for successful revegetation.

4. Conclusions

In this study, vegetation survey and a vegetation test were conducted to investigate the current situation of acid soils and plant growth in mine site and to understand the effects of acid soils on plant growth over time. The results are summarized with the key to successful revegetation in terms of soil acidification and tolerance characteristics of plants as follows:

1. The dissolution of Al under acidic conditions in acid soils which were attributed to the formation of acidic water triggered by the dissolution of sulfides influenced plant growth in mine site. It is necessary to select a proper plant for successful revegetation from the point of view of Al tolerance and the dissolution of Al with the formation of acidic water over time.
2. Not only soil pH but also the assessment of the dissolution of sulfides over time is crucial for successful revegetation, suggesting that net acid producing potential (NAPP) and net acid generation (NAG) pH, which are used for evaluating the formation of acidic water, are useful to evaluate soil conditions for the revegetation in addition to soil pH.
3. The effects of acid soils on plant growth change according to plant species because Al-tolerant mechanism of plants depends on the species. Moreover, plants can survive under acidic conditions by increasing the resistance against acidic conditions with the plant growth. Therefore, the timing of the transplant of plants and acidification of soils over time should be taken into account for the revegetation.

Acknowledgements

The authors would like to express their appreciation to the mine for providing the samples. The experiments were conducted with the kind support of Mr. Shunta Ogata in Department of Earth Resources Engineering of Kyushu University.

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