

Growth of typha grass (*Typha angustifolia*) on gold-mine tailings with application of arbuscular mycorrhiza fungi

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Manuscript received: 12 January 2018. Revision accepted: 28 February 2018.

Abstract. Setyaningsih L, Wulandari AS, Hamim H. 2018. Growth of typha grass (*Typha angustifolia*) on gold-mine tailings with application of arbuscular mycorrhiza fungi. *Biodiversitas* 19: 504-509. Gold mine tailings contain extreme physical and chemical properties, which inhibit plant growth due to lower nutrition and higher heavy metal contaminants. Typha (*Typha angustifolia*) is type of grass growing well on waterlogged area including tailing dam. The objective of this study was to investigate the effect of arbuscular mycorrhizal fungi (AMF) in combination with compost and soil on the typha growth in gold mine tailings. The study was conducted in greenhouse by inoculating two AMF isolates (*Glomus etunicatum* and *G. manihotis*) to typha seedlings grown in pure tailing media, mixed tailing-compost media, and mixed tailing-compost-soil media. The compatibility and growth of typha grass were analysed after 1 month. Results showed that *G. etunicatum* and *G. manihotis* application significantly increased AMF colonization of typha roots up to 16.6% and 21.8% respectively. The length, number of leaves and biomass of typha also increased up to 90%, 50% and 97% respectively compared to those without AMF inoculation. *G. etunicatum* contributed the best growth of typha grown in mixed compost-soil-tailings, resulting in double increase of its length and biomass. The application of *G. manihotis* did not significantly increase the growth of typha in mixed media; however, under pure tailing, this mycorrhiza had the best induction for typha biomass and leaf number. In general, AMF application increased growth of typha grass in tailings media.

Keywords: *Glomus etunicatum*, *Glomus manihotis*, gold-mine tailing, mycorrhiza, *Typha angustifolia*

INTRODUCTION

Gold mining managed by both industry and small-scale public mining caused serious environmental damage by producing contaminants in the form of cyanide compounds (Akci 2003; Hamim et al. 2017) and heavy metals such as Pb (Setyaningsih et al. 2012) and Hg (Hidayati et al. 2009). Heavy metals at certain concentrations are very harmful to living things when they enter to the metabolic system. Lead (Pb), for example, will bind to a number of molecules such as amino acids, hemoglobin, enzymes, RNA, and DNA when it is absorbed by the human body, resulting in metabolic disorders and even brain damage (ATSDR Information Center 1999). Therefore, the unprepared handling management of environmental pollution due to gold mining, will result in a considerable potential danger, especially in tropical areas like Indonesia, which weather and biochemical activities occur so high so that the mobilization of potentially toxic elements will be very fast.

Heavy metal have direct and indirect negative effect to the plants including photosynthesis reduction, nutrient uptake, and the reduction of growth and production (Cambrollé et al. 2011; Dirilgen 2011; Shahid et al. 2011). In many species, heavy metal can induce the dramatic increase of reactive oxygen species (ROS) (Mirza et al. 2010; Radic et al. 2010; Mou et al. 2011), which resulted in lipid peroxidation (Rascio and Navari-Izzo 2011). In

addition, at higher concentrations, heavy metals accumulating to the leaves will cause chlorophyll loss, resulting in metal-induced chlorosis (Marques and do Nascimento 2013). Therefore, considerable effort especially remediation activities should be done to reduce the potential danger of heavy metal contamination to the broader environment.

Phytoremediation is an alternative method to reduce and remediate contaminants from the environment using plants within variety of media (Hidayati et al. 2009). For this reason, the existence of plant species having ability to absorb high metal concentration, known as metals hyper-accumulator, is very important. In addition, the use of microbes to induce plant growth either from bacteria (Mahfouz and Sharaf-Eldin 2007; Altuhaish et al. 2014) or fungi (Augé 2001; Smith et al. 2010) also increased dramatically, which may relate to the important mechanism in phytoremediation process (Rozpadek et al. 2014). Many experiments also suggested that mycorrhizas fungi have important role in phytoremediation process by improving plant growth and inducing metal accumulation (Weissenhorn et al. 1995; Javaid 2011; Khan et al. 2014). Therefore, mycorrhizal fungi can be utilized to improve the plant growth and capability to phytoremediate the gold mining area.

Typha (*Typha angustifolia*) is a type of grass growing very well in the tailing area of gold mining, which may

have potential role in phytoremediation process. However, the intensive utilization of this species in phytoremediation process has not been carried out. In addition, the interaction of this species with microbes including mycorrhizas has also not been elucidated well. Therefore, this experiment aimed to investigate the effect of arbuscular mycorrhizal fungi (AMF) and the addition of compost and soil on growth of typha (*Typha angustifolia*) grown in gold mine tailings.

MATERIALS AND METHODS

Media preparation

In this experiment, typha grass (*Typha angustifolia*) was obtained from tailing dam of Aneka Tambang Inc. (PT Antam), Pongkor, Bogor Indonesia. The experiment was prepared using 3 combinations of growing media, i.e.: pure tailing (T0), mixed tailing-compost media with the proportion of 3: 1 (v/v) (T2), and mixed tailing-compost-soil with the proportion of 4: 2: 1 (v/v) (T3). Sterilization of media was carried out using heat steam drum at 1 atmospheric pressure for 2 hours. Sterilized Media were then put into 5 kg of polybags for planting.

Seedling preparation

Typha grasses were grown in polybags and kept in slightly flooded conditions to mimic the circumstances conditions of the tailing dam. At the beginning, plant seedlings were grown using similar media until they grew and new shoots appeared. The new plants were then watered every 3 days and flooded conditions were maintained until the treatment.

Inoculant and AMF inoculation

There were two inoculants applied in the experiment. i.e. *Glomus etunicatum* and *G. manihotis* obtained from the Forest Biotechnological laboratory, Bogor Agricultural University, Bogor, Indonesia. The inoculation of AMF was carried out twice, during germination period (using 30 g of AMF inoculants) and during seedling transplantation to the treatment media (20 g of inoculants with approximately 50-100 AMF spore). The inoculants were sown and immersed into treatment media around typha root system. The plants were then grown and watered every day for the period of 30 days.

Plant growth analysis and measurements

To understand AMF-compatibility, some parameters such as AMF colonization and mycorrhizae inoculation effect (MIE) (Bagyaraj 1992) were analyzed. Growth parameters including leaf length, leaf number and typha biomass were analyzed after 30 days of the treatment. AMF colonization was calculated using root staining procedure developed by Brundrett et al. (1996) with some modifications. The root of seedlings that have been cleaned and cut into pieces were put into a test tube contained KOH 2.5% for approximately 24 hours until the solution became clear. A 5-10 ml of H₂O₂ solution was added if the solution was still not clear. The roots were then washed and

immersed in 0.1 N of HCl solution for 10 minutes. The excess of HCl solution was discarded, then a dye solution consisting trypan blue 0.02% + Glycerol 70% + 30% of distilled water was added, and then it was waited to stand between 30 minutes to 12 hours. The roots were washed again and put in 50% of glycerol solution. The root cutting were then placed onto the object glass and the colonization of CMA at the root was readily observed using light microscope.

The observation of AMF colonization and mycorrhizae inoculation effect (MIE) or mycorrhizal inoculation affecting root seedlings was calculated at the end of observation (4 weeks). The AMF and MIE colonization (%) was counted by comparing the number of mycorrhizal root with the number of root. The MIE was calculated using Bagyaraj equation (Bagyaraj 1992) by comparing the difference of inoculated biomass of seedlings to non-inoculated divided by the inoculated biomass in percent.

Colonization = $(\Sigma \text{mycorrhizal roots in a viewing area}) / (\Sigma \text{total roots in a viewing area}) \times 100\%$

MIE = $[(\text{Dry weight of inoculated plants} - \text{dry weight of non-inoculated plants}) / \text{Dry weight of inoculated plants}] \times 100\%$

RESULTS AND DISCUSSION

Analysis of AMF colonization in typha grass (*Typha angustifolia*)

Mycorrhizal colonization of typha roots increased significantly ($p < 0.05$) due to the inoculation of both species of mycorrhizal fungi. The colonization value in both tailing treatments (T) and species of mycorrhiza (M) applied in the experiment was varied (Figure 1). The interaction between two factors also showed that the pattern of colonization between those treatments was unclear (Figure 1).

In average, the single factor's effect of media treatments showed that the treatment of pure tailings (T0) had the highest colonization (20.6%), while the addition of compost (T1) and soil and compost (T2) decreased mycorrhizal colonization significantly ($P < 0.05$), i.e. 14.1 % and 8.9% respectively (Table 1). Based on single factor's effect of mycorrhiza treatment, inoculation with *G. etunicatum* (M1) increased mycorrhizal colonization up to 16.6%; whereas with *G. manihotis* (M2) inoculation, it was even higher (21.8%) compared to that without mycorrhizal inoculation (M0), which only gave 5.1% (Table 1). The highest colonization occurred on the typha roots treated with the combination of pure tailings and *G. manihotis* (T0M2), which reached 37.1% (Figure 1).

Analysis of typha growth in various treatments

The typha growth was analyzed after 8 weeks of planting by measuring leaf length, number of leaves and dry biomass. The data showed that the combination treatment of media and mycorrhiza significantly influenced the plant growth (Table 2). The application of mixed compost-tailings (T1) and mixed soil-compost-tailings (T2) significantly ($p < 0.05$) increased the leaf length up to 23% (46.7 cm) and 90% (76.6 cm), respectively compared to

that in pure tailing medium (T0) (37.9 cm). This application also increased the dry biomass by 42% (5.4 g at T1) and 97% (6.3 g at T2), while the number of leaf increased up to 50% (8 leaves) under both treatments (Table 2).

Mycorrhizal application of *G. etunicatum* (M1) significantly ($p < 0.05$) increased the average of leaf length, leaf number and leaf biomass up to 60% (70.8 cm), 12% (8 sheets) and 85% (6.3 g), respectively. While the average of growth parameters of typha applied with *G. manihotis* application (M2) was not significantly different ($p > 0.05$) (Table 2). The highest average of leaf length and dry biomass of typha was shown in typha grown on mixed tailing-compost-soil medium with *G. etunicatum* mycorrhizal application (T2M1), which had 111.9 cm of leaf length, 9 leaves and 5.9 g of total dry weight (Figure 2).

The Figure 2 also shows that the combination of tailing media treatment with mycorrhizal inoculation resulted in variation of growth responses. The highest growth response due to mycorrhizal applications was obtained by pure tailings treatment (T0) in combination with *G. manihotis* inoculation (M2). The addition of compost or soil caused the mycorrhizal application showed less consistent, although the application of *G. etunicatum* (M1) tended to have better result than M2 (Figure 2). The morphology and growth conditions of typha in the treatment of media and mycorrhiza was shown in Figure 3.

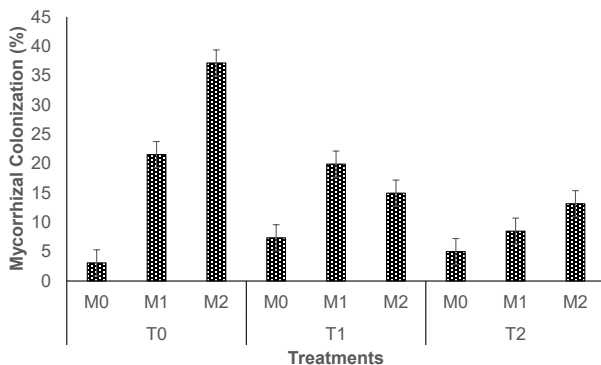


Figure 1. Mycorrhizal colonization of typha roots treated by a combination of pure tailings (T0), tailings-compost (T1) and tailings-compost-soil (T2) without mycorrhizal treatment (M0), with *G. etunicatum* (M1) and *G. manihotis* inoculation (M2)

Table 1. Single factor's effect of media treatments and mycorrhizas treatment on AMF colonization of typha roots

Treatments			
Tailing	Mycorrhizas		
	(%)		
T0	20.6 c	M0	5.1 a
T1	14.1 b	M1	16.6 b
T2	8.9 a	M2	21.8 c

Note: Note: T0: pure tailings, T1: tailings - compost, T2: tailings - compost - soil; M0: without mycorrhiza, M1: with *G. etunicatum* inoculation, M2: with *G. manihotis* inoculation. The number in the similar column followed by similar letter does not significantly different based on Duncan Analysis at 5% of α

Table 2. The average of leaf length, number of leaves and dry weight of typha plant treated with combination of tailing media and mycorrhizal inoculation. The data were calculated from single treatment

Treatments		Leaf length (cm)	Leaf number	Total dry weight (g)
Media	T0	37.9 a	6 a	3.8 a
	T1	46.7 b	8 b	5.4 b
	T2	76.6 c	8 b	6.3 c
Mycorrhiza	M0	44.1 b	7 b	3.4 a
	M1	70.8 c	8 b	6.3 c
	M2	46.3 b	7 b	5.8 b

Note: Note of the treatments: pure tailings (T0), tailings + compost (T1), and tailings + compost + soil (T2), treated without mycorrhiza (M0), by inoculation of *G. etunicatum* (M1) and *G. manihotis* (M2). The number in the similar column followed by similar letter does not significantly different based on Duncan Analysis at 5% of α

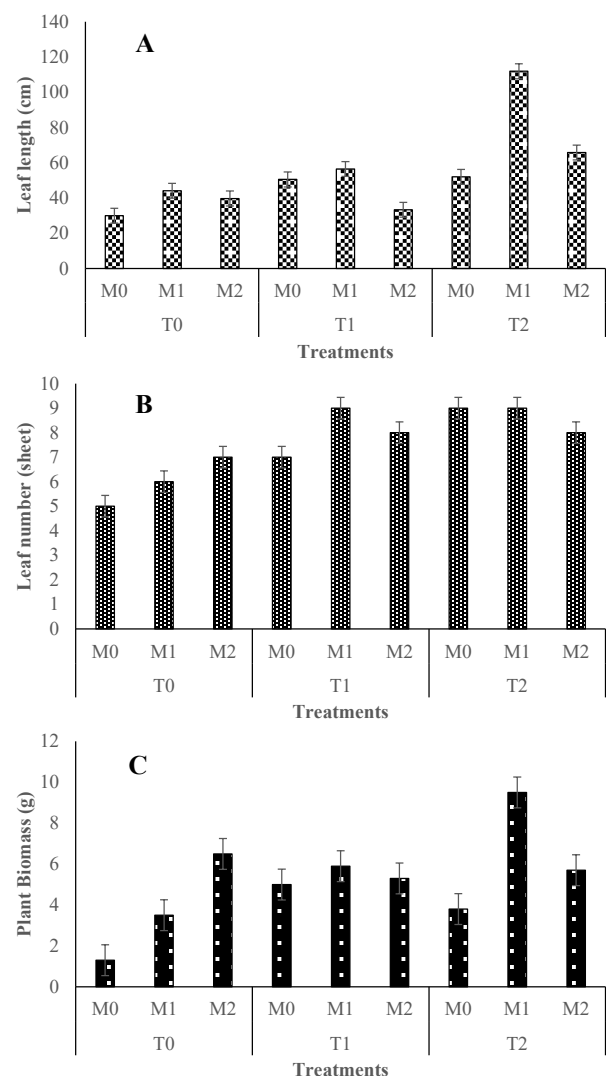


Figure 2. The average of typha growth in response to combination of media treatments and AMF inoculations. A. Leaf length of typha, B. Leaf number of typhi, and C. Plant biomass/dry weight. Note: pure tailings [T0], tailing-compost [T1] and tailing-compost-soil [T2]) in combination with mycorrhizal treatments (without mycorrhiza [M0], with *G. etunicatum* [M1] and *G. manihotis* inoculation [M2])

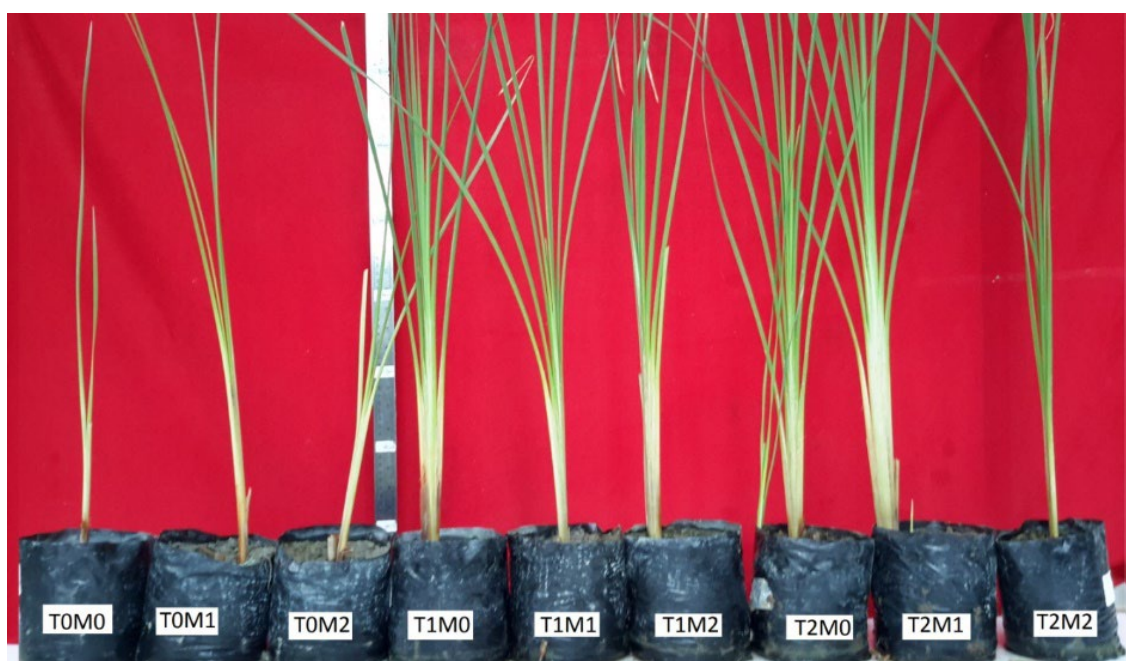


Figure 3. Morphology of typha (*Typha angustifolia*) in various combinations of media treatments. (pure tailings [T0], tailing-compost [T1] and tailing-compost-soil [T2]) in combination with mycorrhizal treatments (without mycorrhiza [M0], with *G. etunicatum* [M1] and *G. manihotis* inoculation [M2])

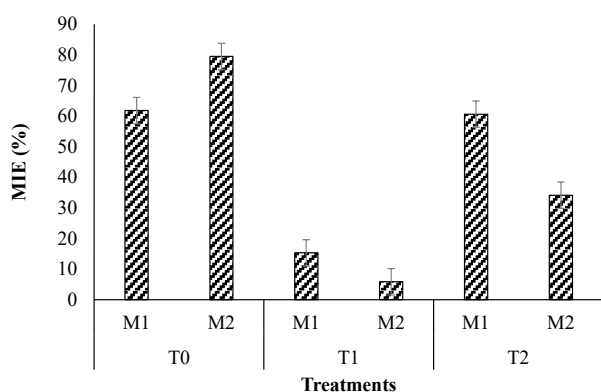


Figure 4. The value of Mycorrhizal Inoculation Effects (MIE) of typha (*Typha angustifolia*) grown on various combinations of plant media treatments (pure tailings [T0], tailing-compost [T1] and tailing-compost-soil [T2]) with mycorrhizal treatments (with *G. etunicatum* [M1] and *G. manihotis* inoculation [M2]).

Analysis of Mycorrhizal Inoculation Effect (MIE)

The effect of mycorrhizal inoculation on the growth of typha plants can be observed from the value of Mycorrhizal Inoculation Effect (MIE) calculated from the biomass of inoculated plants compared to uninoculated plants. The MIE score indicated that the treatment with pure tailings (T0) had the highest MIE value followed by the treatment with the tailing-compost-soil (T2), whereas the tailing-compost treatment (T1) had the smallest MIE value (Figure 4). In pure tailings medium (T0), *G. manihotis* mycorrhiza (M2) had greatest influence on growth, whereas in T1 and

T2 mediums, mycorrhiza application had the major effect on the typha growth was *G. etunicatum* or M1 (Figure 4).

The relationship between inoculation value and growth parameters

To investigate the relationship of both treatments to the growth of typha plants, we analysed the regression of mycorrhizal colonization value and all three-growth parameters. The result showed that the correlation between the percentage of colonization with the three growth components was very low when the regression data was generated using all tailing treatments including T0, T1 and T2 (data not shown). However, the magnitude of mycorrhizal colonization has a very good correlation with the growth component when the data were obtained from the pure tailings treatment only (T0) as shown in Figure 5.

Discussion

Typha grass in Pongkor gold mine region naturally grows along the edge part of tailings dam where slurry and waterlogged tailings were deposited. This gold mine tailings tended to be alkaline (pH 7) with organic carbon (0.15%) and nitrogen (0.06%) content were very low, with the lower availability of macro nutrient such as P (8.34 ppm) and Mg (0.00 cmol/kg), and lower clay content (Setyaningsih et al. 2012). This condition showed that typha grass (*Typha angustifolia*) has good adaptability to gold mine tailings, which is unfavourable to other plant species so that this plant has good prospect to be used in phytoremediation program.

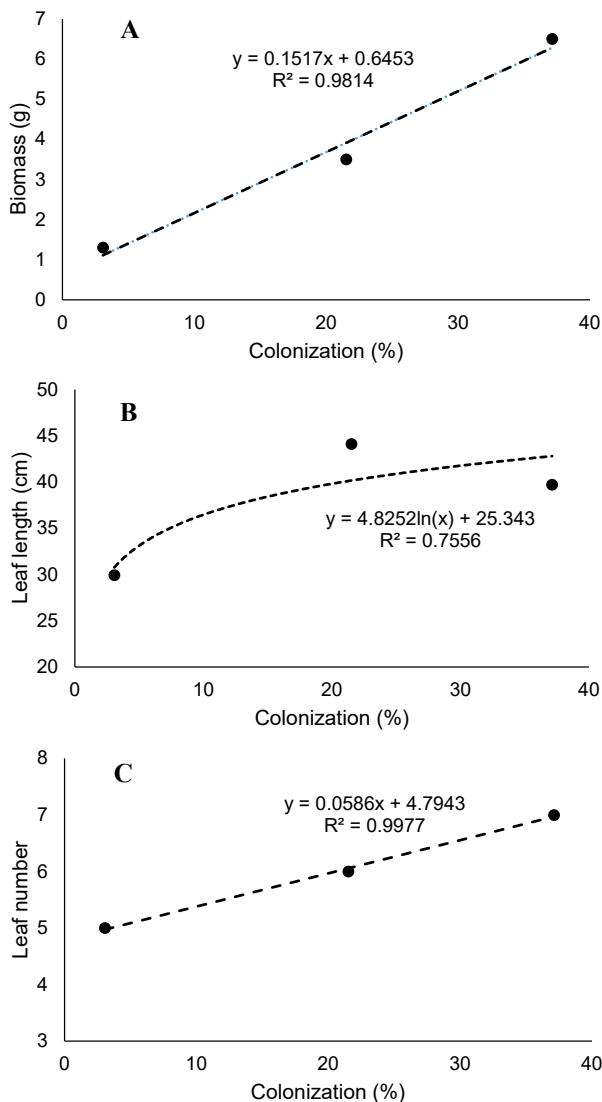


Figure 5. The regression of mycorrhizal colonization with the three growth components. A. Biomass, B. Leaf length, and C. Leaf number

The addition of compost to tailings as a growing medium both with and without soil addition significantly increased the growth of the typha (Figure 2). This suggested the importance of compost application as organic material to improve the physical, chemical and biological properties of tailing media (Diacono and Montemurro 2010). The compost can improve plant growth by promoting granulation of soil aggregate in order to increase the aeration and the ability of media to absorb water and nutrients required by roots (Schulz et al. 2013), leading to the improvement of major processes of plant growth (Diacono and Montemurro 2010).

The experiment showed that in tailing media with the higher water content (flooded conditions), AMF was able to live and infected typha grass, which could be observed from the colonization value (Table 1). AMF colonization and compatibility in tailing media has also been reported in *jabon* (*Anthocephalus cadamba*) seedlings (Setyaningsih et

al. 2017). Different arbuscular mycorrhizal fungi inoculated to typha grass showed different colonization rate and affected to different growth. The largest colonization of typha grass roots was obtained from *G. manihotis* inoculation, although the largest biomass was shown in typha plant inoculated with *G. etunicatum* (Figure 1). These conditions indicated that every type of mycorrhiza has colonization properties and specific effects on certain plant species under certain rhizosphere conditions (Kennedy et al. 2011). Therefore, to get maximum result and more efficient utilization, the specificity of colonization and all requirement need to be further observed.

A significant increase of biomass in mycorrhizal inoculated plants indicated that the growth of typha depended on mycorrhizal symbiosis, which could be observed from the positive MIE values ranging from 5.9% (in T1M2 treatment) to -79.5% (in T0M2 treatment) (Figure 4). The previous experiments indicated that hyphae intensively produced by AMF in its colonization with plant roots was able to increase the capacity of AMF-infected plants in nutrient uptake (Brundrett et al. 1996; Mohammadi et al. 2011; Beltrano et al. 2013). Phosphate is the main nutrient that can be obtained by plant associated by mycorrhiza (Mohammadi et al. 2011). Several indications showed that the uptake of other elements such as Zn, Cu, Ni, NH_4^+ and possibly NO_3^- might also be improved by mycorrhiza (Smith and Read 1997). In addition, to increase the nutrient uptake, the presence of mycorrhiza might improve the structure of media by increasing the compactness of media so that it can improve the rhizosphere leading to the improvement of biological conditions (Borowics 2001).

Nutrition improvements in the presence of mycorrhizal may have an indirect effect on the ability of plants to environmental stress (Beltrano et al. 2013) including heavy metal stresses that may be toxic to the plants (Muhammadi et al. 2011). Through the production of special detoxifying compounds (such as organic acids) or by binding to pollutants in the fungus tissue of roots and forming a physical barrier from the translocation of the pollutant to host plant tissues, the mycorrhiza may help the plant to tolerate particular pollutants (Leung et al. 2013). Extrametrical mycelia has been believed as an important part of all types of mycorrhizas presence in contaminated soil (Vosatka et al. 2006).

In general the experiment showed that typha grass (*Typha angustifolia*) grown in tailing media was able to have mutual symbiotic with arbuscular mycorrhizal fungi (AMF). The application of *G. etunicatum* and *G. manihotis* significantly increased colonization and typha growth compared to that without AMF inoculation. The addition of compost and soil reduced the AMF colonization but it increased the plant growth. The application of *G. etunicatum* on the media mixed compost-soil-tailings had the best plant growth of typha by double increase of typha growth while *G. manihotis* application could induce the best number of typha biomass and leaf number under pure tailings.

ACKNOWLEDGEMENTS

We would like to thank to The Directorate General of Higher Education, Ministry of Research and Higher Education, Republic of Indonesia for supporting this research grant with the scheme of *Riset Strategi Nasional* (STRANAS), grant number: 2412/K4/KM/2016 and 1598/K4/KM/2017.

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