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PLANT RESIDUE BASED-COMPOSTS APPLIED IN COMBINATION WITH *TRICHODERMA ASPERELLUM* IMPROVE CACAO SEEDLING GROWTH IN SOIL DERIVED FROM NICKEL MINE AREA

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ABSTRACT

Cacao is widely grown in Sulawesi close to areas of nickel (Ni) mines indicating a possibility that the plant is affected by this heavy metal. By using soil collected from a Ni mining area, we evaluated three compositions of composted plant residues consisting of firstly gliricidia, billy goat, and rice straw, secondly gliricidia, stalk of palm oil fruit, and rice straw, and thirdly gliricidia, coconut husk, and rice straw, without and with addition of *Trichoderma asperellum,* in supporting growth of cacao seedling. Treatment with the respective compost types without any addition of *T. asperellum* caused an increase of seedling height by 18.9%, 28.5%, and 43.8%, stomata opening area by 17.2%, 4.3%, and 41.3%, stomata number by 13.4%, 22.7%, and 14.3%, and leaf Ni content by 3.8%, 12.8%, and 48.8% respectively. Following treatment with the three compost types included *T. Asperellum,* the increase of seedling height was 36.5%, 45.3%, and 54.7%, stomata opening area 15.9%, 21.3%, and 53.5%, stomata number 5.4%, 7.1%, and 0.0% and leaf Ni content 25.1%, 87.8%, and 161,4% respectively. Leaf analysis indicated that nitrogen content was increased when treated by the combination of composted plant residues and *T. asperellum*, potassium was increased in all treatments without *T. asperellum*, while phosphorus was decreased in all treated seedlings. These data suggest that one cause of cacao seedling growth improvement in soil containing Ni by composted plant residues and its combination with *T. asperellum* was the increase of Ni levels in leaves. Composts treatment could therefore potentially be used for cacao field application around Ni mining areas.

Key words: plant residues composition, plant height, stomata, nickel content, *Trichoderma*.

INTRODUCTION

Plant residues as a raw material of compost are important for improvement of soil organic matter. The presence of organic matter can influence a wide array of environmental and agronomic characteristics (Vallad *et al*., 2003) and composting of plant residues found in the environment can be an attractive and economically sustainable practice to fully recycle resources in the productivity of major cropping systems (Nelson and Boehm, 2002). Compost is formed from stable and mature organic matter derived from induced solid-state aerobic fermentation of biomass with different origins, including agro-industrial or urban wastes (Pane *et al*., 2013). Composting process may be achieved in both small and larger scale plants and the product can be applied to the soil as amendments (Celano *et al*., 2012).

Soil amendment with plant residues based-composts has been extensively proposed to remove pollutants from the environment or to decrease their toxicity. Compared to other techniques, this use of composts has many advantages such as low economic costs and the possibility of being applied to soils, causing a minimum environmental impact (Angelova *et al.*, 2010). Many reports of plant residues such as peat, wood, pine bark, banana pith, rice bran, soybean and cotton seed hulls, peanut shells, hazelnut shell, rice husk, sawdust, orange peel, and shredded maize are composted and used through soil amendment for reducing metal toxicities (Ho *et al*., 2002; Siswanto *et al*., 2013; Beesley *et al*., 2014; Martinho *et al*., 2015; Ahmed *et al*., 2017). They reduce metal mobility in the rhizosphere, induce adsorption, and precipitation processes (Alvarenga *et al*., 2009). Nevertheless, the effectiveness in heavy mineral managements is variable depending on the microbes involved in organic matter decomposition, microbial population dynamics, nutrient content, and chemical and physical factors of plant residues (Gaj and Schnug, 2002; Litterick *et al*., 2004; Avilés *et al.*, 2011; Pane *et al*., 2013). Due to these complexities, researchers still need to test various plant residues in the formulation of compost to optimise reduction of heavy metal impact.

The research describes a step toward characterizing different composted plant residues and evaluating their ability to reduce impact of soil containing nickel on cacao in the laboratory as a mean to develop effective and efficient methods with broader implications for solving this problem in the field in order to support cacao intensification and extensification. In addition, to strengthen their ability to bind nickel, *Trichoderma* was

also combined with these composts. The fungus plays an important role in ecology by taking part in the decomposition of plant residues, as well as in biodegradation of man-made chemicals and bioaccumulation of high amounts of various metals from wastewater and soil (Ezzi *et al*., 2005; Anand *et al*., 2006).

Cacao suffers from a number of diseases both infectious and noninfectious, among them Phytophthora diseases including Phytophthora pod rot, stem canker and leaf blight, vascular streak dieback disease, and abiotic problem such as metal toxicity (Mc Mahon and Purwantara, 2004; Sariwahyuni, 2012; Rosmana *et al*., 2015; Ristanti *et al*., 2016). The last is a serious problem on cacao planted in the area of nickel mining, Sulawesi is an important island in the production of this heavy metal.

MATERIALS AND METHODS

Implementation of research: Research consisted of preparation of compost and cacao seedlings, assessment of the ability of compost and *Trichoderma* to reduce soil impact, analyse of basic soil properties, and measurement of nickel, nitrogen, phosphorus, and potassium content in leaves. This research was carried out from March 2017 until August 2017 in Plant Disease Laboratory, Faculty of Agriculture for first and second part, Soil Fertility Laboratory, Faculty of Agriculture for third part, and Feed Chemistry Laboratory, Faculty of Animal Science, Hasanuddin University for fourth part.

Preparation of compost and cacao seedlings: Plant residues chosen in this work were gliricidia leaves, billy goat plants, rice straws, coconut husks, and broken empty stalks of palm oil fruit. Compost consisting of gliricidia leaves, billy goat plants, rice straws (first compost) was compatible for development of *Trichoderma* according to previous study (Rosmana *et al*., 2018). A second compost was made by replacing billy goat plants with empty stalks of palm oil and a third compost by replacing billy goat plants with coconut husks. The composting process was done by 30 days incubation in covered plastic sheeting followed by routine flicking back for aeration. These composts passed a two weeks curing period at room temperature (27ºC-30ºC) and two weeks mixture with soil before use for planting seedling.

Cacao beans were selected from harvested mature pods of the MCC1 clone in the field. These beans were washed, removing the placenta, and then placed in layers of sterile moist cloth to stimulate germination. Two weeks after incubation, the best seedlings were transferred to poly-bags containing about 0.5 kg mixture of compost and soil and placed in a greenhouse with the temperature range of 27ºC to 32ºC and humidity range of 78% to 90% for around three months.

Assessment of the ability of compost and *Trichoderma* to reduce soil impact: Soil used for the study was collected from a nickel mine area in Luwu Timur Regency, South Sulawesi. Basic soil properties such as texture and pH were determined in accordance with standard procedures (Blume *et al*. 2011). Compost was mixed with respective soil in weight composition of 1: 2 and *Trichoderma asperellum* strain ART-4/G.J.S. 09-1559 was also added to evaluate the role of this fungus in supporting nutrition supply. Therefore, the experiment was established with complete randomized design with six treatments consisting of first compost, second compost, third compost, first compost plus *T. asperellum*, second compost plus *T. asperellum*, and third compost plus *T. asperellum*. Each treatment consisted of five seedlings, therefore the total of seedling used including control was 35.

The impact of the treatments was evaluated through observation of seedling height, stomata number per square centimeter of leaf, measurement of length, width and area of stomata opening, and content of nickel, nitrogen, phosphorus, and potassium in leaves. The third leaf on cacao seedlings of two months old was selected at AM 10:00 and used for calculation of stomata density and measurement of stomata pore length, width, and area. A drop of nail polish was applied to the surface of the leaf and after drying, this drop was covered with clear packing tape. The packing tape was then pressed firmly for about 30 sec, peeled gently, and the impression stuck to a clean slide and observed under the light microscope. The area of stomata opening in square micrometer was calculated by using the formula of $A = \pi RW \times RL$, where A is the area, π is constanta, RW is width radius and RL is length radius (Rosmana *et al*., 2016). The stomata number and size were obtained from three seedlings or six leaves per treatment as well as the control for the MCC1 cacao clone.

Measurement of nickel, nitrogen, phosphorus, and potassium content in leaves: To evaluate the uptake of heavy metal and important nutrition elements by cacao seedling following treatment with composted plant residues alone or in combination with *T. asperellum*, nickel (Ni) and as well nitrogen (N), phosphorus (P), and potassium (K) were analyzed in the third and fourth leaf of cacao. All leaf samples were oven-dried at 60°C for 48 hours and then ground into fine powder in a mortar. The total content of Ni and K was measured after this powder was digested with 65 % of HNO3 and 95% of H2SO4 and then analyzed by Atomic Absorption Spectrophotometer (AAS). The samples were analyzed in triplicate and the concentration was reported in mg kg^{-1} of the dry weight or part per million (ppm) for Ni and as percent for K. The total P was determined colorimetrically using a UV/VIS-Spectrophotometer according to the NaOBr method (Dick and Tabatabai,

1977). N content in leaf was analyzed after dry sample digestion in concentrated sulfuric acid, distillation with 10 mol L^{-1}NaOH and 2% boric acid and titration with 0.1 mol L^{-1} HCl, following the Kjeldahl method (Bremner, 1996). The concentrations of N and P were reported as percent.

Analysis: Plant height, density of stomata per square centimeter, length, width, and area of stomata opening, and content of Ni, N, P, and K in leaves were analyzed without any data transformation. The least significant difference was then used for evaluating significant differences between the treatment means.

RESULTS

Impact of soil treatment on cacao seedling growth: Soil derived from the nickel mine area containing this heavy metal at around 21.7 ppm, has a texture class of clay (27.0% sand, 25.0% silt, and 48.0% clay), and pH of 6.99. A decrease of pH was just observed to around 6.72 and 6.78 in soil containing composted plant residues without and with *Trichoderma asperellum,* respectively.

Plant height increased significantly after the treatment with composted plant residues. At one month after planting, plant height in the control was 13.7 cm and in soil containing first compost (gliricidia, billy goat, and rice straw), second compost (gliricidia, coconut husk, and rice straw) and third compost (gliricidia, empty stalk of palm oil fruit, and rice straw), was 16.3 cm, 17.6 cm 19.7 cm, respectively, indicating an increase of 18.9%, 28.5%, and 43.8%, respectively. Plant height of cacao grown in soil containing these respective types of compost with the addition of *T. asperellum* was 18.7 cm, 19.9 cm, and 21.2 cm, respectively, indicating an increase of 36.5%, 45.3%, and 54.7%, respectively (Figure 1). Therefore, the highest growth observed in the seedling grown in soil treated with third compost plus *T. asperellum* and this was significantly different ($P \leq 0.05$) to other treatments, except for the treatment with the second compost plus *T. asperellum*.

The stomata of leaves grown in untreated soil were relatively small with an opening width of 1.06 µm, opening length of 6.72 µm, and opening area of 5.59 μm^2. Respective composts treatment resulted in increasing the opening width to 1.13 µm (6.2% of increase) , 1.21 µm (14.2%), and 1.32µm (24.5%), the opening length 7.00 µm (4.2%), 6.83 µm (1.6%), and 8.00 (19.0%), and the opening area 6.55 μm^2 (17.2%), 5.83 μm^2(4.3%), and 7,90 μm^2 (41.3%), respectively. In treated respective composts plus *T. asperellum*, the increase of opening width was 1.18 µm (11.3%), 1.23 µm (16.0%), and 1.37 µm (29.2%), opening length 7.00 µm (4.2%), 6.83 µm (1.6%), and 8.00 µm (19.0%), and opening area 6.48 μm^2 (15.9%), 6.78 μm^2 (21.3%), 8.58 μm^2 (53.5%), respectively (Figure 2). Similar impacts of treatment were also observed on the stomata number where the treatment of first compost, second compost, and third compost without any addition of *T. asperellum* increased this number of stomata by 0.0%, 13.4%, and 29.4%, respectively, and in those treated with *T. asperellum*, the increase was 14.3%, 5.3%, and 7.1% respectively (Figure 1). In this regard, the largest stomata opening was observed in seedling grown in soil treated with the combination of third compost and *T. asperellum* and this was significantly different ($P \leq 0.05$) from other treatments, except from third compost minus *T. asperellum*. The highest stomata number was observed in seedlings grown in soil treated with third compost minus *T. asperellum* and this was significantly different to other treatments and control.

Impact of soil treatment on nickel, nitrogen, phosphorus, and potassium content of leaves: Nickel (Ni) as one of the important elements in soil collected from the nickel mine area could be detected at 5.33 ppm on third and fourth leaf of cacao. Its concentration was increased to 5.53 ppm, 6.01 ppm, and 7.93 ppm, indicating the increase of 3.8%, 12.8%, and 48.8%, after soil treatment by first compost, second compost, and third compost without *T. asperellum* respectively. A higher increase to 6.67 ppm, 10.01 ppm, and 13.93 ppm, or an increase of 25.1%, 87.8%, and 161.4% was observed after soil treatment with the combination of each compost and *T. asperellum,* respectively. Therefore, the highest leaf nickel content was detected after soil treatment with the combination of third compost and *T. asperellum* and this content was significantly different ($P \leq 0.05$) to other treatments and control (Figure 3)

Nitrogen (N), phosphorus (P), and potassium (K) content on leaves of cacao grown in soil without any addition of composts and *T. asperellum* were 0.90%, 0.06%, and 1.14% respectively. The content of N was not affected after soil treatment by composts without *T. asperellum*, but when *T. asperellum* was added, an increase of N content by 10.0%, 8.9%, and 10.0%, respectively, was observed. This increase was significantly different ($P \leq 0.05$) for compost treatments minus *T. asperellum* and the control, but not for compost with *T. asperellum* (Figure 3). The content of K increased by 43.1%, 38.3%, 23.7% after soil treatment by three respective composts without *T. asperellum*, and by 20.8%, 16.7%, and 14.0% in those with *T. asperellum,* respectively. The highest content of K was therefore found in leaves after soil treatment with first compost and this content was significantly different ($P \leq 0.05$) to the other treatments and the control (Figure 3). Contrary to the content of N and K, the content of P was reduced after soil treatment both with composts minus *T. asperellum* and with composts plus *T. asperellum*. The decrease by 33.3%, 43.3%, and 46.7% was observed after soil treatment by the respective three composts without

T.asperellum and by 48.3%, 48.3%, and 51.7%, respectively, after treatment with those including *T.asperellum*. The lowest content of P was found in leaves after soil treatment with third compost plus *T. asperellum* and this content was significantly different (P ≤ 0.05) to the control, first compost minus *T. asperellum*, and second compost minus *T. asperellum* (Figure 3).

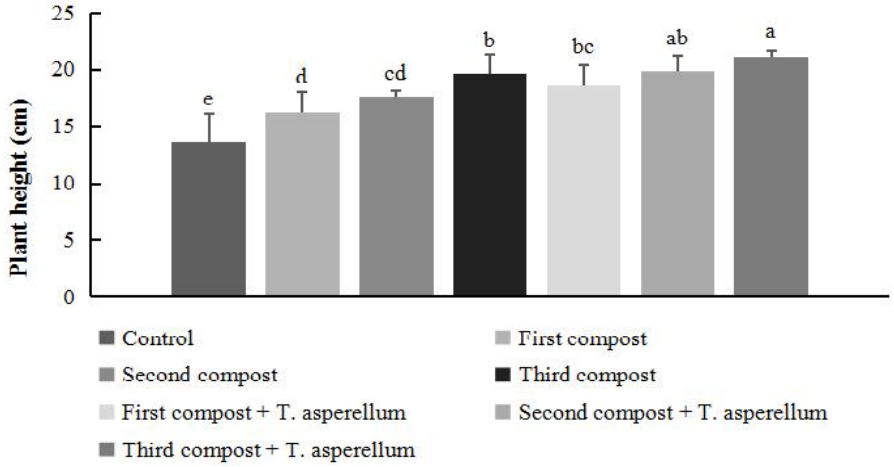

Figure 1. Average height of one month-old cacao seedling after treatment with plant residue based-composts without and with *Trichoderma asperellum*. Means of height followed by same letter are not significantly different according to least significant difference ($p \leq 0.05$).

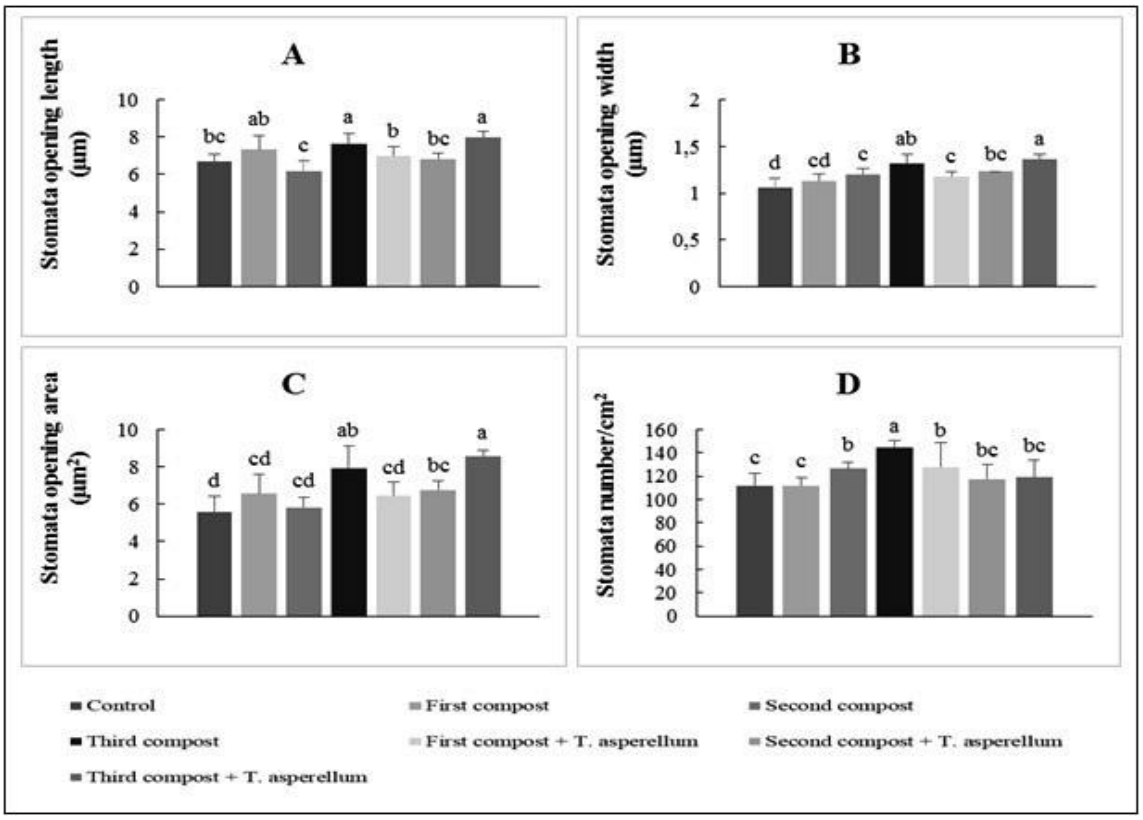

Figure 2. Stomata opening length (A), stomata opening width (B), stomata opening area (C), and total number of stomata (D) on MCC1 clone leaf seedling eight weeks after treatment with plant residue based-composts without and with *Trichoderma asperellum*. Means of each parameter followed by same letter are not significantly different according to least significant difference ($p \leq 0.05$).

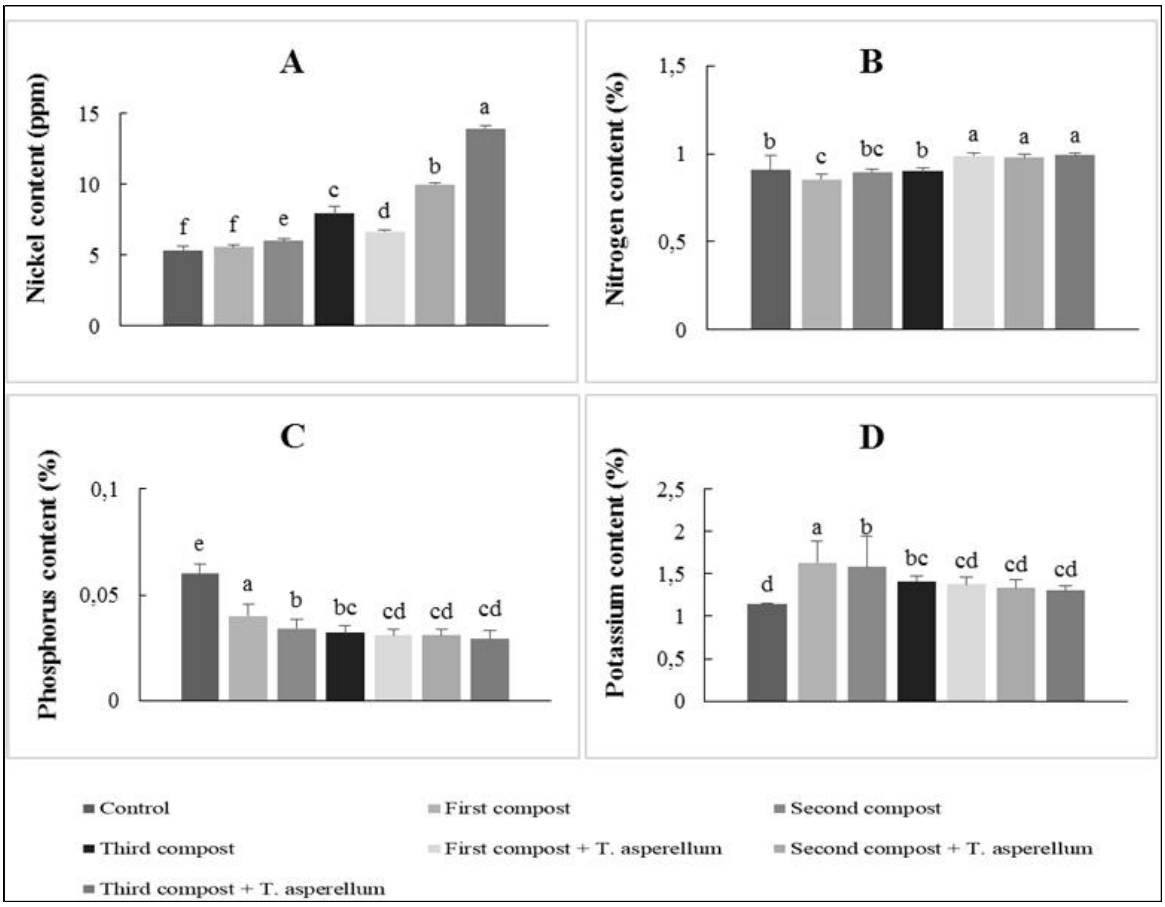

Figure 3. Content of nickel (A), nitrogen (B), phosphorus (C), and potassium (D) on MCC1 clone leaf seedling ten weeks after treatment with plant residue based-composts without and with *Trichoderma asperellum*. Means of each parameter followed by same letter are not significantly different according to least significant difference ($p \leq 0.05$).

DISCUSSION

Nickel (Ni) is a heavy metal and its concentration in soil in certain areas can be increased by human activities such as mining works and phosphate and pesticide application (Gimeno-Garcia *et al*. 1996). At low concentration, this metal can function as an essential micronutrient for plant growth and development, but it becomes toxic at high concentration. Excess Ni impairs photosynthesis and nutrient balance, results in disorder of cell membrane functions, inhibits seed germination, and obstructs plant growth and development (Eskew *et al*., 1983; Moya *et al*., 1993; Nagajyoti *et al*., 2010). In this experiment, cacao seedling grown in soil containing Ni showed symptoms of leaf chlorosis and stunting, smaller stomata area, and lower numbers of stomata. Application of composted plant residues with three alternative formulations, without and with *Trichoderma asperellum,* improved response by reducing of leaf chlorosis, increasing stomata parameters and number, and increasing plant height. The increase of stomata opening (width, length, and area) and number would contribute to increasing photosynthetic rates by enhancing uptake of CO_2 and could be advantageous to seedling growth.

Detection of Ni in seedling leaves indicated that this heavy metal was increased with plant growth, indicated by plant height and stomata opening, so that a higher content of Ni in leaves was correlated with higher seedling growth. Therefore, in this regard cacao seedlings were observed to increase nickel content up to 13.9 ppm and with no apparent toxicity; perhaps additional Ni was used as a micronutrient in supporting seedling growth. One of the important roles of nickel is as an irreplaceable constituent of urease, which assists in the hydrolysis of urea to ammoniacal-N, and which further can be utilized in other metabolic pathways such as synthesis of amino acids, polyamines, and other nitrogen compounds (Gerendás and Sattelmacher, 1999; Witte, 2002; Wood, 2015). In addition, Ni can activate an isoform of glyoxalase I that catalyze an important step in the degradation of methylglyoxal (MG), a compound naturally produced by cellular metabolism that may be lethal to cell functions. It has been suggested also that Ni

may have a key participation in plant antioxidant metabolism, notably in stressful conditions (Fabiano *et al*., 2015).

In soil, Ni is slightly mobile and associated with the residual fraction (Kabata-Pendias and Mukherjee, 2007). Addition of compost can act on a great variety of processes leading to improvements in physicochemical soil properties and fertility status and even alter the heavy metal distribution in the soil (Bernal *et al*., 2007). Some researchers have found that stabilized composted plant residues form complexes with nickel and restricted its mobility and availability for plant absorption (Hanc *et al*., 2012; Jakubus, 2012; Ahmed *et al*., 2017; Navady *et al*., 2017). Furthermore, the present of compost affects soil properties by increasing soil microbial activity (Jordao *et al*. 2006; Hargreaves *et. al*. 2008; Dixit *et al*., 2015). These microorganisms can minimize the bioavailability and biotoxicity of heavy metals through mechanisms such as biosorption and precipitation (Gadd. 2000; Lloyd and Lovely 2001; Bandara *et al.* 2015). However, others show that compost may release contaminating metals from organic combination by degradation of the organometallic complexes, leading to increased bioavailability of these metals (Heyes *et al*., 1998). Our results of the research support the second finding that compost can bind the Ni from soil, which was then released and absorbed by cacao seedling. The content of Ni in leaves was determined by the composition of compost and *Trichoderm*a, and increased with these treatments. The distinction of the three compost formulas was based on billy goat in first compost, fruit stalk of palm oil in second compost and coconut husk in third compost, while the other constituents, gliricidia and rice straw, were the same in the three composts. Treatment with third composition of compost resulted in the highest level of Ni in leaves, due possibly to an active role of coconut husks in sorption and desorption of Ni compared to billy goat and fruit stalk of palm oil. Inclusion of *T. asperellum* in the three compositions of compost resulted in increasing Ni content in leaves following treatment. It has been known that many *Trichoderma* species are tolerant of this metal and fungal biomass production is recorded at Ni concentrations of up to 60 - 150 ppm (Nongmaithem *et al*., 2016). The fungus is capable of solubilizing Ni in soil and enhancing its uptake by *Miscanthus giganteus*, *Panicum virgatum*, and *Phalaris arundinacea* (Kacprzak *et al*., 2014). The *T. asperellum* strain used in this experiment was an endophyte (Rosmana *et al*., 2015), so it is possible that this fungus increases Ni uptake by promoting cacao growth or by directly enhancing Ni uptake into plant tissues

Symptom of Ni deficiency is characterized by chlorosis that tends to be uniform throughout the leaf (Wood *et al*., 2004) and this symptom was shown in cacao seedling. Therefore, one of growth disturbances of cacao in untreated soil was caused apparently by deficiency of Ni. The presence of other metallic compounds such as Fe and Mn-Oxides and also pH are the most important factors affecting the mobility of Ni in soils. Leaching of Ni to the groundwater often occur in acidic condition with maximum ionic $Ni(H_2O)_6^{2+}$ concentration. However, under alkaline condition, Ni may precipitate as hydroxides such as $Ni(OH)_3$ or $Ni_4(OH)_4^{4+}$, with low solubility (Alloway, 2013). As soil used in the experiment was alkaline, nickel hydroxide was probably as well formed. In this regard, we suggest that when Ni is bound to compost or microorganisms including *T. Asperellum* and released to be taken up by the plant. This uptake of Ni restrains possibly availability of phosphate that can be taken up by plant and causes reduction of phosphorus content in leaf. The increase of nitrogen content in leaf treated with all combination of compost and *T. asperellum* was probably related to degradation of compost by microorganisms.

We conclude that the increase of cacao growth planted in the soil of nickel mining area origin after treatment with composted plant residues and its combination with *T. asperellum* was determined by the capacity of plant residue species and *T. asparellum* to adsorb and desorb of Ni from soil for uptake by the plants. This research suggests that compost treatment, especially a combination of particular formulations of compost and *T. asperellum* could potentially be used for cacao field application around nickel mining areas.

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