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Originalarbeit

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A bioremediation process based on the application of *Rhizobium* sp. I3 and Ramie (*Boehmeria nivea* L.) in lead contaminated soils

Ein Bioremediationsprozess basierend auf Applikation von *Rhizobium* sp. I3 und Ramie (*Boehmeria nivea* L.) in Blei-kontaminierten Böden

Abstract

Environmental pollution by heavy metals, especially lead (Pb), is one of the most serious environmental problems. The existence of excessive Pb in the soil will cause soil degradation and threaten the ecosystem of life on the land, therefore remediation needs to be done. Ramie (*Boehmeria nivea* L.) is known to have hyperaccumulatory qualities. Therefore, it can be used as bioremediation agent. This study was done to determine the effect of inorganic fertilizers, *Rhizobium* sp. *I3* and cow manure in an effort to reduce soil Pb levels with Ramie plants. The research was conducted from May to November 2017 in Karanganyar Regency, Central Java Province, Indonesia in paddy fields directly adjacent to the textile industry contaminated by Pb. The experimental design was factorial with a Completely Randomized Block Design as the based design, consisting of 3 factors: inorganic fertilizer (P0: without inorganic fertilizer, P1: with inorganic fertilizer), bioremediation agents (B0: without bioremediation agents, B1: with *Rhizobium* sp. *I3*, B2: with cow manure), and plant (T0: without plant, T1: with Ramie). The experiment was repeated three times. Data were analyzed by statistical analysis using ANOVA significance level of 95% continued by T-test or Duncan Multiple Range Test, significance level of 95%, as well as a correlation test. The results showed that the combination of inorganic fertilizer + *Rhizobium* sp. *I3* + Ramie (P1B1T1) had the highest Pb absorbtion of 29.98 $\mu g\ g^{-1}$ (45% higher than control) and was able to reduce soil Pb levels by 9.29 $\mu g\ g^{-1}$ from initial soil Pb level. P1B1T1 had the most effective bioremediation value of 60.35%, a difference of 12.22% in comparison to the control (48.13%). Bioremediation using *Rhizobium* sp. *I3* was better than using cow manure; because it can reduce soil Pb levels in the soil with the highest effectiveness of bioremediation.

Key words: Ramie, lead, phytoremediation, bioremediation, soil

Zusammenfassung

Umweltverschmutzung durch Schwermetalle, insbesondere Blei (Pb), ist eines der schwerwiegendsten Umweltprobleme. Das Vorhandensein von übermäßigem Pb im Boden führt zu einer Verschlechterung des Bodens und gefährdet das Ökosystem des Landlebens. Daher muss eine Sanierung durchgeführt werden. Ramie (*Boehmeria nivea* L.) hat bekanntlich hyperakkumulative Eigenschaften und kann als Bioremediationsmittel verwendet werden. In dieser Studie wurde die Wirkung von anorganischen Düngemitteln, *Rhizobium* sp. *I3* und Kuhdung getestet, um mit Ramie-Pflanzen den Bleigehalt im Boden zu senken. Die Forschung wurde von Mai bis November 2017 in Karanganyar, Zentral-Java, Indonesien in Reisfeldern durchgeführt, die direkt an die Textilindustrie angrenzen und mit Blei kontaminiert waren. Der Versuchsaufbau

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war faktoriell mit einem vollständig randomisierten Blockdesign und bestand aus 3 Faktoren: Anorganischer Dünger (P0: ohne anorganische Dünger, P1: mit anorganischem Dünger), Bioremediationsmittel (B0: ohne Bioremediationsmittel, B1: mit *Rhizobium* sp. *I3*, B2: mit Kuhdung) und Pflanze (T0: ohne Pflanzen, T1: mit Ramie) und wurde dreimal wiederholt. Die Daten wurden durch statistische Analyse unter Verwendung eines ANOVA-Signifikanzniveaus von 5% analysiert, gefolgt von einem T-Test oder einem Duncan Multiple Range Test mit einem Signifikanzniveau von 5% sowie einem Korrelationstest. Die Ergebnisse zeigten, dass die Kombination aus anorganischem Dünger + *Rhizobium* sp. *I3* + Ramie (P1B1T1) die höchste Bleiaufnahme gegenüber dem anfänglichen Bodenbleiniveau von 29,98 µg g^{-1} (45% höher als bei der Kontrolle) aufwies und die Bleigehalte um 9,29 µg g^{-1} senken konnte. P1B1T1 hatte mit 60,35% den höchsten Wert für die Wirksamkeit der Bioremediation, eine um 12,22% höhere Differenz zur Kontrolle (48,13%). Die Bioremediation unter Verwendung des Bakteriums *Rhizobium* sp. *I3* war besser als bei der Verwendung von Kuhdung, da es den Bleigehalt des Bodens bei höchster Wirksamkeit der biologischen Sanierung verringern kann.

Stichwörter: Ramie, Blei, Phytoremediation, Bioremediation, Boden

Introduction

Pb is often found dangerous when it enters the food chain. Pb is a heavy metal and often found in pollutants and in excess amounts in the environment. Industry is one of the causes of environmental pollution by Pb that do not manage their waste properly. Karanganyar Regency, Central Java Province, Indonesia, is one of the regencies that has many industries in their region, there are textile and chemical industries. Most of these industries are located on agricultural land and the waste has the potential to pollute the environment. Results of initial laboratory analysis showed that Pb content in soil before bioremediation research was 15.385 µg g^{-1}. While the threshold of Pb metal in the soil according to PPRI No. 101 of 2014 is 3,0 µg g^{-1}, thus there is a fivefold exceedance of the threshold.

Bioremediation is an effort to reduce pollutants in the environment by utilizing living organisms. One form of bioremediation is phytoremediation, which uses plants to clean the environment from pollutants (Laghimi et al., 2015).

Ramie (*Boehmeria nivea* L.) (Fig. 1) was used as phytoremediation agent because it has hyper accumulator qualities (Wang et al., 2008). There were several criteria that can be considered in selecting plants for bioremediation, including high ability to absorb heavy metals, rapid growth, being no food crop. Ramie was chosen because it has the mentioned criteria. Wang et al. (2008) said that Ramie also has a high economic value because the fiber can be used as raw material for woven crafts and it is known that the quality of fiber does not decrease when the heavy metal content increases in its plant tissue. Table 1 lists the scientific classification of Ramie.

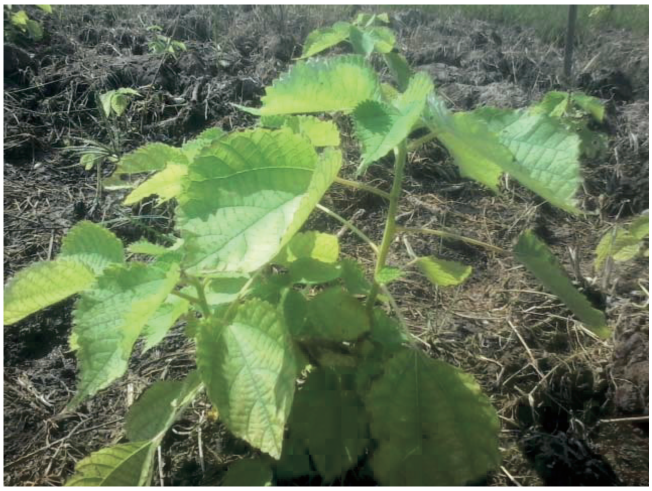

Fig. 1. Ramie two months after planting

Rhizobium was used as bioremediation agent because it can help plants in absorbing and accumulating heavy metals. *Rhizobium* can release secondary metabolites, such as enzymes, organic acids or siderophores, that can enhance the phytoremediation process (Hao et al., 2014). Pajuelo et al. (2008) also explain that there were bacteria that survive in environmental conditions contaminated by heavy metals, one of them was *Rhizobium*.

Manure is an organic material that has chemical compounds in the form of organic acids, such as oxalic acid and citrate (Chen et al., 2016). A study about phytoremediation shows that manure can neutralize soil pH and increase plant height, decrease soil pH and also can help the Pb to be available for plants (Estuningsih et al., 2015).

Inorganic fertilizers are made synthetically by physical and chemical processes. Inorganic fertilizers are useful in increasing the amount of nutrients for plants in the available form directly. But according to Alloway (1995) inorganic fertilizers, such as urea and factory-made phosphate fertilizers, also contain heavy metals. The results of heavy metals analysis showed that inorganic fertilizers also contain Pb. Although the content is below the toler-

Table 1. Scientific classification of Ramie

Division	Magnoliophyta
Class	Magnoliopsida
Ordo	Urticales
Family	Urticaceae
Genus	*Boehmeria*
Species	*Boehmeria nivea* L. Gaudich

ance limit, heavy metals are accumulative and dangerous for the environment.

The purpose of this study was to determine the effect of inorganic fertilizers and bioremediation agents (*Rhizobium* sp. *I3* and cow manure) on Pb absorption in Pb contaminated soil by Ramie and to find out which treatment gives the highest Pb absorption by Ramie and the most reduced soil Pb levels.

Materials and methods

Study area

This research was conducted from May to November 2017 in Kaling Village, Tasikmadu District, Karanganyar Regency, Central Java, Indonesia. The research was located at coordinates 110⁰40'30" E – 07⁰07'30" S. The Analysis of soil samples and plant samples were carried out in the Laboratory of Soil Biology and Biotechnology, Soil Physics and Conservation, Laboratory of Chemistry and Soil Fertility, Universitas Sebelas Maret. Heavy metal analysis measurements were carried out at the Integrated Laboratory at the Agricultural Environment Research Center in Pati Regency, Central Java.

Materials and equipment

The material used included *Rhizobium* sp *I3* isolated by Rosariastuti (2014), cow manure, inorganic fertilizers in the form of urea, KCl, and SP-36 obtained from farm shops, Ramie plant seedlings from Wonosobo, Central Java, and media Luria Bertani that consisted of yeast 5 g l^{-1}, pepton 10 g l^{-1}, NaCl 10 g l^{-1} and destilled water (Sezonov and Joseleau, 2007). Chemicals needed for wet destruction were destilled water, NaCl, Alcohol, HNO_3^- and $HClO_4^-$.

The equipment needed were sample ring, test tube, flacon, pipette, erlenmeyer, pH meter, electric oven, refrigerator, Balance Sheet Analytic, hot plate and stirrer, Autoclave, vortex, shaker, fume hood, AAS (Atomic Absorption Spectrophotometer) and hoe.

Experimental design

The experimental design was factorial with a Completely Randomized Block Design as the based design, consisting of 3 factors: inorganic fertilizer (P0: without inorganic fertilizer, P1: with inorganic fertilizer), bioremediation agents (B0: without bioremediation agents, B1: with *Rhizobium* sp. *I3*, B2: with cow manure), and Plant (T0: without plant, T1: with Ramie), so there were 12 treatment combinations that were repeated 3 times, thus there were 36 treatment units.

Procedure

Bacterial inoculum. One hundred ml of liquid Luria Bertani (LB) media was prepared, then one tab of pure *Rhizobium* sp *I3* isolate was inoculated, then shaken up using a shaker until the bacterial density reached 10^{10} cells ml^{-1}. Furthermore, 100 ml of the liquid inoculum were inserted into 900 ml LB liquid in order to enlarge the inoculum to 1 liter. The inoculum was shaken out using a shaker to a density of 10^{10} cells ml^{-1}. The application dosage of bacteria in the soil was 10^6 CFU ml^{-1} of soil.

Fertilizer preparation. The dosage of fertilizer used was urea 60 kg ha^{-1}, P_2O_5 20 kg ha^{-1} and K_2O 30 kg ha^{-1} (Purwati, 2010). The dosage cow manure was 10,000 kg ha^{-1} (Santoso, 2005). From the results after the conversion, the dosage of fertilizer per plot was urea 17.25 g per plot, SP-36 was 4.5 g per plot, KCl was 11.25 g per plot, and manure 6,25 kg per plot.

Land preparation. The treatment plot was made with a size of 2.5 m × 2.5 m and the distance between plants was 50 cm × 50 cm. The distance between the plots was 25 cm. The application of manure was done one week before planting, the application of bacteria was done three days before planting, while the application of inorganic fertilizer was done one day before planting. Harvesting was done 45 days after planting.

Plantation. One plot contained 25 sub-plots. Each subplot was planted with two Ramie seedlings. Upkeep was done by regulating water irrigation, and weeding the grass. Five samples were randomly selected in each plot.

Observation parameters and laboratory analysis

Observed parameters were soil characteristics including soil pH (potentiometric method), Cation Capacity Exchange (CEC) (ammonium treatment method), Soil Organic Matter (Walkley-Black method), Population of bacteria *Rhizobium* sp. *I3* (Plate Count method) and the level of Pb (wet destruction method followed by reading with AAS) (Balittanah, 2009).

Further observed parameters were plant characteristics including plant height and dry weight (including root and shoot), Pb content and Pb absorption in plant (wet destruction method followed by detecting the metal content using AAS).

Data analysis

Data were statistically analyzed by using ANOVA (95% significance level) continued by Duncan Multiple Range Test (DMRT) (95% significance level) on treatment factor consisting of 3 treatment levels and T-Test (95% significance level) on treatment factor having 2 levels of treatment. Correlation test was done to see the relationship between parameters observed.

Results and discussion

Plant characteristics

Table 2 shows that treatment with inorganic fertilizers and cow manure (P1B2T1) has the highest plant height, but the highest total dry weight was found in inorganic fertilizer treatment with *Rhizobium* sp *I3*. According to the ANOVA results, the interaction between treatments had no significant effect on plant height and total weight but the treatment of inorganic fertilizer and bioremedia-

Table 2. Mean values of plant characteristics

No.	Treatment	Plant Height (cm)	Dry Weight (g)		
			Root	Shoot	Total
1	P0B0T1	12.6	3.86	0.88	4.74
2	P0B1T1	14	4.93	1.17	6,10
3	P0B2T1	14.2	5.04	1.13	6.17
4	P1B0T1	14.6	4.31	1.02	5.33
5	P1B1T1	15.1	6.50	1.97	8.47
6	P1B2T1	15.2	5.88	1.54	7.42

tion agents significantly influenced the total weight of the plant.

Figure 2 shows that the mean value of total plant dry weight of control (P0) was 5.67 g with a standard error of 0.29 g. For the treatment with inorganic fertilizer (P1) the mean value was 7.07 g with a standard error of 0.58 g.

Figure 3 shows that treatment with *Rhizobium* Sp I3 has the highest mean value of total plant dry weight (7.29 g with a standard error of 0.89 g).

Inorganic fertilizers provide nutrients that are essential for plant growth in available form so that they can increase the growth of cultivated plants. The presence of microorganisms, such as bacteria can solubilize soil nutrients so that they can be absorbed by plants and increase plant growth (Ma et al., 2011). Based on the correlation test results, the total weight of plants is positively correlated with Pb levels in plants, Pb absorbtion by plants, effectiveness of bioremediation, CEC, and number of bacterial populations. According to Shahab et al. (2009) bacteria produce Indole Acetic Acid (IAA), which can affect plant height because IAA can control many important physiological processes during plant growth, such as tissue differentiation, cell division and enlargement.

Soil characteristics

Soil pH. Based on the ANOVA results all treatments had a very significant effect ($p < 0.01$) on soil pH. The interaction between the three treatments had a significant effect ($p < 0.05$) on soil pH. The treatment without inorganic fertilizer, without bioremediation agents + Ramie (P0B0T1) had the highest pH and was significantly different from the other treatments. The Correlation test shows that pH has a negative correlation with Pb levels in the shoot ($r = -0.638$) and total Pb absorbtion by Ramie ($r = -0.492$).

As it is a shown in Table 3, pH decreased after the research activities were carried out in all treatments. Before the study, the soil pH was 6.15 and after the study it had a lower pH range from 5.23 to 5.84. The lowest soil pH was found in treatment P1B2T0. Soil pH is known to be one of the factors that can affect the solubility of heavy metals. All treatments experienced a decrease in pH after bioremediation. Decreasing pH values can increase the availability of Pb for plants. According to the USDH (2012) the acidic environment will cause releasing of Pb due to Pb cation dissolution, which in non-acidic environment is strongly bound to soil particles. A decrease in pH will cause Pb cations to be dissolved and oxidized from $Pb^{2+}$to Pb^{4+} (Karmakar et al., 2007). Pb^{4+} is a form

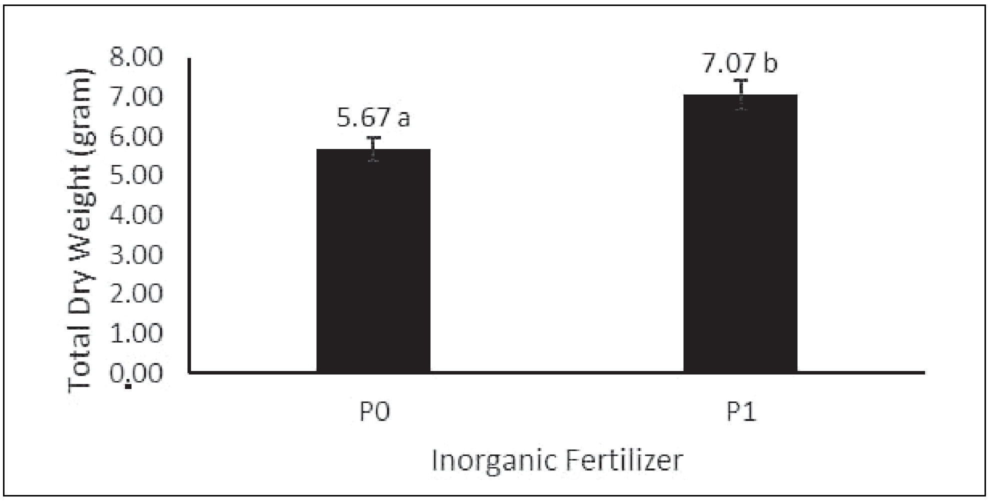

Fig. 2. Histogram of the effect of inorganic fertilizer on Ramie biomass. Bars representing standard errors

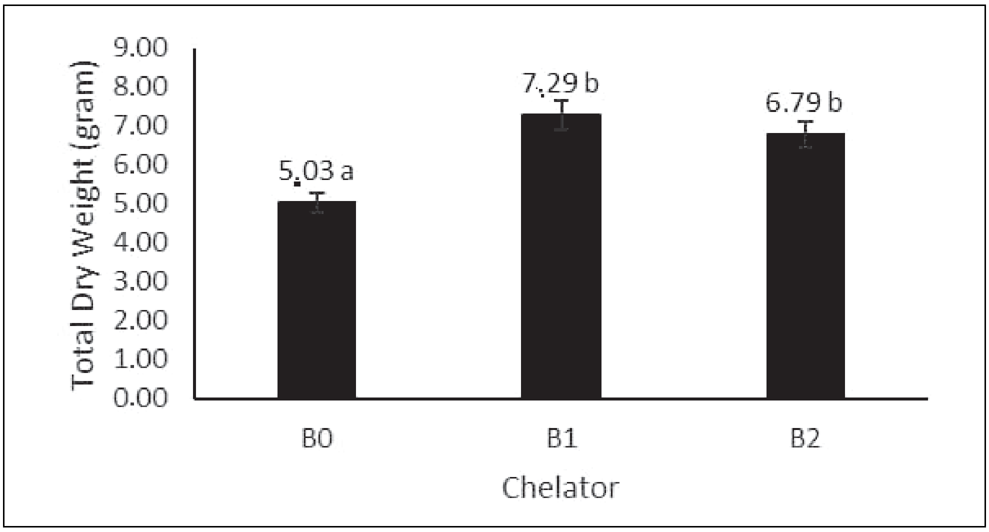

Fig. 3. Histogram of the effect of bioremediation agents on Ramie biomass. Bars representing standard errors

Table 3. Mean values of soil characteristics

Treatment	Soil pH	Cation Exchange Capacity (cmol(+) kg^{-1})	Soil Organic Matter (%)	Number of Bacterial Populations (CFU g^{-1})	Pb Soil Level (µg g^{-1})	Effectiveness of Bioremediation (%)
Initial	6.45	20.22	3.09	2.31	15.39	
P0B0T0 (control)	5.61 g	25.07 a	3.28	3.47 a	7.98 ef	48.13 ab
P0B1T0	5.25 ab	27.58 b	2.95	8.84 h	7.48bcd	51.38 cde
P0B2T0	5.36 bcd	31.13 d	4.21	5.60 c	8.20 f	46.74 a
P1B0T0	5.46 def	26.11 a	3.20	3.74 b	7.82 de	49.15 bc
P1B1T0	5.36 bcd	27.48 b	3.22	8.57 g	7.57 cd	50.80 cd
P1B2T0	5.23 a	28.83 c	4.25	5.88 d	8.15 ef	47.05 ab
P0B0T1	5.84 h	25.91 a	3.28	3.75 b	7.49bcd	51.32 cd
P0B1T1	5.40 cde	28.00 bc	3.74	7.56 e	7.40 bc	51.90 de
P0B2T1	5.64 g	30.52 d	4.28	5.66 c	7.18 b	53.31 e
P1B0T1	5.48 ef	27.38 b	3.56	3.84 b	7.49bcd	51.32 cde
P1B1T1	5.54 fg	33.86 e	3.73	7.87 f	6.10 a	60.35 f
P1B2T1	5.29 abc	34.70 e	4.21	5.63 c	7.22 bc	53.07 de

Note: the same small letters or no letters written in the same column showing no significant difference

of Pb with high mobility and is easily absorbed by plants (Ahmad et al., 2005). Pb^{4+} has a lower toxicity level than Pb^{2+} so that the hyperaccumulator plants can survive the polluted environment (Wicaksono and Lili, 2016). The highest pH value was found in P1B2T0 treatment. A decrease in soil pH value was generally caused by wastes containing free mineral acids and carbonates. The addition of inorganic fertilizers, such as urea, can increase the level of acidity of the soil (Sudaryono, 2009).

Cation Exchange Capacity. Based on the ANOVA results all treatments and interactions between treatments had a very significant effect ($p < 0.01$) on the value of soil CEC. The highest soil CEC was in P1B2T1 treatment with a value of 34.70 cmol(+) kg^{-1} and was significantly different from other treatments except the P1B1T1 treatment, which was not significantly different. The lowest CEC value was found in the treatment of P0B0T0 with a CEC value of 25.07 cmol(+) kg^{-1}.

Soil CEC has a close relationship with total Pb levels in plants ($r = 0.566$) and total Pb absorption in plants ($r = 0.730$). This is in accordance with Tomašić et al. (2013) who explained that metals tend to be easily exchanged on soils with high CEC so that metals are more easily absorbed by plants. The existence of Ramie plants can indirectly increase CEC because the roots of the plant can excrete root exudates as nutrients for microorganisms, such as bacteria. The bacteria itself can produce extracellular enzymes that can decompose organic matter (Sinsabaugh et al., 2009). Regarding decomposition of organic matter, HANAFIAH et al. (2009) explain that a higher amount of decomposed organic matter will increase the CEC value.

Soil organic matter. Figure 4 shows that treatment with cow manure and Rami has the highest mean value of soil organic matter (4.25% with a standard error of 0,47%).

Based on the ANOVA results the interaction between inorganic fertilizers, bioremediation agents and Ramie plants do not significantly effected ($p > 0.05$) the organic matter content. However, the interaction between bioremediation agents and Ramie had a significant effect ($p < 0.05$) on organic matter content. As it is shown in Fig. 4, the interaction between bioremediation agents and Ramie plants with the highest amount of organic matter was found in B2T1 treatment with organic matter content of 4.25%.

Heavy metal pollutants can form a complex bond with organic acids. This reaction is a bond between an organic compound and a coordinated metal ion. Negative charge arises because of the functional dissociation of phenolic and carboxyl (ARIYANTO, 2006).

The results of the correlation test in this study indicate that the content of organic matter has a close relationship with the soil CEC ($r = 0.594$). This means that the higher the content of organic matter in the soil, the higher the value of soil CEC. This is supported by ARIFAH (2013) who states that the addition of organic materials, such as manure, can increase cation absorption more than clay colloids so that it can increase the value of the CEC of the soil. TOMAŠIĆ et al. (2013) also stated that organic matter can bind cation because it is a source of negative charge.

Bacterial population. Based on the ANOVA results, all treatments and interactions between treatments have a very significant effect ($p < 0.01$) on the number of bacteria. The treatment with the addition of inorganic fertilizer, without bioremediation agents and Ramie (P1B0T0) increased the number of bacterial populations more than the control (P0B0T0). This is in accordance with the statement of MUJIYANTO and SUPRIYADI (2009) who state that the use of NPK fertilizer (inorganic) can increase the bacterial population.

The treatment with *Rhizobium* sp. *I3* (B1) resulted in the highest number of bacteria and is significantly different from other treatments. The application of *Rhizobium* sp. *I3* can directly increase the population of soil *Rhizobium* sp. bacteria. Increasing the number of bacterial populations on research land contaminated with heavy metals shows that bacteria have a high tolerance or resistance to the presence of Pb. According to NAIK and DUBEY (2013) bacteria have several biochemical mechanisms to survive in Pb polluted environments, some examples of such mechanisms are surface biosorption, extra-cellular sequestration or organic Pb biotransformation.

Correlation test results showed that the bacterial population had a positive correlation with the effectiveness of bioremediation ($r = 0.620$), total biomass ($r = 0.650$) and soil CEC ($r = 0.531$). Based on the results of the correlation test it can be concluded that the greater the bacterial population is the greater is the total biomass. According to MA et al. (2011) the presence of microorganisms such as bacteria can solubilize soil nutrients to be available to plants that can increase plant growth.

Pb soil level. Based on the ANOVA results, all treatments and interactions between treatments have a very significant effect ($p < 0.01$) on Pb metal content in the soil. DMRT results show that the interaction between inorganic fertilizer treatment, *Rhizobium* and Ramie plants (P1B1T1) had the lowest levels of soil Pb metal and was significantly different from other treatments. A decrease of Pb metal in the soil can be caused by Pb metal uptaking by plants through the roots. The plant's roots can excrete root exudates as nutrients for microorganisms, such as bacteria. The bacteria itself can produce extracellular enzymes that can decompose organic matter (SINSABAUGH et al 2009). *Rhizobium* sp. *I3* can also reduce Pb levels in the soil because *Rhizobium* can increase Pb absorption by plants. This is in accordance with the statement of ROSARIASTUTI et al. (2013) that *Rhizobium* can increase the absorption of heavy metals and translocations from

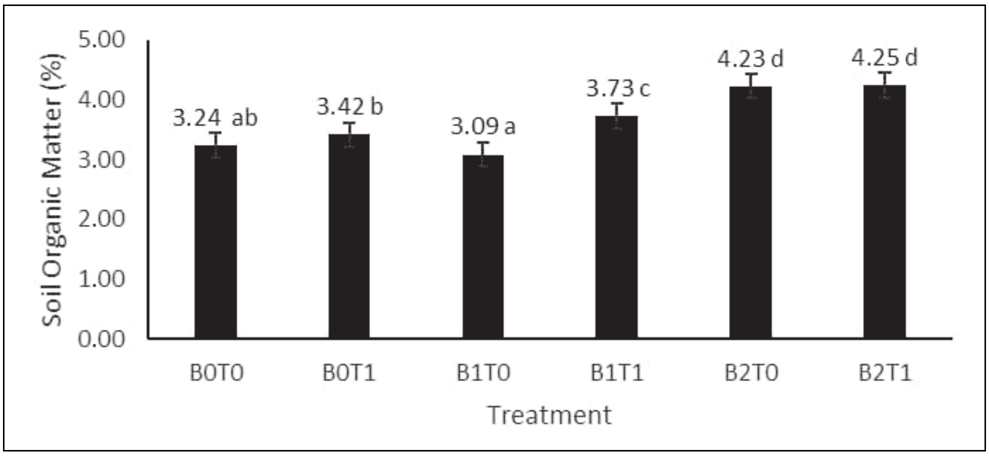

Fig. 4. Histogram of the effect of interaction between bioremediation agents and Ramie plants on soil organic matter. Bars representing standard errors

roots to the shoot. Furthermore, Mäser et al (2001) explained that cells in the roots can adsorb metal, retrieve it and then transport it through the plasma membrane with energy from ATP. Bacteria can also produce organic acids that can oxidize Pb so that the Pb can be absorbed more easily by plant roots.

The correlation test shows that Pb levels in soil were negatively correlated with the effectiveness of bioremediation ($r = -0.977$), total plant weight ($r = -0.591$) and total bacterial population ($r = -0.615$).

Effectiveness of bioremediation. P1B1T1 treatment had the highest bioremediation effectiveness and was significantly different from the other treatments. The control has an effectiveness value of 48.13% and the treatment that has the highest effectiveness was treatment P1B1T1 with a value of 60.35%, 12.22% higher than control. The treatment of *Rhizobium* sp. *I3* (B1) increased the removal effectiveness and was significantly different from the treatment of manure (B2) and control (B0).

According to the correlation test, the effectiveness of bioremediation has a negative correlation with Pb content in the soil ($r = -0.977$), which means that the removal effectivity would increase when the Pb content in the soil decrease at the end of the research. The correlation test also showed that the effectiveness of bioremediation has a very close relationship with soil CEC ($r = 0.635$) and bacterial population ($r = 0.620$). This means that the increase in the effectiveness of bioremediation was also followed by an increase in CEC and bacterial populations. Related to the land CEC and the bioremediation process, Hanafiah et al. (2009) argues that soil CEC will have an effect on increasing metal absorption by plants, because soil with high CEC metals will tend to be easily exchanged so they can be easily absorbed by plants and will increase the effectiveness of bioremediation. The results of the correlation between the effectiveness of bioremediation and the number of bacterial populations are in line with Yulia's (2013) statement that the success of the bioremediation process or the effectiveness of bioremediation is determined by several factors, one of which is the number of microorganisms.

Pb in plants. The Pb content in plants is the indicator of the ability of plants in accumulating heavy metals. The treatment of inorganic fertilizer and bioremediation agent treatment had very significant effect ($p < 0.01$) on root Pb content and shoot Pb content. The interaction between bioremediation agent and inorganic fertilizer had very significant effect ($p < 0.01$) on root Pb content, shoot Pb content and had significant effect on total Pb content.

The ANOVA showed that inorganic fertilizer and bioremediation agent had very significant effect ($p < 0.01$) on root Pb accumulation, shoot Pb accumulation and total Pb accumulation by plants. Interaction of inorganic fertilizer + bioremediation agent + Ramie plants had very significant effect ($p < 0.01$) on root Pb accumulation, shoot Pb accumulation and total Pb accumulation by plants. The treatment of P1B1T1 had the highest root, shoot, and total Pb accumulation by plants and was significantly different from all other treatments except P1B2T1.

Based on Table 4, Pb accumulation was greater in the shoot than in the root. This means that Ramie plants are capable of translocating Pb from root to shoot. Nikolova (2013) said high Pb levels in roots are not always followed by high Pb levels in shoots. This is influenced by the ability of plants in transporting heavy metals from roots to other parts of plants. The Pb level in shoot was greater than the Pb level in root. One of the factors that influence Pb accumulation in roots is phosphate content in the soil. On soils with high phosphate, Pb mobility will decrease because Pb can be bound by phosphate to form the ionic compounds Pb (II) phosphate or $Pb_3(PO_4)_2$, reducing the mobility causing Pb to be absorbed in plants and to be more concentrated in plant roots (Cao et al., 2002). In this research, Pb content in shoot was higher than Pb content in root. Probably, this was because of the low content of phosphate in soil.

Plants with the highest Pb metal content were found in the P1B1T1 treatment with a value of 6.07 µg g^{-1}. Interactions between microorganisms and plants can increase the availability of Pb in the soil so that plants can easily absorb it. Microorganisms and plants will form a mutualism symbiosis where roots of plants can produce exu-

Table 4. Mean values of Pb in plants

Treatment	Content (µg.g^{-1})			Accumulation (µg/plant)		
	Root	Shoot	Total	Root	Shoot	Total
P0B0T1	1.02 a	6.02 a	2.83 a	6.61 b	14.04 a	20.65 b
P0B1T1	2.46 b	6.83 b	2.96 ab	5.74 b	15.94 b	21.68 b
P0B2T1	2.83 b	5.77 a	2.83 a	3.13 a	13.46 a	16.59 a
P1B0T1	4.04 c	6.71 b	4.06 b	5.74 b	15.65 b	21.39 b
P1B1T1	4.34 c	8.5 1 c	6.07 c	10.13 c	19.85 c	29.98 c
P1B2T1	3.00 b	8.5 8 c	4.01 b	9.43 c	20.01 c	29.44 c

Note: the same small letters or no letters written in the same column showing no significant difference dates, which can be used by microorganisms as a source of nutrition to develop, so this symbiosis will increase the number of bacterial populations in the soil. The bacteria themselves can produce metabolic compounds in the form of organic acids, such as oxalic acid or citrate (Chen et al., 2016). This acid can oxidize Pb^{2+} (not available for plants), and becomes Pb^{4+} (available for plants). Ramie even without the combination of inorganic fertilizer and bioremediation agent can still absorb Pb metal in Pb contaminated soil. Plants can release phytochelatin substances, so far phytochelatin is only found if an environment around the plant experiences an excess of heavy metals. Fitochelatin can help detoxifying heavy metals and increase their absorbtion within plants (Salisbury, 1995).

Both bioremediation agents, *Rhizobium* sp. *I3* and manure can increase heavy metal absorbtion as evidenced by the discovery of the highest heavy metal absorbtion in the treatment P1B2T1 with the amount of 29.44 µg/plant. Absorbtion in Ramie plants were higher because of the interaction between inorganic fertilizers and bioremediation agents. Inorganic fertilizers can stimulate plant growth indicated by increasing plant biomass, so Pb absorbtion will also be increased. Manure contains organic ingredients such as citric acid and oxalate, which can chelate and increase the absorbtion of heavy metals by plants. The addition of *Rhizobium* bacteria can also increase heavy metal absorbtion in plants because *Rhizobium* sp. can produce siderophore. Siderophore is better known as a compound that can chelate iron metal ions. But siderophores can also play a role as Pb chelator (Schalk et al., 2011), so Pb can be easily absorbed by plants. Mari et al. (2006) also said that *Rhizobium* sp. can produce organic acids, such as oxalic acid and citrate. These organic acids have the ability to form complexes with metal cations in forms that are available and easily soluble in water to be absorbed by plants.

Ramie plants are suitable for the use as Pb phytoremediation because they can translocate heavy metal from the root to the shoot, after which the metal content in the shoot is higher than in the root. This is a characteristic of the hyperaccumulator plant. But the combination of Ramie plants and bioremediation agents can increase the ability of Ramie to absorb heavy metals and their absorbtion will be higher if combined with inorganic fertilizers. This can be seen from the best combination of treatments P1B1T1.

Conclusion

The results showed that the combination of inorganic fertilizer + Rhizobium sp. I3 + Ramie (P1B1T1) had the highest Pb absorbtion of 29.98 µg g-1 (45% higher than control) and was able to reduce soil Pb levels by 9.29 µg g-1 from initial soil Pb level. P1B1T1 had the most effective bioremediation value of 60.35%, a difference of 12.22% to the control (48.13%). Bioremediation using Rhizobium sp. I3 was better than using cow manure, because it can reduce soil Pb levels in the soil with the highest effectiveness of bioremediation.

As bioremediation is an effective procedure to reduce PB levels in soil, farmers have to be informed better to be able to apply bioremediation and therefore produce healthy and safe plant products for the consumers. In addition, the government plays a role in the process of land remediation by providing compensation as a substitute for the loss of farmer's income. In parallel, it needs further research on the production of fiber from Ramie and business analysis to replace the loss of farmers' income.

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Conflicts of Interest

The authors declare no conflicts of interest.

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