

Remediation of Lead Contaminated Paddy Field Using Ramie Plants Combined with Bioremediation Agents and Inorganic Fertilizer

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ABSTRACT

Industrial waste containing heavy metals used for wetland irrigation will affect the heavy metals content in the soil. The accumulation of heavy metals in the soil may result in reducing microbial activity, soil fertility, soil quality, and heavy metals compounds in agricultural products. Agricultural products contaminated by heavy metals can influence human health. The purpose of this study was to reduce the Pb content in Pb contaminated paddy fields. This research was conducted in a contaminated paddy field using factorial research and a Completely Randomized Block Design consisting of 3 factors : P (Inorganic fertilizer), B (Bioremediation agents), T (Ramie). Every treatment combination was repeated 3 times. This study used a combination of Ramie with *Rhizobium* sp. I3 or manure as bioremediation agents to improve Pb uptake by plants so could decrease soil Pb. The results showed that the bioremediation agents were able to increase Pb uptake by Ramie. *Rhizobium* sp. I3 gave the highest Pb uptake of 103.77 mg.kg-1 or 2 more times than control. The best treatment in decreasing Pb is with inorganic fertilizer, *Rhizobium* sp. I3 and Ramie (P1B1T1) with Pb soil 8.57 mg.kg-1 or 8.4 % than control and with inorganic fertilizer..

Key words: Lead, phytoremediation, *Rhizobium* sp. I3, Ramie

1. INTRODUCTION

Environmental pollution by heavy metals, especially lead (Pb), is one of the most serious environmental problems [1]. Pb is very toxic to living organisms including animals, plants and microorganisms. The existence of excessive Pb in the soil will cause soil degradation and threaten the ecosystem of life on the land [2]. Accumulation of heavy metals in soil can reduce microbial activity, soil fertility, and heavy metal compounds in agricultural products that result in human health problems [3]. Phytoremediation is an environmentally friendly technology that utilizes plants in decontaminating heavy metals from the environment. Phytoremediation is a green technology used to remove pollutants from the environment [4][5]. Plant Growth Promoting Rhizobacteria (PGPR) will colonize planting roots

and other beneficial plants to provide growth promoting substances [6]. The use of Plant Growth Promoting Rhizobacteria (PGPR) can influence the plant growth through their ability to produce fitohormon, siderophore, Indole Acetic Acid (IAA) hormone, nutrients, ACC-deaminase [7]. One of plants that can be utilized for phytoremediation with high economics is Ramie. Ramie (*Boehmeria nivea* L.) was used as phytoremediation agent because it has hyper accumulator qualities 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 [8]. Fertilization, pesticides and other agricultural activities are important activities to increase agricultural production [9]. The study by Rosariastuti [10] based on bioassay on Ramie isolate I3 and combination of treatment of isolate

I3 + compost in grumusol and regosol, effectively increased dry weight and 2-fold uptake of chromium roots and shoots (phytostabilization and phytoextraction). Based on the existing research, it is expected that Ramie can reduce the content of Pb in paddy soil so that inoculated by *Rhizobium* sp. I3 bacteria or manure on Ramie in accumulating heavy metal Pb needs to be known and studied further. The purpose of this study was to determine the effect of inorganic fertilizers and bioremediation agents (*Rhizobium* sp. I3 and 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 content.

2. MATERIALS AND METHODS

2.1. Study Area

This research was conducted from May to November 2017 in Waru Village, Kebakkramat District, Karanganyar Regency, Central Java, Indonesia with the coordinates of Latitude 7°30'36,036 S'' and Longitude 110°54'23,548'' E. 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.

2.2. Materials and Equipment

The material used included *Rhizobium* sp I3 isolated by Rosariastuti [10], 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 broth which has composition: 10g peptone composition from Himedia Laboratories, 5g yeast extract from Unipath ISD, 5g NaCl and 15g agar [11]. Chemicals needed for wet destruction were distilled 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.

2.3. Research Design

This research uses factorial research design with the basic design of Completely Randomized Block Design (CRBD). There are 3 factors used, namely inorganic fertilizer (P0: no inorganic fertilizer, P1: inorganic fertilizer), bioremediation agents (B0: without bioremediation agents ; B1: *Rhizobium* sp.

I3; B2: manure), and Ramie plant (T0; without Ramie; T1; Ramie). These three factors combined to produce 12 combinations of treatment that then repeated 3 times.

2.4. Procedure

2.4.1. Preparation of *Rhizobium* sp.I3

1 slant medium contains the isolate of *Rhizobium* sp. I3 was inoculated to 100 ml Luria Bertani. Starter was shaken for 3 days and then poured into the medium Luria Bertani broth with more volume of 900 ml as a scale up. The media is shaken until bacterial cell density reaches 10^8 cells/ml.

2.4.2. Preparation of Ramie and Fertilizer

Ramie is from Wonosobo, Central Java. Ramie seedlings are propagated by the stem cutting method. Inorganic fertilizers and manure used in purchasing from agricultural production facilities providers. Provision of fertilizer for Ramie is 10.000 kg / ha of manure. In addition to organic fertilizers, inorganic fertilizers are given at the beginning of planting with a dose of 60 kg N + 20 kg P_2O_5 + 30 kg K_2O / ha.

2.4.3. Land preparation and planting

Land preparation consists of clearing land from weeds and making of plot treatment. Plot of treatment was made as much as 36 pieces. Each plot consists of 25 sub plots of treatment. Each treatment combination was applied on a paddy field plot having the size of 2.5 m x 2.5 m. Distance of plant size is 50 cm x 50 cm. One plot contained 25 sub-plots. Each sub-plot 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

2.4.4. 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) [15].

2.4.5. Data Analysis

Data were analyzed using ANOVA F 5% test continued by *Duncan Multiple Range Test* (DMRT) and then for knowing relationships among variables were analyzed using correlation analysis.

3. RESULTS AND DISCUSSIONS

3.1 Effect of Treatment to Soil Variables

Table 1. Soil characteristics

Treatment	pH	OM (%)	CEC (cmol.kg ⁻¹)	<i>Rhizobium</i> sp. I3 population Log10 (CFU.g ⁻¹)	Content Pb (mg.kg ⁻¹)
					9.36de
P0B0T0	6.79c	4.73	37.01	13.2cde	
P0B0T1	6.69c	4.69	45.01	13.13defg	9.26de
P0B1T0	6.45a	4.81	32.54	13.15def	9.75bc
P0B1T1	6.85c	4.44	36.98	13.38ab	9.45de
P0B2T0	6.73bc	4.93	36.85	13.13defg	10.31a
P0B2T1	6.82c	4.82	49.82	13.29abcd	10.56a
P1B0T0	6.6ab	4.75	45.23	12.99fg	9.54cd
P1B0T1	6.76c	4.64	55.28	13.34abc	9.95b
P1B1T0	6.75bc	5.02	47.69	13.28bcde	9.16e
P1B1T1	6.79c	4.69	39.86	13.44a	8.57f
P1B2T0	6.84c	5.21	42.11	13.07efg	9.37de
P1B2T1	6.82c	4.81	45.52	12.96g	9.44de

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

3.1.1 Soil pH

According to the ANOVA results, manure and interaction of inorganic fertilizer, bioremediation agents and Ramie increased soil pH. The highest dose of N fertilizer gave the lowest pH [12]. Table 1 show that the highest increase of pH was in the treatment of without inorganic fertilizer+*Rhizobium* sp I3+Ramie (P0B1T1) of 6.85. A pH value that is close to neutral can support biological activity in the soil and the process of degradation and binding of heavy metals [13]. Soil pH is influenced by both acid and base-forming ions in the soil. Common acid-forming cations (positively charged dissolved ions) are hydrogen (H⁺), aluminum (Al³⁺), and iron (Fe²⁺ or Fe³⁺), whereas common base-forming cations include calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺) and sodium (Na⁺) [14]. Based on the correlation test, soil pH was positively and closely correlated ($p > 0.05$) to *Rhizobium* sp. I3 population ($r = 0.259$). Acidic soil reaction (pH) affects the development of soil microorganisms that live in it. Fungi can live and tolerate soil pH ranges from pH 4 - 6.5 while for bacteria prefer soil conditions with a pH ranging from 6-7 [15].

3.1.2. Soil Organic Matter

According to the ANOVA results, *Rhizobium* sp. I3 and manure increased soil organic matter. Table 1 show that, the highest increase was in P1B2T0 treatment of 5.21%. In order to increase the SOM content and improve soil fertility, organic

fertilization is the most efficient approach [16]. Based on the correlation test, soil organic matter was positively correlated with soil CEC ($r = 0.294$) and negatively correlated with *Rhizobium* sp. I3 population ($r = 0.228$) but the correlation was not close. Organic fertilizers play crucial role for SOM accumulation, aggregate formation and influence microbial community composition and co-occurrence networks in microhabitats [16].

3.1.3. Soil CEC

According to the ANOVA results, manure increased soil CEC. The increase in CEC was caused by the addition of manure which will increase the organic matter content of the soil. The addition of manure into the soil will increase the CEC so that it can increase the absorption of heavy metals in the soil. Based the result of Fitamo et al. of the same soils revealed that considerable proportions of the total levels of heavy metals were extracted from the non-residual fractions, and CEC and pH were key factors in the distribution of heavy metals among these fractions [17]. The increase of CEC will increase the exchangeable metal. Based on soil correlation test, CEC is positively and closely correlated ($p < 0.05$) with soil organic matter ($r = 0.294$) and soil pH ($r = 0.177$). Increase CEC soil along with the increase of soil organic matter. The soil CEC is also negatively correlated ($p > 0.05$) with the population of *Rhizobium* sp I3 ($r = 0.625$). The correlation

between soil CEC and population of *Rhizobium* sp. I3 was very close. Bacteria produce metabolite products such as enzymes, organic acids, EPS etc, which can increase CEC and metal exchangeability.

3.1.4. *Rhizobium* sp. I3 Population

According to the ANOVA results, *Rhizobium* sp. I3, Ramie and their interaction increased *Rhizobium* sp. I3 population. The highest *Rhizobium* sp. I3 population was in treatment P1B1T1, which was 13.44 Log₁₀ (CFU.g⁻¹) (Table 1). The *Rhizobium* sp. I3 significantly increased the total population of *Rhizobium* sp. I3, indicating that *Rhizobium* sp. I3 was able to adapt to polluted soils of Pb heavy metals, to survive in metal contamination, bacteria develop several types of mechanisms to tolerate heavy metal ion removal. The metal ion transfer mechanism out of the cell accumulates and forms metal ion compounds into the cell, thereby converting toxic metals into non-toxic ones. Based on the correlation test, the population of *Rhizobium* sp. I3 was positively correlated with soil pH ($r = 0.259$). The higher the population of *Rhizobium* sp. I3, pH would increase. Total population of *Rhizobium* sp. I3 is negatively and not closely correlated with soil organic matter ($r = 0.228$), CEC ($r = 0.625$), and Pb content in soil ($r = 0.137$).

3.1.5. Soil Pb content

According to the ANOVA results, All treatments and their interaction decreased Pb level in soil. Soil Pb decreasing each treatment. P1B1T1 was able to lower Pb with the highest decrease of 8.57 mg.kg⁻¹ or 8.47 % than control (Table 1). Ramie combined with other treatments has a higher effectiveness than without plants. A decrease of Pb metal in the soil can be caused by Pb metal up taking 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 [18]. *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 [10] that *Rhizobium* can increase the absorption of heavy metals and translocations from roots to the shoot.

3.2. Effect of Treatment to Plant Variables

3.2.1. Biomass of Ramie

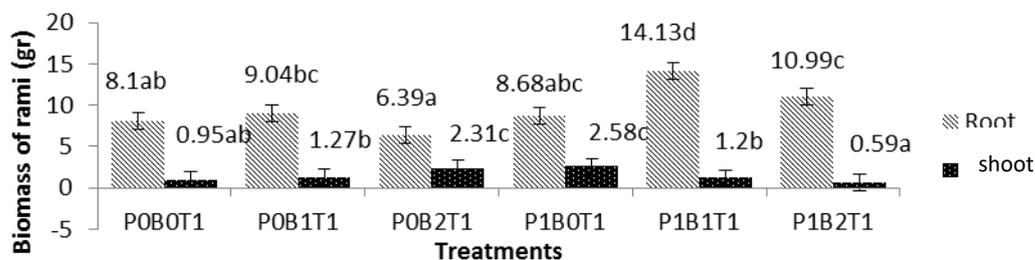


Figure 1. Biomass of Ramie

According to the ANOVA results, inorganic fertilizers and *Rhizobium* sp. I3 significantly influenced Ramie biomass. The inorganic fertilizers are able to provide the nutrients to the plant so as to increase the growth and productivity of the plant. Inorganic fertilizers, *Rhizobium* sp. I3 and manure are able to increase the root biomass of Ramie (Fig. 1). Root biomass is higher than that shoot biomass due to the morphological trait of the Ramie roots in the form of woody stems so as to increase the Ramie biomass. *Rhizobium* sp I3 and manure were able to increase the dry biomass of

Ramie. Rhizobacteria can produce IAA, Siderophores and ACC deaminase that can stimulate the growth of plants and protect plants from heavy metals toxicity [19]. *Pteris vittata* sporophytes inoculated with PG-12 were grown in Pb-contaminated medium and exhibited improved growth, increased P uptake, and reduced Pb uptake into plant tissue compared to the control. Results demonstrated that viable PG-12 cells were responsible for Pb immobilization and plant growth enhancement in *Pteris vittata* [20].

3.2.2 Pb Content and Uptake of Ramie

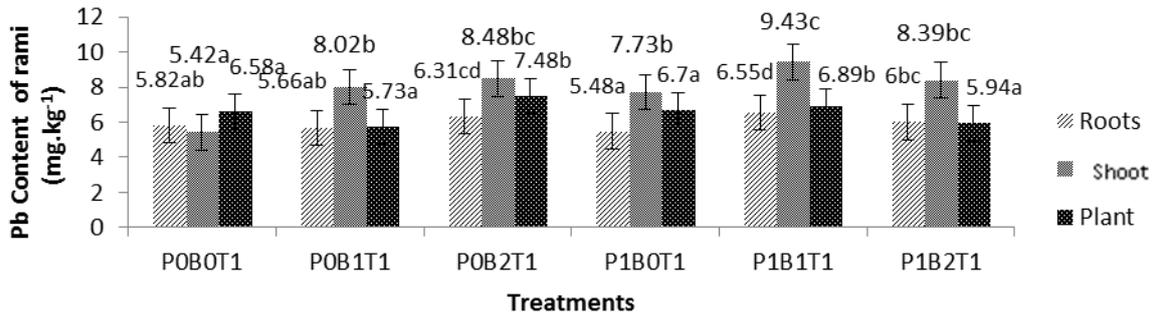


Figure. 2 Pb content of Ramie

According to the ANOVA results, applications of inorganic fertilizers, *Rhizobium* sp. I3 and manure, had significant influence on the level of Pb roots and Ramie shoot (Figure 2). Treatment using inorganic fertilizer+*Rhizobium* sp. I3+Ramie (P1B1T1) gave the highest increase Pb content reached to 9.43 mg.kg⁻¹ or 73.98% than control. Applications of inorganic fertilizers, *Rhizobium* sp I3 and manure, increased the level of Pb roots and Ramie shoot (Figure 2). Treatment using *Rhizobium* sp. I3 and inorganic fertilizer gave the highest increase Pb content reached to 9.43 mg.kg⁻¹ or 73.98 % than control. The resulting increase in trace metal uptake by the plants caused by microbial siderophores might enhance the

effectiveness of phytoextraction processes of contaminated soil. The following section highlights the basic biology and chemistry of siderophores produced by bacteria [21]. Application of rhizosphere bacteria can increase the availability of heavy metals of Pb in the rhizosphere and increase plant growth [22]. Higher levels of Pb in shoot compared with plant roots showed that after planting for two months, Ramie was able to translocate Pb from the root to the shoot. Based on the correlation test, Pb level on Ramie positively correlated ($p < 0.05$) to Pb uptake by Ramie ($r = 0.482$) and Ramie height ($r=0.543$). Correlation between Pb content and Ramie height was close.

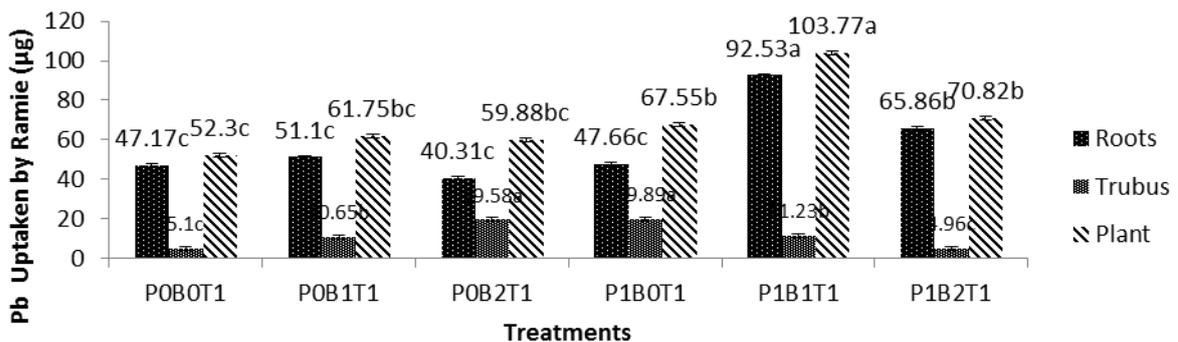


Figure 3. Pb uptake by Ramie

According to the ANOVA results, inorganic fertilizer and *Rhizobium* sp. I3 had significant influence on the uptake of Pb roots and Ramie shoot (Figure 3). The increase in plant biomass and metal concentration means an increase in Pb uptake by Ramie. Inorganic fertilizers and *Rhizobium* sp. I3 increased Pb uptake in the roots and shoots of Ramie. Increased plant biomass and metal concentrations mean increased Pb uptake by

Ramie. *Rhizobium* sp. I3, manure and inorganic fertilizers can increase the uptake of Pb in Ramie. All treatments can increase Pb uptake of Ramie. The highest increase was in the P1B1T1 treatment of 103.77 mg.kg⁻¹ or 2 more times than control. *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 [23]. Based on the correlation test, Pb absorption by Ramie was positively correlated with Pb levels in Ramie ($r = 0.482$), Pb uptake by roots ($r = 0.942$), root dry weight ($r = 0.913$), total dry weight ($r = 0.967$) and plant height ($r = 0.543$), the correlation were very close. It means that the increase of plant biomass would increase Pb uptake.

4. CONCLUSION

Application of inorganic fertilizer, *Rhizobium* sp I3 or manure and Ramie increases soil pH, and *Rhizobium* sp. population I3. The application of inorganic fertilizer, *Rhizobium* sp I3 or manure and Ramie also reduced the Pb content of the soil. The interaction between inorganic fertilizers+ *Rhizobium* sp I3+Ramie decreased soil pH, soil Pb content. The use of a combination of inorganic fertilizers+ *Rhizobium* sp I3+Ramie was able to reduce Pb content in the soil with the highest decrease of 8.57 mg.kg-1 or 8.47% lower than the control. The highest Pb uptake in the combination of inorganic fertilizer+*Rhizobium* sp. I3+Ramie were 103.77 mg.kg-1 or 2 times higher than the control. The best treatments was P1B1T1. By this research, Pb contaminated soil can be handled and safe for planting crops.

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