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Soil Bioremediation of lead (Pb) polluted paddy field using Mendong (*Fimbristylis globulosa*), *Rhizobium* SpI₃, compost, and inorganic fertilizer

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Abstract. Industrial developments cause an increase in lead (Pb) pollution in the paddy field because irrigation water is mixed with industrial liquid waste. This problem needs to be handled. Without proper treatment, lead can enter the human body either directly or indirectly through the food chain cycle, thus endangering human health. One method that can be used is bioremediation; a technology to restore the environment (paddy field) polluted by heavy metals using biological agents. The purpose of this research is to analyze the ability of *Mendong plants* (*Fimbristylis globulosa*) combined with inorganic fertilizers and chelators (*Rhizobium* spI₃ and compost) in absorbing lead in the paddy field. The research was conducted in Jaten Sub-district, Karanganyar Regency in April-October 2016. Randomized block design experimental method completed with the factorial pattern was applied as the research method. There were three treatment factors, namely inorganic fertilizer, chelator, and plant with three replications. The results show that some soil characteristics such as soil pH, cation exchange capacity (CEC), Organic Material (OM), and total microbes impact the availability of lead in the soil to be absorbed by plants. The highest uptake of lead occurred in the treatment of inorganic fertilizer + bacteria + Mendong plants (P1B1T1) amounting to 52.01 mg/kg at the root and 38.6 mg/kg in the shoot section. The Mendong plants combined with *Rhizobium* spI₃ has the potential to be an alternative bioremediation technology on lead-polluted paddy field soils.

1. Introduction

Industrial development causes environmental pollution on paddy field due to the disposal of industrial waste into irrigation channels. The number of the textile industry in Jaten Sub-district of Karanganyar Regency is 85 units. Lead (Pb) is one of the heavy metals found in the textile industrial waste. This results in the accumulation of Pb in the paddy field's soil. Paddy fields are crop-producing land, so it is very dangerous if the food crops produced are contaminated by lead.

Lead (Pb) is an element that is not essential for plants. At high doses, Pb may cause metabolic disorders, inhibit growth, and lead to death [1]. The threshold limit value of Pb metal in plants ranges from 0.2 – 20 mg.kg⁻¹. Excessive accumulation of Pb in the soil causes a decrease in functional microbial populations [2]. Government Regulation No. 101 of 2014 on the Management of Hazardous and Toxic Wastes (HTW) states that the safety limit of Pb accumulation in the soil is 3 mg.kg⁻¹. Lead



ingested into the human body will accumulate in the liver, kidneys, and bones. Symptoms of Pb poisoning include abdominal pain, nausea, neurological disorders, anemia, and inhibition of enzyme activity [3].

Paddy fields should be refined from the contamination of Pb in order to produce food products which are safe and have high quality. One of the methods that can be applied is bioremediation technology. Bioremediation is a rehabilitation process on the polluted environment by utilizing the activity of organisms (microbes and plants) to decompose pollutants into simpler, less harmful substance, and to add value to the environment [4]. The microbes used for bioremediation are functional microbes that can chelate and change the form of Pb^{4+} ions into Pb^{2+} ions in order to be absorbed by accumulator plants. The plants that are optimal to be used in bioremediation are non-food crops which can live on marginal land conditions, accumulate large quantities of heavy metals without impairing the growth, and to add value to the environment [5].

One of the non-food crops that can be used for bioremediation is Mendong plants. The Mendong plants were able to decrease Pb content in paddy fields in Rancaekek by 10.91%. The accumulation of Pb in the Mendong plant's root was 21.69 ppm, while in the shoot was 5.44 ppm [3]. Based on some of the requirements, the Mendong plants are eligible to be used as a phytoremediator. The Mendong plants are suitable to be planted in a wetland. The Mendong plants can also be used as the main material of fabric fiber manufacture. There has not been any publication of the remediation of paddy field polluted by heavy metals using the Mendong plants combined with *Rhizobium* spI₃. Thus, this study aims to analyze the ability of the Mendong plants combined with *Rhizobium* spI₃ in absorbing Pb from the contaminated soil.

2. Material and Method

2.1. Research design

This research was conducted in Ngringo Village of Jaten Sub-district, in Karanganyar Regency, Central Java (7°33'11.4" LS and 110°52'47.2" BT). This is a field experimental research with Randomized Complete Block Design (RCBD) with factorial experiment. There were three treatment factors, namely basic fertilizer (P), chelator (B) and plant (T) with three replications in each treatment. The study design is presented in Table 1.

2.2. Preparation of *rhizobium spi₃* inoculum and carrier

The preparation involved inoculum propagation in liquid medium *Luria Bertani* (LB) and carrier manufacturer. LB compositions are Yeast Extract 5 g, Protease peptone 10 g, NaCl 5 g for each Liter of aquadest [6]. The isolate source was derived from the pure *Rhizobium* spI₃ isolate [7] which has been cultured on slant medium. The carrier used is compost enriched with EM₄ (Effective Microbial) bio-activator.

2.3. Chemical-biological analysis of soil and plant

The chemical-biological analysis includes pH, Cation Exchange Capacity (CEC), Organic Material (OM), and wet destruction. Wet destruction method was used to calculate the metal content in soil and plants. Destruction was conducted using HNO_3^- and $HClO_4^-$ solutions, which then destroyed with a temperature of 200°C. The extract was cooled, then diluted using aquadest to 25 mL and homogenized. The destruction results are ready for analysis using Atomic Absorption Spectrophotometer (AAS).

2.4. Measurement of plant biomass

Crop biomass analysis includes plant height and dry stover. Plant height is measured once every week for 6 weeks. Measurements are made using a ruler, starting from the parts above the soil surface to the top part of the plant.

Table 1. Research design.

No.	Treatments	Explanation
1.	P0B0T0	Without inorganic fertilizers, without chelators, without Mendong plants (control)
2.	P0B0T1	Without inorganic fertilizers, without chelators, with Mendong plants
3.	P0B1T0	Without inorganic fertilizers, with <i>Rhizobium</i> spI ₃ , without Mendong plants
4.	P0B1T1	Without inorganic fertilizers, with <i>Rhizobium</i> spI ₃ , with Mendong plants
5.	P0B2T0	Without inorganic fertilizers, with compost, without Mendong plants
6.	P0B2T1	Without inorganic fertilizers, with compost, with Mendong plants
7.	P1B0T0	With inorganic fertilizers, without chelators, without Mendong plants (control)
8.	P1B0T1	With inorganic fertilizers, without chelators, with Mendong plants
9.	P1B1T0	With inorganic fertilizers, with <i>Rhizobium</i> spI ₃ , without Mendong plants
10.	P1B1T1	With inorganic fertilizers, with <i>Rhizobium</i> spI ₃ , with Mendong plants
11.	P1B2T0	With inorganic fertilizers, with compost, without Mendong plants
12.	P1B2T1	With inorganic fertilizers, with compost, with Mendong plants

2.5. Data analysis

The data were analyzed statistically using ANOVA (Analysis of Variant) analysis with the significance level of 5%. If there was a real difference, the analysis was then followed by DMRT (Duncan Multiple Range Test) with 5% level using SPSS (Statistical Product and Service Solution) application version 16.

3. Result and Discussion

3.1. Characteristics of paddy fields in research sites

A key factor in creating a healthy and environmentally friendly farming system is the availability of fertile and productive soils. Ideal soil should have the capability to support biodiversity, plant growth, and soils chemical and biological characteristics in terms of pH, cation exchange capacity (CEC), organic materials (OM), soil microbes, and should also be free from various pollutants [7].

The paddy fields in the research location are vertisol type soil with a pH value of 7.25 (neutral) because the paddy fields have buffer capacity [1]. Soil acidity impacts the availability of metals. Low soil acidity helps increase the availability of metals for plants. Soil acidity also provides a good place for microbial growth, especially bacteria. Bacteria can generally live well in the neutral pH range. Organic fertilizers potentially neutralize soil pH, thus ensuring optimal soil pH for plant growth. CEC value of 22.4 cmol⁽⁺⁾.kg⁻¹ is a moderate category. The value of CEC in the tropical soil depends on the pH level of the soil since the soil consists of a permanent charge and the charge depends on the pH-dependent charge [8]. Vertisols have clay texture of smectite clay type (2:1) and neutral pH. Smectite mineral has a negative charge which causes this mineral to be highly reactive in the environment and tend to have high CEC [9].

Low humus content is indicated by the low organic materials status (1.92%). The levels of C and N organic soil vertisol are low. This is followed by the total value of microbial colonies present in the soil of 7.55 log 10 CFU.g⁻¹. An important process that is very influential on the chemical and biological properties of paddy field soil is the oxidation-reduction process resulting from the flooding

in the soil. Reduced conditions due to inundation will alter the soil microbial activity in which aerobic microbes will be replaced by anaerobic microbes [9]. The Pb content in paddy field soils was 8.91 mg.kg^{-1} , thus the soil had been polluted by Pb because the threshold value of Pb in the soil according to Government Regulation No. 101 of 2014 is $3 \text{ } \mu\text{g.g}^{-1}$.

3.2. Concentrations of pb in mendong plants parts

The value of Pb uptake is highest at the roots of the Mendong plants in P1B1T1 treatment namely $52.01 \text{ } \mu\text{g.g}^{-1}$ as shown in Figure 1. This is consistent with the value of dried stover on P1B1T1 treatment which has the highest value of 7.33 g which is presented in Figure 2. Meanwhile, the value of Pb uptake is lowest at the Mendong plants root in P0B2T1 treatment, i.e. $24 \text{ } \mu\text{g.g}^{-1}$ sample. This is because the amount of Pb content in the root of the Mendong plants facilitates P0B2T1 treatment to have the lowest Pb content value compared to the other treatment which is $5.41 \text{ } \mu\text{g.g}^{-1}$. When compared with control treatment (P0B0T1), treatment of P1B1T1 has 1.9 times higher absorption value. According to the ANOVA test, it is found that the treatment had no real effect on Pb content in Mendong plants roots.

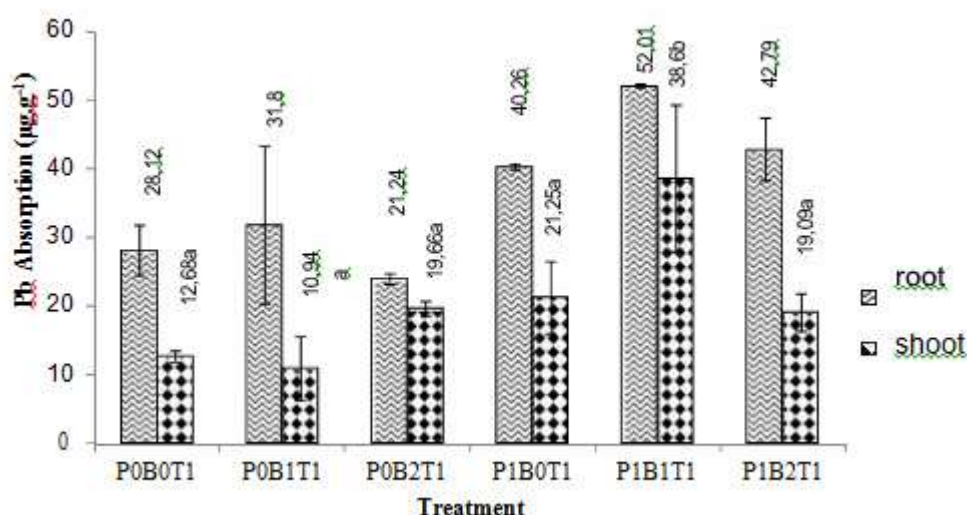


Figure 1. Pb absorption histogram at root and shoot of mendong plants.

The highest uptake of Pb on Mendong shoot was found in the P1B1T1 treatment of $38.6 \text{ } \mu\text{g.g}^{-1}$ as presented in Fig. 1. This is also directly proportional to the highest value of dried stover of the shoot with P1B1T1 treatment, which is 16.57 gr as presented in Figure 2. Meanwhile, the lowest value of Pb uptake in Mendong plants shoots is in P0B1T1 treatment, which is $10.94 \text{ } \mu\text{g.g}^{-1}$. This is because the amount of Pb metal content in the Mendong crown treated P0B1T1 has the lowest Pb metal content value compared to the other treatment, that is $10.94 \text{ } \mu\text{g.g}^{-1}$. When compared with control treatment (P0B0T1), treatment of P1B1T1 has 1.4 times higher absorption value. According to 95% ANOVA test followed by DMRT test, real different results were found in the treatment of P0B0T1 to P1B1T1 treatment.

The treatment of P1B1T1 has the highest absorption value compared to other treatments both at Mendong's root and shoot. Thus it can be inferred that the Pb found in soil solution (ion Pb^{2+}) is not lost due to leaching, absorption by soil particles, or organic matter, instead it was absorbed by Mendong plants. The normal limit of Pb in plants is $0.2\text{--}20 \text{ } \mu\text{g.g}^{-1}$.

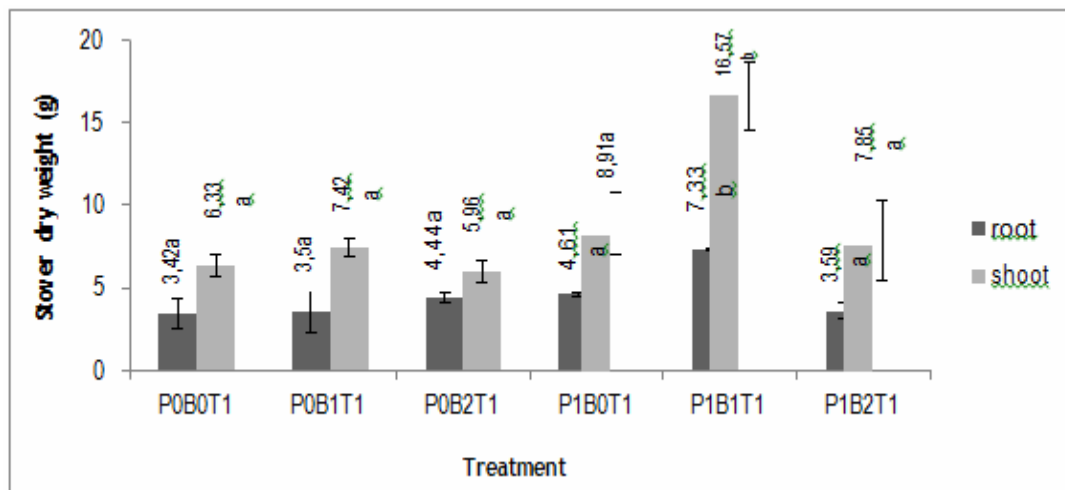


Figure 2. Dry weight histogram of mendong root and shoot stover.

Mendong plants are able to absorb Pb beyond the prescribed normal limits and are able to transmit Pb from root to plant's shoot without deviation or physiological growth restriction shown by dry weight of the plant's stover and its height as presented in Figures 2 and 3. Pb uptake in the root of Mendong is higher than Pb metal uptake in the Mendong shoot. The plant parts that are capable of accumulating metals are in the following order; the largest is the root > stem > leaf > fruit [10]. It can be said that Mendong has the ability as a Pb rhizoremediation plant on paddy field soil. Rhizoremediation plants are plants that can absorb heavy metals exceeding the threshold without experiencing defects and physiological growth restrictions, as well as large biomass. Rhizoremediation plants should be able to transmit heavy metals from roots to other plant tissues [11].

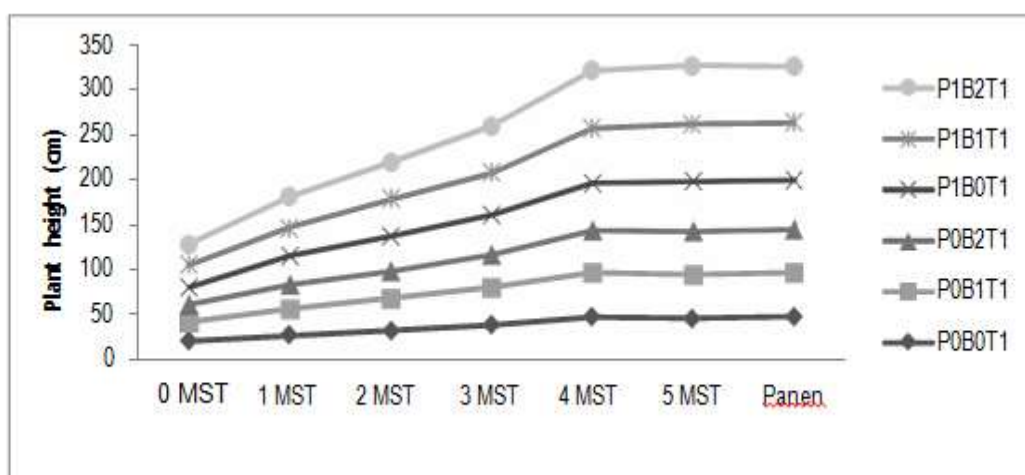


Figure 3. Height histogram of mendong plants.

Inorganic fertilizers contribute to supporting the growth of Mendong plants so that its height is consistently increasing on a weekly basis with very fresh crop conditions. This has an impact on the dry weight stover value of Mendong's root and shoot in P1B1T1 treatment. The treatment of P1B1T1 has the highest dry weight stover value, both at root and shoot. The dry weight of stover indicates that the content of substances or other elements other than water is absorbed by the plants.

Rhizobium spI₃ is a biological agent which has a significant role that determines the success of the bioremediation. *Rhizobium* spI₃ is capable of producing secondary metabolite substances that can transform heavy metal forms. In soil, Pb⁴⁺ ion form is a form that is not available for plant absorption, whereas, Pb²⁺ ion is an ionic form of Pb that can be absorbed by plants. A special mechanism to change the form of the Pb is necessary. *Rhizobium* spI₃ gets the nutrients from root exudate in the form of organic acids released by Mendong root thus symbiotic mutualism occurs. The *Rhizobium* spI₃ isolates are able to increase the absorption of chromium metal (Cr) to the plant shoot. Chromium is absorbed and accumulated both in the roots and shoots of the plants. In addition, the *Rhizobium* spI₃ isolates are also able to increase plant growth [12].

Bacterial viability is the bacteria's ability to keep growing. The total of bacterial colonies of PIBITI treatment is 10.56 log 10 CFU.g⁻¹. This suggests that bacteria can adapt and survive in the conditions of wetland soil contaminated with heavy metals. In addition, the bacteria can perform its function to transform the Pb form, from unavailable for absorption forms into available forms. The organic material values in the PIBITI treatment are also included in the moderate category because the carrier of *Rhizobium* spI₃ is compost. So aside from being a source of nutrition for microbes and a source of nutrients for sustainable crops, compost also impacts the CEC value because the organic material has a negative charge that can increase the CEC value of the soil. CEC and OM have an impact on the mobility of Pb²⁺ ions. Heavy metals in the soil are affected by CEC and OM [13].

4. Conclusion

Rhizobium spI₃ and compost as chelators can increase Pb uptake by the Mendong plants. The combination of the Mendong plants, *Rhizobium* spI₃, and inorganic fertilizers results in highest uptake of soil Pb with root absorption value of 52.01 μg.g⁻¹ and 38.6 μg.g⁻¹ uptakes in the shoot. Mendong plants potential is used as a phytoremediation plant with a major Pb uptake at its root, and *Rhizobium* spI₃ is potential as microbial bioremediation because it has a higher impact in increasing Pb uptake by Mendong plants than compost.

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