Effects of *Agrobacterium* sp. I26, Manure and Inorganic Fertilizers to Pb Content of Rice Grains Planted in Pb Polluted Soil

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ABSTRACT

Chemical waste from textile industries discharged directly into rivers will affect paddy soil irrigation surrounding these factories. Thus, heavy metal pollution may occur in this paddy soil. Bioremediation can remediate polluted heavy metals by removing the pollutant. *Agrobacterium* sp. I26 and manure were studied as a bioremediation agent because both use biological processes in remediation. The effectivity of bioremediation agent (*Agrobacterium* sp. I26 or manure) and inorganic fertilizer in inhibiting the absorption of Lead (Pb) in rice, as well as the production of rice, was studied. This study used a factorial Randomized Completely Block Design (RCBD), which consisted of two factors: a) inorganic fertilizers (P): without inorganic fertilizers (P0) and with inorganic fertilizers (P1); b) bioremediation agents (K): without bioremediation agents (K0), with *Agrobacterium* sp. I26 (K1), with manures (K2). From these two factors, six treatment combinations with four repetitions, resulting in 24 experiment units were obtained. Results of this study showed that *Agrobacterium* sp. I26 and manures are able to inhibit Pb absorption in rice grains. The best treatment of this study was the combination of inorganic fertilizers with *Agrobacterium* sp. I26, which showed the highest weight of 1000 seeds (31.95 g), 14.96% higher compared to control, and was able to inhibit Pb absorption by rice grain up to a threshold (0.29 µg/g), 39.58% lower compared with control.

1. INTRODUCTION

This study was held in Waru, Kebakkramat Sub district, Karanganyar Regency, Central Java Province, Indonesia which has a lot of textile industries. The impact of the textile industry caused lead (Pb) pollution in paddy soil to be 16.18 mg/kg (Kharisma et al., 2018). Because of that, bioremediation research has been carried out in this area to absorb heavy metals in the soil using plants.

Industrial activities play an important role in developing countries, especially economically, socially and culturally (Syaifullah, 2009). Industries in Kebakkramat Sub-district are dominated by textile industries, using melamine as one of its raw materials. Melamine is used in dye coating processes so that textiles become water resistant, are not easily tangled and don't fade. Melamine is synthesized from urea at a temperature between 350-400 °C (Ullman, 2003), hence textile waste contains urea. Textile industries besides having a positive impact on a community also have a negative impact towards the environment from its chemical waste.

Wuana et al. (2010) stated that one of the heavy metals commonly found in industrial waste is lead (Pb). Another source of Pb, other than textile waste, can be found in inorganic fertilizers. Laboratory analysis of Pb in the inorganic fertilizers used in this research showed that urea fertilizer (source of Nitrogen) contained 0.32 µg/g of Pb, SP36 fertilizer (source of Phosphate) had the highest concentration of Pb with 1.63 µg/g and KCl fertilizer (source of Potassium) contained 0.52 µg/g of Pb. The manure used in this research contained 0.32 µg/g of Pb.

Based on Government Regulation of Indonesia number 101 (2014), soil Pb content is allowed at a level of 3 µg/g. Rice grains should not exceed the threshold

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of 0.3 µg Pb/g of rice (Indonesia's Badan Standardisasi Nasional, 2009). Darmono (2001) explained that rice with heavy metal pollution, if consumed and accumulated in the human body will cause chronic toxicity and damage internal organ functions.

Bioremediation is the development in the field of environmental biotechnology that utilizes biological processes in controlling pollution with agents called bioremediation agents. Darmono (2001) found organic compounds can prevent heavy metal ion absorption in plant tissue. Another effort to minimize Pb absorption is application of bacterium *Agrobacterium* sp. I26. A previous study by Rosariastuti et al. (2013) found, addition of this bacterium will minimize Cr absorption to shoots. Pramono et al. (2013) also said that *Agrobacterium* sp. I26 can produce the reductase enzyme that can reduce chromium from toxic (available for plant) to non-toxic (non-available for plant) forms. Therefore, it is possible that the reductase enzyme can reduce Pb^{4+} (available for plants) to Pb^{2+}/Pb II (not available for plants). *Agrobacterium* sp. I26 and manure are included as bioremediation agents because both are using biological processes in remediation.

However, research on the effect of *Agrobacterium* sp. I26 and manures to heavy metals in soil is needed. *Agrobacterium* sp. I26 and manures play roles as bioremediation agents to minimize Pb absorption to shoots or rice grains, hence rice produced will be safe for consumption. This study compared the effectivity of bioremediation agents (*Agrobacterium* sp. I26 or manure) and inorganic fertilizers in inhibiting the absorption of Pb in rice and the production of rice.

2. METHODOLOGY

2.1 Study area

This study was held from April to September 2018 in Waru, Kebakkramat Sub-district, Karanganyar Regency, Central Java Province, Indonesia (7º30'36'26"S and 110º54'23'72'E) with an area of 651 m^2.

Figure 1. The study site

2.2 Materials

The materials used in this study were manure that comes from cow feces (produced by Jatikuwung Inovation Center), *Agrobacterium* sp. I26 isolated by Rosariastuti et al. (2013), inorganic fertilizer: Urea (source of Nitrogen), SP36 (source of Phosphate) and KCl (source of Potassium) (Figure 2), Luria Bertani medium (Tryptone 10%, NaCl 5%, Yeast extract 5%, Agar 15%), sulfuric acid, nitric acid, acid percolate, NaCl, $K_2Cr_2O_7$, Alcohol, Atomic Absorption Spectrophotometer (AAS), Autoclave, vortex, shaker, etc.

This study used 3 types of inorganic fertilizers: Urea, SP36, and KCl because the Nitrogen, Phosphate, and Potassium are essential macro nutrients which are more needed by plants than other nutrients.

Figure 2. Fertilizers used in this study: a) Manure; b) Urea; c) SP-36; d) KCl.

2.3 Experimental design

This study used a factorial and Randomized Completely Block Design (RCBD) consisting of two factors: a) inorganic fertilizers (P): without inorganic fertilizers (P0) and with inorganic fertilizers (P1); b) bioremediation agents (K): without bioremediation agents (K0), with *Agrobacterium* sp. I26 (K1), with manures (K2). From these two factors, six treatment combinations with four repetitions, resulting in 24 experiment units were performed.

Table 1. Treatments combination

Treatments	Information
P0K0	Without any treatment (Control)
P0K1	Without inorganic fertilizers + *Agrobacterium* sp. I26
P0K2	Without inorganic fertilizers + Manures
P1K0	Inorganic fertilizers + without bioremediation agents
P1K1	Inorganic fertilizers + *Agrobacterium* sp. I26
P1K2	Inorganic fertilizers + Manures

2.4 Procedure

2.4.1 Agrobacterium sp. I26 inoculum preparation

Bioremediation agents used in this study were *Agrobacterium* sp. I26 and manure. Phenotypic characterization such as cell morphology/cells type, gram stain reaction, motility, aerobicity, and ability to grow at 40 °C was recorded. Propagation of *Agrobacterium* sp. I26 isolates was done using pure isolates of *Agrobacterium* sp. I26 grown in solid LB media on new slope media. After it was grown, it was transferred to 100 mL LB liquid media per one tube pure isolate of *Agrobacterium* sp. I26. Media was shaken until the density of its bacterial cells measured using a hemacytometer was 10^{10} CFU/mL. The dosage of bacteria was 10^{6} CFU/g soil, therefore the quantity of bacteria needed were 1.25×10^{10} CFU/mL per sub plot obtained from the calculation results of the soil weight (Bulk density × Volume of soil).

2.4.2 Land preparation

The soil was hoed manually on the rhizosphere, the lower rhizosphere was moved to the upper part so that the nutrients in lower part of the rhizosphere were moved to the top where they could be used by plants. Twenty four plots (same as experimental units) at 1.25 × 1.25 m per-plot were prepared. The plots were made higher than the surrounding land to keep the land in a dry condition. The plant distance of this study was 25 × 25 cm (sub plots), so there were 25 sub plots per-plot. Three paddy seeds were planted per plot.

One application of manure and bacterial inoculum was performed seven days before planting. The application of bacterial inoculum was done by pouring the inoculum into the planting holes (per-sub plot). Inorganic fertilizers used in this study were urea (N), SP36 (P) and KCl (K), with dosages of: Urea 300 kg/ha, SP36 100 kg/ha and KCl 100 kg/ha (Minister of Agriculture Regulation Republic of Indonesia, 2007). The application of fertilizer was done by spreading out and was done three times; five days after planting (3/5 of dosage), 25 days after planting (1/5 of dosage) and 45 days after planting (1/5 of dosage). Manure was applied at 10,000 kg/ha (Rauf et al., 2000) and the application was spread out.

2.4.3 Harvesting

Harvesting was done three months after planting by taking all parts of the plant. Plant samples were separated into their roots, shoots, and grains. After that, everything was air dried, then dried in an oven until constant weight. Next, each sample was ground prior to laboratory analysis.

Figure 3. Sample of plants.

2.5 Sample collection and analysis

Soil and plant samples were chosen randomly from the inner sub plots of each plot (Figure 4). Five soil and plant samples were taken from each plot. The samples were decomposed into one as a sample from the plot. It took two weeks to prepare the soil and plant samples, while laboratory analysis took 1 month.

The parameters measured and laboratory analysis methods, based on Indonesia's Balai Penelitian Tanah (2009), were pH (Electrometric method), Soil CEC (Ammonium Acetate Saturation Method), Soil Organic Matter that calculated from C-Organic (Walkey and Black Method), soil bacteria colonies (Plate Count Method), Soil Pb levels (Wet Destruction Method continued by reading the metal content using AAS), Removal Effectiveness (calculated from differences in initial heavy metal levels and final heavy metal content after treatment), Weight of 1000 Seeds (Direct Measurement Method using digital scales), and Pb content (roots, shoot, grain) in Plants (Wet Destruction Method continued by reading the metal content using AAS).

2.6 Data analysis

Data were analyzed by statistical analysis using ANOVA with a confidence level of 95%. If significant, then continued by the LSD or T-test with a level of 95%.

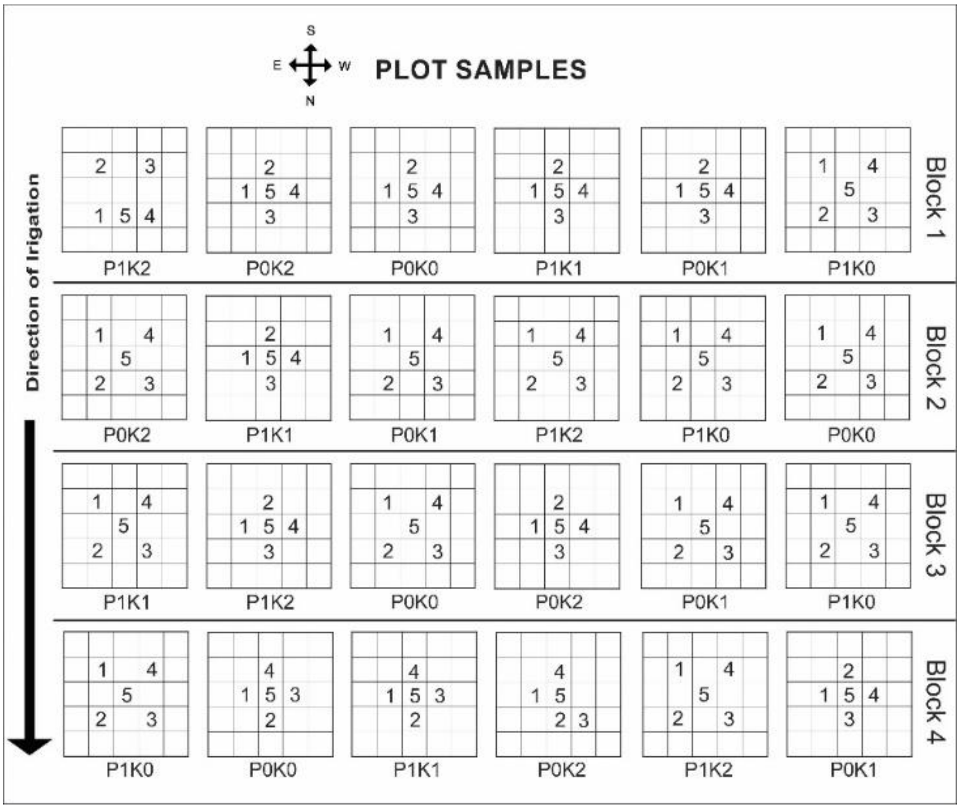

Figure 4. Samples of each sub plot

3. RESULTS AND DISCUSSION

3.1 Soil characteristics

3.1.1 Soil pH

Result of soil pH analysis can be seen in Figure 5 below.

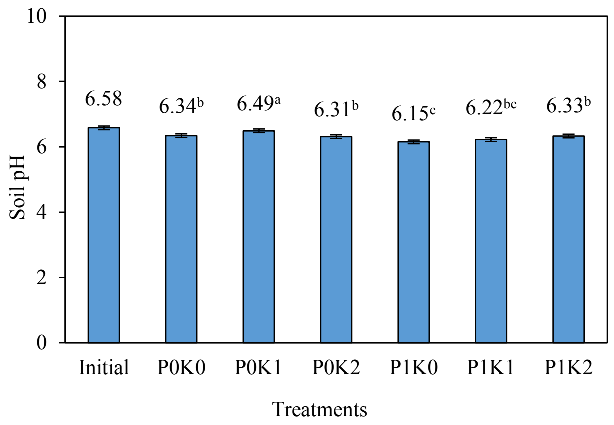

Figure 5. Soil pH

Figure 5 shows decreases of soil pH from its initial soil pH in all treatments. This is due to the nitrogen content in textile industry waste that flows in irrigation water. Foth (1995) stated that nitrogen in the form of ammonia or other can turn into nitrate because of the nitrification process that produces hydrogen ions. Based on the results of the laboratory analysis showed that Urea pH of 5.5; SP36 of 6 and KCl of 6. This causes the soil pH to drop from the initial soil.

ANOVA results showed that inorganic fertilizer treatment had a very significant effected on soil pH, whereas bioremediation agent treatments did not significantly affect the soil pH. The interaction between inorganic fertilizer and bioremediation agent treatments significantly affected the soil pH. Figure 5 shows the treatment P0K1 has the highest soil pH value and was significantly different with other treatments. The treatment has the lowest decrease of pH compared to initial soil pH by 1.37%. Meanwhile the control decreased by 3.64% from the initial soil pH. Soil pH in P0K1 treatment was higher than the control, this is in accordance with the study by Li and Zhang (2012) which found that microbial biology activity can increase Ca^{2+} cation availability, which can increase soil pH. Based on the soil bacterial colonies it can be seen the P1K1 treatment was the highest treatment followed by the P0K1 treatment, but in the P1K1 treatment there was an inorganic fertilizer that can reduce soil pH due to the substitution of H^+ in plant roots with cations in the soil, so the concentration of H^+ in the soil increases.

George et al. (2016) stated that biosorption process will be optimum around pH 5 for Pb ion. However, the soil pH in this study was greater than 6. Therefore, statistical analysis showed that soil pH had low correlation with soil Pb level.

3.1.2 Soil CEC (Cation Exchange Capacity)

Results of soil CEC analysis are shown in Figure 6 below.

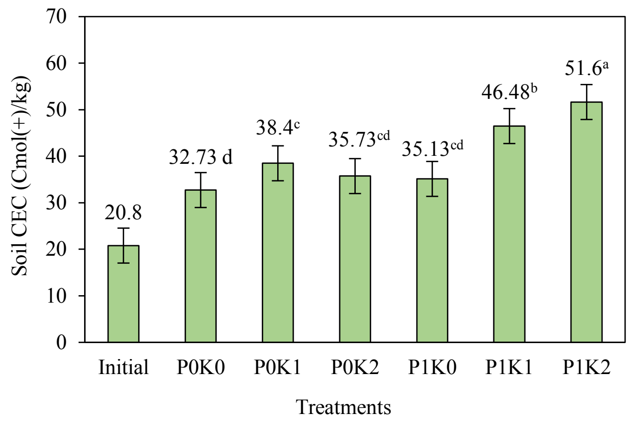

Figure 6. Soil CEC

Figure 6 shows soil CEC increase compared to its initial soil CEC in all treatments. This is caused by soil bacterial activities in soil organic matter decomposition thus it can increase soil CEC. Ferina et al. (2017) stated that soil bacterias can increase soil CEC with decomposition of soil organic matter. Hameed et al. (2015) stated soil microbes have an important role in immobilization of soil cations. The results of observations in this study showed that *Agrobacterium* sp. I26 has a dominant growth and its characteristics are stem, short, round, convex, flat, yellow colony and fine structure.

The ANOVA showed that inorganic fertilizer treatment, bioremediation agents and the interaction between these treatments had a significant affect on soil CEC. The P1K2 treatment had the highest soil CEC and was significantly different from all other treatments (51.65 Cmol(+)/kg), while the treatment with the lowest soil CEC was P0K0 (32, 73 Cmol(+)/kg) which was not significantly different with the P0K2 and P1K0. Walker et al. (2003) states that organic matter can increase CEC in soil. Treatment combinations of inorganic fertilizer and bioremediation agents made the increasing of soil CEC higher than treatment without inorganic fertilizer. Mujiyati and Supriyadi (2009) and Su et al. (2014) also stated that inorganic fertilizers can increase the activity of plant roots in producing root exudates as a source of energy for bacteria to decompose organic matter, thereby increasing soil CEC.

The decomposition result of soil organic matter is organic acids which have a negatively charged functional group, thus it helps increase soil CEC (Kargar et al., 2015). Soil CEC have an important role in the exchange of cations in colloidal soil. Soils that have a lot of clay content can minimize the availability of Pb. In this study, *Agrobacterium* sp. I26 and fertilizer also act as bioremediation agents to immobilize Pb metal in the soil. Bacteria produce secondary metabolites consisting of many organic compounds such as enzymes, organic acids, hormones, etc., which can also increase soil CEC.

3.1.3 Soil organic matter

Result of soil organic matter analysis could be seen in Figure 7 below.

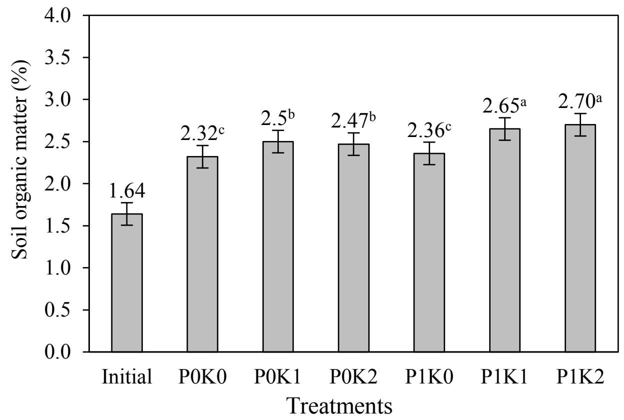

Figure 7. Soil organic matter

Figure 7 shows the content of organic matter increased from the initial soil in all treatments. This was due to soil bacterial activity in decomposing soil organic matter in the soil. The ANOVA showed that the treatment of inorganic fertilizers and bioremediation agents significantly affected the soil organic matter. The interaction between the treatment of inorganic fertilizers and bioremediation agents significantly affected the content of organic matter in the soil. Figure 7 shows that the treatment with the lowest soil organic matter was P0K0 (control) treatment at 2.32% which was not significantly different from P1K0 treatment.

The treatment with the highest organic matter content was P1K2 at 2.70% and not significantly different from P1K1 treatment. This suggests that bioremediation agents can increase the soil organic matter. The combination of inorganic fertilizer and bioremediation agents could increase the plant growth so plant would produce root exudate more than other treatments. Therefore the soil organic matter is also higher than other treatments. This is in accordance with the study of Johanto et al. (2019) and Rosariastuti et al. (2018) that revealed the combination of inorganic fertilizers with *Agrobacterium* bacteria can increase the highest value of organic matter compared to other treatment combinations.

Decomposition of organic matter will produce simple organic acids. According to Huang and Schnitzer (1997) and Ding et al. (2016), effective organic acids in soil mineral binding are the result of decomposition of organic matter with high molecular weight acid complexes, for example humic acid. Johanto et al. (2019) revealed that the process of decomposition of organic matter would release cation bonds in complex compounds of humic acid or fulvate and be replaced by Pb, so that Pb in the soil formed Pb II - Humic complexes.

3.1.4 Soil bacteria colonies

Result of soil bacteria colonies analysis could be seen in Figure 8 below.

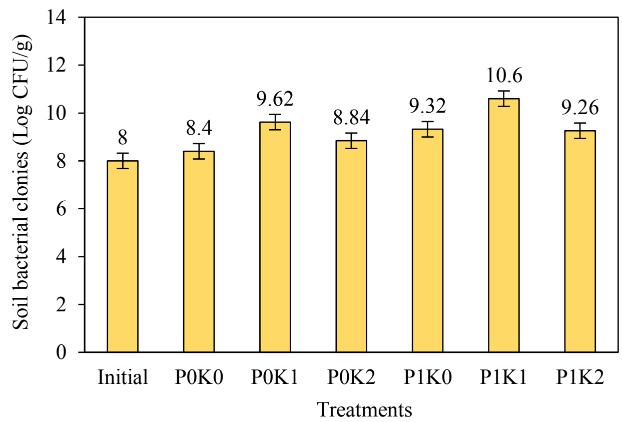

Figure 8. Soil bacteria colonies

Figure 8 shows that the average number of soil bacteria colonies increased compared with the initial soil bacteria colonies in all treatments including P0K0 (control). This was possible due to the bacterial activity from previous research. The bacteria colonies showed that *Agrobacterium* sp. I26 looked to be dominant with characterization such as cell rod morphology/cells type, negative gram stain reaction, and motility. The colonies morphology was spherical, convex, and the inner structure was smooth.

The ANOVA showed that the treatment of inorganic fertilizers, bioremediation agents and interactions between the two treatments had no significant affect on the soil bacteria colonies. Although the treatments had no significant affect, the treatment that used *Agrobacterium* sp. I26 had soil bacteria colonies higher than the other treatments.

According to Rosariastuti et al. (2013), the bacteria *Agrobacterium* sp. I26 is able to grow in aerobic conditions and temperatures around 40 ºC. Paddy soil conditions in this research tended to be wet for several days due to rain, so that bacterial growth was less optimal and each treatment was not significantly different. The highest soil bacterial colonies was in P1K1 treatment of 10.6 Log CFU/g, while the treatment with the lowest soil bacterial colonies was P0K0 (control) treatment of 8.4 Log CFU/g.

Rosariastuti et al. (2018) stated that the combination of inorganic fertilizers and *Agrobacterium* sp. I3 can increase the highest total bacterial colonies compared to other treatments. Narayani and Shetty (2013) said that secondary metabolites produced by bacteria in soil can efficiently eliminate Pb through bioaccumulation. Bacteria can bind Pb ions in the soil by extracellular polymers or extracellular poly-saccharides produced by bacterial cells and have a reactive side of negatively charged cells.

3.1.5 Soil Pb level

Results of soil Pb level analysis are shown in Figure 9 below.

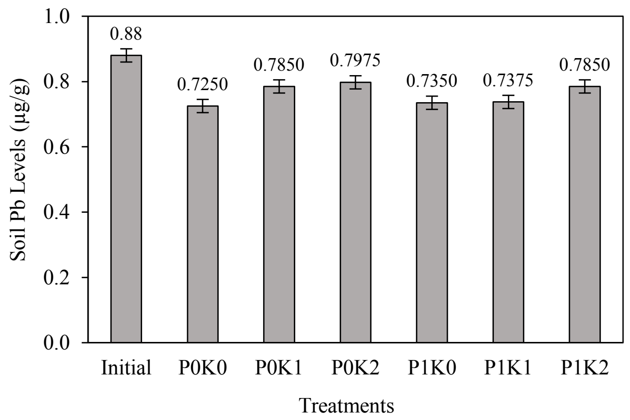

Figure 9. Soil Pb levels

Figure 9 shows that Pb soil levels decreased from the initial Pb soil level in all treatments. Factors that causes a decrease of Pb soil levels are Pb absorb by plant and Pb leaching caused by rainwater. The weather in the study area was rainy, so the rainfall intensity tends to be high. Johanto et al. (2019) stated decreasing Pb levels in soil was possible due to the presence of leaching factor. Based on Figure 9 it can be seen that the decrease in soil Pb level from the initial soil to the final soil was not high, this may be due to the influence of the soil type at the study site, namely vertisol (sand 6.294%, dust 25.756%, clay 67.950%). The texture that was dominant by clay will cause Pb to be bound by colloidal soil.

The result of ANOVA showed that inorganic fertilizer treatment had no significant affect on soil Pb levels, whereas the bioremediation agents treatment had a significant affect on soil Pb levels. The interaction between inorganic fertilizers and bioremediation agents treatments did not significantly affect soil Pb levels. Treatment with the highest soil Pb level was P0K2 treatment (0.7975 µg/g), while the treatment with the lowest soil Pb levels was P0K0 treatment (0.7250 µg/g).

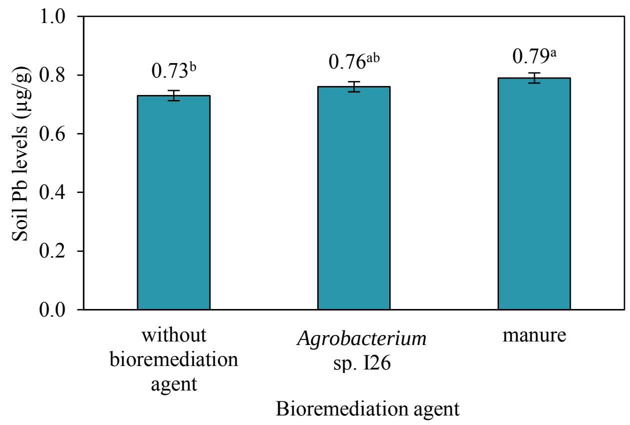

Figure 10. Effect of bioremediation agent on soil Pb level.

The treatments without bioremediation agents were significantly different from treatment using manure (sig 0.009). Treatment using *Agrobacterium* sp. I26 had no significant affect to other treatments. Although treatment with *Agrobacterium* sp. I26 was not significantly different from treatment without bioremediation agents, the soil Pb levels in treatment with *Agrobacterium* sp. I26 was higher than without bioremediation agents. This means the two bioremediation agents could inhibit the soil Pb absorbed by plants or immobilize the Pb in soil. Rosariastuti et al. (2013) stated that the addition of *Agrobacterium* sp. I26 can minimize Pb absorption so that it does not enter into the shoot of plants. Narayani and Shetty (2013) also said that bacteria can produce secondary metabolites which can efficiently eliminate Pb through bioaccumulation.

Decreasing Pb on land caused by phytoremediation activities can be used to calculate the effectiveness of phytoremediation. The effectiveness of bioremediation or often called Removal Effectiveness (RE) is the success of the phytoremediation process in soil which is calculated from differences in initial heavy metal levels and final heavy metal content after treatment

$$RE = \frac{\text{InitialPb level} - \text{final Pb level}}{\text{Initial Pb level}} \times 100\%$$

Removal effectivity values can see in the Table 1 below.

Table 2. Removal effectivity (RE)

Treatments	Removal effectivity (%)
P0K0	17.04
P0K1	10.39
P0K2	8.96
P1K0	16.10
P1K1	15.81
P1K2	10.39

Table 2 shows the decrease of soil Pb levels in all treatments. In this study, the treatment with the lowest effectiveness was the best treatment because this study aimed to minimize the absorption of soil Pb to the plant tissue, especially grain. Table 2 shows the best treatment with the lowest RE of 8.96% was P0K2 treatment. This phenomenon was in accordance with the soil organic matter that can bind the Pb in the soil with humic being Humic - Pb (II).

3.2 Plant characteristics

3.2.1 Weight of 1,000 seeds

Result of weight of 1,000 seeds analysis could be seen in Figure 11 below.

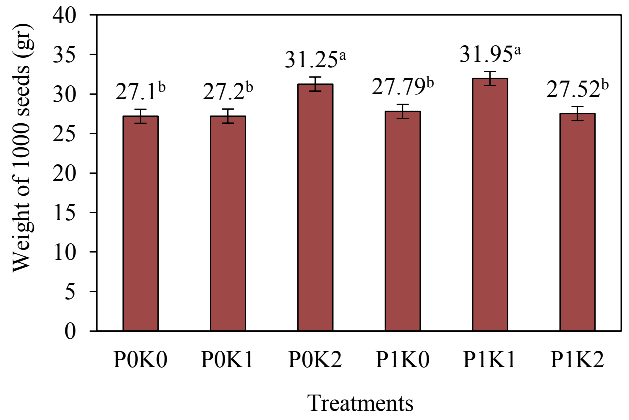

Figure 11. Weight of 1,000 seeds

Figure 11 shows the P0K2 and P1K1 treatments can increase the weight of 1,000 seeds compared with control. The lowest weight of 1,000 seeds was P0K0 treatment of 27.17 g, while the highest weight of 1000 seeds was P1K1 treatment of 31.95 g, but it was not significantly different from the P0K2 treatment. This showed that *Agrobacterium* sp. I26 affects the weight of 1,000 seeds of rice.

The ANOVA showed that inorganic fertilizer treatment did not significantly affect the weight of 1,000 seeds of rice, but bioremediation agents and interaction between inorganic fertilizer and bioremediation agent fertilizer had a very significant affect on the weight of 1,000 seeds of rice.

Manure can increase the weight of 1,000 seeds, but when combined with inorganic fertilizer it had a lower weight of 1,000 seeds than without inorganic fertilizer. This is because the immature organic matter in manure which have high molecular weight can bind the nutrients in soil. Nutrients that are bound by immature organic matter will be immobilized in soil. *Agrobacterium* sp. I26 can increase the weight of 1,000 seeds, but when combined with inorganic fertilizer it can increase more optimally the weight of 1,000 seeds. This is because the inorganic fertilizer can promote plant growth, so that the plant produced many root exudates that can be energy for bacteria (Vigliotta et al., 2016). Bacteria could produce enzymes which will increase the process of metabolism, so it could increase the weight of 1,000 seeds.

According to research conducted by Mulsanti et al. (2014) about the weight of 1,000 seeds in various varieties of paddy showed that the weight of 1,000 seeds of Ciherang varieties showed the highest weight of 1,000 seeds was 26.19 g. In this study the weight of 1,000 seeds were better than Mulsanti's research, including in the control treatment.

3.2.2 Pb Content in plant

Table 3 shows the average Pb content in roots higher than in plant shoot and rice grain. It means that paddy plants accumulated Pb in their roots and did not translocate the Pb in plant shoot.

ANOVA showed that inorganic fertilizers significant affected Pb content in roots, but not significantly affect Pb content in plant shoots and rice grain. Bioremediation agent treatments significantly affected Pb content in roots and rice grain, but did not significantly affect Pb content in plant shoots. Interaction between inorganic fertilizers and bioremediation agent had significantly effected to Pb content in roots, but not significantly effected to Pb content in plant shoot and rice grains.

Table 3. Pb content in plant

Treatments	Pb content in plant (μg/g)			
	Roots	Shoots	Grains	Totals
0K0	0.84[b]	0.0280	0.48	0.31[b]
P0K1	0.93[b]	0.0080	0.30	0.22[d]
P0K2	0.87[b]	0.0009	0.41	0.26[bc]
P1K0	1.10[a]	0.0255	0.43	0.31[a]
P1K1	0.26[c]	0.0155	0.29	0.17[e]
P1K2	0.31[c]	0.0009	0.42	0.23[cd]

P1K1 treatment had the lowest Pb content: 0.17 μg/g, while the treatment with the highest Pb content was P0K0: 0.31 μg/g. In general, bioremediation agents can inhibit the entry of Pb into plant shoots, especially in the rice grain, but *Agrobacterium* sp. I26 can inhibit the entry of Pb more than manure. This is in accordance with Rosariastuti et al. (2013), which found that the application of *Agrobacterium* sp. I26 was able to minimize Pb absorption to plant tissue and accumulate in roots. Narayani and Shetty (2013) also said that bacteria application induced secondary metabolites that efficiently eliminate Pb through bioaccumulation. *Agrobacterium* sp. I26 can bind Pb ions in soil by extracellular polymer or extracellular polysaccharide produced by bacteria and has reactive cell with negative charges. Pramono et al. (2013) also said that *Agrobacterium* sp. I26 can produce the reductase enzyme that can reduce the chromium from toxic (available for plant) to non-toxic (non-available for plant). Therefore, it is possible that the reductase enzyme can reduce the Pb^{4+} (available for plants) to Pb^{2+}/Pb II (not available for plants).

The initial soil showed that soil Pb level was 0.88 μg/g and the final soil has an average that was not much different from the initial soil, while the soil Pb level showed the Pb that enters the plant tissue was higher than the difference between the initial and final soil. This is due to the addition of inorganic fertilizers thereby increasing soil pH, while initial soil pH sampling came before the application of inorganic fertilizers.

Based on Indonesia's Badan Standardisasi Nasional (2009), the maximum allowable Pb content in rice grains is 0.3 μg/g. This study (Table 3) showed that the only treatment that inhibited Pb absorption to the threshold or below was treatment using bioremediation agent *Agrobacterium* sp. I26, and the best combination treatments was P1K1 treatment.

4. CONCLUSION

Based on the results of this study it can be concluded that the highest production weight of 1000 seeds was P1K1 (inorganic fertilizer + *Agrobacterium* sp. I26) treatment of 31.95 g (14.96% higher than the control) followed by P0K2 (without inorganic fertilizer + manure) treatment of 31.25 g (13.06% higher than control). The treatment that can inhibit the absorption of Pb in rice grains to below the threshold was P1K1 (inorganic fertilizer + *Agrobacterium* sp. I26) treatment of 0, 29 μg/g (39.58% lower than control) followed by P0K1 (without inorganic fertilizer + *Agrobacterium* sp. I26) treatment of 0.30 μg/g (37.5% lower than control).

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