EFFORT TO GET SAFE RICE FOR CONSUMPTION THROUGH BIOREMEDIATION TECHNOLOGY IN PADDY FIELD CONTAMINATED BY LEAD†

[ESFUERZO PARA CONSEGUIR ARROZ SEGURO PARA EL CONSUMO A TRAVÉS DE LA TECNOLOGÍA DE BIORREMEDIACIÓN EN PADDY CONTAMINADO POR PLOMO]

Ahmad Johanto[1], Retno Rosariastuti[2,]* and Vita Ratri Cahyani[3]

[1]*Master Study Program of Environmental Science, Graduate School, SebelasMaret University, Surakarta 57126, Central Java*
[2]*Soil Science Study Program, Faculty of Agriculture, Sebelas MaretUniversit , Surakarta 57126, Central Java. Email: retnobs@staff.uns.ac.id*
[3]*Doctoral Study Program of Agricultural Science, Graduate School, Sebelas Maret University, Surakarta 57126, Central Java*
**Corresponding author*

SUMMARY

Textile industry that has less optimal waste management due to the absence of a wastewater treatment plant (WWTP) tends to dispose the waste directly into the water or river. Most farmers near industrial area use liquid waste for irrigation water. This generates negative impacts of complicated problems in the agricultural sector, especially heavy metals contamination in paddy soils. In such case, bioremediation can be done to handle the contaminated soils by heavy metals to create a healthy farming environment. The purpose of this study was to determine the effectiveness of the chelating agents (*Agrobacterium* sp. I_{37} or compost) to the characteristic of soil and the level of Pb in rice plant. This study was conducted between June - September 2017 in Tasikmadu, Karanganyar, Indonesia. The experimental design was factorial using randomized completely block design (RCBD) as the based design. There are two factors in this research: a) inorganic fertilizer (P): without inorganic fertilizer (P0) with inorganic fertilizer (P1); b) chelator (B): without chelator (B0), with chelator *Agrobacterium* sp I_{37} (B1), with chelator compost (B2). There were six treatment combinations then replicated 3 times for each treatment, so there were 18 unit treatments. The results showed that *Agrobacterium* sp. I_{37} or compost can decrease soil Pb. The best treatment in decreasing soil Pb was treatment using *Agrobacterium* sp. I_{37}, which soil Pb decreased of 42.91% from 8.04 mg/kg to 4.59 mg/kg; could enhance accumulation of Pb in the roots of 69, 63% and decrease Pb levels in rice grains around 55.67% higher than control treatment.
Keywords: Bioremediation; lead; metal chelator; safe rice; soil.

RESUMEN

Una industria textil que tiene un manejo de desechos poco recomendado, debido a la ausencia de una planta de tratamiento de aguas residuales (EDAR) tiende a tirar los desechos directamente al agua o al río. La mayoría de los agricultores cerca del área industrial utilizan los desechos líquidos para el agua de riego. Esto genera impactos negativos de problemas complicados en el sector agrícola, especialmente la contaminación por metales pesados en los suelos de arrozales. En tal caso, se puede realizar una biorremediación para manejar los suelos contaminados con metales pesados para crear un ambiente agrícola saludable. El propósito de este estudio fue determinar la efectividad de los agentes quelantes (Agrobacterium sp. I37 o compost) para la característica del suelo y el nivel de Pb en la planta de arroz. Este estudio se realizó entre junio y septiembre de 2017 en Tasikmadu, Karanganyar, Indonesia. El diseño experimental fue factorial utilizando diseño completamente aleatorizado de bloques (RCBD) como el diseño base. Hay dos factores en esta investigación: a) fertilizante inorgánico (P): sin fertilizante inorgánico (P0) con fertilizante inorgánico (P1); b) quelante (B): sin quelante (B0), con quelante Agrobacterium sp I37 (B1), con compuesto quelante (B2). Hubo seis combinaciones de tratamiento que luego se replicaron 3 veces para cada tratamiento, por lo que hubo 18 tratamientos de unidad. Los resultados mostraron que Agrobacterium sp. I37 o el compost pueden disminuir el Pb del suelo. El mejor tratamiento en la disminución del Pb del suelo fue Agrobacterium sp. I37, cuyo Pb del suelo disminuyó 42,91%, de 8,04 mg / kg a 4,59 mg / kg; podría aumentar la acumulación de Pb en un 69,63% y dismiuir los niveles de Pb en los granos de arroz alrededor 55,67%, más que el tratamiento de control.
Palabras clave: Biorremediación; plomo; quelante de metales; arroz seguro; suelo.

† *Submitted September 12, 2018 – Accepted March 12, 2019.*
ISSN: 1870-0462

INTRODUCTION

Rapid development of textile industry has negative impacts in the agricultural sector, especially because of the pollution of heavy metals in paddy fields. This is due deficient wastewater management of the industry where they dispose the wastewater directly into waterbodies or rivers. Textile industry in its production process uses a lot of water, especially in the process of coloring and rinsing. In the process of dyeing textile products, most workers use chemical substances (Khatri et al., 2015) and other metal compounds that make the textile color last long (Paul et al., 2012). In the staining process, it is very likely that textile wastewater does not only contain dyes but also toxic and hazardous heavy metals (Dixit et al, 2015). Zille (2005) found some heavy metals in textile liquid waste such as Cr 3,0 $mg.L^{-1}$, copper 35 $mg.L^{-1}$, Pb 28 $mg.L^{-1}$, mercury 0,5 $mg.L^{-1}$etc. Heavy metals contaminated soil leads to toxic plants. This will affect the ecosystem and leads to plant growth retardation. Some fields in Karanganyar are in textile industrial area; one of them is in Kaling, Tasikmadu District. Most of Kaling farmers use textile wastewater from the textile industry for irrigation. The farmers in Kaling assume that the liquid waste has fertility benefits in agricultural areas. The initial analysis results indicated that the wastewater sample is containing ammonium and nitrate, with the amount of 0.04 $mg.L^{-1}$and 0. 004 $mg.L^{-1}$ respectively. Although, these values are very low, when the textile wastewater is continuously used for irrigation can cause the accumulation of heavy metals (Pb, Cd, Zn, Cu and As) in agricultural fields (Shahid et al, 2012).

Lead (Pb) is a heavy metal that is released from toxic and hazardous industrial waste textiles (Zille, 2005). If Pb levels exceed the water quality standard for irrigation, the metals will be absorbed by plant roots, and then translocate to shoot of plants. Plants that are contaminated by Pb have the risk of poisoning if they are consumed by humans. Based on Indonesian Government Regulation No. 101 of 2014 on The Waste Management of Hazardous and Toxic, that soil contains Pb heavy metals of no more than 0, 2 – 3 ppm. In this research, based on the previous laboratory analysis of the soil, the Pb level was 8.04 mg. kg^{-1}. It means, that the level of soil Pb in paddy soil has exceeded, hence the soil is necessary to be remediated.

Soil that is contaminated by heavy metals causes problems for living things and it must be addressed immediately. The removal of heavy metals pollution in the field can be done through bioremediation. Bioremediation is a process of handling harmful pollutant contamination using biological processes and microbial catabolism activities by producing products such as carbon dioxide, water, inorganic salts, and microbial biomass (Elekwachi et al., 2014). Environmental recovery by microorganisms is considered a potential strategy in reducing the heavy metals contamination in environment (Gandjar et al, 2006; Olaniran et al, 2013; Elekwachi et al., 2014). Bioremediation is an alternative strategy to cope with the toxicity of heavy metals in the soil (Dixit et al 2015; Akcil et al, 2015) to achieve a healthy agricultural environment.

Bioremediation in this research use microorganisms as chelating agents to absorb the heavy metals in the soil and alienate them in the roots. The microbes commonly used for bioremediation are rhizobacteria (Pramono et al, 2012). Besides bacteria, the addition of organic material (compost) to the soils can be a chelating agent to reduce heavy metal ions (Singh and Kalamdhad, 2013).The P element in compost is useful to stimulate root growth and compose an excellent root system, thus the plants get more nutrients.The use of compost as a chelating agent is also expected to increase absorb of Pb from the soil and to accumulate it in the roots (Pramono et al, 2012).

The purpose of this study was to determine the effectiveness of the chelating agents (*Agrobacterium* sp. I_{37} or compost) to the many characteristic of soil (pH, CEC, organic matter, total colony of bacteria and Pb level) and the level of Pb in rice plant especially in the root, shoot and rice grain.

MATERIALS AND METHODS

This research was conducted in Kaling, Tasikmadu, Karanganyar, Central Java, Indonesia from June to September 2017. The experimental design was factorial using randomized completely block design (RCBD) as the based design. There are two factors in this research: a) inorganic fertilizer (P): without inorganic fertilizer (P0) with inorganic fertilizer (P1); b) chelator(B): without chelator (B0), with chelator *Agrobacterium* sp I_{37} (B1), with chelator compost (B2). There were six treatment combinations then replicated 3 times for each treatment, so there were 18-unit treatments. Each treatment combination was applied on a paddy field plot having the size of 1.25 m x 1.25m; with the distance between the planting holes was 50 cm and the distance between plots was 100 cm. the number of planting holes in each plot was 25.

The six treatment combinations i.e:

P0B0 = Without inorganic fertilizer + without chelator (control)

P0B1 = Without inorganic fertilizer + with chelator *Agrobacterium* sp. I_{37} $10^6 g^{-1}$ soil

P0B2 = Without inorganic fertilizer + with chelator compost 781,25 g per plot

P1B0 = With inorganic fertilizer N (31,25 g); P (15,625 g); K(15,625 g)/ plot + Without chelator
P1B1 = With inorganic fertilizer N (31,25 g); P (15,625 g); K(15,625 g) / plot + with chelator *Agrobacterium* sp. I_{37} $10^6 g^{-1}$ soil
P1B2 = With inorganic fertilizer N (15,625 g); P (7, 8125 g); K (7, 8125 g) /plot + compost 390,625 g /plot

The NPK fertilizer used in this study were Urea for N, SP-36 for P, and KCl for K. The fertilizers were from an agricultural supply store at Nongko Market of Surakarta. The dose comparison of NPK applied was N: P: K = 2:1:1 = 200:100:100 kg. ha^{-1} (Rauf et al., 2000). The chemical fertilization was done three times, i.e. at 5 days after planting rice (N, P, and K), 35 days (N and K), and 55 days (K).

The metal chelators used in this study were *Agrobacterium sp.* I_{37} (B1) isolated by Rosariastuti *et al*. (2013), and compost (B2) obtained from an agricultural supply store at Nongko Market of Surakarta. The preparation of *Agrobacterium* sp. I_{37} was started by making an LB (*Luria Betani*) medium. The medium was a mixture of 5 g of yeast extract, 10 g of proteose peptone, 5 g of NaCl, and 1000 mL of distilled water. Furthermore, the 100 ml LB medium consisted of making a starter by adding pure *Agrobacterium* sp. I_{37} isolate using needle. The starter was shaken for 72 hours. Then, it was scaled up by pouring the starter into a flask which contained 650 mL LB and shaken for 72 hours or until the density of bacteria reached to 10^8. ml^{-1}. The dose of the *Agrobacterium sp.* I_{37} was 10^6 .g^{-1} soils and was applied 12, 5 mL for inoculum each rice-planting hole and it was incorporated into the soil 1 week before planting rice.

Parameters observed in this study were soil properties at initial and at 90 days after planting, i.e. pH (electrometric method); CEC (base saturation extract ammonium acetate method); organic matter (Walkley and Black method); total microbial colonies soil (medium luria betani agar with a hand colonies counter), levels of Pb (wet digestion method) in soil and plant tissue (root, shoot, and grain). Data obtained were statistically analyzed using One-way Anova at 5% level, followed by Duncan Multiple Range Test (DMRT) at 5% using SPSS 16.0.

RESULTS AND DISCUSSION

Soil Characteristics

1. Soil pH

The analysis of soil pH was presented in Figure 1. The initial soil analysis results showed that the pH value was 6, 89, the value was categorized as slightly acidic. The last soil analysis showed the decrease in all pH values of the treatments (Figure 1). The lowest pH value (5.74) was in the treatment without chemical fertilizer with the chelator of *Agrobacterium* sp I_{37} (P0B1); while the highest pH value (6.15) was in the control treatment (P0B0). It showed a similar value with the initial soil pH.

The results of ANOVA showed that the interactions of chemical fertilizer, the interactions of chelator and the interactions of both chemical fertilizer and chelator significantly affected the soil pH ($p < 0.05$). Further DMRT showed that P0B1 treatment was not significantly different from P0B2 but it was significantly different from other treatments.

Inorganic fertilizers, bacteria and composts will affect the pH value. In the application of urea, N is then supplied to the plants in the form of $NH4^+$. According to Liu et al. (2010) pH reduction in N fertilizer is due to the release of ion H^+ caused by oxidation. Zhou, et al (2013) state there is a negative correlation between pH and soil bacteria. The smaller the bacteria present in the soil, the higher the pH value. The application of compost as fertilizer can increase the value of soil organic material. Whilst the pH increases along with the addition of decomposed organic material (mature), as stated by Atmojo (2010) this is due to the base cations release from mineralized organic material.

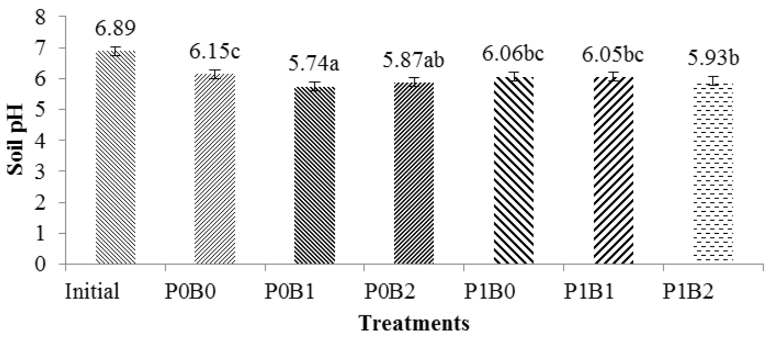

Figure 1. Soil pH.

The degree of soil acidity affects the availability of metals in the soil. The metal will be widely available in soils with low pH (acid) (Yoon *et. al*., 2006). Wuana, et al (2010) mentioned that soil pH has important roles in absorb of heavy metals in which the pH regulates the solubility and hydrolysis of metal, carbon and phosphate hydroxides. The mobility and bioavailability of heavy metals increases when the pH decreases (Zeng, et al 2011), this raises the high absorb of heavy metals in plant roots.

2. Soil CEC (Cation Exchange Capacity)

The analysis of the cation exchange capacity is presented in Figure 2. Initial soil analysis results indicated that soil CEC was 20.22 cmol (+).kg^{-1}. This value is categorized as medium. The final soil analysis showed that the treatment given lead to the CEC enhancement of the soil. The CEC value of soil that has been observed ranged in high category. The lowest CEC value of soil (28.08 cmol (+).kg^{-1}) was found in the treatment of inorganic fertilizer without chelator (P1B0). Meanwhile the highest CEC value (33.12 cmol (+).kg^{-1}) was found in the treatment without inorganic fertilizer, specifically compost as the chelator (P0B2).

The result of ANOVA test indicated that the application of chemical fertilizer, chelators and chemical fertilizer and chelator interaction proved significant effects to the value of CEC ($p<0.05$). Further results of DMRT showed that P0B1 treatment was significantly different from other treatments. P0B0 was insignificantly different from P1B0; P1B1 and P1B2.The highest of CEC value (33, 12 cmol(+).kg^{-1}) especially was compost. Hanum et al. (2016) mentioned that the treatment of compost, straw and NPK on paddy cultivation with SRI system increased soil CEC. The organic matter contained organic acid which are the result of decomposition of organic materials by microorganisms; which in turn increases the content of soil organic materials as well as the CEC (Margolang et al., 2015). According to Hartati et al (2013) CEC by type and number of colloids, types of clay minerals, texture, and levels of organic matter determine the value of CEC. CEC has an important role in terms of the absorption of cations exchanged in the soil. Soils with high clay content have a high affinity for heavy metals which make heavy metals less available. In this study, *Agrobacterium* sp. I_{37} and compost were added with the aim to increase CEC so that the presence of heavy metals will be easily absorbed by the root plant tissue and exiled in the roots.

3. Soil Organic matter

The analysis of the organic matter was presented in Figure. 3. Initial soil analysis showed 3% of organic matter content. This value is considered medium. The final soil analysis showed that the treatments increased the value of organic matter. The highest value of organic material (4.49%) was found in the treatment of *Agrobacterium* sp I_{37} (P0B1) as the chelator whilst the lowest value organic matter (2.62%), was observed in the control treatment (P0B0).

Based on ANOVA test, fertilizer, chelator, and interaction of fertilizer and chelator, gave a significant effect on the organic matter value ($p <0.05$). Further test of DMRT showed that P0B1 treatment was not significantly different from P0B2 but it was significantly different from other treatments. This means that the application of compost or *Agrobacterium* Sp I_{37} had insignificant differences and showed that the average value of organic matter was higher than on other treatments.

Organic matter increases with the application of compost. Compost will help the process of decomposition of the soil as a result of the microbial decomposers in it. Compost also supplies organic matter which becomes a source of colloid humus and increases soil fertility (Hanun et al., 2016). High organic matters provide nutrients for plants and it will affect plant growth. According to Minardi (2006), organic matter can produce organic compounds in the form of humic acid and fulvic acid which have a role in P release and increase P in soil.

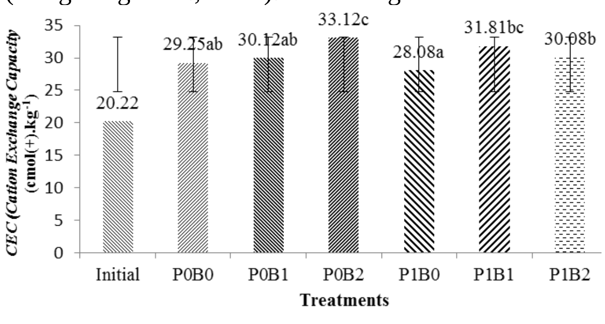

Figure 2. Cation Exchange Capacity (CEC).

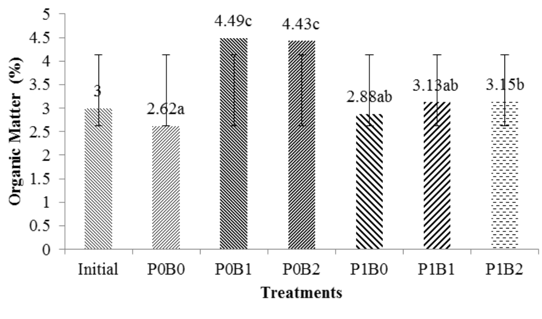

Figure 3. Soil Organic Matter.

The release process of P will bind with metal ions and change bonds with humic acid and fulvic acid, this bond is what indicates the occurrence of whitening. Chelation by organic matter can regulate the availability of metals in the soil. Complex compounds between metals and organic acids cause increased metal mobility to be absorbed by plant root tissues.

4. Total Microbial Colonies

Initial soil analysis showed that the total soil microbial colonies was 7.31 Log 10 CFU.g^{-1} (Figure 4). The final soil analysis showed that the treatments generated an increase in the value of organic materials. The highest total value of microbial colonies (12.69 Log 10 CFU.g^{-1}) was found in the treatment without chemical fertilizers *Agrobacterium* sp I_{37} (P0B1) whilst the lowest microbial colonies (11.36 Log 10 CFU.g^{-1}) was found in the treatment of chemical fertilizer without chelator (P1B0).

The results on ANOVA test indicated that fertilizer, chelator, and interaction of fertilizer and chelator gave a significant effect to the total value of microbial colonies ($p < 0.05$). Further test of DMRT showed that the P0B1 treatment had insignificant difference compared to P0B2, otherwise the test result had significant difference from other treatments. It means that the soil with *Agrobacterium* sp I_{37} or compost fertilizer showed no significant difference, and also showed the average value of total microbial colonies that was higher than other treatments.

Both chelators increased the microbial colonies. This was due to the addition of *Agrobacterium* spI_{37} that reproduce through fragmentation (Mazur and Koper, 2012). Therefor *Agrobacterium* sp I_{37} added to the soil increased total microbial colonies in the soil. The more total microbial colonies in the soil, the greater the likelihood of interaction between microorganisms and root exudates, thereby enhancing the ability of plants to absorb heavy metals (Gadd, 2010; Agustiyani et al., 2004). The use of compost and inorganic fertilizers can supply C-organic in the soil thereby increasing the population of microorganisms (Zhang et al., 2015).

5. Soil Pb level

The analysis of Pb levels in soil is presented in Figure. 5. Initial soil analysis showed Pb levels by 8. 04 mg.kg^{-1}. The final soil analysis showed that the treatments decreased Pb levels in the soil. The lowest concentrations of Pb (4.59 mg.kg^{-1}) were found in the treatment without chemical fertilizers with *Agrobacterium* Sp I_{37}, whilst the highest levels of Pb (6.97 mg.kg^{-1}) and Cr (2.3 mg.kg^{-1}) were found in the control treatment. Based on Indonesian Government Regulation No. 101 of 2014 on The Waste Management of Hazardous and Toxic, that soil contains Pb heavy metals of no more than 0, 2 – 3 ppm. Pb levels in both initial and final analyzes of the soil showed that they were exceeded.

Considering heavy metals are hazardous and toxic if they accumulate in the soil, they will disturbances function of soil to plants, hence the soil is necessary to be remediated by bioremediation. Bioremediation using *Agrobacterium* sp I_{37} as chelator and compost can reduce the Pb level in soil and accumulate absorption of heavy metals in plant roots.

The results of ANOVA test chelator and interaction of fertilizer and chelator showed a significant effect ($p < 0.05$) to the Pb levels in soil. Further test of DMRT showed that for Pb grade analysis, P0B1 treatment was different from other treatments. This means that *Agrobacterium* sp I_{37} had better capability to reduce the value of Pb level that was lowest than other treatments.

The addition of both chelators was also included as organic matter. The presence of organic matter is a major factor in improving the soil's ability to retain heavy metals in an exchangeable form (Zenget al., 2011). McCauley et al. (2009) mentioned that organic matter supplies organic chemicals such us organic acid, Exopolisacharides (EPS), enzyme, and siderophore to the soil, to hold and increase the availability of metals for plants. Soil Organic matter can increase the mobility and absorption of heavy metals in plant roots.

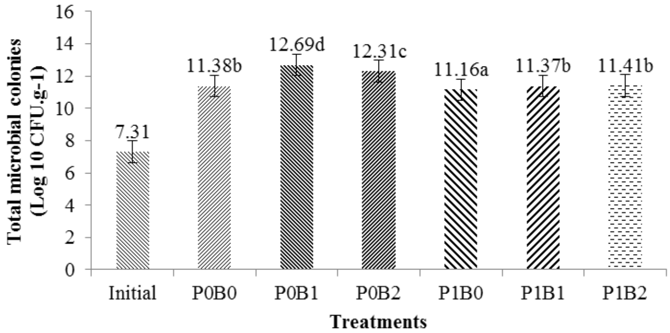

Figure 4. Total Microbial Colonies.

The increase of soil organic matter would decrease the concentration of soil heavy metals (Saidy and Badruzsaufari, 2009). According to Narayani and Shetty (2013), secondary metabolites microorganisms produced by microorganisms efficiently remove heavy metals in soluble form as well as in particle form, through bioaccumulation; the use of microorganisms provides an alternative in the remediation of contaminated soil of heavy metals. The effectiveness of soil Pb decrease was indicated by the value of Removal Effectivity (RE) (Figure 6).

Removal Effectivity (RE) was written in order to compare the initial soil with final analysis each treatment, so there were change data on the control treatment. Decreasing Pb levels in the control treatment was possible due to the presence of leaching factor and several natural factors that were not controlled during the research process. In this condition it would be dangerous because the metal Pb could not be controlled to change its place. Based on the RE, the *Agrobacterium* sp. I_{37} could reduce Pb levels of 42.91% respectively, greater than other treatments.

The bioremediation process will convert Pb metal ions into other cations that can be absorbed by plants that will decrease the levels in the soil (Pramono, et al 2012). The process of nutrient absorption by plants will be easier if the process is supported by soil microorganisms. Soil microorganisms act as decomposers of compost into nutritional elements that can be absorbed by plants (Hanafiah et al., 2009; Ferina and Rosariastuti, 2017).

Pb metals are absorbed in plant tissue by sulfate mechanisms (Pramono, et al 2012). Pb and Cr will resemble nutrients so they can be absorbed by plant tissues through the roots. The decrease of metal content shown by the RE must be accompanied by an observation of the location of metal accumulation in plant tissues. Where for food crops, it is not desirable to have the metal accumulation in the parts of plants that will be consumed.

Plant (Root, Shoot and Rice grain) Pb levels

Data presented in figure 7 show that the highest level of Pb in the root (10.26 mg. kg^{-1}) was on the treatment combination of chemical fertilizer and *Agrobacterium* sp. I_{37} (P1B1), while the lowest Pb level in the root (8.58 mg. kg^{-1}) was found in the treatment of *Agrobacterium* sp. I_{37} (P0B1). The highest level of Pb in the shoot (2.93 mg. kg^{-1}) was found in the treatment combination of chemical fertilizers and *Agrobacterium* sp. I_{37} (P1B1), while the lowest Pb level in the shoot (1.08 mg. kg^{-1}) was found in the control treatment (P0B0). The highest level of Pb in the grain (5.37 mg. kg^{-1}) was in the control (P0B0) and the combined chemical fertilizer and compost treatments (P1B2), while the lowest Pb level in the grain (2.38 mg. kg^{-1}) was found in the treatment of *Agrobacterium* sp. I_{37} (P0B1).

Result of ANOVA showed that interaction of between fertilizer and chelator showed a significant effect ($p < 0.05$) to Pb in the root, shoot and grain. Furthermore DMRT results on Pb levels in the root showed that the use of *Agrobacterium* sp. I_{37} was significantly different from all treatments. The Results on shoot Pb levels showed that the treatment of *Agrobacterium* sp. I_{37} was insignificantly different with the treatment of combination of compost and chemical fertilizers. The Result on rice grain Pb levels showed that application of *Agrobacterium sp.* I_{37} was not significantly different with the compost treatment, but it was significantly different with other treatments.

Based on figure 7, the levels of Pb in the control treatment were higher compared to the treatment of chelator and its combination with inorganic fertilizer. It means that without treatments, heavy metals can easily enter the plant tissue, so it will threaten the yield of rice plants. The levels of Pb were higher in the roots than in the shoots and grains. Furthermore, Hidayanti (2013) stated that the rhizosphere interaction at the root zone as the place of processing the elements in the soil, processed non absorbable form into absorbable form by involving several exudates produced by roots.

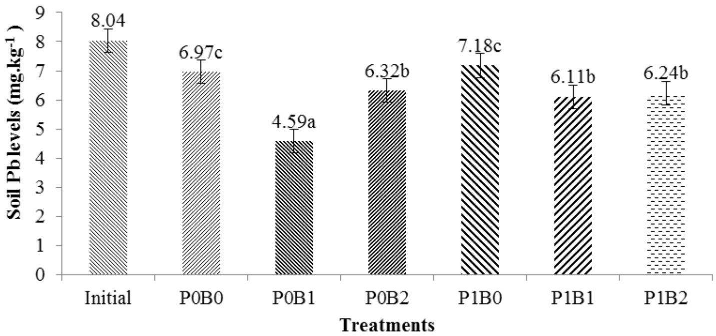

Figure 5. Soil Pb levels.

Results of this study showed that the use of *Agrobacterium sp.* I_{37} and compost in bioremediation process enhanced the accumulation of Pb by 69.63% in root tissue compare with control treatment. Decomposition of organic matter in the soil will produce fulvic, humic, and humin acid containing a large amount of anion charge. Decomposition of organic matters will produce humic compound that produces a negatively charged colloid of soil. This negative charge comes from a carboxyl group (COOH) and hydroxyl (OH) in organic compounds. Pb in soil are elements having positive charges that will react with negatively charged colloids produced by organic matters. This causes Pb to concentrate in the rhizosphere and they become easier to be absorbed by the root of rice plants (Saptiningsih and Haryanti, 2015; Siregar et al., 2017).

The presence of *Agrobacterium sp.* I_{37} or compost was also able to reduce levels of Pb in the rice grains. The concentration of Pb in the rice was equal to 2.38 mg. kg^{-1}, this number is above the threshold permitted by the WHO that equals to 2 mg/kg and it does not meet the permitted values by SK Dirjen POM No. 03725/B/Sk/VII/89; for rice / flour 1.0 mg. kg^{-1}. Pb level in rice grain with *Agrobacterium* sp. I_{37} treatment was 2. 38 mg. kg^{-1}. Pb level in grain without treatment (control) was 5.37 mg/kg. It means that although the treatment of *Agrobacterium* sp. I_{37} cannot reduce the concentration of lead in rice reach to permitted values yet, *Agrobacterium* sp. I_{37} can decrease Pb levels around 55.67% compared to the control treatment. According to this result, it is recommended to clean up paddy fields before planting the paddy plant. One method that can be used along to bioremediation technology is planting non-food plants such as Rami (*Boehmeria nivea*) to absorb the metals, before planting the soil by food crop.

In agriculture, soil has a very important role, in addition to climate and water. All plants and crops yields are indispensable to human growth and development, and it is highly dependent on the soil conditions other than climates and water. If the paddy fields polluted by heavy metals, there will be unexpected changes in physical, chemical, and biological properties of the soil. The accumulation of heavy metals can degrade soil quality, reduce yields and quality of agricultural products, and thereby adversely affect human health, animals and ecosystems (Nagajyoti et al., 2010). A healthy soil must be attained to achieve healthy farming, thereby producing healthy food for human beings. Efforts to achieve healthy yields and human must be started with a healthy soil (Atmojo, 2010).

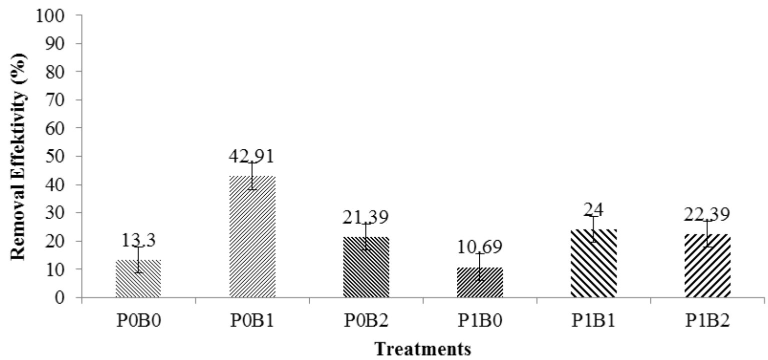

Figure 6. Removal effectivity.

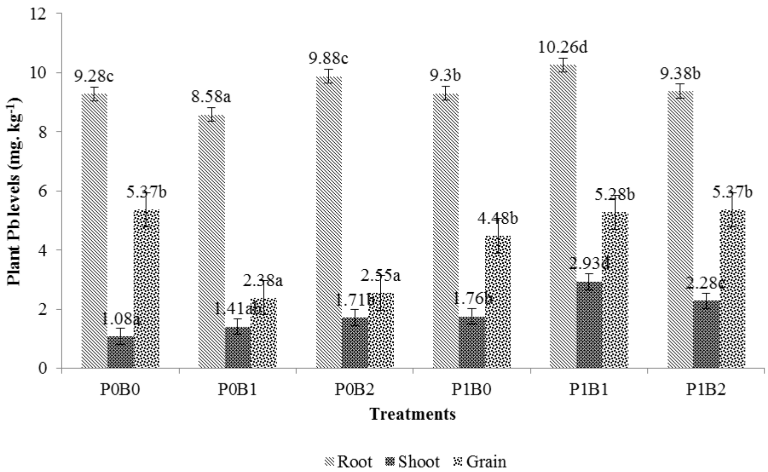

Figure 7. Plant Pb levels.

CONCLUSION

The results showed that the use of *Agrobacterium* sp. I_{37} and compost decreased pH soil value from 6.15 to 5.7. The CEC value of soil was greatest by using compost than any other treatments, from 20.22 cmol/kg to 33.12 cmol(+)/kg. The use of *Agrobacterium sp.* I_{37} and compost increased the soil organic matter from 3% to 4.49% and increased the microbial content from 7.31 Log 10 CFU.g^{-1} to 12.69 Log 10 CFU.g^{-1}. Bioremediation using *Agrobacterium sp.* I_{37} or compost could reduce soil Pb level. Use *Agrobacterium sp.* I_{37} had the highest reduction on Pb levels of the soil around 42.91% (from 8.04 mg/kg to 4.59 mg/kg); could enhance accumulation of Pb by 69,63% in the plant roots and can decrease Pb levels in rice grains around 55.67% higher than control treatment. *Agrobacterium* sp. I_{37} or compost as chelate agents can be used as an effort to get safe rice for consumption in paddy field contaminated by lead. Excessive use of chemical fertilizers can also increase the levels of heavy metal absorbed by plants, so it is not recommended for long time using. In addition, there needs government regulation for industry to make wastewater management installations according to quality standard.

REFERENCES

Agustiyani, D. W. I., Imamuddin, H. and Faridah, E. N. U. R. 2004. The Effect of pH and Organic Substrate on the Growth and Activity of Ammonia Oxidizing Bacteria. Biodiversitas 5: 43–47. https://doi.org/10.13057/biodiv/d050201

Akcil, A.; Erust, C.; Ozdemiroglu, S.; Fonti, V.; Beolchini, F. A review of approaches and techniques used in aquatic contaminated sediments: Metal removal and stabilization by chemical and biotechnological processes. *Journal Clean Production* 86: 24–36. DOI: 10.1016/j.jclepro.2014.08.009

Atmojo, S., W. 2010. *Sustainable Land Resource Management.* Surakarta: UNS Press

Dixit, R.,Malaviya, D.,Pandiyan, K., Singh, U.B., Sahu, A., Shukla, R., Singh, B.P., Rai, J.P.,Sharma, P.K., Lade, H. 2015. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability* 7: 2189–2212. https://doi.org/10.3390/su7022189

Elekwachi, C.O., Andresen, J. and Hodgman, T.C. 2014. Global use of bioremediation technologies for decontamination of ecosystems. *Journal of Bioremediation and Biodegradation* 5: 1–9. https://www.omicsonline.org/open-access/global-use-of-bioremediation-technologies-for-decontamination-of-ecosystems-2155-6199.1000225.php?aid=26995

Ferina, P. and Rosariastuti, R. 2017. The effectiveness of Mendong plant (*Fimbrystilisglobulosa*) as a phytoremediator of soil contaminated with chromium of industrial waste. *Journal of Degraded and Mining Lands Management* 4(4): 899–905. https://doi.org/10.15243/jdmlm.2017.044.899

Gadd, G.M. 2010. Metals, minerals and microbes *geomicrobiology and bioremediation. Microbiology* 156: 609-643, DOI: https://doi: 10.1099/mic.0.037143-0.

Ganjar I, Syamsurizal W, Wutari A. 2006. Mycology Basic and Applied. Jakarta: Yayasan Obor Indonesia.

Hanafiah, M.A.K.M., Zakaria, H., Wan Ngah, W.S. 2009.Preparation, Characterization, and Adsorption Behavior of Cu(II) Ions onto Alkali-Treated Weed (*Imperatacylindrica*) Leaf Powder. *Water Air Soil Pollut* 201: 43. https://doi.org/10.1007/s11270-008-9926-2

Hanum, H, Hardy, G., Jamilah. 2016. The Influence of Inorganic and Organic Fertilizer on Soil Chemical Characteristics in Wetland with SRI and Conventional Cultivation System. Proceeding of National Seminar of Land of Suboptimal 2016, Palembang. ISBN: 979 – 587 -659-7

Hartati, S., Minardi, S., Aruyanto, P. 2013. Zero Point Charge of Various Organic Fertilizer: The Effect on Soil Cation ExchangeCapacity in Degraded Lands. Journal of Soil Science and Agroclimatology 12 (1). ISSN 2356-1424. http://oaji.net/articles/2014/1067-1405994956.pdf

Hidayati, N. 2005. Phytoremediation and Potency of Hyperaccumulator Plants. Hayati 12 (1) 35–40. ISSN 0854-8587. http://dx.doi.org/10.4308/hjb.12.1.%25x

Hidayati, Nuril., 2013. The physiological Mechanism of Heavy Metal Hyperaccumulator Plants. *Pusat Penelitian Biologi LIPI*

Khatri A., Peerzada M.H., Mohsin, M., White, M. 2015. A review on developments in dyeing cotton fabrics with reactive dyes for reducing effluent pollution. *Journal Clean*

Production 87: 50-57. https://doi.org/10.1016/j.jclepro.2014.09.017

Liu, E., Yan, C., Mei, X., He, W., Bing, S.H., Ding, L., Liu, Q., Liu, S., Fan, T. 2010. Longterm effect of chemical fertilizer, straw and manure on soil chemical and biological properties in northwest China. *Geoderma* 157: 173 -180. https://doi.org/10.1016/j.geoderma.2010.04.029

Margolang. R. D., Sembiring, M., Jamila, J. 2015. Characteristics of Some Physical, Chemistry, and Biological Qualities of Soil on Organic Farming System. Online Journal of Agroecotechnology, 3(2), 717–723. ISSN 2337- 6597. https://jurnal.usu.ac.id/agroekoteknologi/article/view/10358

Mazur, A and Koper, P. 2012. Rhizobial plasmids replication structure and biological role.*Central European Journal of Biology* 7 (4): 571-586 DOI: 10.2478/s11535-012-0058-8

McCauley, A., Jones, C., Jacobsen, J. 2009. Soil pH and Organic Matter.Nutrient management modules no.8. Montana State University Extension Service, Bozeman, Montana, pp. 1-12.

Minardi, Slamet, Joko Winarno dan Abror Hanif Nur Abdillah. 2009. Effects of balancing organic and inorganic fertilizers on the soil chemical properties of andisol tawangmangu and the results of carrot plants (*Daucus carota L*.). Journal Soil Science and Agroclimatology 6(2): 111 – 116. http://dx.doi.org/10.15608%2Fstjssa.v6i2.59

Ministry of State for Population and Environment Republic of Indonesia and Dalhousie University Canada. 1992. Environmental Management in Indonesia. Report on Soil Quality Standards for Indonesia

Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M. 2010. Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters 8 (3): 199–216. DOI: 10.1007/s10311-010-0297-8

Narayani, M. and Shetty, V. 2013. Chromium-Resistant Bacteria and Their Environmental Condition for Hexavalent Chromium Removal: A Review. Critical Reviews in Environmental *Science and Technology* 43: 955 – 1009. DOI: 10.1080/10643389.2011.627022.

Olaniran, A.O., Balgobind, A., Pillay, B. 2013. Bioavailability of heavy metals in soil: Impact on microbial biodegradation of organic compounds and possible improvement strategies. International Journal Mollecular Science 14: 10197–10228. doi:10.3390/ijms140510197

Paul, S.A., Chavan, S.K., Khambe, S.D. 2012. Studies on characterization of textile industrial waste water in solapur city. International Journal of Chemical Sciences 10: 635-642. https://www.researchgate.net/publication/267783788_Studies_on_characterization_of_textile_industrial_waste_water_in_Solapur_city

Pramono, A., Rosariastuti, M.M.A.R., Ngadiman, Prijambada, I.D. 2013. Bacterial Cr (VI) reduction and its impact in bioremediation. *Jurnal Ilmu Lingkungan* 11 (2): 120-131.

Pramono, A., Rosariastuti, R., Ngadiman, dan Prijambada, I.D. 2012. Peranrhizobakteriadalamfitoekstrasilogamberatkromiumpadatanamanjagung.Ecolab 6(1): 38-50.

Rauf, A. W., Syamsuddin, Sihombing, S.R. 2000. The Role of NPK Fertilizer on Rice Plant. Department of Agriculture Agency for Agricultural Research and Development. Workshop on Assessment of Agricultural Technology Kota Barat Irian Jaya.

Rosariastuti, R., Prijambada I.D., Ngadiman, Prawidyarini G.S., Putri, A.R. 2013.Isolation and Identification of plant growth promoting and chromium uptake enhancing bacteria from soil contamined by leather tanning industrial waste. Journal of Basic and Applied Sciences.9: 243-251. http://www.lifescienceglobal.com/pms/index.php/jbas/article/viewFile/769/539

Saidy, A.R. and Badruzsaufari. 2009. The relationship between Cr (VI) concentration and soil chemical qualities. Journal of Tropical Soils 14(2): 97-103.

Shahid, M., Arshad, M., Kaemmerer, M., Pinelli, E. 2012. Long-term field metal extraction by pelargonium: phytoextraction efficiency in relation to plant maturity, *International Journal of Phytoremediation* 14 : 493-505.

Singh, J. and Kalamdhad, A.S. 2013. Reduction of bioavailability and leachability of heavy metals during vermicomposting of water hyacinth. *Environmental Science and Pollution Research* 20: 8974-8985. https://doi.org/10.1007/s11356-013-1848-x

Siregar, Prengki, Fauzi dan Supriadi. 2017. Pengaruh pemberian beberapa sumber bahan organik dan masa inkubasi terhadap beberapa aspek kimia kesuburan tanah ultisol. Jurnal Agroekoteknologi FP USU 5(2):256-264.E-ISSNNo.2337-6597. https://media.neliti.com/media/publications/109880-ID-pengaruh-pemberian-beberapa-sumber-bahan.pdf

Wuana, R.A., Okieimen, F.E., Imborvungu, J.A. 2010. Removal of heavy metals from a contaminated soil using organic chelating acids*International Journal Environmental Science Technology* 7: 485. https://doi.org/10.1007/BF03326158

Yoon, J., Cao, X., Zhou, Q., Ma, L.Q. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site.*Science of the Total Environment* 368: 456–464. https://doi.org/10.1016/j.scitotenv.2006.01.016

Zeng, F.,Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., Zhang, G. 2011. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Journal of Environmental Pollution* 159: 84-91. doi:10.1016/j.envpol.2010.09.019.

Zhang, H., Ding, W., Yu, H., He, X. 2015. Linking organic carbon accumulation to microbial community dynamics in a sandy loam soil: result of 20 years compost and inorganic fertilizers repeated application experiment. Biology and Fertility of Soils 51: 137-150. https://doi.org/10.1007/s00374-014-0957-0

Zhou, X., Guo, Z., Chen, C., Jia, Z. 2013. Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. Biogeosciences14: 2106 https://doi.org/10.5194/bg-14-2101-2017

Zille, A. 2005. *Laccase reactions for textile applications*. Universidade do Minho