MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.13, No.3, September 2019, p 75-82

Available online at
http://jurnal.permi.or.id/index.php/mioline
DOI: 10.5454/mi.13.3.1

The Use of *Agrobacterium* sp. I_3 and Compost as Chelator Combined by NPK Fertilizer and Mendong Plant (*Fimbristylis* sp.) in Bioremediation of Paddy Soil Contaminated by Lead (Pb)

RETNO ROSARIASTUTI[1*], ABDI LEONARDO SARAGIH[1], SUDADI[1], SUPRIYADI[1], AND WIWIN WIDIASTUTI[2]

[1]*Soil Science Department, Faculty of Agriculture, Universitas Sebelas Maret, Jalan Ir Sutami 36A, Kentingan, Surakarta, 57126, Indonesia;*
[2]*Regional Planning Research and Development Board, Central Java Province, Jalan Pemuda 127-133, Semarang 50132, Indonesia.*

Industrial waste supplies contains heavy metals such as Pb which will cause pollution in paddy fields. Remediation of paddy soil contaminated by Pb heavy metal must be done by simple, environmental friendly, cheap and sustainable technology, that is bioremediation. The purpose of this study was to study the effectiveness of bioremediation using *Agrobacterium* sp. I_3 and compost as chelator combined by Mendong plant and NPK fertilizer, and learn the ability of Mendong in uptaking metal soil Pb. This was field experimental research, had a factorial patern, using Completly Randomized Block Design as the base design, with three factors: (1) NPK fertilizers (P0: no NPK fertilizers, P1: with NPK fertilizers), (2) Chelator (K0: no chelator; K1: with chelator *Agrobacterium* sp. I_3 ; K2: with chelator compost); and (3) Plant (T0: without plant; T1: with Mendong plant). The results showed that *Agrobacterium* sp. I_3 and compost were increasing Pb uptake in shoot, but decreasing Pb uptake in root. Mendong plant has highly ability in uptaking soil Pb, so decreased soil Pb, and effective as the phytoremediator. NPK fertilizer increased plant growth so increased Pb uptaken by plant. The highest Pb uptake was in treatment combination of NPK fertilizer + Mendong plant: 80.916 µg, followed by NPK fertilizer + *Agrobacterium* sp. I_3 + Mendong Plant: 76.363 µg. The highest decreased of soil Pb (42.41%) was found in treatment combination of compost + Mendong Plant.

Key words: *Agrobacterium* sp. I_3, compost, *Fimbristylis* sp, Pb, phytoremediation

Limbah industri mengandung logam berat seperti Pb, menyebabkan polusi di sawah. Remediasi tanah sawah terkontaminasi Pb harus dilakukan dengan teknologi sederhana, ramah lingkungan, murah dan berkelanjutan, yaitu bioremediasi. Tujuan penelitian ini adalah untuk mempelajari efektivitas bioremediasi menggunakan *Agrobacterium* sp. I_3 dan kompos sebagai chelator yang dikombinasikan dengan tanaman mendong dan pupuk NPK, serta mempelajari kemampuan mendong dalam menyerap Pb tanah. Penelitian ini merupakan penelitian lapangan, memiliki pola faktorial, menggunakan rancangan dasar Rancangan Acak Kelompok Lengkap, dengan tiga faktor: (1) pupuk NPK (P0: tanpa pupuk NPK, P1: dengan pupuk NPK), (2) Chelator (K0 : tanpa chelator; K1: dengan chelator *Agrobacterium* sp. I_3; K2: dengan chelator kompos); dan (3) Tanaman (T0: tanpa tanaman; T1: dengan tanaman Mendong). Hasil penelitian menunjukkan bahwa *Agrobacterium* sp. I_3 dan kompos meningkatkan serapan Pb pada tajuk, tetapi menurunkan serapan Pb pada akar. Mendong memiliki kemampuan tinggi dalam menyerap Pb tanah, sehingga dapat menurunkan Pb tanah, dan efektif sebagai fitoremediator. Pupuk NPK meningkatkan pertumbuhan tanaman sehingga dapat meningkatkan serapan Pb oleh tanaman. Penyerapan Pb tertinggi adalah pada kombinasi perlakuan : pupuk NPK + mendong: 80,916 µg, diikuti oleh kombinasi perlakuan pupuk NPK + *Agrobacterium* sp. I_3 + mendong: 76,363 ug. Penurunan tertinggi Pb tanah (42,41%) pada kombinasi perlakuan : kompos + mendong.

Kata kunci: *Agrobacterium* sp. I_3, bioremediasi, *Fimbristylis* sp, kompos, Pb

Paddy fields area in Kebakkramat sub-district based on the Central Agency of Indonesia Statistics (2013) is 2,258 hectares. Wiyono and Widodo (2004) states that 80% of paddy fields has been polluted by heavy metals in Kebakkramat District, Karanganyar. The source of Heavy metal such as lead (Pb) were industrial waste disposal, fertilizer, pesticide, and mining waste. The metal will be accumulated by plants, consequently can affect the nutritional value and the quantity of crops in food plants, causing harm to farmers from the economic and health aspects of its consumers.

Ari Susanti and Purnawi (2013) mentioned that heavy metals are uptaken by plant roots in the form of water-soluble ions such as nutrients that come along with the water flow. Sari *et al.* (2014) stated that plant heavy metals can inhibit metabolic processes and cell

*Corresponding author: Phone: +62-82185455812; Email: retnobs@staff.uns.ac.id

growth. Its because the mechanism of reaction of heavy metals to proteins that generally attack sulphide bonds. The invaded sulphide bond always leads to its protein molecule so that it will cause structural damage. One of the ways to restore the soil environment that has been contaminated by heavy metals is by planting plants that are able to uptake the metal from the soil. This method is known as phytoremediation. In this study the plants used was mendong plants and its combination treatment with NPK fertilizers, bacteria, and compost.

Pramono *et al.* (2012) described that rhizobacteria can affect the availability of soil Pb. So it can cause Pb easily or difficult to be uptaken by plants. In general, each plant has the ability to accumulate different heavy metals. Mendong plants are known in uptake heavy metals such as Pb (Dewi and Hindersah 2009). According to Charlena (2004) the content of dangerous heavy metals such as Pb and Cd are available in the soil and uptaken by food crops commonly consumed by humans as a result of excessive use of fertilizers. The purpose of this study was to study the effectiveness of bioremediation using *Agrobacterium* sp. I_3 and compost as chelator combined by Mendong plant and NPK fertilizers, and learn the ability of Mendong in uptaking Pb soil and study the most affective combination among the treatments.

MATERIALS AND METHODS

The research was held on May-November 2016. The research place was located in Waru Village, Kebakkramat Sub-District, Karanganyar District, Central Java (7030'36,4"SL and 110054'21,4 "EL, ± 108m above sea level). Laboratory analysis was carried out in the chemistry, physics and biology laboratory of Agriculture Faculty, Universitas Sebelas Maret, and Central Agricultural Research Institute of Central Java.

Materials. Materials needed include seedlings of mendong plants, inoculum of *Agrobacterium* sp. I_3 (10^6cell.g^{-1}soil), compost for mendong plant that was 5 t.ha^{-1} (3.125 kg.m^{-2} = 0.75 kg/plot), NPK fertilizer for mendong plant with dose of each fertilizer that was: Urea: 27 kg.ha^{-1} (19.56 g/plot) ; SP-36: 21.6 kg.ha^{-1} (25 g/plot); KCl: 36 kg.ha^{-1} (15 g/plot). Media and chemicals needed for making inoculum of *Agrobacterium* sp. I_3 include: LB (luria betani: 10 g pepton, 5 g NaCl, 5 g yeast, 18 g agar) agar and liquid, bacterial carrier (15 kg compost, 7.5 rice bran, 750 mL EM-4 , And 15 L water.) and chemicals for destruction, such as percloric acid dan nitric acid.

Methods. This research was conducted by field experiments. This was factorial research using Completely Randomized Block Design (RCBD) as the based design, with three factors (1) NPK fertilizers (P0: withoutNPK fertilizers, P1: with NPK fertilizers), (2) Chelator (K0: without chelator; K1: with chelator Agrobacterium Sp I_3; K2: with chelator compost), and (3) Plant (T0: without plant, T1: with mendong plant) to obtain 12 treatment combinations, each treatment combination was repeated 3 times. The observed variables were Pb concentration in soil (Wet Destruction and detected by AAS), soil parameters analyzed were CEC (Amonium Asetat Saturation), C-Organic (Walkley and Black), pH H_2O (Electrometric), and total bacterial colonies (Plate count), content and uptake of Pb roots and shoot of mendong plant (Wet Destruction detected by AAS), plant height, and dry weight of biomass.

Preparation of Bacteria Carrier. Materials of carrier used in this study consist of : 15 kg compost, 7.5 kg rice bran, 750 mL EM-4 , And 15 L water. These materials were mixed well and then incubated for 2 months, becoming carrier of bacteria. Then this carrier was sterilized using a presto pan for keeping the carrier sterile from undesirable bacteria or fungi.

Preparation of *Agrobacterium* sp. I_3 Inoculum. Propagation of *Agrobacterium* sp. I_3 inoculum was started by preparing the LB (Luria Bertani) medium with the composition of 10 g tripton, 10 g NaCl, 5 g yeast extract, 100 mL destilled water; 15-20 g NA (Nutrient Agar) medium with the composition of 10 g beef extract, 10 g pepton, 5 g NaCl, 1000 mL destilled water, and 15 g agar L^{-1}. After obtaining pure isolate of *Agrobacterium* sp. I_3, purification was done in Luria Bertani liquid in Erlenmeyer and and shaked out to gain the density of 10^{10} cells mL^{-1}. Sterile Carrier was then enriched with squirted *Agrobacterium* sp. I_3. The comparison was 600 mL *Agrobacterium* sp. I_3 for 2 kg of the carrier.

Implementation of the Study. The size of the plot of land was1.5 m x 1 m (sub plot : 0.5 m x 0.5 m). Seeds of Mendong plant needed were six per plot (one seed per sub plot), with spacing of plant per sub plots was 50 cm x 50 cm. Inoculum of *Agrobacterium* sp. I_3 and compost were added 7 days before planting. NPK fertilizer was added one day before planting. Harvest was carried out 30 days after the Mendong added by bacterial innoculum. Field observations were plant height of one time every week and plant dry weight after harvesting. Plant dry weight devided into two parts : root and shoot of Mendong plant.

The data were analyzed using anova test of 95%

and 99% confidence level, with the continued test of DMRT 95% confidence level and correlation test.

RESULTS

Characteristics of Soil Before Treatment. The land used was paddy soil that has a fairly abundant water with the type of soil that is vertisol (USDA 1999). The characteristics of initial soil (Table 1) showed that the soil used in this study has soil pH of 7.55 (netral). CEC of 19.614 cmol (+) kg^{-1} was classified in medium grade. The soil C-organic was high at 3.31%, which could create good environment in providing nutrients for bacteria or plants. The total population of bacterial colonies in this initial soil condition was 12.62 Log 10 CFU.g-1 (10^{12} g^{-1}) and Pb concentrations in this initial soil was 12.203 µg g^{-1}. Based on GR (Government Regulation) no. 101 (2014), its value has exceeded the allowable limit.

Pb Concentrations in Soil. Lead (Pb) concentration in initial soil was 12.203 µg g^{-1} (Fig 1). Soil Pb concentrations of all treatment including control were decreasing from soil Pb concentrations of initial soil (Fig 1). The results of Anova showed that chelator and mendong significantly affected the decreasing concentration of Pb soil. Control P0K0T0 treatment has the lowest decreased of Pb soil (27.18%) from initial soil with value of 9.129 µg g^{-1}, while P0K2T1 treatment has the highest decreased of Pb soil, that was 42.41% with value 7.028 µg g^{-1} (Fig 2).

The effect of compost significantly different with treatment without chelator (Fig 3). Compost and Mendong could decrease Pb concentration in soil. Mendong plant (P0K0T1 treatment) also has highly decreased in concentration of soil Pb, it was 39.55% from initial soil, the value was 7.376 µg g^{-1}. This suggests that Mendong has high ability in uptaking Pb in soil. Based on the correlation test of soil chemical characteristics, it is suggested that Pb concentration in the soil was positively correlation with Pb concentration and Pb uptake in plants.

Pb Concentration and Uptake in Mendong. The mean values of Pb concentrations in roots and shoots of mendong have exceeded the normal limit allowed for food crops ranging from 0.5 to 3 µg g^{-1} (Widaningrum *et al.* 2007). The highest Pb concentration in the root was in P1K0T1 treatment which was 7.527 µg.g^{-1}, while the highest Pb concentration in the shoot was in P0K2T1 treatment which was 4.593 µg g^{-1}. The average Pb concentrations in roots was higher than that of shoots. Based on the correlation test, Pb concentration in plant was negatively correlated to height and biomass of Mendong plant.

The highest Pb uptake in root was in P0K0T1 treatment which was 58 856 µg (Fig 4). The highest Pb uptake in shoot was found in P0K2T1 treatment which was 31.581 µg. Generally, chelator, *Agrobacterium* sp. I_3 and compost, influencing the increasing of Pb uptake in shoots, so it could increase total Pb uptaken by plant (including Pb uptake in root and Pb uptake in shoot), the uptake higher than plant treatment only. Therefore, it is very recommended to do when the aim of bioremediation is uptake much soil metal. Based on the results of Anova, chelator significantly effected to Pb uptaken by plant, but no interaction between fertilizer and chelator.

The result of DMRT, was that mendong plant has high ability in uptaking Pb in root of plant, with the highest Pb uptake in root 58.856 µg (Fig 5). Different with plant, the influence of chelator treatment, especially compost caused the lower of Pb uptake in root than that with plant treatment only which was 22.456 µg.

P1K2T1 treatment tend to has low Pb concentration and uptaken by plant (2.720 µg g^{-1}) with an uptake value 38.707 µg. P1K0T1 treatment tend to has high Pb concentration in plant (5.234 µg g^{-1} with uptake value of 80.916 µg (Table 2). Chelator tend to have low Pb concentration and Pb uptaken by Mendong plant.

Height and Dry Biomass of Mendong Plant

Plant Height. Based on result of Anova, fertilizer treatment very significantly effected ($P < 0.01$) to plant height of Mendong plant. But the interaction between fertilizer and chelator did not effected to plant height. The height of Mendong with P0K0T1 (control) treatment, was 50 cm and the highest height of Mendong found in P1K2T1 treatment that was 65.64 cm (Table 3). NPK Fertilizer treatment, influencing the average of plant height, higher than treatment without NPK fertilizer.

Plant Dry Weight (Plant Biomass). Based on the result of Anova of NPK fertilizers, chelator, and its combination were not significant effect to plant biomass. The dry plant biomass of the mendong with a combination of P1K1T1 treatment (15.80 gr) tended to be higher than control (13.96 gr) and other treatments (Table 3). Fertilizer treatment higher effected to plant biomass, it was 15.16 g. Based on the correlation test, the height of mendong plant have a positive correlation

Table 1 Characteristics of initial soil

Parameters	Unit	Value	Grade
pH	-	7,55	Netral*
C-Organic	%	3,31	High*
Cation Exchange Capacity (CEC)	cmol(+).kg^{-1}	19,61	Medium*
Bacteria Colonies Total	Log 10 CFU.g^{-1}	12,62	High*
Lead (Pb)	μg.g^{-1}	12,203	High*

Source: Laboratory Analysis (Primer)
Information: *) According to Soil Research Institue 2009
*) Government Regulation No. 101 (2014)

Table 2 Total Pb in Mendong Plant

No.	Treatment	Pb Concentration in Plant (μg.g^{-1})	Pb uptaken by Plant (μg)
1	P0K0T1	5,191	72,449
2	P0K1T1	4,387	63,179
3	P0K2T1	4,362	59,355
4	P1K0T1	5,234	80,916
5	P1K1T1	4,834	76,363
6	P1K2T1	2,720	38,707

Source: Primer

Table 3 Plant height and plant dry weight (plant biomass)

No.	Treatment	Plant Height (cm)	Plant Dry Weight (Plant Biomass) (g)
1	P0K0T1	50.00	13.96
2	P0K1T1	51.42	14.40
3	P0K2T1	49.78	13.61
4	P0K0T1	53.56	15.46
5	P0K1T1	61.72	15.80
6	P0K2T1	65.64	14.23

Source: Primer

with plant biomass.

DISCUSSION

Characteristics of Soil Before Treatment. The mobility of Pb absorbed by plant could be influenced by soil pH (Table 1). Soil with low pH (acid conditions) make the metal become dissolved, and easily absorbed by root of plants. The high value of soil C-organic indicated that the soil fertility rate was high. Generally the content of C-organic in land that was administered become paddy fields (wet soil) is higher (1.19-3.63%) than in dry soil (0.59-2.65%). The high C-organic in paddy field soil is superseded by the addition of organic material derived from the remnants of the roots of paddy plant also because of the slow decomposition process of soil organic matter. Haryanti (2013) described that the content of soil Pb was influenced by the fertility and soil organic matter content. Pb in the soil was almost always strongly bounded by a precipitated organic or colloidal material. Paddy field soils has higher CEC than dry soil. This is due to the low washing of the soil bases (Rahayu *et al.* 2014). Soil bacterias can increase soil CEC with decomposition of soil organic matter. Soil microbes have an important role in immobilization of soil cations (Rosariastuti *et al.* 2020).

Soil Pb Concentration. The Pb concentration of control also decreased (Fig 1). It happened maybe because of the Pb leaching by rainwater or water flow in the ground. Leaching is the process by which contaminants are transferred from a stabilized matrix to liquid medium, such as water or other solutions (Zheng *et al.* 2014). This phenomenon was unwanted because where lead will accumulate in the soil was unpredicted and it could cause environment pollution including soil in the newplace of Pb. High Pb concentration in soil can cause the decreasing quality of crop yield, because of the absorbing Pb by plant. This was due to the normal levels of Pb in plants ranging from 0.5 to 3 μg g^{-1} (Widaningrum *et al.* 2007). When Pb entering food chain cycle, it can cause a

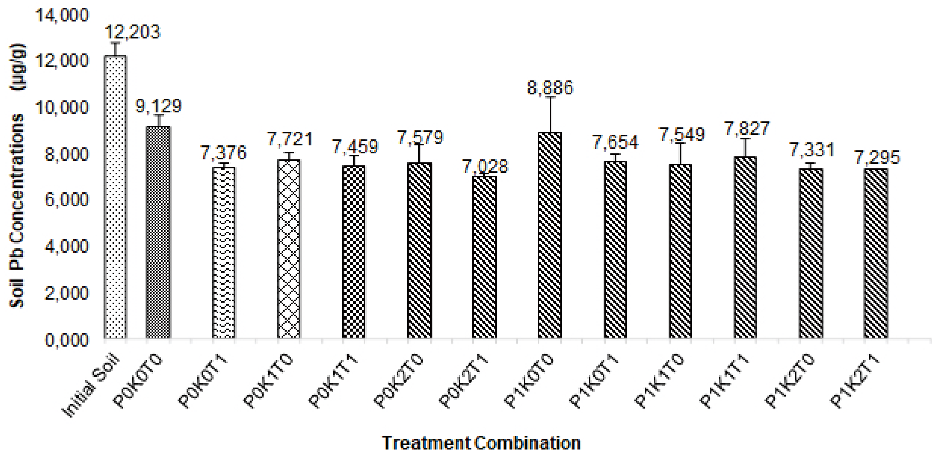

Fig 1 Histogram of soil Pb concentrations.

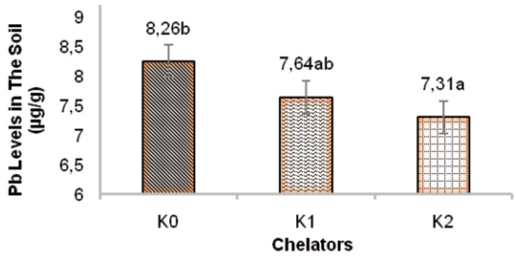

Fig 2 Histogram of the effect of Chelator to soil Pb concentration.

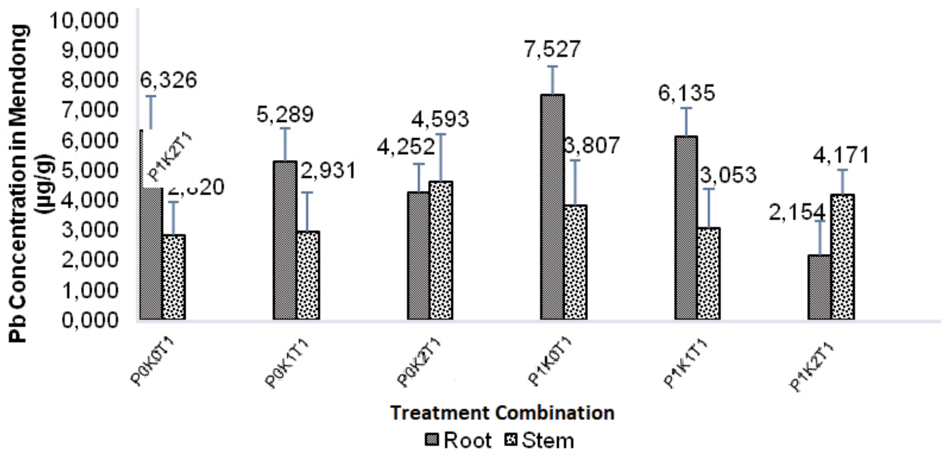

Fig 3 Histogram of Pb concentration in root and shoot of plant.

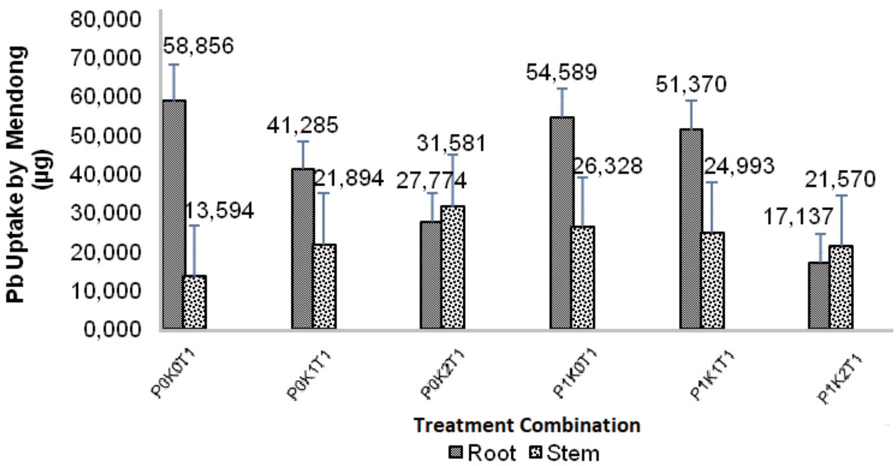

Fig 4 Histogram of concentration of Pb uptake by Mendong plant.

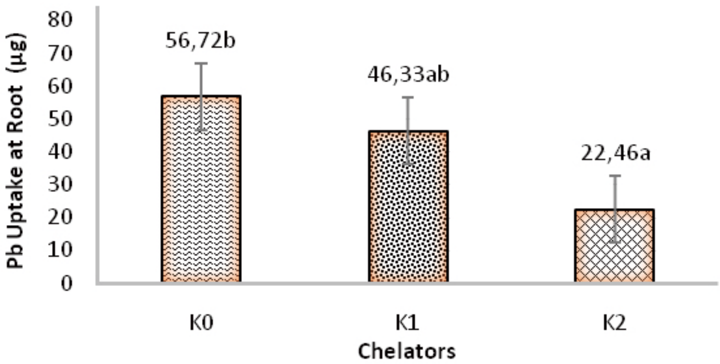

Fig 5 Histogram of the effect of Chelator to Pb uptake in root of plant.

problem to humans health. Heavy metals are transferred from abiotic envieronment to living organisms, accumulated in biota at different trophic levels, and thus contaminate the food chains. Trophic transfer, bioaccumulation, and biomagnification of hazardous heavy metals in food chains have important implications on wildlife and human health (Ali and Ezzat 2019).

Compost and mendong decreased Pb concentration in soil (Fig 2). Kucasov and Guvener (2009) described that compost contains humus substance (fulvic acid, humic acid, and humin) which could minimize soil heavy metals. Decreasing of soil Pb is not only caused by compost, but also by plant uptake. From this research, it is known that Pb concentration in the soil was positively correlation with Pb concentration and Pb uptake in plants. Schmidt (2003) explained that high concentrations of heavy metals in soil may lead to the increase of metal uptaken by plants.

Pb Concentration and Uptake in Mendong. The average Pb concentrations in roots was higher than shoots (Fig. 3). According to Indrasti *et al.* (2006) inhibited metal translocation from the roots to shoots, would make plant become easy to detoxify the metal. Pb concentration in plant was negatively correlated to height and biomass of mendong plant, because of Pb cation uptaken by the roots, would enter into the plant, and it would be an enzyme-forming inhibitor then would inhibit the metabolic process of the plant (Amelia *et al.* 2015).

Generally, chelator, *Agrobacterium* sp. I_3 and compost, influencing the increasing of Pb uptake in shoots, so it could increase total Pb uptaken by plant (including Pb uptake in root and Pb uptake in shoot), higher than plant treatment only (Fig 4). It is very recommended combination when the aim of bioremediation is uptake much soil metal. Compost application induced a different behavior in both species. Compared to wheat and irrespective of As doses, in compost amended soils, barley plants showed an enhanced As translocation to the aerial part. The different bacterial communities structure found for each species, according to the PCR-DGGE cluster analysis, suggested that specific rhizobacteria of barley may have increased As bioavability, and would therefore enhance its translocation to aerial parts (Gonzales *et al.* 2019).

Mendong plant has high ability in uptaken Pb in root of plant, with the highest Pb uptaken in root, but *Agrobacterium* sp. I_3 and compost treatment decreased Pb concentration and Pb uptaken by plant (Fig5, Table 2). Humic acid in compost has ability in bonding either metal ion, or organic compound bonding. According to Tan (2003) the negative charge in humic acid has ability to react and interact with positively charged of metal ions, thereby decreasing Pb uptaken by plants, but on the other hand it could increase the metal traslocation from root to shoot of plant.

Height and Dry Biomass of Mendong Plant. NPK fertilizer treatment, influencing the average of plant height , higher than treatment without NPK fertilizer (Table 3). Fertilizer adduction was very effective to increase fertility and plant growth. NPK Fertilizer, chelator treatment and NPK fertilizer+chelator interaction had a significant effect on plant biomass (Rosariastuti *et al.* 2019). Wasis and Fathia (2010) explained that the use of NPK fertilizers would provide substantial N supply to the soil, which would help plant growth. The average metall content in plants in all treatment combination showed the high levels of metal content in mendong plant, but the plants did not show significant damage such as chlorosis and necrosis. It suggests that mendong could be good plant for phytoremediation of Pb metals.

Dry plant biomass of the mendong plant with a combination of P1K1T1 treatment tended to be higher than control and other treatments. Fertilizer treatment higher effected to plant biomass than without fertilizer. Plant biomass of the mendong were influenced by soil plant nutrients, so fertilizer influenced plant biomass of mendong. The height of mendong plant have a positive correlation with plant biomass. According to Ekowati and Nasir (2011) plant biomass was the result calculation of all plant organs. High plant which has many leaves assumed that the plant biomass would be also high. The result of correlation test also showed that higher Pb levels in plant would cause the low of plant biomass. Metal contamination, as a consequence of anthropogenic activities, poses threat to plants and their ecosystems. Plant growing on metal contaminated soils show severe aberration in their metabolism leading to stunded plant growth and low biomass production (Saboor *et al.* 2019).

Conclusion of this research are that treatment combination of compost and mendong plants (without artificial fertilizer) was the best treatment in the lowering soil Pb concentration from initial soil Pb (7.028 µg g^{-1}) so that there was a decrease of soil Pb concentration from the initial soil by 42.41%. Mendong plant has high ability in uptaken Pb, especially in root. Chelator (*Agrobacterium* sp. I_3 and compost) tends to increase Pb uptake in root of plant, but decrease Pb uptake in shoot. Compost decreased total Pb concentration and uptake in plant. NPK fertilizer tend to increase plant dry weight (biomass), and by its combination with other treatments, the treatment combination tend to increase of Pb uptaken by plant. The highest Pb concentration (5.234 µg.g^{-1}) and Pb uptake in plant (80.916 µg) was in treatment combination of NPK fertilizer and Mendong plant, followed by treatment combination of NPK fertilizer and *Agrobacterium* sp. I_3 and Mendong plant which has Pb uptaken by plant of 76.363 µg.

REFERENCES

Ali H, Ezzat K. 2019. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Hum Ecol Risk Assess. 25(6):1353-1376. doi: 10.1080/10807039.2018.1469398.

Amelia RA, Rachmadiarti F, Yuliani. 2015. Analisis kadar logam berat Pb dan pertumbuhan tanaman padi di area persawahan Dusun Betas, Desa Kapulungan, Gempol-Pasuruan [Analysis of heavy metal Pb and the growth of paddy in paddy field area Dusun Betas, Desa Kapulangan, Gempol-Pasuruan]. Lentera Bio. 4(3): 187–191.

Ekowati D, Nasir M. 2011. Pertumbuhan tanaman jagung *(Zea mays L.)* varietas Bisi-2 pada pasir *Reject* dan pasir asli di pantai Trisik Kulonprogo [The growth of maize (*Zea mays L.*) variant Bisi-2 in rejected and original sand in Trisik Beach, Kulonprogo]. J Manusia Lingkungan. 18(3): 220-231. doi: 10.22146/jml.18445.

Gonzales A, Pilar GG, Mar GD, Juan A, Carmen L. 2019. Compost-assisted phytoremediation of As-polluted soil. J Soils Sediments. 19(7):2971-2983. doi: 10.1007/s11368-019-02284-9.

Haryanti D, Dedik B, Salni. 2013. Potensi beberapa jenis tanaman hias sebagai fitoremediasi logam timbal (Pb) dalam tanah. [Potential of several types of ornamental plants as phytoremediation of Lead Metal (Pb) in soil]. J Penelitian Sains. 16(2): 52-58. doi: 10.36706/jps.v16i2.72.

Indrasti NS, Suprihatin, Burhanudin, Aida. 2016. Penyerapan logam Pb dan Cd oleh Eceng Gondok : Pengaruh konsentrasi logam dan lama waktu kontak. [Pb and Cd metal absorption by Eceng Gondok: Effects of metal concentration and contact duration]. J Tek Ind Pert.16(1): 44-50.

Kucasov G, Gunvener Z. 2009. Efficiency of compost in the removal of heavy metals from the industrial wastewater. Environ Geol. 57: 291-296. doi: 10.1007/s00254-008-1372-3.

Pramono A, Rosariastuti R MMA, Ngadiman, Prijambada. 2012. Peran rhizobakteri dalam fitoekstraksi logam berat Kromium pada tanaman jagung [The role of Rhizobacteria in the heavy metal chromium extraction in corn plants]. J Ecolab. 6(1):1-13. doi: 10.20886/jklh.2012.6.1.38-50.

Rahayu A, Utami SR, Rayes ML. 2014. Karakterisitik dan klasifikasi tanah pada lahan kering dan lahan yang diswahkan di Kecamatan Perak, Kabupaten Jombang [Characteristics and classification of soil on dry and leased land in Perak District, Jombang Regency]. J Tanah Sumber Daya Lahan. 1(2):79-87.

Rosariastuti R, Muhamad Sf, Purwanto, And Suntoro. 2020. Effects of *Agrobacterium* sp. I_{26}, manure and inorganic fertilizers to Pb content of rice grains planted in Pb polluted soil. Environ Nat Resour J. 18(1):75-84. doi: 10.32526/Ennrj. 18.1. 2020.08.

Rosariastuti R, Selly M, Sudadi Sudadi, Sri H, Purwanto P. 2019. Remediation of chromium contaminated soil by phyto-bio system (Pbs) application. Sains Tanah - J Soil Sci Agroclimatol. 16(1):90-102. doi: 10.20961/stjssa.v16i1.24932.

Saboor A, Ghulam M, Muhammad A, Muhamad A, Saiiad H. 2019. Seed priming and metal/metalloid stress tolerance in plants. In: Priming and pretreatment of seeds and seedling. Springer, Singapore. 287-311. doi: 10.1007/978-981-13-8625-1_14.

Schmidt U. 2003. Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. J Environ Qual.32(6):1939-1954. doi: 10.2134/jeq2003.1939.

Tan KH. 2003. Humic matter in soil and the environment. CRC Press: New York.

Wasis B, Fathia N. 2010. Pengaruh pupuk NPK dan kompos terhadap pertumbuhan semai Gmelina (*Gmelina arborea* roxb) pada media tanah bekas tambang emas (tailing) [The effect of NPK fertilizer and compost on the growth of Gmelina seedlings (*Gmelina arborea* roxb) on the land of the former gold mine (tailings)]. J Ilmu Pertanian Indonesia. 16(2):123-129. p-ISSN: 0853-4217.

Widaningrum, Miskiyah, Suismono. 2007. Bahaya kontaminasi logam berat dalam sayuran dan alternatif pencegahan cemarannya [Danger of heavy metal contamination in vegetables and alternative prevention of contamination]. Buletin Teknologi Pascapanen Pertanian. 3:17-27.

Zheng SA, Zheng X, Chen C. 2012. Leaching behavior of heavy metals and transformation of their speciation in polluted soil receiving simulated acid rain. PLoS One. 7(11):e49664. doi: 10.1371/journal.pone.0049664.