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Research Article

Phytoremediation of Pb contaminated paddy field using combination of *Agrobacterium sp*. I3, compost and ramie (*Boehmeria nivea*)

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Abstract : Industry sector exerts a negative effect on the environment. Industrial waste is often disposed to the rivers. The industry contributes to the accumulation of heavy metals in the environment. In farming sector, the accumulation of heavy metals can result in water pollution and be washed into the ground. Therefore, the farming product can be contaminated with heavy metals and they can be harmful to human health. The objective of this research was to reduce the Pb heavy metal content in paddy soil. This research was taken place in the Pb contaminated paddy soil using a randomized complete block design with three factors and three replicates. This research employed combination of inorganic fertilizer, ramie and *Agrobacterium sp*. I_3 or compost as a chelator to improve Pb uptake. The results showed that combination of the three treatments increased Pb uptake. The combination of chemical fertilizers, ramie with compost increased the Pb uptake of 11.93 μg/g or 45.9%. The combination of chemical fertilizers, ramie with *Agrobacterium sp.* I_3 resulted in the highest Pb uptake of 12.85 μg/g or 49.8%. The combination also decreased the soil Pb level by 7.8 μg/g or 23.5% of the control.

***Keywords**: Agrobacterium, chelator, heavy metal, industry, paddy field*

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Introduction

Industrial sector exerts not only a positive effect on the economy but also negative one on environment. Industrial waste is often disposed to rivers (Chen and Chen, 2001). Considering the result of previous studies, the industry contributes to the accumulated heavy metal in an environment (Taiwo et al., 2010). Heavy metal accumulation in the soil could to the decreased microbial activity, soil fertility, soil quality, and hazardous compound entering into agricultural product yield impacting on the impaired human health (Qing et al., 2015). In agricultural sector, the accumulation of heavy metal can result in water pollution and be washed into the ground (He et al., 2004), inhibit the plant growth, reduce biomass production thereby resulting in some economic loss (Nagajyoti et al., 2010); therefore the heavy metal pollution in Agricultural soil should be improved (Huang et al., 2016). Lead (Pb) is a less degradable metal in nature and very toxic to animal and human being when it enters into the food chain (Papanikolaou et al., 2005). Out of many heavy metal types, Pb is the one found most widely in an environment (Patra et al., 2004). Pb can be accumulated in the soil, plant or human body thereby harmful to health particularly in children (Li et al., 2016). Health impairment includes impaired growth and development in children, reduced intelligence, lost short-term memory, and cardiovascular disease risk (Dixit et al., 2015). Pb can exert an adverse effect on seed sprouting, plant growth and photosynthesis (Ruley et al., 2006). Bioremediation is a solution to degrade and to transform the pollutant existing in the soil and phytoremediation to degrade and to transform the pollutant or heavy metal in the soil

(Moosavi and Seghatoleslami, 2013). In situ technique is popular and often used a technique to remedy the soil contaminated with heavy metal (He et al., 2015; Khan et al., 2015).

The plants that can be used for phytoremediation with sufficiently high economic value are, among others, ramie (*Boehmeria nivea*). Ramie is a fibrous plant tolerant to various extreme environments such as drought, infertility, disease, pest invasion and heavy metal contamination. Ramie has been recorded as the plant living dominantly in many different metal-contaminated areas and can accumulate Cd in a large number (Wei S et al., 2011). Compost can affect heavy metal immobilization in the soil (Huang et al., 2016). The addition of compost can reduce Pb concentration in the soil (Beesley et al., 2014) and increase the metal by increasing pH of the soil (Karami et al., 2011).

In a previous study, Rosariastuti et al. (2013) found that *Agrobacterium sp* could improve heavy metal absorption in the soil and yielded a good plant growth. Considering the result of research existing about phytoremidiation, Ramie is expected to reduce Pb content in the farming land so that inoculation of *Agrobacterium sp* or compost in ramie plant in affecting accumulation of Pb should be identified and studied further. The objective of current research was to explore the potential phytoremediation of combined *Agrobacterium sp* or compost and Ramie plant applied in the Pb-contaminated farming land.

Materials and Methods

Agrobacterium sp inoculant used was obtained from results of a study conducted by Rosariastuti et al. (2013).The bacterium is later called as *Agrobacterium sp* I3. The bacteria cells were grown on 200 mL Luria-Bertani broth medium with the composition of 10g peptone, 5g yeast extract, 5g NaCl and 15g agar (Rajkumar et al., 2006), and were centrifuged overnight. In the following day, inoculums were grown on 10 pcs. of 200 mL Luria-Bertani broth and were centrifuged overnight as well or until the culture contained 1010 cell/g bacterial population. Then, the culture containing 1010 cell/g bacteria was poured into sterile compost so that bacteria inoculum was obtained in compost containing 109 cell/g bacteria. Compost carrier was prepared using 7.5 kg compost, 750 mL EM-4 and 15 L water.

The materials used were mixed and incubated for 2 months. Compost sterilization used presto pan. Ramie plant seed was grown with stem cutting method. The ramie planting media used for seedling were an Alfisol and compost in a ratio of 1:1. The planting media were then put into a polybag in 15x15 cm dimension. Inorganic fertilizer and compost used were bought from the provider of farming production media. Fertilizer application for ramie plant was 5 t/ha compost. In addition to inorganic fertilizer, N, P, and K fertilizers were given in the beginning of planting period at doses of 60 kg N + 30 kg P_2O_5 + 30 kg K_2O/ha. The research was conducted using a completely randomized block design and factorial treatment design with three factors of inorganic fertilization, inoculation of *Agrobacterium sp.* I3 or compost and ramie. Thus, twelve treatment combinations were obtained with three replicates so that thirty-six experimental pots were obtained. The research design included P0 (without fertilization), P1 (inorganic fertilization), B0 (without *Agrobacterium sp*. I3 and compost), B1 (application of *Agrobacterium sp* I3), B2 (compost application), T0 (without plant), and T1 (ramie).

This research was taken place in a Pb-contaminated farming soil in Ngringo of Jaten Sub-District, Karanganyar Regency, Central Java7°33'32.05"S-110°52'2.05"E. Based on USDA's classification, soil of the study location is a Vertisol (BPS Karanganyar). The characteristics of soil are as follows: pH of 7.25 (neutral), organic material of 1.95% (low), cation exchange capacity of 22.4 cmol/kg (moderate), Pb level of 8.01 mg/kg (normal), and total soil microorganism of 7.60 Log 10CFU/g (high).

Soil and plant analyses

Samples of soil deriving from research location were stored using clear plastic, and then air dried. The samples used for biological analysis were stored in a refrigerator. The soil samples were sieved into 0.05 mm fraction and then stored at room temperature to be analyzed for pH, C-organic, CEC (cation exchange capacity), Pb (lead) concentration. The soil pH was determined in 1: 2.5 w / v soil: water mixture. Particle-size distribution was determined by sedimentation method (Day 1965). C-organic was determined using Walkley and Black (1934) method. CEC was determined by the neutral normal ammonium acetate method (Richards1954).The Plant samples were washed using water and then divided into root and shoot. The samples were dried using oven at 65^{o}C until constant weight and then ground to be analyzed. The analysis included Pb content and absorption by root and shoot. Pb concentrations in rhizosphere soils of the plants were determined by AAS (Jiang et al., 2008).

Statistic analysis

Data obtained from observation were analyzed using SPSS 16.0 application. The data of research was analyzed using ANOVA with F-test 5%. To find out the difference of treatment, Duncan Multiple Range Test (DMRT) was done and to test the inter-variable relationship, correlation analysis was conducted.

Result and Discussion

Effect of treatments on soil variables

Soil pH

pH of soil changed after the treatment (Table 1). The three treatments increased pH of soil compared with control. Wie et al. (2011) stated that pH close to neutral could support the biological activity of soil, degradation process and lead binding. P0B2T0 increase 2.5 % of pH compared with the control. Beesley et al. (2010) also reported that biochar and compost could increase the pH of soil. Farrell et al. (2010) stated that compost increased pH and organic material of soil.

Organic material will improve Ca^{2+} ion content in soil solution. The increase of Ca^{2+} ion in soil solution will replace Al^{3+} and H^+ and bind Al^{3+} becoming unsolvable Al^{3+} complex so that pH of soil will increase, thereby decreasing Pb concentration in the soil solution. Uchimiya et al. (2010) also stated that the increase of soil pH could increase heavy metal immobilization.

Soil organic matter

Soil organic matter content increased after the treatment (Table 1). The treatment of *Agrobacterium sp.* I3or compost increased significantly ($p < 0.05$) organic matter content compared with control. Such the increase was due to the presence of compost in the two treatments. The highest increase (3.22%) occurred in P0B2T1 treatment. The results showed that the addition of compost into soil increased soil organic matter content. Compost is rich of organic material content, thus the material addition into soil can affect the organic material of soil. Compost can increase heavy metal content in soil solution (Angelova et al., 2013; Taiwo et al., 2016).

Cation exchange capacity

CEC is a key soil chemical property of the adsorption capacity of a soil. Cation exchange capacity increased after treatments (Table 1). The highest increase of cation exchange capacity (62.2 cmol/kg) occurred in P1B2T0. The increase of soil cation exchange capacity was due to the addition of compost that could increase the organic material content of the soil. Kargar et al. (2015) stated that the addition of compost to soil would increase cation exchange capacity, thereby increasing the soil's absorption of heavy metal. Walker et al. (2003) reported that the use of compost and manure in heavy metal-contaminated soil could increase the cation exchange capacity by 21%. The increase of soil cation exchange capacity will increase the exchangeable metals.

Table 1. Chemical characteristics of soil

Treatments	pH	Organic Matter (%)		Cation Exchange Capacity (cmol/kg)		Total bacterial colony Log10 (CFU/g)		Pb concentration (mg/kg)	
P0B0T0	7.56	2.25	a	50.77	bcd	8.55	bc	10.23	cde
P0B0T1	7.64	2.24	a	55.88	de	7.31	ab	10.67	f
P0B1T0	7.66	3.05	b	54.38	d	9.89	dc	9.19	bc
P0B1T1	7.70	2.78	b	51.48	bcd	7.23	ab	9.67	cde
P0B2T0	7.75	2.71	b	50.64	bcd	7.32	ab	9.49	de
P0B2T1	7.58	3.22	b	53.91	bcd	6.98	a	10.36	de
P1B0T0	7.65	2.07	a	45.65	abc	9.83	dc	13.07	f
P1B0T1	7.72	2.43	a	38.40	a	9.30	dc	8.36	ab
P1B1T0	7.64	2.87	b	40.00	a	10.73	d	9.81	cde
P1B1T1	7.73	2.53	b	45.59	abc	10.65	d	7.81	a
P1B2T0	7.73	3.05	b	43.71	ab	10.25	d	7.87	a
P1B2T1	7.69	2.78	b	62.24	e	9.26	dc	9.54	cd

Notes : P0 (without fertilization) P1 (Inorganic fertilization), B0 (without *Agrobacterium sp*. I3 and compost) B1 (application of *Agrobacterium sp* I3) B2 (compost application), T0 (without plant) T1 (ramie). Values followed by different letters in each column of each treatment differ significantly with P<0.05.

Total bacterial colony

Total bacterial colony changed after the treatments (Table 1). The highest total microorganism (0.73 Log10 (CFU/g) existed in the P1B1T0 treatment. Su et al. (2014) reported that the use of inorganic fertilizer in long-term could increase the soil nutrient content and microbial activity. Inorganic fertilizer can change the functional gen diversity and microbial diversity significantly. The change of functional gen diversity includes the gen involved in C and N, P, S cycles. Chelator of *Agrobacterium sp.* I3 ($p > 0.05$) increased bacterial colony significantly; it showed that *Agrobacterium sp.* I3 is adaptable to the Pb-contaminated soil (Vijayaraghavan and Yun, 2008). To survive in the metal contaminant, bacteria develop some mechanism types to tolerate the heavy metal ion uptake. The mechanism of transferring metal ion to outside cell accumulates and creates metal ion compound in the cell thereby changing the toxic into non-toxic metal.

Heavy metal concentration

Pb concentration of soil is shown in Table 1. The application of *Agrobacterium sp.* I3or compost and Ramie affected significantly ($p < 0.05$) the Pb concentration of soil. P1B0T0 treatment increased Pb concentration up to 13.04 mg/kg or 38.4 %. This increase was expectedly due to the lead carried by irrigation water irrigating the farmland during planting season. The use of wastewater as irrigation source can accumulate heavy metal significantly (Ma et al., 2015). P1B1T1 and P1B2T0 treatments decreased Pb concentration of soil by 7.8 mg/kg or 2.6 %. Compost increases organic material content of soil. Organic material will increase the Pb availability in the soil solution due to ligand and complex compound (Park et al., 2011; Paradelo and Barral, 2017). Lang and Kaupenjohann (2003) state that ligand will chelate the heavy metal ion and increase its mobility. Metal ion adoption is highly dependent on soil pH. Pb adoption increases at pH 5-8 and increases slightly at pH 6-8. Soil will adopt Pb leading to its decreased concentration in the solution. Walker et al. (2003) reported that the use of compost and manure in heavy metal-contaminated soil could increase cation exchange capacity by 21%. The increase of soil cation exchange capacity will increase the content of exchangeable metals. The compost decomposition provides humus as the source of negative load in the soil. Karger et al. (2015) stated that the addition of compost into soil would increase the cation exchange capacity, thereby improving the soil heavy metal absorption.

Plant growth

Agrobcaterium sp. I3, compost and inorganic fertilizer increased significantly ($p< 0.05$) the height of Ramie plant (Table 2). Pramono et al. (2013) state that *Agrobcaterium sp* I3 is rhizobacter group that can support the plant growth by synthesizing precursor phytotohormone, vitamin, enzyme, siderophore and antibiotic. The improvement of plant growth, according to Hindersah and Matheus (2015), can occur due to the presence of biofertilizer and biostimulant by rhizobacteria. Rhizobacteria can serve as phosphate solvent fixating N and stimulating the growth through producing phytothormone such as auxin. Inorganic fertilizer given can provide nutrition available in the plant, thereby improving the plant growth and productivity. In addition, basic fertilizer can increase root development thereby absorbing nutrient maximally. The use of compost can improve soil fertility, microbial growth, physical property, aeration and drainage as well (Brunetti et al., 2012). *Agrobcaterium sp*. I3 and compost decreased biomass of ramie plant roots and improved the shoot biomass (Table 2).

Table 2. Plant growth

Treatments	Plant Height (cm)		Dry weight (g)			
			Root	Shoot	Plant	
P0B0T1	17.09	a	0.28	3.98	4.26	a
P0B1T1	18.59	b	0.22	5.16	5.35	b
P0B2T1	18.17	a	0.08	5.33	5.41	ab
P1B0T1	18.44	a	0.09	5.07	5.16	a
P1B1T1	27.34	a	0.16	5.80	5.96	b
P1B2T1	18.46	a	0.08	5.06	5.17	ab

Notes : P0 (without fertilization) P1 (Inorganic fertilization), B0 (without *Agrobacterium sp*. I3 and compost) B1 (application of *Agrobacterium sp* I3) B2 (compost application), T0 (without plant) T1 (ramie). Values followed by different letters in each column of each treatment differ significantly with P<0.05.

The increase of plant height that was not followed by the increase of plant dry weight indicates that the photosynthesis dids not run well (Hindersah and Matheus, 2015). Pb cation absorbed by roots will be the inhibitor in enzyme creation that can harm the plant's metabolism process. The inhibited metabolism activity involves respiration process producing ATP. ATP can be used in the photosynthesis process of plant (Amelia et al., 2008). Overall *Agrobcaterium sp*. I3 and compost increased significantly ($p<0.05$) dry weight of ramie (Table 2).

Rizhobacteria can produce IAA, siderophores and ACC deaminase which can stimulate the growth of plants and protect plants from heavy metal toxicity (Ma et al., 2011). Chen et al. (2013) reported that bacterial inoculation in plant rhizosphere grown on Cd contaminated soil significantly increased dry weight of the plant. Dary et al. (2010) also reported that inoculation of *Bradyrhizobium sp.* 750 to plant rhizosphere *Lupine luteus* could increase plant biomass by 29%.

Rodriguez-Vila et al. (2015) reported that application of 95% compost and 5% biochar increased biomass of *Brassica juncea* L. Mahmoud and Abd El Kader (2015) stated that adding compost to soil contaminated with heavy metals could improve dry weight of the kanola plant. Brunetti et al. (202) stated that the addition of compost and / or *Bacillus* might increase the dry weight of the brassicaceae plant. Compost can provide nutrients for plant growth. Courtney and Harrington (2012) stated that the addition of compost could increase nutrients such as N, P, K and Mn in soil.

Pb concentration in plant

The successful of phytoremediation is dependent on the increase of plant biomass and heavy metal concentration (Usman and Mohamed, 2009; Rosariastuti et al., 2013). The results showed that the application of *Agrobacterium sp.* I3, compost, and inorganic fertilizer did not affect significantly ($p > 0.05$) the Pb concentration of ramie plant root and shoot. However, the treatment using *Agrobacterium sp.* I3and inorganic fertilizer provided the highest increase (12.59 mg/kg). Bacteria inoculation in the plant potentially improves phytoextraction. Microorganism produces organic extracellular chelator, sidorephores and ligand that can improve the metal mobility so that it is absorbable to the plant (Sessitsch et al., 2013). The similar result was reported by Rosariastuti et al. (2013 that the addition of rhizosphere bacteria into maize plant improved Cu content of maize plant. Sheng et al. (2008) said that the application of rhizosphere bacteria could improve the availability of Pb availability in rhizosphere and improve the plant growth. The use of compost improved Pb concentration in the plant tissue (Figure 1).

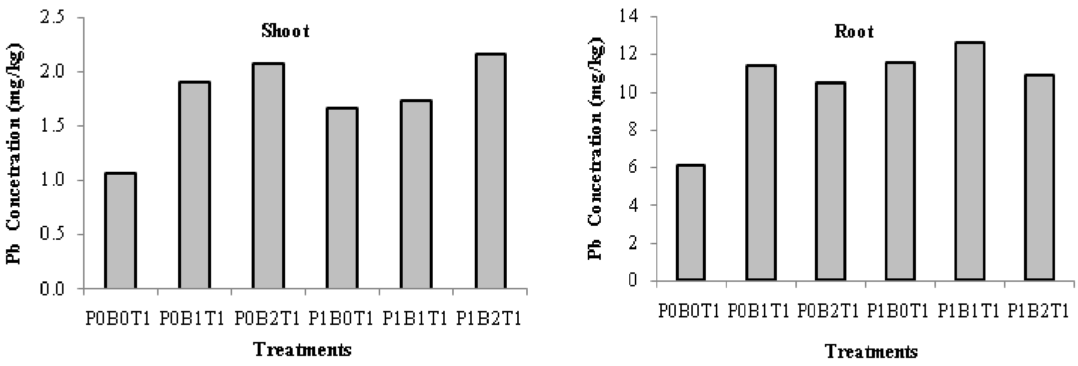

Figure 1. Effect of compost on Pb concentration in the plant tissue

Hattab et al. (2015) studied the effect of fresh and mature organic material on phytoremediation technique. They concluded that the use of compost improved the plant growth and Zu and Cu concentrations in the peanut. Pb concentration in the shoot that is lower compared with that in the root of plant indicates that its translocation value is low. Pb storage in the root occurs due to the change and extracellular deposition of ions. These two mechanisms occur in cellular wall (Jarvis and Leung, 2002). Pb not always penetrates into endoderm layer of root and stele. Endoderm can inhibit absorption and penetration into stele in order to prevent transportation (Weis and Weis, 2004). The increase in plant biomass and metal concentration increases the uptake of heavy metal by the plant (Rosariastuti et al., 2013). The application of *Agrobacterium sp.* I3, compost and inorganic fertilizer improved the Pb uptake by ramie plant (Figure 2). All treatments increased the absorption of ramie. The highest increase (12.53 mg/kg) occurred in the P1B1T1 treatment. Luo et al. (2012) reported that inoculation of rhizobacter *Bacillus sp.* improved sorghum plant biomass and absorption of Mn and Cu. Inoculation of *Bacillus* affects growth, harvest output and nutrient absorption. *Bacillus sp* can increase the nutrients such as P, Fe, S, Cu and reduce the toxic effect of heavy metal by secreting acid and protein (Saharan and Nehra, 2011).

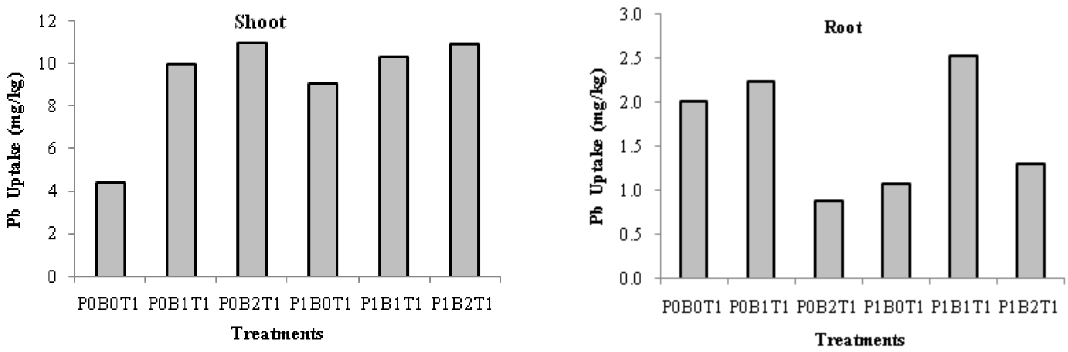

Figure 2. Effect of *Agrobacterium sp.* I3, compost and inorganic fertilizer on Pb uptake by ramie plant.

Conclusion

The application of inorganic fertilizer, *Agrobacterium sp.* I3 or compost and Ramie affected significantly the soil cation exchange capacity, Pb content and total microorganism but the application did not affect significantly the soil pH. The administration of inorganic fertilizer *Agrobacterium sp.* I3 or compost affected significantly the height and dry weight of ramie but it did not affect significantly Pb concentration and uptake in Ramie plant. But the use of combined inorganic fertilizer *Agrobacterium sp.* I3 or compost and ramie increased the absorption of Pb (84.8-99.2 %) in the Ramie. The Pb concentration of soil can be decreased to 7.81 mg/kg.

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