

Research Article

Application of *Agrobacterium sp. I₃₀* and vermicompost to suppress lead (Pb) uptake by rice in Pb polluted soil

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Abstract: Irrigation with textile wastewater and fertilization with an inorganic fertilizer containing lead (Pb) have negative impacts for agricultural sectors, especially lead contamination in a rice field and its uptake by rice plant. The rice grain that contains Pb is dangerous if consumed by humans, so it needs treatment to suppress Pb uptake by rice. *Agrobacterium sp. I₃₀* and vermicompost have a role as chelating heavy metals agents. The purpose of this study was to determine the effect of the chelating agents to suppress Pb uptake by rice in Kebakkramat, Karanganyar of Central Java. The method used was the quantitative method through a completely randomized block design with two factors (inorganic fertilizer and chelating agents). The results showed that application of *Agrobacterium sp. I₃₀* without inorganic fertilizer decreased Pb content in rice from 5.03 mg/kg to 4.97 mg/kg with a decrease in Pb uptake of 6.28%. Application of vermicompost without inorganic fertilizers decreased Pb content in the rice from 5.03 mg/kg to 1.61 mg/kg, with a decrease in Pb uptake of 72.18%. The use of both chelating agents can be recommended to suppress Pb uptake by rice in polluted soil and increases the safety of rice for consumption.

Keywords: *Agrobacterium sp. I₃₀*, Pb, rice, vermicompost

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Introduction

The progress of development with the use of various existing resources, the increasingly diverse activities by humans, and various industrial activities carried out, as one manifestation of the progress of civilization is always directly proportional to the increase in pollution to the environment. One of the pollutions that come from industrial activities is pollution of liquid waste. The textile industrial waste contains toxic materials that are harmful to the environment, water, soil, and human health. The textile industry in its productions process uses much water, especially in the process of dyeing textile products, most workers use chemical substances (Khatri et al., 2015). In the staining process, it is ensured that textile waste does

contain not only dyes but also toxic and hazardous heavy metals (Dixit et al., 2015). According to the Ministry of The Environment (2010), many of heavy metals that are produced by the textile industry are Ag, Cu, Cr, Pb, Cd, Hg, Ni and Zn. Zille et al. (2005) reported that heavy metals such as Cr 3.0 mg/L, Pb 28 mg/L, mercury 0.5 mg/L etc. were found in textile liquid waste.

Some fields in Karanganyar are located in a textile industrial area; one of them is Waru, Kebakkramat District. Industries that stand around agricultural land near the river or water source have the potential to cause pollution in agricultural lands (Wardhana, 1999). Textile industrial liquid waste is commonly discharged into the river around the industrial area that is used as one of the irrigation sources of rice fields in Waru. If the Pb level exceeds the water quality

standard for irrigation, then the metal will be absorbed by plant roots, and later stored in the body of the plants. Plants contaminated by a heavy metal such as Pb have the risk of poisoning if humans consume them. Food plants contaminated with Pb metal will be very harmful to human's health as consumers (Hirasawa, 1999).

Initial analysis of this study indicated that irrigation water containing Pb metal was 0.53 mg/kg, where its use will continue to increase the accumulation of Pb in the soil. Heavy metal pollution in rice fields is not only caused by industrial waste but also the behaviour of farmers in doing fertilization. Phosphate fertilizer as an inorganic fertilizer contains 5-156 mg Pb /kg, and 7 mg Cd /kg which can increase the availability of heavy metals in the soil, and increase heavy metals uptake by plant tissue if it is used repeatedly (Kurnia, 1999). Preliminary analysis on inorganic fertilizers (urea, phosphate, and KCl fertilizers) commonly used by the local farmers in farming indicated that urea fertilizer contains 0.0024 mg Pb/kg, phosphate fertilizer contains 0.3946 mg Pb/kg, and KCl fertilizer contains 0.0195 mg Pb/kg. They all contribute to the accumulation of Pb in the rice fields. Pb accumulated in the rice fields will be potentially absorbed by plant tissue. Through the food chain, Pb is deposited in the body parts of a living thing at one particular size that can cause toxicity (Mulyani, 2004).

Rice fields that have been irrigated with water from rivers contaminated with textile industry liquid waste in a textile industrial area of Kebakkramat contains 16.183 mg Pb/kg, yet the Limit of Threshold Value of Pb permitted under Reg. 101 the Year 2014 on the Management of Hazardous and Toxic Waste is 3 mg/kg. The Pb in the rice fields will be potentially taken up by rice plant tissue such as roots, shoots and rice (grains). The application of *Agrobacterium sp. I₃₀* and vermicompost as chelating agents can minimize this pollution problem by suppressing and sequestering heavy metals in root tissues (Hidayati, 2005). Environmental recovery by microorganisms is considered a potential strategy in reducing the heavy metals contamination in an environment (Gandjar et al., 2006). Rosariastuti et al. (2013) isolated *Agrobacterium sp. I₃₀* that can act as a chelating agent in rice fields exposed to heavy metals. The bacterial isolate can bind Pb in the soil and hold Pb that accumulates in the roots of the plant, thus suppressing the Pb content in the rice grains to be consumed by humans.

Another organic material that can be used as a chelating agent is vermicompost derived from worm rearing. Besides bacteria, the addition of vermicompost as a chelating agent to the soils can

reduce heavy metal ions (Sing and Khalamdhad, 2013). Vermicompost has good benefits in the field of agriculture to improve fertility, and improve the physical, chemical and biological properties of the soil, so that plant growth increases and suppresses Pb uptake is more optimal (Suntoro, 2010).

The purpose of this study was to explore the effect of the application of *Agrobacterium sp. I₃₀* and vermicompost as chelating agents to suppress Pb uptake by rice (grains) in Pb polluted soil.

Materials and Methods

The study was conducted in Waru, Kebakkramat, Karanganyar which is geographically located at coordinates 7°30'36.4" S-110°54'21.4" E, from June to September 2017. The tools used were microbial insulation equipment, destruction equipment, AAS (Atomic Absorption Spectrophotometer), pH analysis equipment, organic material analysis equipment, CEC analysis equipment. The materials used were rhizobacterial inoculums of *Agrobacterium sp. I₃₀* of Rosariastuti et al. (2013), vermicompost from local farmers, IR64 rice seedlings, inorganic fertilizers ((N as Urea, P as SP-36, and K as KCl), and chemical reagents.

This research used a factorial design with two factors, namely inorganic fertilizers (P) and chelating agents of *Agrobacterium sp. I₂₆* (B1) and vermicompost (B2). There were six treatment combinations as follows: control (P0B0), application of *Agrobacterium sp. I₃₀* without inorganic fertilizers (P0B1), application of vermicompost without inorganic fertilizers (P0B2), application of inorganic fertilizer without *Agrobacterium sp. I₃₀* and vermicompost (P1B0), application of inorganic fertilizer and *Agrobacterium sp. I₃₀* (P1B1), and application of inorganic fertilizer and vermicompost (P1B2). The six treatments were arranged in a completely randomized block design with three replicates.

There were 18 treatment plots used in this study. Plot size was 1.25 m x 1.25 m, with the distance between the planting holes was 50 cm, and the distance between plots was 100 cm. The number of planting holes at each plot was 25 holes. Dose of NPK inorganic fertilizer used ratio of N: P: K = 2: 1: 1 = 200 kg: 100 kg: 100 kg per ha (Rauf et al., 2000). There were two doses used for NPK fertilizers in this study. One dose for P1B0 and P1B1 treatments (N = 31.250 g; P = 15.635 g; K= 15.625 g), while the half dose for P1B2 treatment (N= 15.625 g; P= 7.812 g; K= 7.812 g). Preparation of *Agrobacterium sp. I₃₀* was begun by making LB (Luria Bertani) media. The composition was 5 g yeast extract, 10 g protease

peptone, 5 g NaCl, and 1,000 mL of distilled water. Subsequently, the LB was used to make a starter by adding bacterial inoculum into LB and shaking the starter for 3 x 24 hours. After that, the starter was scaled up by poured starter into a flask containing 650 mL of LB, and it was then shaken again for 3 x 24 hours or until the bacterial density reached 10^8 . The dose of *Agrobacterium sp. I₃₀* was 10^5 per gram of soil with application in the rice field was 12.5 mL per hole. The dose used for vermicompost was 5 t/ha. The chelating agents (*Agrobacterium sp. I₃₀* and vermicompost) were applied once at one week before planting.

Parameters observed in this study were soil properties (pH, cation exchange capacity, organic matter content, and total microbial colonies), and plant parameters (plant dry weight, Pb content, and Pb uptake). The methods used for measuring pH, cation exchange capacity, organic matter content, and total microbial colonies were the standard methods used in the Soil Laboratory of Sebelas Maret University. Pb content in plant tissues was determined using wet destruction method. The data obtained were subjected to analysis of variance at 95% significance level followed by the Duncan Multiple Range Test at 95 % significance level.

Results and Discussion

Soil pH

Results of analysis of variance showed that application of inorganic fertilizers and chelating agents affected the soil pH, but their effects were not significant (Table 1). The P0B1 treatment (without inorganic fertilizer, with the application of *Agrobacterium sp. I₃₀*) showed the lowest pH value among others. Application of urea supplied N to the plants in the form of NH_4^+ . Soil pH reduction in N fertilizer treatment was due to the release of ion H^+ caused by oxidation (Liu et al., 2010). Application of *Agrobacterium sp. I₃₀* to the soil would increase the number of soil microbes that during their life metabolism produced organic acid compounds. The availability of large amounts of organic acids increases the concentration of H^+ ions and makes the soil pH decreased. Application of vermicompost could increase the value of soil organic matter leading to pH increase due to base cations released from mineralized organic matter (Atmojo, 2010). Metals will be widely available in soils with low pH (acid) (Yoon, 2006). Results of this study showed that the highest level of acidity was in the P0B1 treatment. Application of *Agrobacterium sp. I₃₀* decreased the soil pH to 5.88 from the initial soil pH of 6.39. Application of inorganic

fertilizers and vermicompost (P1B2 treatment) yielded a pH value of 5.89. Soil pH has essential roles in the uptake of heavy metals in which the pH regulates the solubility and hydroxides (Wuana et al., 2006). Decreasing soil pH will increase solubility, availability, and mobility of Pb so that it can be absorbed and sequestered by root plants.

Soil cation exchange capacity

Cation exchange capacity (CEC) has an essential role in the bonding of cations in the soil. In general, CEC values reflect the level of soil fertility. If the soil has a high CEC, it can be said that the soil has a high fertility level (Hartati et al., 2013). Data presented in Table 1 show that generally after treatment, soil CEC has increased from the initial CEC level of 20.29 cmol (+)/kg especially on the P1B1 and P1B2 treatments. The application of *Agrobacterium sp. I₃₀* in the P1B1 treatment yielded the CEC value of 26.72 cmol (+)/kg.

The application of vermicompost in the P1B2 treatment yielded a higher CEC value of 28.72 cmol (+)/kg. As vermicompost contains organic acids which are the result of decomposition of organic matters by worms and microorganisms, application of vermicompost in this study increased soil organic matter content as well as soil CEC. However, results of the analysis of variance indicated that application of inorganic fertilizer did not have a significant effect on soil CEC, while the application of *Agrobacterium sp. I₃₀* and vermicompost, as well as the interaction between inorganic fertilizers and chelating agents, showed a significant effect on soil CEC. Therefore, the application of these two chelating agents was able to increase the value of soil CEC to support continuous soil health, and improve soil fertility and productivity.

Healthy soil can support biodiversity, plant growth, soil characteristics in the form of soil acidity, organic matter, cation exchange capacity, and soil microbes that are good and free from pollutants (Suntoro, 2010). The fertile soil will increase the fertility of plants so that the mechanism of Pb suppress can be more advantageous. The higher CEC will increase the ability of the soil to exchange heavy metal cations for supporting the suppression of Pb uptake by rice (Simamora et al., 2016).

Soil organic matter

The existence of organic matter is essential in this study because it is related to the supply of nutrients and the maintenance of soil structure (Gugino et al., 2007). According to Suntoro

(2010), organic materials can be derived from animal waste, plant residues, green manure, organic waste, and compost. Organic materials can improve soil structure, increase soil ability to bind water, increase the availability of N, P, and S elements, increase cation exchange capacity

(CEC), and increase soil microbial activity (Leiwakabessy et al., 2003). Organic matter affects soil improvements, both from physical, chemical, and biological indicators of soil (Nardi et al., 2004).

Table 1. Soil characteristics

No	Treatments	Soil pH	Soil CEC (cmol (+)/kg)	Soil Organic Matter (%)	Total Soil Bacterial Colonies (Log ₁₀ CFU/g)
1.	P0B0	5.89	21.92 d	4.78 b	12.25 a
2.	P0B1	5.88	23.98 c	5.28 a	12.24 a
3.	P0B2	5.96	26.58 b	4.51 c	12.15 a
4.	P1B0	5.91	24.27 c	5.09 a	12.49 a
5.	P1B1	5.92	26.72 b	4.39 c	12.50 a
6.	P1B2	5.89	28.72 a	5.12 a	11.78 b

Remarks: P0B0 = control, P0B1 = application of *Agrobacterium sp. I₃₀* without inorganic fertilizers, P0B2 = application of vermicompost without inorganic fertilizers, P1B0 = application of inorganic fertilizer without *Agrobacterium sp. I₃₀* and vermicompost, P1B1 = application of inorganic fertilizer and *Agrobacterium sp. I₃₀*, and P1B2 = application of inorganic fertilizer and vermicompost. Numbers with the same letters in the same columns show no significant difference at p<5%; CEC = cation exchange capacity.

Data presented in Table 1 show that in general after treatments, soil organic matter content increased from the initial organic matter value of 1.84%. In this study, the highest organic matter content was in the application of *Agrobacterium sp. I₃₀* (P0B1 treatment) with the value of 5.28% and at the application of vermicompost (P1B2 treatment) with the value of 5.12%. Results of analysis of variance showed that the application of inorganic fertilizer had no significant effect on the soil organic matter content, while the application of *Agrobacterium sp. I₃₀* and vermicompost, as well as the interaction between inorganic fertilizers and chelating agents showed a significant effect on the soil organic matter content. The existence of high organic matter content will increase the cation exchange capacity of the soil, and by the treatment, it will support the mechanism of suppressing Pb uptake by rice.

Total soil microbial (bacteria) colonies

Results of analysis of variance on the total soil microbial colonies indicated that the initial total microbial colony of 8 Log¹⁰ CFU/g had increased (Table 1). The largest total microbial colonies in this study (12.50 Log¹⁰ CFU/g) was found in the P1B1 treatment with application of *Agrobacterium sp. I₃₀*, while for best application of vermicompost was observed for the P0B2 treatment (12.15 Log¹⁰ CFU/g). *Agrobacterium sp.* living in plant roots can help the process of soil formation, plant growth, pathogen biological control, and affect other microorganism activities. *Agrobacterium sp. I₃₀* as rhizobacteria can reduce

the toxic effects of heavy metals to plants with the resulting organic acids, as well as various other chemical compounds capable of optimizing the adsorption of heavy metals (Rosariastuti et al., 2013).

Bacteria can affect the availability of metals in the soil through the secretion of their metabolite products. In addition, metabolite products can also increase plant growth. Secondary metabolite products from *Agrobacterium* such as citric, oxalic, gluonic, fumaric and malic acid can chelate toxic metals causing the formation of metallo-organic molecules, and they are then sequestered in the part of the root. Results of analysis of variance showed that the application of inorganic fertilizer and chelating agents partially had no significant effect on the total soil microbial colonies, while the interaction between inorganic fertilizers and chelating agents showed a significant effect on the total soil microbial colonies. The higher total microbial colony in the soil, the greater likelihood of interaction between microorganisms and root exudates, thereby enhancing the ability of plants to absorb heavy metals (Agustiyani et al., 2004)

Pb content in soil

Results of analysis of variance showed that the Pb content in the soil had decreased by more than 50% in compared to the initial Pb content of 16.183 mg/kg (Figure 1). The application of *Agrobacterium sp. I₃₀* and vermicompost in the P0B1 and P1B2 treatments yielded the highest

decrease in Pb content compared to other treatments. Both chelating agents made Pb in the soil available to be easily absorbed by the plant roots and then exiled to the part of roots so that it is not expected to be absorbed up to the rice grains. Based on analysis of variance, it is known that the application of inorganic fertilizers has no significant effect on Pb levels in the soil, while the application of *Agrobacterium sp. I₃₀* and vermicompost and the interaction between inorganic fertilizer and chelating agents had a significant effect on Pb levels in the soil. The DMRT results showed that there were three

different spaces for the controls, POB2, P1B0 and P1B1 treatments. The decrease of Pb content in the soil could occur because of Pb uptake by rice plants. This indicates that rice as a food crop can accumulate heavy metals. The entry of heavy metals to rice is indeed not a condition that is expected to occur, so it is necessary to suppress or absorb metal in tissues that are not consumed by humans so that the rice produced is safe for consumption. The application of both chelating agents increased organic matter that improving soil's ability to retain heavy metals in an exchangeable form (Zhang et al., 2015).

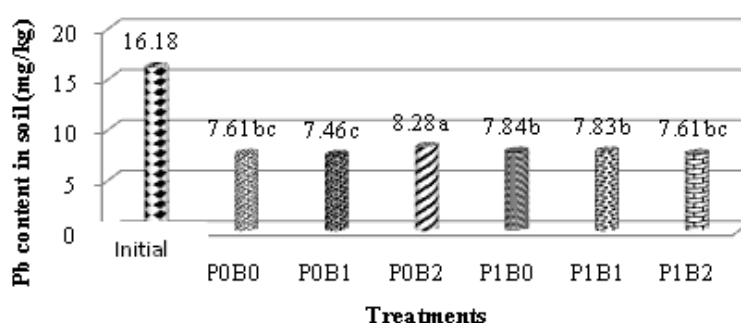


Figure 1. Pb content in the soil studied

Remarks: POB0 = control, POB1 = application of *Agrobacterium sp. I₃₀* without inorganic fertilizers, POB2 = application of vermicompost without inorganic fertilizers, P1B0 = application of inorganic fertilizer without *Agrobacterium sp. I₃₀* and vermicompost, P1B1 = application of inorganic fertilizer and *Agrobacterium sp. I₃₀*, and P1B2 = application of inorganic fertilizer and vermicompost.

Plant dry weight

Based on the results of analysis of variance on the plant dry weight, it was found that generally after treatment showed an increased value of dry weight compared to plants that were not treated with the application of *Agrobacterium sp. I₃₀* and vermicompost (Table 2). The highest plant dry weight was found in the POB2 treatment with root dry weight of 7.05 g, shoot dry weight of 33.26 g, and rice dry weight of 3.26 g. Without inorganic fertilizers (P0), the average dry weights of the root, shoot, and rice were 6.01 g, 27.46 g, and 3.62 g, respectively. With inorganic fertilizers (P1), the average dry weights of root, shoot and rice were 7.47 g, 33.68 g, and 2.78 g, respectively. Without the chelating agents (B0), the average dry weights of the root, shoot, and rice were 7.38 g, 28.7 g, and 3.39 g, respectively. With *Agrobacterium sp. I₃₀* (B1) the average dry weights of the root, shoot, and rice were 5.71 g, 30.72 g, and 3.4 g, respectively. With vermicompost application (B2), the average dry weights of the root, shoot, and rice were 7.15 g, 32.28 g, and 2.82 g. Results of analysis of

variance showed that application of inorganic fertilizers and chelating agents, and their interactions significantly affected the dry weight of rice plants. The higher level of organic matter combined with inorganic fertilizers would increase the dry weight of the plant.

Table 2. Plant dry weight

Treatments	Dry Weight (g)		
	Root	Shoot	Rice
POB0	5.069 d	22.6471 d	3.714 a
POB1	5.9351 c	26.484 c	3.912 a
POB2	7.0532 b	33.265 ab	3.265 b
P1B0	9.67 a	34.777 a	3.084 b
P1B1	5.4933 cd	34.975 a	3.892 b
P1B2	7.2682 b	31.3087 b	3.3835 c

Remarks: POB0 = control, POB1 = application of *Agrobacterium sp. I₃₀* without inorganic fertilizers, POB2 = application of vermicompost without inorganic fertilizers, P1B0 = application of inorganic fertilizer without *Agrobacterium sp. I₃₀* and vermicompost, P1B1 = application of inorganic fertilizer and *Agrobacterium sp. I₃₀*, and P1B2 = application of inorganic fertilizer and vermicompost.

The administration of vermicompost could expand the root range in nutrient absorption, increase the number of roots and increase the amount of food reserves absorbed by plants, so that the dry weight of the roots would increase. The shoot is the most dominating part of the rice plant so that its good growth will dominate the dry weight. The provision of inorganic fertilizers combined with vermicompost in the right dosage could increase the dry weight of the plant.

Pb content in plant

Data presented in Figure 2 show that the lowest Pb content in the rice (grains) was on the POB2 treatment (without inorganic fertilizer, with application vermicompost). Results of analysis of variance showed that application of *Agrobacterium sp. I₃₀* and vermicompost, and the interaction between inorganic fertilizers and chelating agents showed a significant difference in Pb content in plants (roots, shoot and rice). Some treatments were significantly different from control (Figure 2). Analysis of Pb content in plant tissue showed that the best treatment in the application of *Agrobacterium sp. I₃₀* was the POB1 treatment with Pb values in the root, shoot, and rice were 10.77 mg/kg, 3.36 mg/kg, and 4.97 mg/kg, respectively. The best treatment in the application of vermicompost was the POB2 treatment with Pb values in the root, shoot, and rice were 10.59 mg/kg, 3.48 mg/kg, and 1.61 mg/kg, respectively. The best treatment for the Pb content in this study was indicated by the high Pb content in the root and the low Ph content in the

shoot and rice. The low level of Pb in the rice is an indication of the safety of rice production for human consumption. Application of *Agrobacterium sp. I₃₀* and vermicompost indicated the mechanism of suppressing Pb. It was shown by high level of Pb in the root and low levels of Pb in shoot and rice. Both chelating agents (*Agrobacterium sp. I₃₀* and vermicompost) could sequester Pb in the root and minimized its absorption to other parts of the plant, especially in the rice grains. *Agrobacterium sp. I₃₀* as a rhizosphere bacterium maximizes its ability to absorb Pb as much as possible and proceeds with the sequestration of Pb as toxic metal in the root, so that when the mechanism of mineral nutrient transport continues, Pb is expected to decrease in the rice. Vermicompost containing various nutrients performs an essential function as chemical energy to store and transfer energy in all plant metabolisms. Vermicompost as a chelating agent could decrease Pb content in rice plant tissue because Pb element tended to be bound by organic material to form the metal chelate complex so that the Pb content could still be absorbed by the rice grains in a low amount (Suparno et al., 2013). The results showed that treatment of *Agrobacterium sp. I₃₀* (B1) and vermicompost (B2) had levels of 11.13 mg Pb/kg whereas Pb in the B2 (9.34 mg Pb/kg) was lower than that in B1 (10.62 mg/kg) treatments. The value of the calculated Pb content in the root that was much higher than that in the shoot and rice proved the passage of the Pb sequester process in the roots.

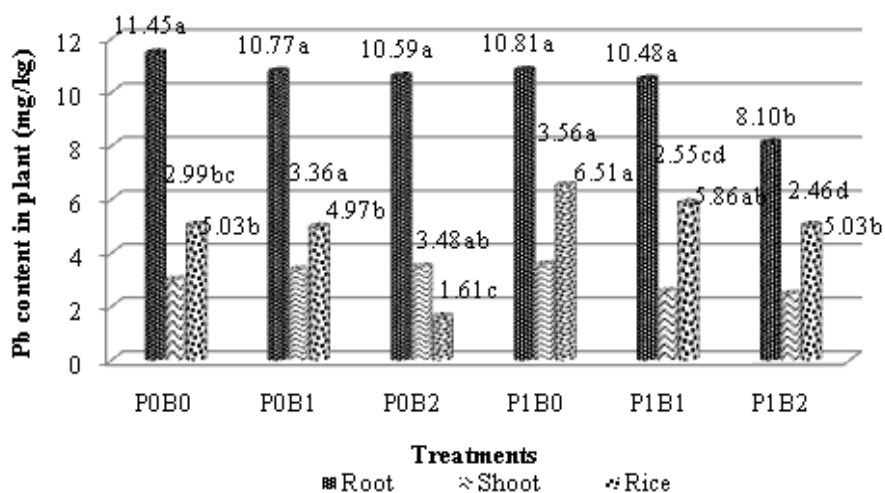


Figure 2. Pb content in plant tissue

Remarks : POB0 = control, POB1 = application of *Agrobacterium sp. I₃₀* without inorganic fertilizers, POB2 = application of vermicompost without inorganic fertilizers, P1B0 = application of inorganic fertilizer without *Agrobacterium sp. I₃₀* and vermicompost, P1B1 = application of inorganic fertilizer and *Agrobacterium sp. I₃₀*, and P1B2 = application of inorganic fertilizer and vermicompost.

Pb uptake by plant

Data presented in Figure 3 show that the application of *Agrobacterium sp. I₃₀* and vermicompost, and the interaction between the inorganic fertilizers and the chelating agents showed a significant difference in Pb uptake by plant tissues (root, shoot and rice). Pb uptake analysis in rice plant tissue showed that the application of *Agrobacterium sp. I₃₀* in the P1B1 treatment yielded 62.39 µg, 98.31 µg, and 16.96 µg Pb in the root, in the shoot, and in the rice, respectively. The application of vermicompost in the P0B2 treatment yielded 71.39 µg, 97.92 µg, and 5.18 µg Pb in the root, in the shoot, and in the rice, respectively. The best treatment for the Pb uptake analysis in this study is shown by the high Pb uptake tendency by the root and the low Pb by the rice. Pb uptake by the shoot showed a higher value than in the roots and rice because shoot dry weight tended to be high, so it affected the absorption rate. The low Pb uptake by the rice is an indication of low Pb level in the rice, thus

increasing the safety of rice production for human consumption. The amount of Pb taken up by rice in the best treatment of P0B2 decreased to 3.5 times from the control, which was about 72.18%. The decrease in Pb uptake by rice of more than 50% showed suppression of Pb uptake by the root and the shoot. In the P0B2 treatment, Pb uptake by root increased up to 28.72%, and Pb uptake by shoot increased up to 72.41% (Figure 3). Decomposition of vermicompost in the soil will produce fulvic, humic and humin acid containing a large amount of anion charge (Septiningsih et al., 2015). Decomposition of vermicompost will produce a humic compound that produces negatively charged colloid of soil (Siregar et al., 2017). The negative charges come from COOH and OH inorganic compounds. Pb in soil has a positive charge that will react with negatively charged colloid produced by organic matters. This causes Pb becomes easier to be absorbed by the root, and thus reduces Pb level in the rice.

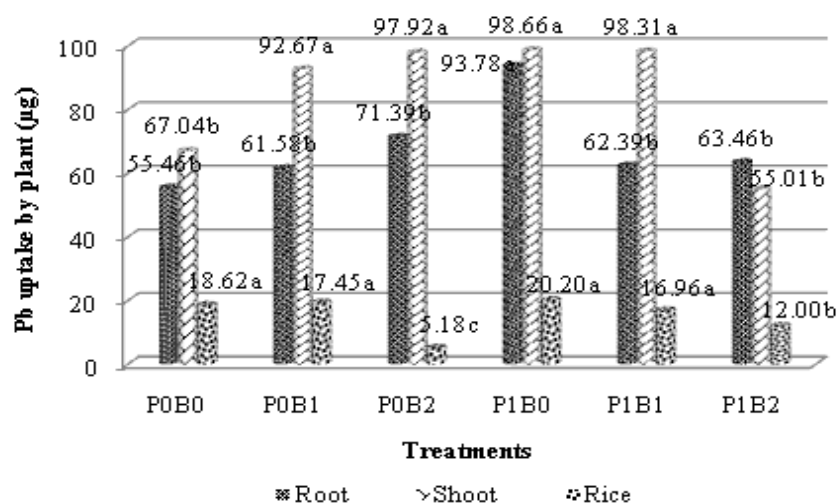


Figure 3. Pb uptake by plant tissue

Remarks: P0B0 = control, P0B1 = application of *Agrobacterium sp. I₃₀* without inorganic fertilizers, P0B2 = application of vermicompost without inorganic fertilizers, P1B0 = application of inorganic fertilizer without *Agrobacterium sp. I₃₀* and vermicompost, P1B1 = application of inorganic fertilizer and *Agrobacterium sp. I₃₀*, and P1B2 = application of inorganic fertilizer and vermicompost.

Conclusion

Application of *Agrobacterium sp. I₃₀* and vermicompost on rice cultivation can support the mechanism of suppressing Pb by rice. The decrease of soil acidity level and the increase of cation exchange capacity value, organic matter content, total soil microbe, and plant dry weight due to the application of *Agrobacterium sp. I₃₀*

and vermicompost were capable of supporting Pb in the root and reducing Pb concentration in the rice. The best treatment was the P0B2 with the application of vermicompost, Pb uptake by rice decreased to 72.18% compared to control, while the application of *Agrobacterium sp. I₃₀* without inorganic fertilizers (P0B2) decreased Pb content in the rice from 5.03 mg/kg to 4.97 mg/kg with a decrease in Pb uptake by 6.28%.

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