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RESEARCH ARTICLE

ENHANCING CHROMIUM PHYTOSTABILIZATION USING CHELATOR (*Agrobacterium sp.* I_{26}, and Manure) TO SUPPORT GROWTH AND QUALITY OF RICE (*Oryza sativa* L.)

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ABSTRACT

There are many of rice field which is located near the industrial area. The industrial waste contains heavy metals (chromium) which will cause contamination of rice if the waste isn't treated properly. The used of chemical fertilizers can cause chromium contamination. It needs an effort to do remediation, such as using phytostabilization mechanism. This research aimed to determine the role of chelator in chromium phytostabilization and its influence on the growth and quality of rice. The study was conducted in Waru village, Karanganyar in May to October 2018. This research was factorial design used completely randomized block design with two factors, namely chemical fertilizers and chelator (*Agrobacterium* sp. I_{26}, and manure). The parameters observed are chromium content and uptake by plant tissues (roots, shoot and rice), plant height and a number of clumps. Research output was without chemical fertilizer-chelator *Agrobacterium* sp. I_{26} that can increase the chromium uptake in roots as big as 95.38 %, increased up to 10 % in the shoot and decreased up to 92.38 % in rice compared to control. Application of *Agrobacterium* sp. I_{26} can be recommended to produce good quality and quantity of rice (good growth and free from harmful pollutants such as chromium metal).

Keywords: *Agrobacterium* sp. I_{26}, Chromium, Manure, Phytostabilization, Quality of Rice

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INTRODUCTION

Indonesia is an agricultural country, where most people are farmers (Anggriawan & Indrawati, 2013). The staple food majority of Indonesian people are rice, it is necessary to pay attention to the quality of rice that produced. The rice must be free from contaminants that can be harmful to health. One of the contaminants that are harmful to health if consumed is chromium (Syahfitri et al., 2011; Gururajan & Belur, 2018). The threshold value for chromium in foodstuffs was 0.1-0.5 ppm (Alkhalaf et al., 2007). Chromium can enter into various strata of the environment such as air, water, and soil. In the environment, chromium comes from the combustion of oil and coal, petroleum from refractory Ferro chromate, catalysts, and the weathering of rocks containing metal (Abbassi et al., 1998). Chromium contamination can be also caused by an industrial process. Chromium

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was widely used in the textile industry, metal plating, leather tanning, manufacture of paints, wood preservation, nuclear reactors and manufacture of stainless steel (Sembel, 2015). Chromium is a heavy metal that is easily soluble, strong oxidizing agents, high mobility, very toxic, carcinogenic, dermatoxic, teratogenic, mutagenic and in large quantities in the organism can cause death. Chromium in the form of Cr^{6+} is more dangerous than the Cr^{3+} (Cervantes et al., 2001).

Kebakkramat is subdistricts in Karanganyar district which is one of the largest rice-producing areas in Central Java. One of the villages in Kebakkramat, that the most land used for agriculture is Waru (BPS, 2017). Most of the rice field in Waru get water from a river that is contaminated by industrial waste. One of the contaminants in the irrigation water is chromium. That can cause the chromium contamination of rice field.

Chromium contamination in the rice field has come from irrigation water that is contaminated by industrial waste and accumulation of residual chemical fertilizers (Syahfitri et al., 2011). Based on the results of laboratory tests is known that the water used to irrigate rice field contained chromium as big as 0.37 ppm. Chromium content in urea, Sp-36, and KCl was 0.004 ppm, 0.692 ppm, and 0.004 ppm. Chromium content in the rice field would be dangerous if not be surmounted immediately, because chromium may move to the rice plant tissue (Agrawal & Sharma, 2006; Etesami, 2018). Chromium can enter the food chain with the consumption of the plant by living things (Nagarajan & Ganesh, 2015). Chromium will accumulate in the living body and endanger health.

According to the Regulation of Government Republic Indonesia No. 101 of 2014 about management of hazardous and toxic waste, the threshold value for chromium in the soil was 2.5 ppm. At the beginning of the study, the initial soil has tested and it has known that soil Cr content was 6.61 ppm. The contents already exceeded the specified threshold. The remediation process was needed to overcome the heavy metal contamination in the rice field. One of the methods commonly used as remediation was bioremediation.

Bioremediation is one of a method for handling soil contaminated by heavy metal utilizing biological agents such as plants (phytoremediation) and microorganisms. Phytoremediation has two mechanisms, namely phytostabilization and phytoextraction. This research used the phytostabilization mechanism, wherein the microorganism (chelator agent) will be helped the roots absorbed heavy metals from soil and sequestered them at the roots (Hidayati, 2005; Radziemska et al., 2017; Handayanto et al., 2018). In this research used *Agrobacterium* sp. I_{26} (rhizosphere bacterial) as chelator agents (Pramono et al., 2012). *Agrobacterium* sp. I_{26} was bacteria isolate that isolated by Rosariastuti in 2013 (Rosariastuti et al, 2013). As the comparison in this study also used cow manure as a chelator agent. Cow manure is an organic fertilizer derived from cow manure that is good for improving fertility, physical, chemical, and biological soil, improving macro and micronutrients, increase water holding and increasing the cation exchange capacity (Hadisumitro, 2002; Alvernia et al., 2017). The use of manure as chelator agents are also expected to increase the chromium uptaken from soil and sequestered them in plant roots. The used of manure and *Agrobacterium* sp. I_{26} was expected can support the phytostabilization mechanism (increasing the chromium uptaken in root tissue).

The aimed of this research was to determine the role of chelator (*Agrobacterium* sp. I_{26}, and manure) in chromium phytostabilization and its influence on the growth and quality of rice (*Oryza Sativa.* L).

MATERIALS AND METHOD

Place and Research Time

The research was conducted in Waru village, Kebakkramat subdistrict, Karanganyar district of Central Java province in May to September 2017. This research used a factorial design with two factors, namely chemical fertilizers (P) and chelator (B)(*Agrobacterium* sp. I_{26}, and manure). There are six treatment combinations which were repeated 3 times and arranged in a completely randomized block design (CRBD). Combination treatments are presented in Table 1.

Plots treatment used in the research were 18 plots. Plot size was 1.25 m x 1.25 m, with the distance between the planting hole was 50 cm and the distance between plots was 100 cm. The number of planting holes in each plot was 25.

Preparation Chemical Fertilizer

Chemical fertilizers used in this study were NPK (Nitrogen = Urea, Phosphorus = SP-36, Potassium = KCl). Dose of NPK fertilizer used ratio of N: P: K = 2: 1: 1 = 200 kg.ha^{-1}: 100 kg.ha^{-1}: 100 kg.ha^{-1} (Rauf et al., 2000). There were two doses used for NPK in this research, according to the treatment in each plot. One dose for treatment P1B0 and P1B1 (N= 31.250 g; P= 15.635 g; K= 15.625 g), while the half dose for the treatment P1B2 (N= 15.625 g; P= 7.812 g; K= 7.812 g). Fertilization was done 3 times they were at the age of plant was 5 DAP (Day After Planting) (N, P, K), 35 DAP (N, K) and 55 DAP (K) (Rauf et al., 2000).

Preparation of Chelator Agents

Chelator agent that used in this research were *Agrobacterium* sp. I_{26} (B1) and manure (B2). Preparation of chelator *Agrobacterium* sp. I_{26} is started by making LB media (Luria Betani). The composition was 5 g yeast extract, 10 g protease peptone, 5 g NaCl, and 1000 mL of distilled water. Subsequently, the LB used to make a starter by adding bacterial inoculum into LB. The starter was shaken for 3 x 24 hours. After that, the starter was poured into the flask containing 650 mL LB to scale up this and shake again during 3 x 24 hours or until the density of bacterial was 10^8. The dose for chelator *Agrobacterium* sp. I_{26} is 10^5 per gram soil with applications in the rice field as much as 12.5 mL per hole. As a comparison, manure was also used as chelator agents. The dose used for manure was 5 tonnes.ha^{-1}. Chelator agent application time (*Agrobacterium* sp. I_{26}, and manure) was doing at the same time, that was one week before planting.

Table 1. Combinations

Treatment	Explanation
P0B0	Without chelator and without chemical fertilizers (control)
P0B1	Without chemical fertilizer and chelator *Agrobacterium* sp. I_{26}
P0B2	Without chemical fertilizer and chelator manure
P1B0	Chemical fertilizers and without chelator
P1B1	Chemical fertilizers and chelator *Agrobacterium* sp. I_{26}
P1B2	Chemical fertilizer and chelator manure

Table 2. The Result of Initial Soil Analysis

Parameter	Method	Value	Unit	Rate
Soil Cr content	Wet destruction	6.61	ppm	Exceeded the threshold value (*)
pH	Potentiometric	6.9	-	Acid (**)
CEC	Ammonium acetate washing	25.29	$cmol(+).kg^{-1}$	High (**)
Organic matter	Walkey and Black	1.84	%	Low (***)
Total soil microbial colonies	Hand Colony Counter	8	$Log10CFU.g^{-1}$	High

Remarks:
(*): Government Regulation Republic of Indonesia Number 101 (2014)
(**): Soil Research Institute (2009).
(***): Sumaryo (1982).

Data Analysis

Data of this research was the chromium content and uptake by plant tissues include the roots, shoots, rice, and the rice growth (height, number of clumps, and dry weight). The chromium content was determined by using wet destruction method. The growth of the rice was determined by using direct observation method. The data obtained were subjected to the statistical test of Anova at 5 % level (to know the effect of treatment) and continued with the test of Duncan Multiple Range Test (DMRT) at 5 % level (to determine differences between treatments). Analyzes were performed using software SPSS 16.

RESULTS AND DISCUSSION

Analysis of Initial Soil

The initial rice field was analyzed before treatment. Analysis of initial soil includes soil chromium content, pH, cation exchange capacity (CEC), organic matter (BO) and total soil microbial colonies. The results of initial soil analysis were presented in Table 2.

Chromium Contents in Plant Tissue

The results of the laboratory test at initial paddy soil showed that soil Cr content was 6.61 ppm. The contents already exceed the threshold values of soil Cr content according to Government regulation number 101 of 2014 that was 2.5 ppm. Bioremediation used chelator *Agrobacterium* sp. I_{26} and manure can support the phytostabilization mechanism. It was proved by Cr content at the root of all treatments was higher than the Cr content at the shoot and rice (Figure 1).

ANOVA test toward the chelator treatment (*Agrobacterium* sp. I_{26} and manure) and the interaction between the two treatments (chemical fertilizers and chelator) showed a significantly on Cr content in plants (roots, shoot and rice). Furthermore, the DMRT test was done and there were some treatments that significantly different from control. DMRT test result was showed in Figure 1. The treatments were significantly different from the control have a different letter notation with control.

The treatment P0B1 shows the best results among others. The used of *Agrobacterium* sp. I_{26} can minimize the Cr content in the rice. Cr absorbed by the plant was accumulated in the root tissues. Agrobacterium sp. I_{26} is a biological agent that can interact with the roots. *Agrobacterium* sp. I_{26} helped roots to maximize the Cr uptake and sequestered them in the root (Rajkumar & Freitas, 2008). Applications of *Agrobacterium* sp. I_{26} will increase the number of soil microorganisms. Metabolism of soil microbial will produce secondary metabolites such as organic acids. Increasing the content of soil organic acid will increase the concentration of H^{+} ions so that the pH of the soil decreased. Acidic soil will increase the mobility of Cr so it can be absorbed by plants (Yoon et al., 2006). Soil microorganisms will also help the process of decomposition of organic matter in the soil into inorganic material that is readily absorbed by the plant. The plant who absorbed much of the inorganic material, the plant growth will increase too (Figure 3).

Bioremediation process will change Cr into a form that is more easily absorbed by the plant that was Cr (VI). Cr entered the plant tissue through sulfate pathway (Cervantes et al., 2001; Shanker 2005; Rosariastuti et al., 2013; Aji et al., 2017). The phytostabilization mechanism would be sequestered Cr that absorbed by plants at the roots. Rhizobacteria can help reduce the toxic effects of heavy metals in plants by produced acids, protein and several chemical compounds that will be maximized the isolation of heavy metals in the roots of plants (Rosariastuti et al., 2013). Plant roots can absorb Cr up to 10-100 times higher than shoot and rice (Cervantes et al., 2001). It makes other plant parts such as shoot and rice were safe for consumption.

Treatment P0B1 showed the best Cr uptake in the plant tissue among others. Cr uptake by rice was minimum compared with the other treatments (Figure 2). P0B1 treatment can increase chromium uptake in the roots as big as 95.38 %, increased the uptake in the shoot up to 10 % and decreased the uptake in rice up to 92.38 % compared to the control. It shows the phytostabilization mechanism, whereby heavy metals were absorbed into plants will sequester at the roots (Radziemska et al., 2017; Rosariastuti et al., 2013). The used of *Agrobacterium* sp. I_{26} can support the phytostabilization process at the plant by produce organic acids through the process of metabolism.

ANOVA test toward the chelator treatment (*Agrobacterium* sp. I_{26} and manure) and the interaction between the two treatments (chemical fertilizers and chelator) showed a significantly on Cr uptake in plants (roots, shoot, and rice). Furthermore, the DMRT test was done and there were some treatments that significantly different from control. DMRT test result was showed in Figure 2. The treatments were significantly different from the control have a different letter notation with control.

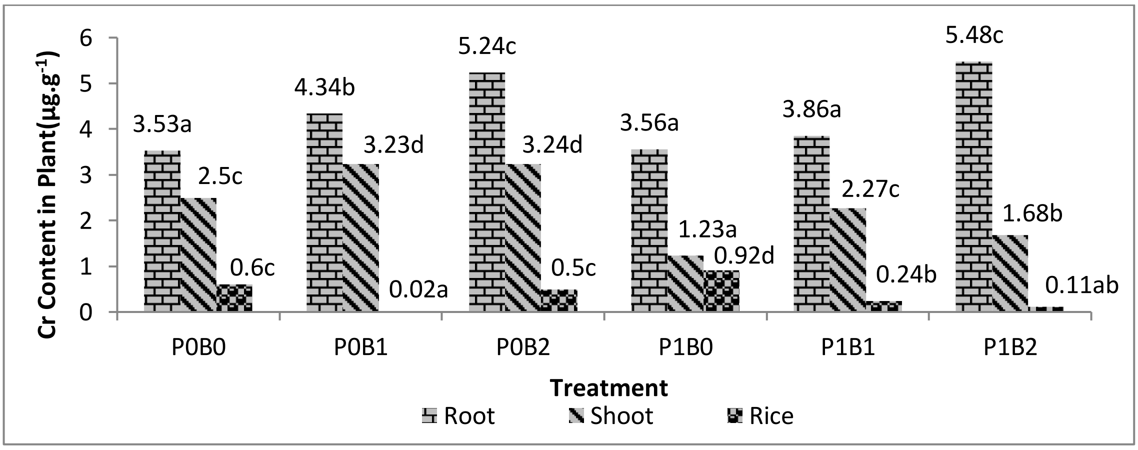

Figure 1. Chromium Content in Plant Tissue

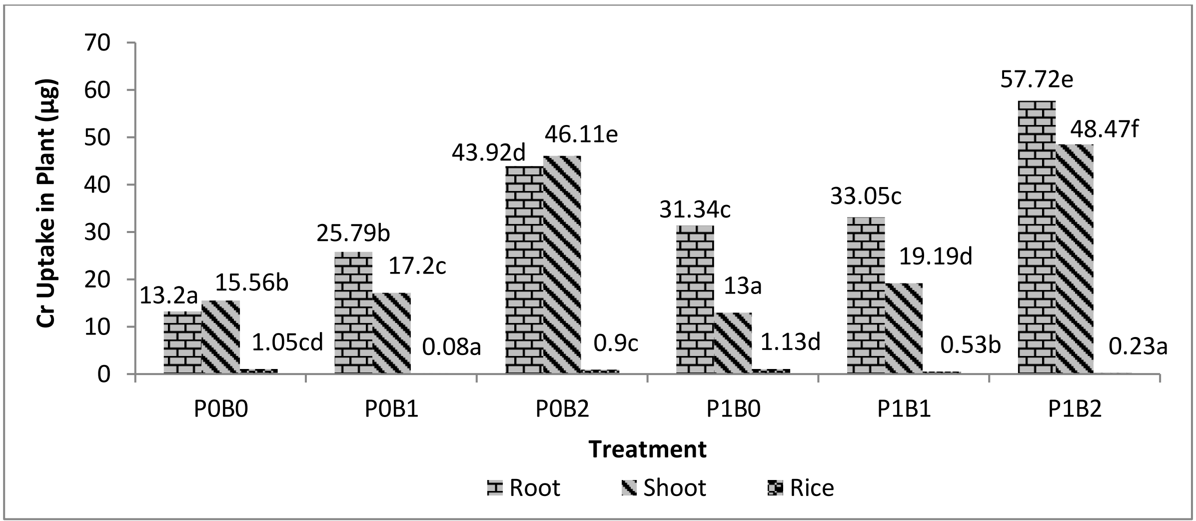

Figure 2. Chromium Uptaken by Plant Tissue

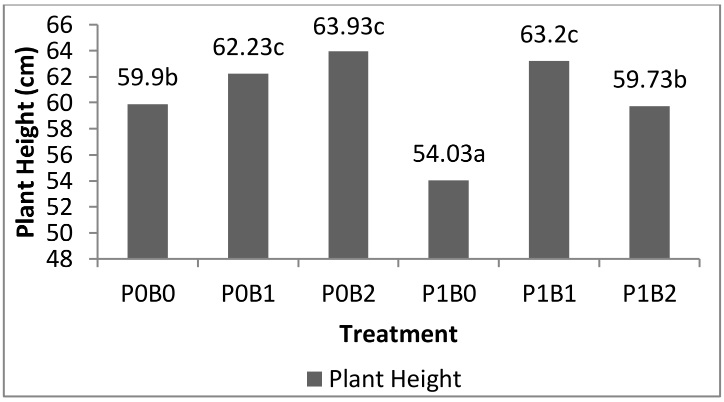

Figure 3. Plants Height

Rhizobacteria such as *Agrobacterium* sp. I_{26} will help the plant to absorb nutrients. Treatment using *Agrobacterium* sp. I_{26} (B1) showed a higher value of plant height (62.71 cm) than treatment using manure (B2). Rhizosphere bacteria such as *Agrobacterium* sp. I_{26} can increase plant growth by synthesizing precursors of phytohormones, vitamins, enzymes, siderophores, and antibiotics Applications of *Agrobacterium* sp. I_{26} showed that can increase the growth of plants compared to control (Figure 3, Figure 4, and Figure 5). Rhizobacteria can enhance the growth, yield and nutrient uptake in corn plants that grow in soil contaminated by heavy metals. metabolic processes of rhizosphere bacterial produced plant growth regulators such as auxin, cytokinin, and gibberellin which helps the growth of plants (Pramono et al., 2012; Antonius et al., 2014; Sharma, 2015). The symbiosis between *Agrobacterium* sp. I_{26} and the roots will increase the uptake of N_2 from the air by plants. The growth and productivity of plants will be increased along with the absorption of N (Patola et al, 2017; Supriyadi et al., 2017).

ANOVA test toward the chelator treatment (*Agrobacterium* sp. I_{26}, and manure) and the interaction between the two treatments (chemical fertilizers and chelator) showed a significantly on plant height, the number of clumps and dry weight. Furthermore, the DMRT test was done and there were some treatments that significantly different from control. DMRT test result was shown in Figure 3, Figure 6, and Figure 7. The treatments were significantly different from the control have a different letter notation with control.

The used of chelator (*Agrobacterium* sp. I_{26}, and manure) can increase the number of clumps compared with controls (Figure 6). Both of chelator contain organic material that will be decomposed by soil microbes into inorganic material that will be absorbed by plants. The plant growth will increase along with the absorption of the inorganic material. The used of *Agrobacterium* sp. I_{26} shows the higher average number of clumps (10.1) than treatment used manure. This indicated that *Agrobacterium* sp. I_{26} is included as PGPR bacteria (Plant Growth Promotion Rhizobacteria) group.

Figure 4. Shoots of P0B1

Figure 5. Shoots of P0B0 (Control)

Remarks: The plant height of P0B1 was higher compared with control.

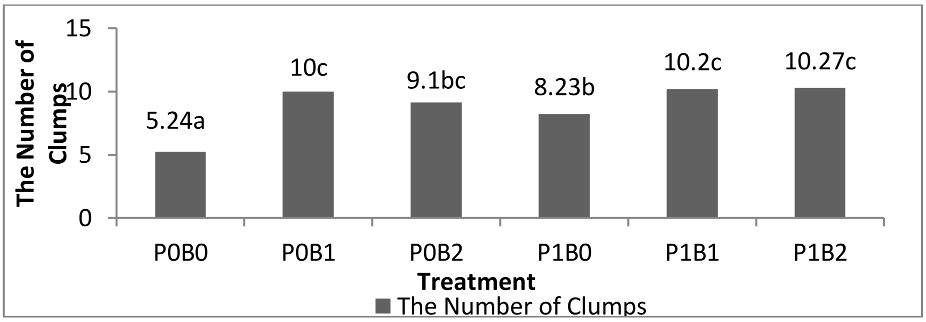

Figure 6. Number of Clumps

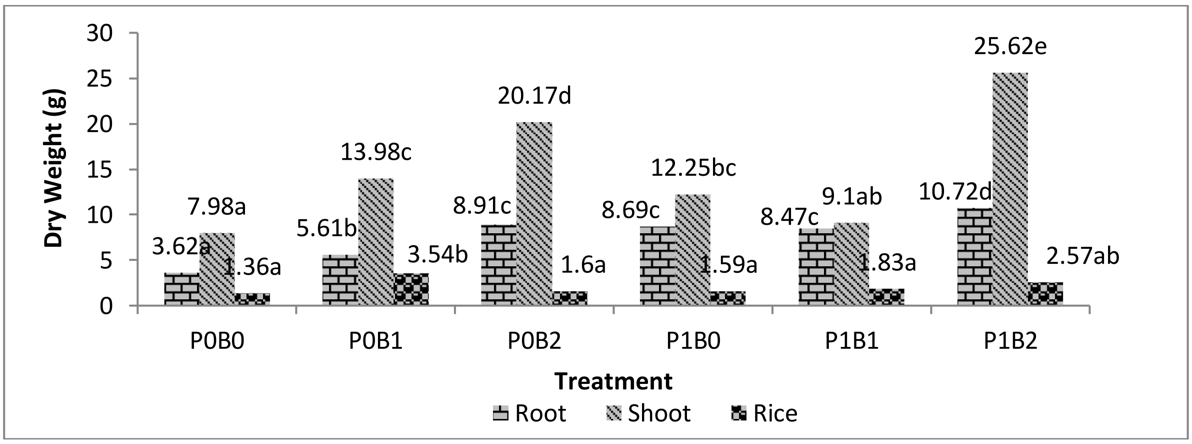

Figure 7. Dry Weight of Plant

Figure 8. Roots Plant of P1B2 treatment

Figure 9. Roots Plant of P0B0 (Control)

Remarks: the roots of P1B2 treatment was bigger compared with control.

Both chelators can increase plant dry weight (Figure 7). The used of manure showed a higher average value of dry weight than *Agrobacterium* sp. I_{26}. This is because of the organic matter content of the manure directly usable by the plant because it has been through the process of decomposition by bacteria. Provision of organic material by *Agrobacterium* sp. I_{26} needs more time because it must go through the process of metabolism first. Application of manure can extend the reached of roots in absorbed nutrients and water. In addition, it can also add the number of roots (Figure 8) and can increase the number of food reserves were absorbed by the plant, so that the root dry weight increased (Hatta, 2012; Pratiwi, 2016). P1B2 treatment showed the highest dry weight compared to all treatments. The combination of chemical fertilizer and organic fertilizer (manure) in the right combination can increase plant dry weight. Chemical fertilizers and manure provide nutrient is required by plants in a form that is readily available and can directly be absorbed by plants (Pratiwi, 2016).

CONCLUSIONS

The used of both chelator (*Agrobacterium* sp. I_{26}, and manure) can support the phytostabilization mechanism of chromium metal in rice. The used of *Agrobacterium* sp. I_{26} was proven to be more effective than manure in minimizing the Cr metal uptaken by rice. Research output was the treatment without chemical fertilizers-chelator *Agrobacterium* sp. I_{26} (P0B1) can increase the chromium uptake in roots as big as 95.38 %, uptake in the shoot increased up to 10 % and the uptake in the rice decreased up to 92.38 % compared with control. In addition *Agrobacterium* sp. I_{26} can increase plant growth. Plant height and number of clumps in plots without chemically fertilizers-chelator *Agrobacterium* sp. I_{26} shown a higher average value compared with control and the plot using manure. Average value of plant height in the plot using *Agrobacterium* sp. I_{26} was 62.71 cm (higher than the treatment using manure and control). Average value of the number of clumps in the plot using *Agrobacterium* sp. I_{26} was also higher than the plot using manure and control that was 10.1. This showed that *Agrobacterium* sp. I_{26} is included in the group of PGPR bacteria. Application of *Agrobacterium* sp. I_{26} can be recommended to produce rice with good quality and quantity (good in plant growth and the rice free from harmful pollutants such as chromium metal).

REFERENCE

Abbassi, S. S., Abbassi, N., & Soni, R. (1998). *Heavy metals in the environment*. New Delhi, India: Mittal Publication.

Agrawal, V., & Sharma, K. (2006). Phytotoxic effects of Cu, Zn, Cd, and Pb on in vitro regeneration and concomitant protein changes in Holarrhena antidysenterica. *Biologia Plantarum*, *50*(2), 307–310. https://doi.org/10.1007/s10535-006-0027-z

Aji, A., Masykuri, M., & Rosariastuti, R. (2017). Phytoremediation of Rice Field Contaminated by Chromium with Mendong (Fimbristylis globulosa) To Supporting Sustainable Agriculture. In *Borderless Communities and Nations with*

Borders: Challenges of Globalisation. Yogyakarta: the International Indonesian Forum for Asian Studies (IIFAS).

Alkhalaf, N. A., Osman, A. K., & Salama, K. A. (2007). Monitoring of aflatoxins and heavy metals in some poultry feeds. *African Journal of Food Science.*, *4*(4), 192–199.

Alvernia, P., Minardi, S., & Suntoro, S. (2017). Zeolite and Organic Fertilizer Application to The Improvement of Available P and Soybean (Glycine max L) Seed Yield in Alfisols. *Sains Tanah - Journal of Soil Science and Agroclimatology*, *14*(2), 83–89. https://doi.org/10.15608/stjssa.v14i2.839

Anggriawan, & Indrawati, T. (2013). Peranan Komoditi Gambir Terhadap Perekonomian Kabupaten Lima Puluh Kota Provinsi Sumatera Barat. *Jurnal Ekonomi*, *21*(2), 1–21.

Antonius, S., Agustyani, D., Imamuddin, H., Dewi, T. K., Laili, N. (2014). Kajian Bakteri Penghasil Hormon Tumbuh IAA Sebagai Pupuk Organik Hayati dan Kandungan IAA Selama Penyimpanan. Prosiding Seminar Nasional Pertanian Organik. 279–285.

BPS. (2017). *Kecamatan Kebakkramat Dalam Angka 2017*. Karanganyar: Badan Pusat Statistik Kabupaten Karanganyar.

Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J. C., & Moreno-Sánchez, R. (2001). Interactions of chromium with microorganisms and plants. *FEMS Microbiology Reviews*, *25*(3), 335–347. https://doi.org/10.1016/S0168-6445(01)00057-2

Etesami, H. (2018). Ecotoxicology and Environmental Safety Bacterial mediated alleviation of heavy metal stress and decreased the accumulation of metals in plant tissues : Mechanisms and future prospects. *Ecotoxicology and Environmental Safety*, *147*(July 2017), 175–191. https://doi.org/10.1016/j.ecoenv.2017.08.032

Government Regulations Republic of Indonesia Number 101 year 2014 about Management of Hazardous and Toxic Wastes.

Gururajan, K., & Belur, P. D. (2018). Screening and selection of indigenous metal tolerant fungal isolates for heavy metal removal. *Environmental Technology & Innovation*, *9*, 91–99. https://doi.org/10.1016/J.ETI.2017.11.001

Hadisumitro, L. (2002). *Membuat Kompos*. Jakarta: Penebar Swadaya.

Handayanto, E., Krisnayanti, B., & Muddarisna, N. (2018). Potensi Pohon Lokal untuk Fitostabilisasi Logam Berat pada Tanah Tercemar Limbah Sianidasi Emas di Lombok Barat. *Jurnal Lahan Suboptimal*, *4*(1), 71–80.

Hatta, M. (2012). Uji Jarak Tanam Sistem Legowo terhadap Pertumbuhan dan Hasil Beberapa Varietas Padi Pada Metode SRI. *Jurnal Agrista*, *16*(2), 87–93.

Hidayati, N. (2005). Fitoremediasi dan Potensi Tumbuhan Hiperakumulator. *HAYATI Journal of Biosciences*, *12*(1), 35–40. https://doi.org/10.1016/S1978-3019(16)30321-7

Nagarajan, M., & Ganesh, K. S. (2015). Growth and Nutrient Uptake of Paddy (Oryza sativa L.) under Chromium(VI) Treatment. *International Journal of Environment and Bioenergy Int. J. Environ. Bioener*, *10*(2), 115–121.

Patola, L. N. P., Supriyono, S., & Pardjanto, P. (2017). Effect Use Biofertilizer and Differences Type Soil on Growth and Yield Arrawroot. *Sains Tanah - Journal of Soil Science and Agroclimatology*, *14*(1), 29–35. https://doi.org/10.15608/stjssa.v14i1.600

Pramono, A., Retno Rosariastuti, M., Ngadiman, N., & Prijambada, I. D. (2012). Peran Rhizobakteri Dalam Fitoekstraksi Logam Berat Kromium Pada Tanaman Jagung. *Jurnal Ecolab*, *6*(1), 38–50.

https://doi.org/10.20886/jklh.2012.6.1.38-50

Pratiwi, S. H. (2016). Growth and Yield of Rice (Oryza sativa L.) on various planting pattern and addition of organic fertilizers. *Gontor AGROTECH Science Journal*, *2*(2), 1–19. https://doi.org/10.21111/agrotech.v2i2.410

Radziemska, M., Vaverková, M. D., & Baryła, A. (2017). Phytostabilization-Management Strategy for Stabilizing Trace Elements in Contaminated Soils. *International Journal of Environmental Research and Public Health*, *14*(9), 1–15. https://doi.org/10.3390/ijerph14090958

Rajkumar, M., & Freitas, H. (2008). Influence of metal resistant-plant growth-promoting bacteria on the growth of Ricinus communis in soil contaminated with heavy metals, *71*, 834–842. https://doi.org/10.1016/j.chemosphere.2007.11.038

Rauf, A. W., Syamsuddin, T., & Sri, R. S. (2000). *Peranan pupuk NPK pada tanaman padi*. Irian Jaya: Derpartemen Pertanian, Badan Penelitian dan Pengembangan Pertanian.

Rosariastuti, R., Prijambada, I. D., & Prawidyarini, G. S. (2013). Isolation and Identification of Plant Growth Promoting and Chromium Uptake Enhancing Bacteria from Soil Contaminated by Leather Tanning Industrial Waste, 243–251.

Sembel, D. T. (2015). *Toksikologi Lingkungan Dampak Pencemaran dari Berbagai Bahan Kimia dalam Kehidupan Sehari–hari*. Yogyakarta: Andi Offset.

Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International*, *31*(5), 739–753. https://doi.org/10.1016/J.ENVINT.2005.02.003

Sharma, G. (2015). Review of Plant Growth Regulators - Control. *International Journal of Preclinical & Pharmaceutical Research*, *6*(3), 155–159.

Soil Research Institute. (2009). Technical Guidelines for Chemical Analysis of Soil, Plants, Water, and Fertilizers.

Sumaryo. 1982. *Ilmu Kimia Tanah.* Surakarta: Fakultas Pertanian UNS

Supriyadi, Purnomo, D., & Mangkulla, Y. D. (2017). Organic matter and root development of soybean in agroforestry of bengawan solo sub watershed Wonogiri Indonesia. *Sains Tanah - Journal of Soil Science and Agroclimatology*, *14*(1), 1–6. https://doi.org/10.15608/stjssa.v14i1.471

Syahfitri, W., Damastuti, E., & Kurniawati, S. (2011). Penentuan Logam Berat Cr , Co , Zn , dan Hg Pada Beras dan Kedelai dari Wilayah Kota Bandung. In *Prosiding Seminar Nasional Sains dan Teknologi Nuklir* (pp. 213–219). Bandung: PTNBR – BATAN.

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*(2–3), 456–464. https://doi.org/10.1016/J.SCITOTENV.2006.01.016