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Research Article

The effectiveness of Mendong plant (*Fimbrystilis globulosa*) as a phytoremediator of soil contaminated with chromium of industrial waste

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Abstract : The textile industry produces sideline output in the form of dangerous waste. The textile industrial waste containing heavy metal, one of which is Chromium (Cr). Chromium is very dangerous metal for environment, especially chromium hexavalent that has properties of soluble, carcinogenic, and toxic. The pollution of chromium in soil is a problem that the action to be taken with the technology of bioremediation. Phytoremediation of soil contaminated with chromium using Mendong plant (*Fimbrystilis globulosa*), combined with association of microorganisms *Agrobacterium* sp I_3 and compost. This study was conducted in field experiment plots using a completely randomized block design. Data were analyzed using Anova followed by Duncan and correlation tests. The results showed that the Mendong plant was an effective phytoremediator of soil contaminated with chromium and it can be used as a chromium accumulator plant. The highest decrease of soil chromium content of 58.39% was observed on the combined artificial fertilizer, *Agrobacterium* sp I_3 and Mendong plant treatment (P1B1T1). Removal effectiveness of chromium at the treatments using Mendong plant was higher than without the Mendong plant. Chromium uptake in shoots was higher than in roots of Mendong plant. Bioremediation increased the total bacterial colonies, decreased soil pH, and increased cation exchange capacity of the soil. The growth of the Mendong plant was in a good condition during the process of bioremediation.

***Keywords**: bioremediation, chromium, Fimbrystilis globulosa, phytoremediation, rhizoremediation*

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Introduction

A growing population increases human needs to meet the human life. One the measures to enhance the welfare of human life is the existence of industrial activities. One of industrial activities is the textile industry that produces output in the form of waste. Textile industrial waste contains toxic materials that are harmful to the environment, water, soil, and human health. According of 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. One of the dangerous heavy metals is Chromium (Cr). Chromium comes from industrial activity, textile, tanning, manufacturing of paints, iron coating, wood preservation, and mining activities.

Chromium is hazardous to the environment, especially chromium hexavalent / Cr (VI) because it has properties of soluble, carcinogenic, and toxic, that cause death of microorganisms, animals, and humans if concentration is above of standard quality (U.S. Department of Health and Human Service 2012). In Jaten District, Karanganyar Regency, Central Java Province, there are many agricultural lands that have been irrigated with industrial wastewater in Jaten containing Cr between 0.531-3.99 ppm (Widyastuti et al., 2003). In general, standard quality of chromium in soils that is allowed by the Indonesian Government is 2.3 ppm (Ministry of Environment, 2010). Soils in Kebakkramat sub district, Karanganyar Regency are suspected to be polluted with Cr above the standard quality because there are many industries at Kebakkramat areas. The textile industries in the Kebakkramat area are near agricultural lands and rivers. Textile industrial waste that is commonly discharged to the river and agricultural land becomes the source of contamination on the surrounding agricultural lands.

Technology to recover quality of soil contaminated with heavy metal that is now being developed is bioremediation. Bioremediation is a way to degrade, move, and change harmful compounds into more simple and harmless (Kamaludeen et al., 2003). Bioremediation that uses plants is called phytoremediation. Phytoremediation is a technology for reducing, degrades, and isolates pollutants of the environment by using plant (Pramono et al., 2013). Plant that can be used as a hyperaccumulator is a plant that has high durability, rapid growth, ability to do phytoextraction of heavy metal, and it is not a food crop. Mendong plant is a non-food plant for human or animal consumption that is easily cultivated. The plant that can survive in flooded condition has high economic value of craft materials. The Mendong plant can be selected as a plant in phytoremediation. One of phytoremediation techniques that can be employed to clean up soils contaminated with heavy metals is rhizoremediation. Rhizoremediation is a process that involves the association of mutualism rizosphere plants with microorganisms, which can release exudates and oxygen into the soil to decrease chromium (Pramono et al., 2013). The bacteria used in the remediation of chromium are resistant to chromium and can survive in the chromium-contaminated environments. One of the bacteria that is resistant to the environmental conditions contaminated with heavy metals is *Agrobacterium* sp I_3. This bacterium can increase the uptake of the Cr to plant shoots. Addition of *Agrobacterium* sp I_3 isolate can increase the growth of rami plant (Rosariastuti et al., 2013).The purpose of this study was to explore the ability of Mendong plant in absorbing chromium in chromium contaminated soil.

Materials and Methods

This study was carried out on paddy fields contaminated with chromium at Waru village, Kebakkramat sub district, Karanganyar regency of Central Java Province, from May to October 2016. Treatments tested in this study consisted of three factors, i.e. artificial fertilizers treatment (P), chelators (*Agrobacterium* sp I_3; or compost) (B), and Mendong plant (T). Twelve treatments (Table 1) were arranged in a completely randomized block design with three replicates.

Table 1. Treatments

No.	Treatments	Description
1.	P0B0T0	Without artificial fertilizers, without chelators, without Mendong plant (control)
2.	P0B0T1	Without artificial fertilizers, without chelators, with Mendong plant
3.	P0B1T0	Without artificial fertilizers, with *Agrobacterium* sp I_3, without Mendong plant
4.	P0B1T1	Without artificial fertilizers, with *Agrobacterium* sp I_3, with Mendong plant
5.	P0B2T0	Without artificial fertilizers, with compost, without Mendong plant
6.	P0B2T1	Without artificial fertilizers, with compost, with Mendong plant
7.	P1B0T0	With artificial fertilizers, without chelators, without Mendong plant (control)
8.	P1B0T1	With artificial fertilizers, without chelators, with Mendong plant
9.	P1B1T0	With artificial fertilizers, with *Agrobacterium* sp I_3, without Mendong plant
10.	P1B1T1	With artificial fertilizers, with *Agrobacterium* sp I_3, with Mendong plant
11.	P1B2T0	With artificial fertilizers, with compost, without Mendong plant
12.	P1B2T1	With artificial fertilizers, with compost, with Mendong plant

Preparation of bacteria carrier

Carrier materials used for this study were 7.5 kg of bran compost, 750 mL of EM-4, and 15 L of water. The materials were mixed well and then incubated for 2 months. After the incubation period, the material mixture was sterilized using a presto pan for keeping the carrier sterile from undesirable bacteria or fungi.

Preparation of Agrobacterium sp I_3 inoculum

Duplication of *Agrobacterium*sp I_3 inoculum was started with the preparation of the LB (Luria Bertani) medium with the composition of 10 g tripton, 10 g NaCl, 5 g yeast extract, 100 mL distilled water, 15-20 g NA (Nutrient Agar) medium with the composition of 10 g beef extract, 10 g peptone, 5 g NaCl, 1000 mL distilled water, and 15 g agar/L. After obtaining pure isolate, purification was done in Luria Bertani liquid in Erlenmeyer and mixed up to gain density of 10^{10} cells/mL. The carrier was then enriched with squirted *Agrobacterium* sp I_3 to sterile carrier. The comparison was 600 mL *Agrobacterium* sp I_3 for 2 kg of the carrier.

Implementation of the study

This study used compost with a dose to the Mendong plant of 5 t/ha, while a dose of NPK fertilize used for Mendong plant was 400 kg/ha (Darini 2012). The dose of compost applied to Mendong plant treatment was 0.75 kg/plot of land. The dose of compost applied to the control treatment (without Mendong plant) was 1.125 kg/plot of land. Artificial fertilizers applied to Mendong plant treatment were 19.59 g Urea/plot of land, 25 g of SP-36/plot of land, and 15 g KCl/plot of land. Artificial fertilizers applied to control treatment (without Mendong plant) were 19.56 g Urea/plot of land, 18.75 g SP-36/plot of land, and 11.25 g KCl/plot of land. Application of compost and artificial fertilizers was done 1 day before planting the Mendong plant. The size of the plot of land was1.5 m x 1 m. Seeds of Mendong plant needed were six in one treatment or one plot of land, with spacing of plant per sub plots was 50 cm. Field observations were plant height of every week and plant dry weight at harvest. Plant dry weight consisted of parts of the root and the shoot of Mendong plant. Harvest was carried out at 30 days after the Mendong planting with bacterial isolate treatment. Analysis of chromium content in soil, roots, and shoots of the Mendong plant was done using a wet destruction method with AAS (Atomic Absorption Spectrophotometer). Soil parameters analyzed were CEC (ammonium acetate saturation), C-Organic (Walkley and Black), pH H_2O (Electrometric), and total bacterial colonies (nutrient agar medium with the hand colony counter). Removal effectiveness or phytoremediation effectiveness of Cr was calculated with the following formula: Removal Effectiveness (%) = [(initial concentration-final concentration)/initial concentration] x 100%. The data obtained were subjected to statistical test of Anova at 5 % level , continued test of Duncan at 5 % level, and correlation test.

Results and Discussion

Chromium content in the soil

Bioremediation decreased soil chromium content in all treatments (Figure 1). Before bioremediation, the soil contained chromium 2.46 mg/kg that was above the standard quality of 2.3 mg/kg (Ministry of Environment, 2010). The decrease of chromium content in the soil was caused by decrease of soil pH (Table 2).The high H^+ ions increased the solubility of chromium hexavalent so chromium become soluble and easily to be taken up by plant. Soil pH of all treatments decreased after bioremediation (Table 2). The increase of acidity is usually caused by waste containing free mineral acids and carbonic acid (Sontang, 2004). As compost contains free mineral acids, the compost treatment reduced soil pH value.

The artificial fertilizers caused soil pH become lower, it could happen because the fertilizers are soluble in water or hygroscopic that can cause content of H^+ ions in the soil becomes high. High H^+ ion on water content causes the solubility of hexavalent chromium higher, so it has high solubility and more easily taken up by plants (Yunilda 2008). Treatment with Mendong plant (T1) had lower soil chromium content than without Mendong plant (T0) (Figure 1). Treatment with *Agrobacterium* sp I_3 or compost had lower soil chromium content than control treatment (P0B0T0). It was proven by the increase of C-organic after bioremediation (Table 2). High C-organic in the soil caused high availability of nutrient elements that affected the increase growth of Mendong plant. The compost on P1B2T1 treatment could decrease Cr content by 1.55 µg/g. Results of Anova test at 5 % level showed that the treatments of artificial fertilizers, *Agrobacterium* sp I_3, compost and Mendong plant significantly decreased chromium content in the soil. Based on the correlation tests, chromium content in the soil correlated positively to soil pH. Content chromium in the soil correlated negatively to total soil bacterial colonies, soil CEC, and C-organic. The treatment of artificial fertilizers, *Agrobacterium* sp I_3, compost and Mendong plant decreased chromium content in the soil by 1.023 mg/kg (58.39%) in the P1B1T1 treatment, whereas treatment with low chromium content decrease was in the control treatment of P0B0T0 with chromium content in the soil was 2.438 mg/kg.

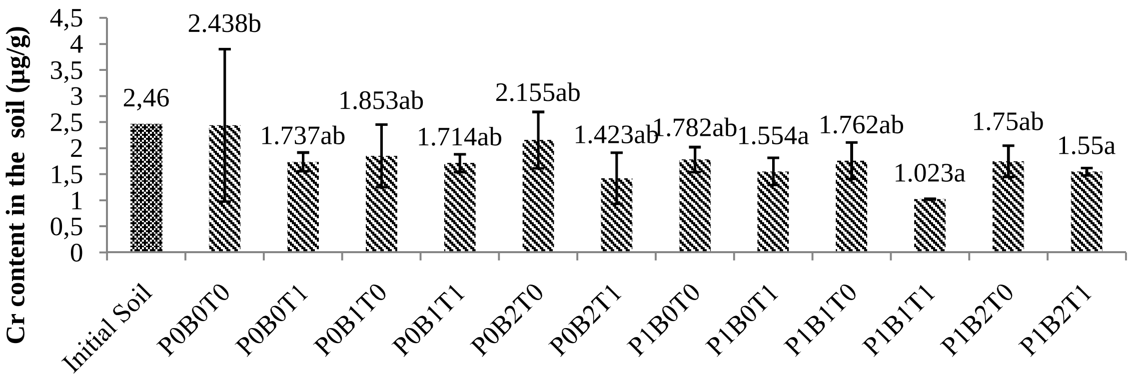

Figure 1. Chromium content in the soil.

Mendong plant ability in decreasing chromium in the soil can be calculated to detect effectiveness as a phytoremediator. Phytoremediation effectiveness for soil contaminated with chromium was high in the treatment of Mendong plant with artificial fertilizers, with *Agrobacterium* sp I_3 (P1B1T1) of 58.39% (Table 3). Whereas other treatment combinations using Mendong plant had chromium removal effectiveness more than without Mendong plant. The Mendong plant could increase CEC (Table 2). The process of bioremediation make Cr^{6+} cation is exchanged with other cations, Cr in the soil are exchanged with other cations then Cr in the soil could be reduced because of the Cr in the soil is uptake by plants. Bacteria acted elaborate compost into the nutrient elements ready to be uptake by the plant (Hanafiah et al., 2009).

Table 2. Soil characteristics

Treatment *)	Soil pH		Soil CEC (cmol(+)/kg)		C-organic (%)		Total Soil Bacterial Colonies (log 10 CFU/g)	
Initial Soil	7.55		19.614		3.31		12.62	
P0B0T0 (control)	6.96	bc	30.22	ab	2.91	a	12.65	a
P0B0T1	6.59	a	26.43	ab	2.99	a	12.18	a
P0B1T0	6.76	abc	32.24	b	3.28	a	16.18	b
P0B1T1	7.02	c	21.67	a	3.72	a	16.85	c
P0B2T0	6.88	abc	22.39	ab	3.11	a	12.66	a
P0B2T1	6.90	bc	23.91	ab	4.14	a	12.88	a
P1B0T0	6.59	a	29.98	ab	2.85	a	14.54	a
P1B0T1	6.68	ab	22.79	ab	2.71	a	12.98	a
P1B1T0	6.88	abc	25.73	ab	3.47	a	14.54	a
P1B1T1	6.81	abc	24.97	ab	3.05	a	14.57	a
P1B2T0	6.69	ab	26.79	ab	3.23	a	12.24	a
P1B2T1	6.59	a	24.21	ab	3.32	a	12.10	a

Remarks: Numbers followed by the same letter at the same column show no significant different in LSD test at 5% level. *) see Table 1.

Treatment combinations of artificial fertilizers, bacteria *Agrobacterium* sp I_3 and Mendong plant decreased chromium concentration in the soil. *Agrobacterium* sp I_3 treatment that made symbiosis with root of Mendong plant decreased chromium concentration in the soil because *Agrobacterium* sp I_3 has high tolerance to hexavalent chromium (Rosariastuti et al., 2013). In phytostabilization mechanism, *Agrobacterium* sp I_3 helped Mendong plant to accelerate chromium in rhizosphere areas or chromium uptake by root but it could not be toxic for root of Mendong plant. Total soil bacterial colonies on all treatments increased, except on the P0B0T1 (Table 2). *Agrobacterium* sp I_3 treatment had more total bacterial colonies than the compost treatments. The treatment with the highest number of total soil bacterial colonies of 16.85 log 10

CFU/g was the P0B1T1 treatment (Table 2). Application of *Agrobacterium* sp I_3 also increased the total soil bacterial colonies.

Table 3. Chromium removal effectiveness

Treatment *)	Phytoremediation Effectiveness (%)
P0B0T0	0.87
P0B0T1	29.37
P0B1T0	24.65
P0B1T1	30.32
P0B2T0	12.38
P0B2T1	42.15
P1B0T0	27.55
P1B0T1	36.98
P1B1T0	28.38
P1B1T1	58.39
P1B2T0	28.84
P1B2T1	36.98

*) see Table 1.

The resilience of bacteria can be seen from the number of colonies, if number of total soil bacterial colonies before bioremediation is low, and they become high after bioremediation, so the *Agrobacterium* sp I_3proved capable of adapting and a good tolerance in those plots. Treatment that had lowest removal effectiveness of 0.87% was the control treatment (P0B0T0). Mendong plant was effective as a phytoremediator of soil contaminated with chromium if it was combined with artificial fertilizers, *Agrobacterium* sp I_3, and compost. The Mendong plant treatment without artificial fertilizers and chelators could only decrease chromium concentration by 29.37%. Therefore, a better strategy for bioremediation of Cr contaminated soil is a combination treatment that can do maximum absorption of chromium.

Chromium content and uptake by Mendong plant

Chromium content in roots was lower than chromium content in shoot of Mendong plant (Figure 2). Mendong plant with control treatment (P0B0T1) had chromium content in shoot of plant that was lower than the other treatments. Treatment of compost application (B2) had chromium content in the shoot that was higher than the other treatment. Based on the Anova test at 5% level, chelator treatments influenced differently to chromium content in shoot of Mendong plant. This indicates that addition of compost improved C-organic in the soil. The high content of C-organic in the soilled to the high chromium uptake, because C-organic affected chromium uptake processes in plant roots and shoot. Chromium content in root of Mendong plant was lower than that in the shoot.

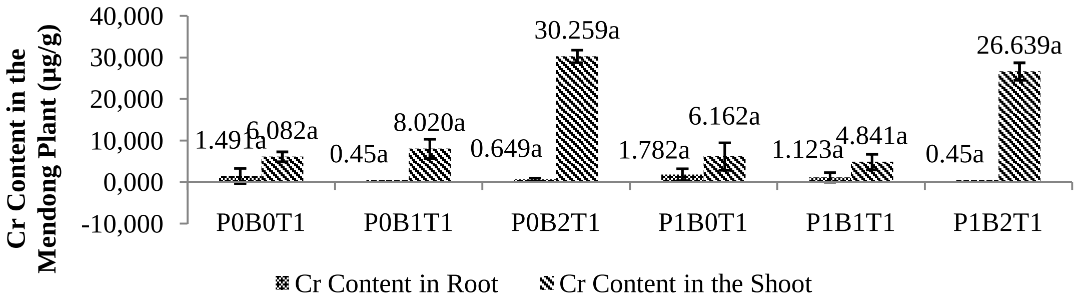

Figure2. Chromium content in Mendong plant root and shoot

The highest Chromium uptake in the shoot of Mendong plant the (214.92 µg) was observed in the P0B2T1 treatment (Figure 3). Based on the Cr uptake, Mendong plant can be considered as a Cr hyperaccumulator plant. A plant can be considered hyperaccumulator if it can uptake more than 100 ppm for Cd, Cr, Pb, and Co (Baker et al., 1994). The lowest chromium uptake in the root was observed in the P1B2T1 treatment (Figure 3). It occurred because chromium was translocated in the shoot of Mendong plant. Hexavalent chromium gets into the root from epidermis, then crosses a series of cells and breaks through the endodermis to xylem shoot of the plant. The highest chromium uptake in the root is (11.83µg) in the P1B0T1 treatment (Figure 3). The uptake process occurs because of accumulation of Cr in the root cells. The root cells are closely related to soil CEC. Soil CEC increased during the bioremediation process, so a high soil CEC caused the high chromium uptake in the root. Based on the Anova test at 5 % level, chelator treatment resulted in significantly different Chromium uptake in the shoot of the Mendong plant.

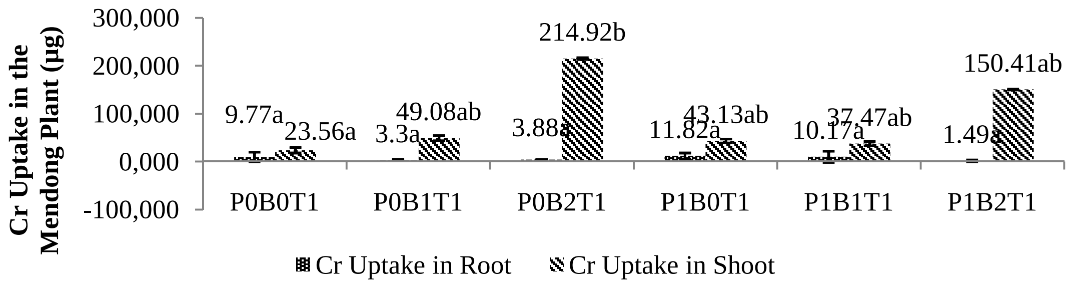

Figure 3. Chromium uptake in the Mendong plant

Height of Mendong plant

Plant height was measured at a time of maximum vegetative period. Application of artificial fertilizers increased the height of the Mendong plant. Data presented in Figure 4 show that the growth of Mendong plant varied. The highest Mendong plant of 65.6 cm was in the P1B2T1 treatment, while the lowest plant height of 49.8 cm was in the P0B2T1 treatment. Based on the correlation test, the height of Mendong plant was correlated positively to chromium content in the root, chromium content in the shoot, chromium uptake in the root, chromium uptake in the shoot, and dry weight of the plant. Based on the Anova test at 5 % level, the artificial fertilizers treatment provided significantly different height of Mendong plant. Figure 4 shows that artificial fertilizers treatment (P1) made Mendong plant higher than that without artificial fertilizers (P0). Artificial fertilizers applied in this research were Urea, KCl, and SP-36 to accelerate the growth of plant, increase the rate of photosynthesis, plant cells, stimulates enlargement the cells, and make stronger plant shoot. The Mendong plant has self-defense against the toxicity chromium in the environment. This is shown by the high growth of Mendong plant. Therefore, Mendong plant can be use as a hyperaccumulator plant for chromium, because chromium uptake in the plant was high and plant vegetative growth did not suffer interface.

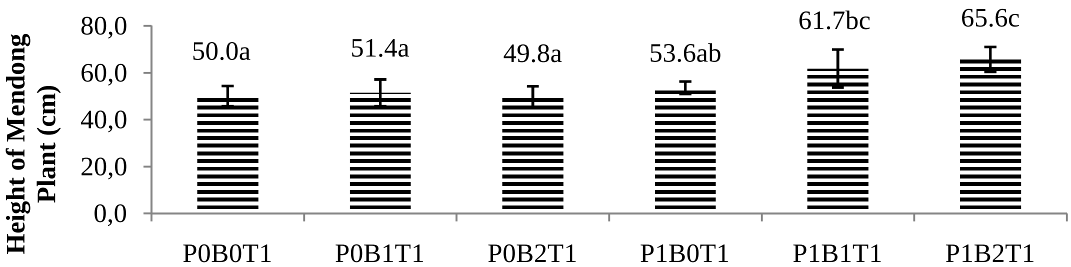

Figure 4. Height of Mendong Plant

Dry weight of Mendong plant

Figure 4 shows that the P1B2T1 treatment had the highest growth, but the treatment had the lowest dry weight (Figure 5). This occured because of the different in the number of clumps in the Mendong Plant. Mendong plant has many numbers of clumps then it automatically has high dry weight. Based on Figure 5, it can be seen that the highest plant dry weight of16.725 g on the P1B1T1 treatment. The lowest plant dry weight of7.943 g was on the P1B2T1 treatment. Hyperaccumulator plant can be tolerance against heavy metals at least 10-20 times of normal plant and still produce high biomass (Baker et al., 1994). Plant dry weight correlated positively to chromium content in the root, chromium uptake in the root, and height of Mendong plant. However, plant dry weight correlated negatively to chromium content in shoot and chromium uptake in shoot. Based on Anova test, chelators significantly increased plant dry weight.

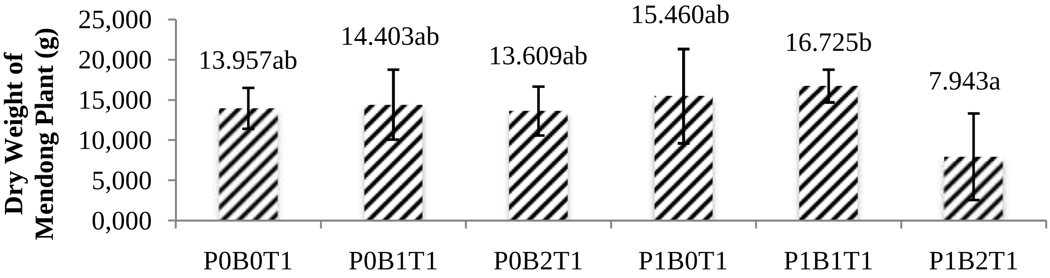

Figure 5.Dry weight of Mendong plant

Conclusion

The Mendong plant effective as a as phytoremediator of soil contaminated with chromium and can be used as plant of chromium hyperaccumulator. Bioremediation decreased soil pH, increased soil CEC, increased soil C-organic, and increased total soil bacterial colonies. Effective combination treatments to decreased chromium in the soil was application artificial fertilizers, *Agrobacterium* sp I_3, Mendong plant (P1B1T1) had highest phytoremediation effectiveness of 58.39 %, 42,15 % in (P0B2T1) treatment, and 36,98 % in (P1B0T1) treatment. Chelator treatments (*Agrobacterium* sp I_3 or compost) had lower chromium content in the soil than in the control treatment (P0B0T0).Removal effectiveness of chromium in treatment using Mendong plant was higher than without Mendong plant. Chromium uptake in the root was less than chromium uptake in the shoot of plant. Artificial fertilizers, *Agrobacterium* sp I_3 and compost increased the growth of Mendong plant. The growth of Mendong plant was in a good condition during the bioremediation process.

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