

Effect of bioremediation in soil of paddyfield contaminated by chromium (Cr) on soil fertility and chromium uptake by plant in Karanganyar, Central Java

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Abstract

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This research aimed to study the effect of bioremediation of soil contaminated by chromium on soil fertility and studying the Chromium uptake of rami and mendong during the bioremediation process. Field experiment was conducted in Waru Village, Kebakkramat, Karanganyar on May to November 2016. This research design was factorial using Completely Randomized Block Design as the based design consist of three factors: inorganic fertilizers (P), chelator (B), and plant (T). Variables observed were: N total, P available, K exchange, C organic, soil pH, total Cr, colony of bacteria, and plant biomass. Data was analyzed by statistical analysis using Anova test then continued by Duncan Multiple Range Test and Pearson's correlation test. The results showed that bioremediation treatment affected to several parameters. If it compared with control, the effect of bioremediation to soil fertility was an increase of N total by 40.9%, P available by 11.34%, K exchange by 2.71 %, C organic by 34.78%, and soil bacteria by 8.76% and decrease of soil Cr levels by 12.35% and soil pH by 3.03%. Rami plants uptaking of chromium was higher than that in mendong plants by 37.29%, i.e. with the average Cr uptake of rami 92.85 µg and mendong 72 µg.

Keywords: paddyfield; bioremediation; chromium; soil fertility; rami; mendong

Abbreviations: DMRT – Duncan Multiple Range Test

Introduction

Pollution of heavy metals in the soil becomes an increasingly concerning issue because of its great influence on human health and ecological systems (Alghanmi et al., 2015). This pollution occurs due to the absence of sewage treatment systems at the existing plant so that they immediately dispose of waste into the water channel and eventually enter the farm. Incoming waste to agricultural land containing heavy metals, causing soil to be contaminated. According

to the United States Environment Protection Agency (US EPA, 1997) heavy metals that are categorized as dangerous major pollutants, namely Sb, Ag, Be, Cd, Cr, Cu, Pb, Hg. Karanganyar Regency, Central Java Province, is one of the developing districts that has many industries. The type of industry which is developed is textile industry. This industry produces liquid waste that is not environmentally friendly and contains heavy metals. So far the waste management has not met the standards. These poorly managed wastes were discharged into waterways and eventually enter the farm.

As a result agricultural land in this region has contaminated by chromium with a range of levels between $2.13 \mu\text{g g}^{-1}$ to $7.43 \mu\text{g g}^{-1}$ (Junaedi, 2004). This can be seen from the growth of abnormal plants and the yield are not optimal. Therefore, it is necessary to restore the soil condition in one way, namely bioremediation. According to Muller et al. (1996) bioremediation is the use of microorganisms or other biological systems to degrade / transform the pollutant under controlled conditions. Turner and Rust (1971) found that chromium (VI) reduced K, Mg, P, Fe and Mn uptake in soybean roots and also reduces the concentration of Ca, K, Mg, P, B, and Cu in the soil. Based on these findings, this study aimed to study the effect of bioremediation on soil fertility and to assess the growth of rami and mendong plants during the bioremediation process.

Materials and Methods

Materials and experimental design

The research was conducted from May to November 2016, located in the Aluvial soil Waru village, Kebakkramat, Karanganyar. Soil and tissue analysis was performed at Soil Chemistry and Fertility Laboratory, Physics and Soil Conservation Laboratory, Biology and Soil Biotechnology Laboratory Faculty of Agriculture, Sebelas Maret University, Surakarta. The tools used include soil cultivators, tools for analysis of physical, chemical and microbiological properties of soil and stationery. Materials used include rami and mendong seedling, inoculum *Agrobacterium Sp I₃* isolated by Rosariastuti (2013), carrier inoculum, urea fertilizer, KCl fertilizer, SP-36 fertilizer, compost, LB media and other chemicals for analysis of physical, chemical and soil microbiology.

The study was prepared using Randomized Complete Block Design (RCBD) with three factors. The first factor was inorganic fertilizer (P) with two treatment levels: (P_0) no inorganic fertilizer; (P_1) with inorganic fertilizer – rami (urea 130 kg ha^{-1} ; SP-36 55 kg ha^{-1} ; KCl 50 kg ha^{-1}), mendong (urea 130 kg ha^{-1} ; SP-36 166 kg ha^{-1} ; KCl 100 kg ha^{-1}) (Sudjindro et al., 2005; Darini, 2012). The second factor was the chelator (B) with three levels of treatment: (B_0) without chelator; (B_1) inoculum *Agrobacterium Sp I₃* with dosage of $10^6 \cdot \text{g}^{-1}$ soil; (B_2) compost – rami (10 t ha^{-1}), mendong (5 t ha^{-1}) (Adjie, 2005; Sudjindro et al., 2005). The third factor was plant (T) with three treatment levels: (T_0) without plant; (T_1) rami; (T_2) mendong. Observational variables include total N, available P, K exchange, soil pH, C organic, Cr content and Cr uptake, soil bacteria, plant height and biomass.

Implementation and analysis

The implementation of the research consisted of several progress: first, propagation of *Agrobacterium Sp I₃* isolate until

density 10^{10} g^{-1} solid media (Celantis Analitical, 2003); second, preparation of rami and mendong seedlings; third, initial soil analysis was conducted to obtain initial soil chemical characteristics comparison. Then, soil tillage was done based on the soil fertility also makes 54 plots each plot size around $1.5 \text{ m} \times 1 \text{ m}$ per plot with plant spacing $50 \times 50 \text{ cm}$. After that, all treatments were given at the beginning of planting except for *Agrobacterium Sp I₃* isolates given a week after planting to ensure the plant was alive. Plants were planted for a month and measured weekly. Harvesting was done 30 days after inoculation by removing the roots and taking some soil around rhizosphere.

Soil analysis included the following methods: chromium (wet destruction method then Cr detected with using Atomic Absorption Spectrophotometer), N total (Kjeldahl's method), P available (Olsen's method), K exchange (base saturation method), soil pH (electrometric method), C organic (Walkey and Black's method), and soil bacteria (nutrient agar with plate count method). Plant height was measured using ruler, and biomass measured with dried in an oven with a temperature of 70°C within 48 hours. The data were analyzed using Anova (F test of 5% level), and if it was significant, continued by Duncan Multiple Range Test (DMRT) of 5% level, and also Pearson correlation test to determine the correlation of each dependent variable.

Results and Discussion

The soil type at the research site was Alluvial with neutral pH, medium total N content, low P available, medium K exchange, medium CEC and high C organic content with loamy sand texture (Table 1).

According to Hardjowigeno (2003) that Alluvial soils have a uniform fertility rate or vary from low to high, me-

Table 1. Characteristics of initial soil

Variable observation	Result	Class
pH H ₂ O	7.53	Neutral*
C organic (%)	3.31	High*
N total (%)	0.23	Medium*
P available (ppm)	9.57	Low*
K exchange (cmol(+) kg ⁻¹)	0.59	Medium*
CEC (cmol(+) kg ⁻¹)	19.61	Medium*
Indigenous Bacteria (log 10 CFU g ⁻¹)	12.55	–
Cr ($\mu\text{g g}^{-1}$)	2.36	Below the threshold**
Sand (%)	86.86	
Silt (%)	6.38	Loamy sand*
Clay (%)	6.76	

Classification by *Eviati and Sulaeman (2009), **Government Regulation of the Republic of Indonesia No 101 (2014)

dium to rough textures, and organic content from low to high and soil pH ranges from acid to neutral to alkaline, saturation bases and cation exchange capacity also vary as it depends on the parent material.

Effects of bioremediation on soil chemical characteristics

Based on Anova, there was an interaction between P treatment (inorganic fertilizer) and B (chelator) also an interaction of P treatment (inorganic fertilizer) and T (plant) to K exchange, but no interaction between inorganic fertilizer, chelator and plant. The interaction of inorganic fertilizers and the chelator to the K exchange are presented in Table 2.

Table 2. Interaction effect of inorganic fertilizers and chelator to K exchange

Levels of treatment	Content cmol(+) kg ⁻¹		
	B0	B1	B2
P0	0.184abc	0.187bc	0.179ab
P1	0.184abc	0.178a	0.189c

The numbers followed by the same letter were not significantly different by DMRT 5%.

Table 2 showed that bioremediation treatment has a significant effect on K exchange for the interaction of inorganic fertilizers and chelators. The highest yield was obtained by the combination of inorganic fertilizer and compost fertilizer (P_1B_2) treatment ie 0.189 cmol (+) kg⁻¹ and the lowest inorganic fertilizer treatment with *Agrobacterium Sp I₃* (P_1B_1) ie 0.178 cmol (+) kg⁻¹. Based on Anova, interaction of inorganic and plant fertilizer also showed significant result. The interaction of inorganic and plant fertilizers presented in Table 3.

Table 3. Interaction effect of inorganic fertilizers and plant to K exchange content

Levels of treatment	Content cmol(+) kg ⁻¹		
	T0	T1	T2
P0	0.185 ab	0.183 ab	0.182 ab
P1	0.182 ab	0.189 b	0.180 a

The numbers followed by the same letter were not significantly different by DMRT 5%.

The highest result for the interaction of inorganic and plant fertilizers was obtained by treatment of inorganic fertilizer and rami plant (P_1T_1), i.e. 0.189 cmol (+) kg⁻¹. Initial K content value 0,588 cmol (+) kg⁻¹ (medium) indicated that after treatment was given precisely, K exchange level decreased. Decreased K levels were probably due to absorption by plants, erosion, and leaching (Kaddar et al., 1984).

Erosion caused 86% of the soil was a sand fraction and also causes leaching. Compared with the control of biore-

mediation treatment of inorganic fertilizer and compost fertilizer (P_1B_2) as well as inorganic fertilizer and rami plant (P_1T_1) tends to increase the K exchange about 2.71% since the addition of organic material will increase the negative charge so that it will increase the cation absorption (Suntoro, 2003). Inorganic fertilizers in the form of KCl also affect the availability of K exchanged soil.

Based on the result of Anova, factor B (chelator) give an effect to organic C content. The results of the organic C analysis were presented in Table 4. Table 4 shows that bioremediation treatment has significantly effect on organic C.

Table 4. Effect of chelator on organic C content

Levels of treatment	Organic C (%)	Organic matter (%)
Factor B		
B0	2.76 a	4.75
B1	3.70 b	6.37
B2	3.74 b	6.44

The numbers followed by the same letter were not significantly different by DMRT 5%.

Based on DMRT, the highest result was treatment compost fertilizer (B_2) which was 3.74%, and the lowest result was by treatment of B_0 which was 2.76%. Bioremediation treatment tends to increase the organic C content around 34.78%. NCAT (2015) states that compost contributes to soil fertility by increasing organic materials, water holding capacity, and soil nutrient availability.

The giving *Agrobacterium Sp I₃* given with the carrier also gives a significant effect. As Davidson et al. (2002) explains that the high rate of respiration reflects a high C investment in the soil. After correlation tested, organic C correlated quite positively to total N ($r=0.326^*$), P available ($r=0.453^{**}$). This was in accordance with several roles of organic materials such as adding nutrients.

Based on the result Anova there was an interaction among P treatment (inorganic fertilizer), B (chelator), and T (plant) to soil total N. The result of soil N total content analysis presented in Table 5. N total content before treatment was 0.23% (mean) and at treatment control $P_0B_0T_0$ was 0.22% (medium). The highest result was $P_0B_2T_2$ treatment that was 0.32% followed by $P_1B_0T_0$ treatment ie 0.31%. Bioremediation treatment tends to increase the soil total N by 40.9%. An increase of organic matter will affect the availability of soil total N as evidenced by positive correlation test results with C organic ($r=0.326^*$). This was in accordance with the statement of Tisdale and Nelson (1975) which organic material was a source of nitrogen (protein) first the organic material will be decomposed into amino acids, then decomposed by heterotrophic bacteria converted into ammonium. Inorganic fertilizers such as urea also affect the availability of soil total

Table 5. Interaction effect of inorganic fertilizers, chelator and plants to soil chemical characteristics

Treatment	Total N (%)	P available (ppm)	Soil pH
Initial	0.23	9.57	7.53
P0B0T0 (control)	0.22 a	9.15 a	6.96 bc
P0B0T1	0.25 ab	9.65 ab	6.82 abc
P0B0T2	0.26 abc	9.56 ab	6.58 a
P0B1T0	0.28 abc	9.69 ab	6.76 abc
P0B1T1	0.26 abc	9.42 a	6.76 abc
P0B1T2	0.23 a	10.05 bc	7.01 c
P0B2T0	0.25 ab	9.48 a	6.88 abc
P0B2T1	0.31 bc	10.32 c	6.87 abc
P0B2T2	0.32 c	9.40 a	6.90 abc
P1B0T0	0.31 bc	9.23 a	6.58 a
P1B0T1	0.25 ab	9.25 a	6.92 bc
P1B0T2	0.23 a	9.44 a	6.68 ab
P1B1T0	0.25 ab	9.36 a	6.87
P1B1T1	0.28 abc	9.56 ab	6.75 abc
P1B1T2	0.24 a	9.34 a	6.80 abc
P1B2T0	0.25 ab	9.70 ab	6.69 ab
P1B2T1	0.28 abc	9.52 a	6.66 ab
P1B2T2	0.26 abc	9.43 a	6.59 a

The numbers followed by the same letter were not significantly different by DMRT 5%.

N because urea was an inorganic nitrogen source (Indriyati et al., 2008).

Based on the result of Anova there was an interaction among P treatment (inorganic fertilizer), B (chelator), and T (plant) to soil P available. The results of the analysis of Soil P available levels were presented in Table 5. The results of DMRT, the highest level obtained by the combination treatment without inorganic fertilizers with compost and rami plant ($P_0B_2T_1$) ie 10.32 ppm. The lowest result was obtained by control treatment ie 9.15 ppm. Initial P soil value was 9.57 ppm (low). This indicates that bioremediation treatment was able to increase the soil P available about 11.34%. Although without giving the inorganic fertilizers, there was the treatment of compost and rami plants. The interaction between organic matter and plant roots in rhizosphere will affect biogeochemical processes such as organic matter decomposition, dissolution and transport P, N fixation, and biocontrol of soil pesticides (Larsen et al., 2015).

Based on result of correlation test of P correlation quite positive with organic C ($r = 0.453^{**}$). Organic matter was a complete source of nutrients. The effect of organic matter on the availability of P can be directly through the mineralization process or indirectly by assisting the release of fixed P (Suntoro, 2003).

Based on the result of Anova there was an interaction among P treatment (inorganic fertilizer), B (chelator), and T (plant) to soil pH. Soil pH analysis results were presented in table 5. In general bioremediation has a significantly effect in decreasing soil pH. After further DMRT, the highest result obtained by the interaction of treatment without inorganic fertilizers + *Agrobacterium Sp I₃* isolates + mendong plants ($P_0B_1T_2$), which was 7.01. Treatment with the lowest results was inorganic fertilizer, compost, and mendong ($P_1B_2T_2$) 6.59. Initial soil pH before treatment ie 7.53 and control ie 6.96. This happened because the application of fertilizer could decrease soil pH. Soil flooding also affects the whole plot of treatment.

Agustina (1990) states that too much nitrogen fertilizer, like ZA, urea also causes the soil to become more acidic because its reaction in the soil causes an increase in the concentration of H⁺ ions. The giving of organic materials can increase or decrease the pH depending on maturity level. If the maturity level was still low then it will decompose which produce organic acid which decrease the pH and if the mature organic material will release the cations that will raise the soil pH (Suntoro, 2003).

Effect of bioremediation on soil Cr contents and plants

Based on the result of Anova there was significant interaction between P treatment (inorganic fertilizer) and T (plant) on soil Cr content. The result of analysis of soil Cr content is presented in Table 6. It showed that bioremediation treatment has significant effect on soil Cr content.

Table 6. Interaction effect of inorganic fertilizer and plants to soil Cr level

Levels of treatment	Soil Cr content ($\mu\text{g g}^{-1}$)		
	T0	T1	T2
P0	1.70 ab	1.58 ab	1.91 b
P1	1.90 b	1.78 ab	1.49 a

The numbers followed by the same letter were not significantly different by DMRT 5%.

The lowest results were shown by the treatment of P_1T_2 (inorganic fertilizers, mendong) of $1.49 \mu\text{g g}^{-1}$. The highest result was obtained by treatment of P_0T_2 (without inorganic fertilizer, mendong). This indicates that chromium levels were reduced during the bioremediation process. Decrease level caused by the bioremediation process itself was 12.35%. Mendong itself was capable of being used as a phytoremediator for Cr and Cd (Dewi and Hindersah, 2009). Inorganic fertilizer itself also affects as a stimulant for plants

to grow and absorb chromium. The interaction of bioremediation treatment also had an effect on the Cr content of the plants is presented in Table 7.

Although the Cr content of soil was lowest in the treatment of mendong plant, but as can be seen from Table 7, the average rate and uptake of Cr in rami was higher than in mendong. The decrease of Cr levels was caused by plant absorption but also because soil pH reduced from 7.53 to 6.58. The soil reaction strongly affects the Cr level. At high pH, Cr was in stable form and not available, but at low pH Cr will be very soluble in water and available for plants (Palar, 1994).

Table 7. Interaction effect of inorganic fertilizers, chelator and plants to plant Cr content and Cr uptake

Treatment	Plant Cr content ($\mu\text{g g}^{-1}$)	Plant Cr uptake (μg)
P0B0T1	0.23	68.07 ab
P0B0T2	0.22 a	63.90 ab
P0B1T1	0.25 ab	128.15 bc
P0B1T2	0.26 abc	159.27 cd
P0B2T1	0.28 abc	63.38 ab
P0B2T2	0.26 abc	25.94 a
P1B0T1	0.23 a	236.17 d
P1B0T2	0.25 ab	72.44 ab
P1B1T1	0.31 bc	39.11 ab
P1B1T2	0.32 c	53.56 ab
P1B2T1	0.31 bc	22.22 a
P1B2T2	0.25 ab	56.89 ab

The numbers followed by the same letter were not significantly different by DMRT 5%. All indicated that plant Cr levels had exceeded the 1-2 $\mu\text{g g}^{-1}$ (Notohadiprawiro, 2006)

As shown in Figure 1 (comparison levels and plant uptake), Cr content in rami plant was higher by 166.18% than in mendong (14.56 g g^{-1} and 5.47 g g^{-1} , respectively). Rami itself can be used as a heavy metal absorber (Rosariastuti et al., 2013). Based on the statistical analysis, for the Cr uptake of plants, both types of plants showed no significant differences although the Cr content of the plants of both types was significantly different, i.e. rami $92.85 \mu\text{g}$ and mendong $72 \mu\text{g}$. The biomass of mendong was higher than that of rami, but with low accumulation of heavy metals. However, rami Cr uptake was 37.29% higher than mendong Cr uptake.

Plant growth affects the soil Cr content also proved by the correlation test results. Plant Cr content positively correlated very strong with Cr uptake of plants ($r=0.886^{**}$). Cr uptake correlates quite positively with plant height ($r=0.274^*$), and quite positive with biomass ($r=0.378^{**}$). That was in accordance with Shukla et al. (2013) which states that phytoremediation of crop used to degrade toxic contaminants in the environment involves a number of processes including

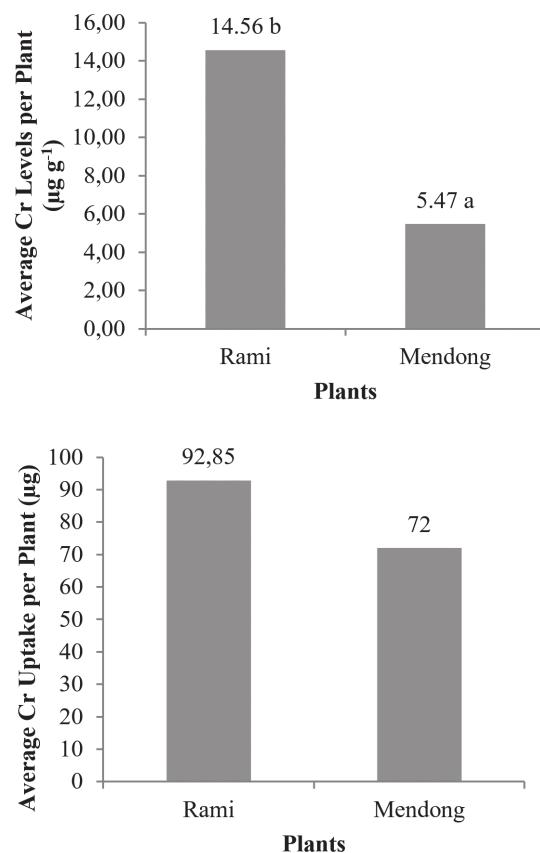


Figure 1. Comparison of plants Cr content and plants Cr uptake

The numbers followed by the same letter were not significantly different by DMRT 5%. Cr uptake was not tested because Duncan had no significant ($P>0.05$)

phytoextraction, fitotransformation, fitostabilization, fitovolatilization and rhizofiltration. Phytoextraction (or phytoaccumulation) involves the absorption and concentration of pollutants into harvested biomass for absorption or incineration. Phytotransformation involves enzymatic modification resulting in inactivation, degradation (phytodegradation), or immobilization (phytostabilization) of pollutants. Phytovolatilization involves the removal of pollutants from the soil and their release through the leaves through the evapotranspiration process and rhizofiltration involves filtering water through the root mass to remove pollutants.

Effect of bioremediation on biological characteristics

Based on the result of Anova there was an interaction among P treatment (inorganic fertilizer), B (chelator), and T (plant) on the number of soil bacteria. The results of the analysis of the number of soil bacteria were presented in Table 8.

Table 8. Interaction effect of inorganic fertilizers, chelator and plants to soil bacteria.

Levels of treatment	Bacteria (\log_{10} CFU g ⁻¹)		
	T0	T1	T2
P0	B0 17.64efgh	14.35abcd	12.52a
	B1 18.66fgh	20.02h	17.80efgh
	B2 12.81ab	14.06abcd	13.88abcd
P1	B0 16.18cdefg	16.73defg	13.33abc
	B1 12.79ab	14.30abcd	18.35gh
	B2 15.07abcde	15.76bcdef	16.25cdefg

The numbers followed by the same letter were not significantly different by DMRT 5%.

Based on the results of further DMRT, the highest result obtained by the interaction of treatment without inorganic fertilizers, *Agrobacterium Sp I₃*, and rami plant (P₀B₁T₁) ie 20.02 log 10 CFU g⁻¹. The lowest results were obtained by treatment interaction without inorganic fertilizer, without chelator, and mendong plants (P₀B₀T₂) ie 12.52 log 10 CFU g⁻¹. The number of soil bacteria before the treatment was worth 12.55 log 10 CFU g⁻¹. Increasing the number of soil bacteria after the bioremediation process the average ranges from 8.76 %.

In general, factors affecting bacterial growth were energy sources, carbon sources, nitrogen sources, minerals, and other growth factors (Irianto, 2006). However, from the data showed that the results with fertilizer and without fertilizer was not significantly affected, the influence was more caused by the addition of isolate *Agrobacterium Sp I₃* so that the amount of bacteria increased in quantity in bioremediation called bioaugmentation (Shukla et al., 2013). Isolate *Agrobacterium Sp I₃* was discovered by Rosariastuti et al. (2013) and was otherwise able to increase chromium uptake to rami plant canopy.

Effect of bioremediation on plant biomass

The use of rami and mendong plants because both were able to be used as plants hyperaccumulator. Rami has been noted as a dominant plant that lives in many different contaminated metal areas, and can accumulate large amounts of Sb, Cd and Hg. Mendong plants can be used to rehabilitate the heavily polluted soil wastes (Kurnia et al., 2003). The selection of rami and mendong itself because the end result of bioremediation used as a craft so it was not consumed by living things. Characteristics of hyperakumulator plants were: (i) resistant to metal elements in high concentrations in root and crown tissues; (ii) high absorption rate of soil elements compared to other plants; (iii) has the ability to translocate and accumulate metallic elements from root to shoot at a high rate. This translocation was a component that must be considered in the determination of hyperakumulator plants (Agunbiade, 2009 cit Hidayat, 2011).

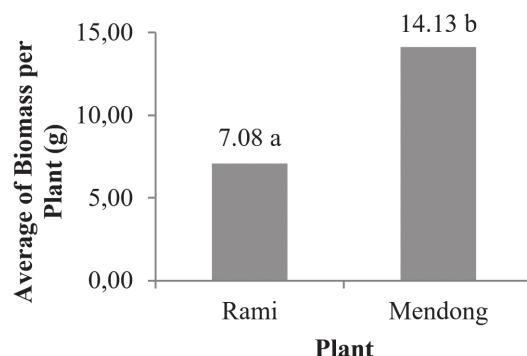


Figure 2. Graph of plant biomass
The numbers followed by the same letter are not significantly different by DMRT 5%.

Shanker et al. (2005) states that chromium has an impact on plant growth, among others, decreasing percentage of germination and decreasing shoot number, reducing root length and dry biomass, increasing root diameter and root hair, and reducing plant height and causing crop reduction. The rami biomass was not very high compared with mendong biomass of 7.08 g and the mendong has a biomass of 14.13 g. It caused by the land used itself was a paddy field with the characteristics always flooded and the permeability was very slow. Water circulation was not good and always flooded. Mendong plants with a clump-like and elongated shape also grow well in high water availability suitable for use in paddy fields by rami environment due to requiring good water circulation (Adjji, 2006).

Conclusions

Compared with control, the effect of bioremediation to soil fertility was an increase in uptaking of N total by 40.9%, P available by 11.34%, K exchange by 2.71%, C organic by 34.78%, soil bacteria by 8.76% and decrease in soil Cr level by 12.35% and soil pH by 3.03%. Rami plants uptaking of chromium was higher than mendong plants with an increase by 37.29%, i.e. with the average Cr uptake of rami 92.85 µg and mendong 72 µg.

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