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CHROMIUM EXTRACTION FROM SOIL BY USING GREEN MUSTARD (*Brassica juncea)*

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ABSTRACT

The extraction of chromium (Cr) from soil by using green mustard (*Brassica juncea)* have been studied. The effect of various experimental parameters have been investigated too. The results showed that the amount of Cr from soil by *Brassica juncea* plants affected by soil pH and plant age, the highest withdrawal occurred at of pH 7.0 - 7.5 at the age of 7 weeks. Cr withdrawal from soil by using green mustard without induction with cadmium showed that the accumulation in roots was 412.287 mg / kg of dry mustard and in the dry weight of leaves was 270.634 mg/kg mustard. The results also showed that there was no influence between the size of the pot with the accumulation of Cr on the plant because of the statistical analysis showed that there was no significant difference between the size of the pot (0.5 kg soil) with the other size larger. While the withdrawal of Cr from soil using cadmium induction showed that the Cr accumulation in roots was 873.30 mg / kg of dry mustard and in the leaves was 545.28 mg / kg dry weight of mustard. Thus, translocation of chromium metal ions from soil into roots and leaves of green mustard with the addition of the cadmium as an inductor may increase significantly which is two times greater than the accumulation of chromium without the addition of inductors.

Key words : extraction, chromium, soil, green mustard, *Brassica juncea.*

1. Introduction

There is a few kind of hyper accumulator plants that can attract Chromium (Cr). So far only *Medicago sativa* and *Dactylis glomerate* that have been identified as hyper accumulator plants of Cr (Shanker, et al, 2005), but both of these plants are very difficult to obtain and has not been developed in Indonesia. Therefore, in order to locate and identify other plants that serve as hyper accumulator of Cr is relatively more easily available and cheap, so this research studied the using of mustard green as hyper accumulator plants of Cr. Long-term goal of this research is to find / produce optimum conditions on the withdrawal of Cr from soil by using green mustard (*Brassica juncea*), with or without cadmium induction, so that this green mustard plants can be used as hyper accumulators of heavy metal chromium.

A hyper accumulator plants are plant species that can move pollutants/metal contaminants into the plant shoots more (with a high concentration) of the translocation in the roots without having symptoms of toxicity and to accumulate more than 10 ppm Hg, 100 ppm Cd, 1000 ppm Co, Cr, Cu and Pb, 10,000 ppm Ni and Zn (Lasat, 2000). Types of hyper accumulator plants that can accumulate Cr are very limited..

Consideration of selection of green mustard plants as an alternative hyper accumulator plant are, it has been shown to be used as hyper accumulator plant of lead (Pb) (Jerald, 1997), green mustard is including in Brassicaceae family, the same family of the most hyper accumulator plants of heavy metals that have been identified and green mustard plants are easily available and cheap so that application in the field are more likely to be done

Chromium, a toxic element, in principle dispersed in nature both in the water, air and soil and environmental industries and metal electroplating industry mainly through atmospheric emissions from industrial activities and residential city. Although Cr is not essential for plant growth, but can be taken by most species of plants. The advantages of this metal is poisoning can induce symptoms such as reduced growth and death of plants (Barcelo and Poschenrieder,1990), the destruction of the membrane (Kennedy and Gonsalves, 1987) and alteration of enzyme activity. Chromium intensively used in electroplating, other anthropogenic activities such as industrial metal, metal plating, mining, production, use and disposal of batteries, metal contaminated waste and sludge disposal, will cause a broad spread of Cr into the environment, including soil (Alloway, 1995).

To make the soil clean from Cr metal, required an effective method. Many conventional methods that can be used such as the methods of flushing, dilution and chemical stabilization. However, these methods are not efficient because it will cause new pollution problems at a time when that will come. For example, the method of rinsing / washing the soil causes the cation / anion leached will only move to a deeper layer of soil to reach groundwater. An alternative method that can be used and has proven effective is to use plants that have very high ability to transport metal pollutants from soil (fitoremediation/fitoextraction).

Phytoremediation approach is divided into five general categories namely phytodegradation, rizofiltration, phytovolatilization, phytostabilization and phytoextraction. Phytoextraction is the use of hyper accumulator plants to remove toxic substances like heavy metals from soil and store it in the bud or leaf (Brooks, et.al., 1979, Jerald, 1997). Intake of metals by the roots is an important stage for the main phytoextraction. The efficiency of this technology depends on many factors, among others, the capacity of root systems which retrieve and transfer the metal into the upper plant parts and the interaction between the soil with its physical-chemical characteristics, microbial and others. These complex interactions are influenced by various factors such as specific sections, climatic conditions, hydrology and geology.

Green mustard plant, *Brassica juncea*, is a heavy metal accumulator plants with high biomass, making it a good candidate for application in phytoplankton remediation strategies (Pilon-switches and Pilon, 2002). Strong -ECS or glutathione synthetase (GS) inγexpression of bacterial. *Brassica juncea* increased synthesis of phytoplankton chelats. This phytochelatin compound that can bind heavy metal ions through brown ties, which in turn can distributed from roots to this part of the other plants.

Research for the use of metal cadmium to induce increased phytochelatin synthetase in leaves of green mustard (*Brassica juncea*) has been done. (Hammer, D. and Keller, C., 2002). The results showed that when the green mustard plants were induced with 25 mM Cd, the accumulation of phytochelatin synthetase increased significantly after more than six days and the concentration of phytochelatin synthetase in leaves of three times higher than in roots.

The use of chelate compounds (EDTA, DTPA) to increase the withdrawl of metals by plants *Salix*, and *Thlaspi Sp* has been carried out (Hammer, D. and Keller, C., 2002). The results showed that the soil is acidic or alkaline, metals Cd decreased by *Salix*, *Sp*.withdrawal, In addition, withdrawal of Cd by *Thlaspi* , *Sp* in the acidic soil significantly increased while the soil is alkaline withdrawal increased very little. So there is no general conclusions can be drawn whether the addition of EDTA can increase Cd withdrawal because it depends on the type of plants used and soil acidity. For that we need also determined the optimum soil pH on the addition of EDTA in the withdrawal of cadmium by mustard green.

1.1. Phytochelatin

Phytochelatin peptides are molecules with the structure (g-Glu-Cys) n-gly, (g-Glu-Cys), nb-ala, (g-Glu-Cys) n-ser, (g-Glu-Cys) n-Glu, (g-Glu-Cys) n-Gln, (g-Glu-Cys) n, where n varies between 2-11 (Grill, et.al., 1985) and was first identified in 1983 in the yeast *Schizosaccharomyces pombe* (Cobbett , 2000)

Phytochelatin synthesized from GSH and catalyzed by the enzyme transpeptidase, called phytochelatin-synthetase, where this enzyme has shown activity only in the presence of heavy metal ions, especially Cd, Ag, Pb, Cu, Hg, Zn, Sn, Au, and As both vivo or in vitro (Cobbett, 2000). Several studies of physiology, biochemistry and genetics have confirmed that the tri-peptide glutathione is a substrate for biosynthesis of phytochelatin.

Although -Glu-Cys)γseveral structural variants of phytochelatin, for example: (γ -Glu-Cys) n-Glu have been identified inγ-Glu-Cys) n-ser and (γ-ala, (βn- several plant species, they are assumed to have an analog functions and synthesized through the same biochemical pathway essential (Rauser, 1999). In tobacco leaves, heavy metal-phytochelatin complexes are reported to accumulate in the vacuole (Vogeli-Lange and Wagner, 1990).

1.2.Metal Ion Mobilization in Plants

Acidification of the rhizosphere and carboxylate exudation are two things that are considered as potential targets for increasing the accumulation of metals (Clemens, et.al., 2002). The degree to which higher plants can attract metal ions in the soil depends on the concentration and bioavailability, modulated/regulated by the presence of organic matter, pH, redox potential, temperature and concentration other elements. Withdrawal of metal ions appear to compete with carriers of the same trans-membrane to nutrients such as K, Ca, Mg, Fe, Mn, Cu, Zn and Ni (Clarkson and Luttge, 1989). Cell membrane plays a role in metal homeostasis, prevent or reduce entry into the cell.

Apoplast of the root epidermis and cortex is permeable to solute. The cell walls of endodermal layer serves as a barrier to diffusion appoplastic into the vascular system. In general, solutes have been drawn into xymplasma roots before it can enter into the xylem (Tastet and Leigh, 2001). Due to the withdrawal of the metal into the xymplasma roots, three processes that determine the movement of metals from the root to the xylem are: metal-metal sequestration in root cells, transport simplastic into the Stele and eventually get into the xylem (Clemens, et.al. , 2002)

On the general environmental conditions, metal ions enter the first time to the roots. Metal ions easily penetrated into the roots through a network of cortical and distributed on the network of the top soil . Immediately after the metal ions into the roots, it can reach the xylem through the lane or path apoplastic and xymplastic (Salt, et al, 1995), complex compound formed by several ligands such as organic acids and / or phytochelatin (Senden, et.al ., 1992). In general, metal ion content in plant parts decreased in the order as follows: roots> stems> leaves> fruits> seeds (Blum, 1997)

1.2. Effect of Soil pH

Solubility of metals depends on a number of characteristics of the soil and is often strongly influenced by soil pH (Harter, 1983). As a consequence of the higher solubility of heavy metals at low pH, making more have been observed in some plant species such as wheat grass (Eriksson, 1989) for metal ions and plant the rice mountain to capture metal ions (eg Cd) of ground rock phosphate (Iretskaya, S.,N., et. al., 1998). However, several studies reported that the withdrawal of heavy metals by plants is influenced not only by soil pH but also by the cation exchange capacity, oxidation reduction potential and chemical composition of soil solution (Bingham, et. al., 1979). On the other hand, soil pH and other factors such as availability of nutrients may also affect root growth characteristics.

Dependence of pH on the release of cadmium, copper and lead from soil has also been observed. The results showed that with decreasing pH (range 2.9 to 7.1) the rate of release of the three metals increased, where the rate of release of cadmium> lead>copper (Sukreeyapongse,O,.et.al.,2002). For phytoextraction strategy develop ment, in addition to parameters such as type and degree of pollution, plant selection associated with the potential withdrawal and time of cleaning is needed, as well as parameters such as other forms of physical-chemical bonding of heavy metals should be considered. The various bond fractions of heavy metals can be determined through a sequential chemical extraction (Ahnstrom and Parker, 1999)

2. Method

2.1.Materials and Instruments

The materials to be used in this research is green mustard (*Brassica juncea*), soil, KH_2PO_4 0,1 M, 0.1 M NaOH, fertilizers TSP, KCl, concentrated HNO_3, H_2O_2 35 %, $Cd(NO_3)_2 . 2H_2O$, Whatman-42 filter paper, universal pH paper, distilled water, buffer solution pH 5, 6, 7.0, 7.5, 8.0.

The instruments will be used in this study is a tool commonly used glass in the laboratory, oven, hotplate, magnetic stirrer, porcelain cup, UV-Vis Spectrophotometer Genesys-10uv, furnace, desiccator, pH meters, stative and clamp, analytical balance , a number of pots of various sizes, syringe, and spoon

2.2.Procedure

2.2.1.Determination of soil volume Effect

To study the effect of soil volume, the optimum pH 7.0 to 7.5 and the optimum age of 7 weeks green mustard are implemented as follows. Plastic pots filled with 500, 1000, and 1500 grams of soil contaminated with Cr 25 ppm. Then the soil pH is set between 7.0 to 7.5 using phosphate buffer and incubated for 6 weeks before the experiment began. In the beginning of the experiment, into the pots experiment added 200, 120, 200 and 100 ppm of N, P, K and Mg as a fertilizer. Green mustard (*Brassica juncea*), which had been grown for 2 weeks (in the same condition) were implanted into the pot experiment and placed in a controlled room (temperature and sunlight intensity only). Every day, the plants were given the same amount of distillated water and crops harvested after 7 weeks.

Once harvested, the plants were washed with double distillated water and dried at 60 ^{o}C for 48 hours and dry weight recorded. Before analysis, dried samples of crushed and refined a number of samples (0.5 g) was dissolved in a mixture of 5 mL HNO3 (65%) and 4 mL of H_2O_2 (30%). Then the concentration of Cr solution was measured using UV-Vis spectrophotometer. As a control, some mustard plants grown in soil with an appropriate variation of soil pH, but the land is not contaminated with Cr.

2.2.2.Determination of Cd Induction Effect

Basically all the same treatment, only green mustard plants were induced with Cd to increase the production of phytochelatin in the mustard green, so the amount of chromium that can be drawn from the soil will multiply.

Plastic pots filled with a number of land (optimum volume) contaminated with Cr in the form of salt with a certain amount. Then the pH (optimum) of land be set using phosphate buffer and incubated for 4 weeks before the experiment began. At the beginning of the experiment, into the pots experiment added 200, 120, 200 and 100 ppm of N, P, K and Mg as fertilizer.

Green mustard (*Brassica juncea*), which had been grown for 2 weeks (in the same conditions) was induced by Cd 25 mM and planted/transplanted into experimental pots and placed in a controlled room (temperature and sunlight intensity only). Every day, the plants were given water with the same amount and plants harvested after a certain time (optimum age). As a control, we use of green mustard without Cr induction. Once harvested, the plants were washed with water and dried at 60 ^oC for 48 hours and dry weight recorded. Then 0.5 gram sample is dissolved in a mixture of 5 mL HNO3 (65%) and 4 mL of H_2O_2 (30%). Then the concentration of Cr was measured using UV-Vis Spectrometer.

Treatment: the influence of pot size and the induction of Cd ions is repetition 6 times, then the data obtained were statistically calculated.

3.Results and Discussion

3.1.Determination of soil volume effect .

Research findings in the first year, which is the optimum pH 7.0 to 7.5 and green mustard optimum age 7 weeks later, in the second year followed up to determine the the influence of soil volume. The results of the research presented in Table 1 and Table 2.

Table.1 The content of Cr (VI) in the leaves in various sizes pot experiment (n= 3)

NO	Heavy of soil (Kg)	The average weight of samples (g)	Absorbance	Average concentration (mg/L)	Average levels For dry weight of samples (mg Cr/Kg)
1	0.5	0.5056	0.983	2.761	273.065
2	1.0	0.5001	0.986	2.769	276.910
3	1.5	0.5008	0.984	2.764	275.972
4	2.0	0.5010	0.983	2.761	275.572

n = the number of repeat analysis

Table.2 The content of Cr (VI) in roots at different size of the pot experiment (n= 3)

NO	Heavy of soil (Kg)	The average weight of samples (g)	Absorbance	Average concentration (mg/L)	Average levels For dry weight of samples (mg Cr/Kg)
1	0.5	0.5050	1.473	4.137	409.181
2	1.0	0.4960	1.456	4.090	412.287
3	1.5	0.5030	1.465	4.115	409.062
4	2.0	0.5040	1.463	4.109	407.693

n = number of repeat analysis

Table 1 and Table 2 above are the result of chromium metal accumulation in green mustard (*Brassica juncea*), which was analyzed by UV-Vis spectrophotometer. Chromium metal accumulation in leaves of green mustard with the highest variation of the pot size is 276.910 ppm in the size of the pot 1.0 kg, while the lowest was 273.065 ppm at 0.5 kg pot size. While the accumulation of chromium in the highest green mustard root is 412.287 ppm in pot size 1.0 kg and the lowest was 407.693 ppm at 2.0 kg pot size. It seems that there is no relationship between the size of the pot with chromium metal accumulation in green mustard because of the statistical analysis showed that there was no significant difference between the size of the pot (0.5 kg soil) with the other size larger. Only real difference is shown between the concentration of chromium greater in roots than in leaves.

3.2. Determination of Cd Induction Effect

At this stage, the conditions of treatment in research adapted to the conditions which have obtained the optimum at pH 7.0 to 7.5 and harvested 40 days of age, while the concentration of cadmium ions are added to the soil is 25 mM. The results in this study can be seen in Tables 3 and Table 4.

Tabel 3. The content of Cr (VI) in leaves of Cd-induced ion

NO	Heavy of soil (Kg)	The average weight of samples(g)	Absorbance	Average concentration (mg/L)
1	0.2577	0.987	2.77	537.93
2	0.2554	0.979	2.75	538.37
3	0.2512	0.984	2.76	550.17
4	0.2514	0.983	2.76	549.17
5	0.2499	0.980	2.75	550.78
Average				585.45

n = number of repeat analysis

Table 4. The content of Cr (VI) in roots of Cd-induced ion

NO	Heavy of soil (Kg)	The average weight of samples(g)	Absorbance	Average concentration (mg/L)
1	0.1568	0.984	2.764	881.39
2	0.1536	0.947	2.660	865.92
3	0.1529	0.942	2.650	865.29
4	0.1535	0.953	2.680	871.98
5	0.1540	0.967	2.720	881.91
Average				873.30

n = number of repeat analysis

The results in the first year of Cr metal accumulation in roots and leaves of green mustard (*Brassica juncea*) is 412.287 mg/ kg and 276.910 mg/kg dry weight. In Table 3 and Table 4 shows that the accumulation of metal ions of Cr in the roots and the leaves on green mustard (*Brassica juncea*), which was induced by cadmium is 873.30 mg/kg and 585.45 mg/kg dry weight. Thus, the inductor of Cd can increase the translocation of metal ions Cr significantly by more than two times than the accumulation of chromium without the addition of inductors. The highest accumulation of cadmium metals contained in the root is an average of 873.30 ppm.

. 4.Conclusions

Based on the results of experiment, observation and discussion that has been done, it can be drawn several conclusions.

1. There is no influence between the size of the pot with cadmium metal accumulation in plant mustard greens because of the statistical analysis showed that there was no significant difference among the size of the pot.
2. Trans location of chromium metal ions from soil into roots and leaves of green mustard (*Brassica juncea*) with the addition of the inductor metal cadmium into the soil to increase significantly which is two times greater

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