Removal of Heavy Metal Cadmium with Phytoremediation by Using Vetiver Plant

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Abstract: Conventional method in removing heavy metal in contaminated soil is bringing some issues such destroying the soil structure and too expensive. Phytoremediation is a new technique that use plant to remove the heavy metal in the contaminated soil without disturbing the soil structure plus saving the cost. Vetiver plant is believe has the capability to act as the hyperaccumulator plant which is being use in phytoremediation of heavy metal in contaminated soil. This study was conducted in order to study the potential of vetiver plant in removing Cd in soil. A chelating agent that is *Ethylene Diamine Tetraacetic Acid* (EDTA) also being used in this experiment in order to study the effect on the removing of heavy metal by vetiver plant. A total number of 50 vetiver plants were sown during the two months period at the roofed shed near the Environmental Engineering Laboratory, Faculty of Civil Engineering, UTM Skudai, Johor. The vetiver plants grown in soil that was mix from soil with organic fertiliser at a ratio of 2 : 1 in polybag with 15 cm high and 10 cm in diameter dimension. 42 plants were selected and divided into 3 group with according to the concentration of heavy metal. Each of the groups had plants to be put heavy metal solution for about 15 and 30 days without EDTA, 30 days with addition EDTA 0.1 g/kg and 0.3 g/kg of soil. The remaining 6 plants were controlled plants for this experiment. Controlled plants were watered with tap water for the same period of time. Plants were weighted for fresh and dry weight, the length of the root and shoot also recorded before digested. Plants that reached the specified time were digested and the samples were analysed by using *Atomic Absorption Spectrophotometer* (AAS) with flame method. Translocation factor (BCF) and bioconcentration factor (BCF) were calculated based on the results from the analysis of the samples. Effect on the addition of EDTA also been analysed from the results. Overall results show that vetiver plant can be use as hyperaccumulator plant in removing Cd by using phytoremediation. Addition of EDTA also enhances the uptake of Cd by vetiver plant.

Introduction

Urbanisation process that happened on the earth gives us many impacts either in positive or the other way around. One of the negative impacts is contaminated soil by the heavy metal. One of this source is come from the industrial waste that is not well managed by the industries. There are many actions taken by the authorities and one of them is remediation technique. This technique is intended to eliminate any environmental contamination from materials such as soil, water and air for the benefit of mankind.

Phytoremediation is one of the solutions that have been found and adopted. This method is a combination of the words phyto which means tree or plant and remediation means restoring process. This method is using plants to reduce the problem without having to dig up the contaminated material. The research aims to study the potential of vetiver plant in removing Cd in contaminated soil and the effect on addition of *Ethylene Diamine Tetraacetic Acid* (EDTA) as chelating agent for the plant to remove heavy metal. Vetiver plant was chosen for this experiment as it is easy to obtain and it has high resistance towards the temperature, so the plant is suitable to be used in this experiment as it may fit any surrounding condition. Heavy metal that used in this experiment is $Cd(NO_3)_2$.

Previous Study

Phytoremediation of contaminated sites is interesting because it is relatively cheap and aesthetically pleasing to the public than with alternate remediation strategies involving excavation, removal or chemical in situ stabilization, conversion. There are some many types of phytoremediation technique such as phytoextraction, phytodegradation, degradation rhizosphere, rhizofiltration, phytostabilization, phytovolatization and phytorestoration [1]. Phytoextraction are techniques that reduce the concentration of contaminants in the soil by using a high capacity plant which are high ability to accumulate metals in shoots and can produce high biomass. Pollutants contained in the soil can be removed by harvesting the plants grown in the contaminated area [2].

When categorizing plants that can grow in the presence of toxic elements, the terms tolerant, indicator and hyperaccumulator are usually used. Hyperaccumulator plant takes up particularly high amounts of a toxic substance in their shoots during normal growth and reproduction [3]. The effectiveness of a phytoremediation technique is dependent on the selection of the appropriate plant. The plant must be able to adapt with the local climate, insects and diseases. Any plant used in this technique must be able to tolerate high concentrations of the toxic [4].

Selection of suitable chelating agents for the extraction of heavy metals from a contaminated site is another thing to be considered. The solubilisation of heavy metals must be enhanced to increase the efficiency of heavy metal extraction which is mainly based on the capacity of chelating agents to form water soluble organic complexes [5]. Ethylenediamine tetraacetic acid (EDTA) is the most effective chelating agent used for phytoremediation because it has a strong chelating ability for different metals and it also increases the bioavailability and plant uptake of the metals in the soil [6]. EDTA also allow plants which are not considered as hyperaccumulators to be usable for phytoremediation purposes because it induce plants to take up more heavy metals than they normally accumulate [7].

Methodology

Growing Process of Plant

A total number of 50 vetiver plants were sown during the two months period at the roofed shed near the Laboratory of Environmental Engineering, Faculty of Civil Engineering, UTM Skudai, Johor before the experiment was run. It was essential to obtain a uniform size. The vetiver plants grown in soil that was mix from soil with organic fertiliser at a ratio of 2:1. The plants were planted in the polybag with dimension of 15 cm high and 10 cm in diameter dimension and placed under a roofed shed. After two months, total of 42 plants with healthy condition and have about uniform size were selected for experiment.

Figure 1: Vetiver plant being put in polybag with mixed soil

Figure 2: Vetiver plant located at roofed shed

Preparation of Experiment Plant

A total of 36 pieces from the total of 42 plants were divided into 3 groups and the remaining of 6 plants were controlled plant for this experiments. Plants were divided into 3 main groups that was based on the concentration of heavy metals which included the 50 mg/L, 100 mg/L and 200 mg/L. Each major group had 12 vetiver plants which were then re-divided into 4 subgroups according to the time of experiment conducted. One small group were watered with cadmium solution for 15 days, while the remaining three small groups were watered with cadmium solution for about 30 days. 2 out of 3 from the subgroups were watered with the cadmium solution with EDTA (0.1 g/kg and 0.3 g/kg) each for 30 days. EDTA was included after 15 days of experiment. The remaining 6 controlled plants were watered with tap water from the first day until the 30th day of experiment.

Table1: Grouping of samples

Time	**Samples**		
15 Days	50 mg/L	-	-
	100 mg/L	-	-
	200 mg/L	-	-
30 Days	50 mg/L	50 mg/L + 0.1 g/kg EDTA	50 mg/L + 0.3 g/kg EDTA
	100 mg/L	100 mg/L + 0.1 g/kg EDTA	100 mg/L + 0.3 g/kg EDTA
	200 mg/L	200 mg/L + 0.1 g/kg EDTA	200 mg/L + 0.3 g/kg EDTA

Starting from the first day of the experiment, all plants except for the controlled plants were put the heavy metal solution with different concentrations in the large groups and alternated with tap water in the next day with the proportion of 50 ml for a prescribed period of time. The amount of 50 mL solution was chosen because to make sure it did not leech out of the polybag after poured into the soil.

Preparation of Sample for Analysis

Plants that have reached a specified period of experimentation time was cleaned using distilled water before being dried in the oven at 70 °C for three days. Soil and plants were weighed first before being put in the oven to determine the fresh weight. After three days, the soil and the plants were weighed again to get their dry weight before going through the process of digestion.

Plants that will be through the digestion process were cut into 2 parts, roots and shoots. Soil, roots and shoots were weighed in advance to get as much as 1 g per portion. All samples were then

added a 10 ml mixture of HNO_3- acid, H_2O_2 and distilled water with a ratio of 6 : 2 : 2. The sample was heated in the heating plate in the fume chamber cupboard for about 2 hours. After 2 hours the sample was cooled before it was mixed with 25 ml of distilled water before the filtration process.

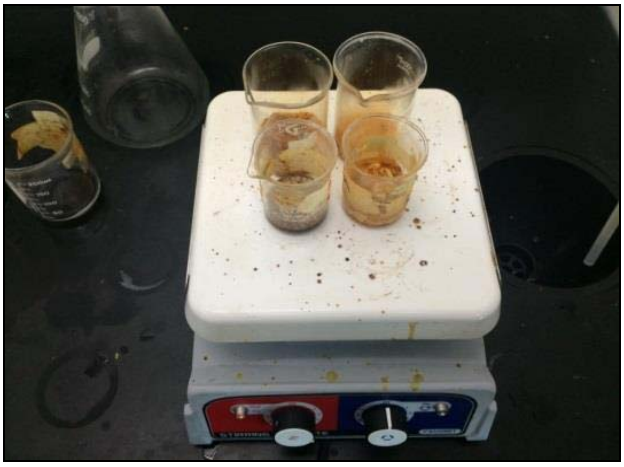

Figure 3: Sample is digested for about 2 hours using hot plate

Figure 4: Sample before digestion process

Figure 5: Sample after digestion process

Each sample that was mixed with distilled water was filtered twice. The first filtration process used the ordinary filter paper to get rid of any bulky waste from the samples. The second filtering

process used syringes with 0.2 micrometre sized filter to obtain a clear sample. This step should be carried out before the sample was taken for analysis by using Atomic Absorption Spectrophotometer (AAS) in the Laboratory of Environmental Engineering, Faculty of Civil Engineering, UTM Skudai, Johor. The sample then stored in a cool room before the analysis was carried out to protect the sample from damaged.

Figure 6: Samples is kept in the cold storage before being analyzed

Analysis of Sample

The filtered samples then analysed by using AAS machine. This machine can determine the quantity of chemical elements in a sample by absorption of light radiation of atoms in a gaseous state. Flame method is one of the methods commonly used to determine the quantity of chemical elements is chosen. This method uses flame at a temperature of 2700^{o}C. Samples will be sucked into the AAS machine and turned into gas through several processes before the quantity of chemical elements can be determined. Before the analysis of sample started, standard solution had to be prepared first. The standard solution concentration was in range from 0.1 mg/L to 0.75 mg/L.

Result and Discussion

In this chapter we discuss about the results get from the analysis of the samples. Translocation Factor (TF) and Bioconcentration Factor (BCF) can be calculated by using data from the analysis results. TF is the efficiency of the plant to translocate metal from its root to shoot at contaminated soil. If the percentage is greater than 100 %, it is said that the plant is a hyperaccumulator plant. Here are the formula of TF:

$$\text{Translocation Factor} : \frac{Shoot\ concentration}{Root\ concentration}\ x\ 100\%$$

BCF of a plant is the efficiency for the plant to uptake metal from the contaminated soil to its root and shoot. It is said that the plant is a hyperacumulator plant when the BCF is more than 100%. Here is the formula to calculate the BCF of plant :

$$\text{Bio concentration Factor} : \frac{Plant\ tissue\ concentration}{Soil\ concentration}\ x\ 100\%$$

The impact on the amount of EDTA being put in the plant also can be analyse in this topic. EDTA is functioning in increasing heavy metal solubility in the soil which will help the plant to translocate more amount of heavy metal. The length of plant section which are root with shoot and the fresh and dry weight of the plants are recorded in order to analyse the impact of heavy metal to the plant growth.

The results from the digestion of plant in laboratory are being compared by some factors such as the total time of experiment being conducted, amount of EDTA, fresh and fry weight of plant and size of plant section. Table 2 shows that translocation factor in percentage for plant with 15 and 30 days length of experiment according to their respective concentration of heavy metal. From the data, Figure 7 is plotted.

From the Figure 7, the translocation factor for 15 days plant is lower than 30 days plant without EDTA for all concentration of the heavy metal. The highest translocation factor for 15 days plant is about 57.59 percent on the 50 mg/L $Cd(NO_3)_2$ while the highest translocation factor for 30 days plant is 131.07 percent on the 50 mg/L $Cd(NO_3)_2$. The lowest translocation factor is on the 200 mg/L $Cd(NO_3)_2$ for both 15 and 30 days plant which are 13.82 and 60.86 percent. The translocation factor is clearly visible to be lower when the concentration is being double for each group of plant.

Based on this result, it shows that 30 days plants can translocate higher amount of heavy metal compare with the 15 days plant. So the longer time is needed in order to increase the translocation of heavy metal by the plant. The plant also can translocate much more amount of heavy metal in the lower concentration compare to the higher concentration of $Cd(NO_3)_2$

Table 2 : Translocation factor of 15 days and 30 days sample without EDTA

Concentration of heavy metal (mg/L)	Translocation Factor (%)	
	15 days sample	30 days sample without EDTA
50	57.59	131.07
100	21.98	123.22
200	13.82	60.86

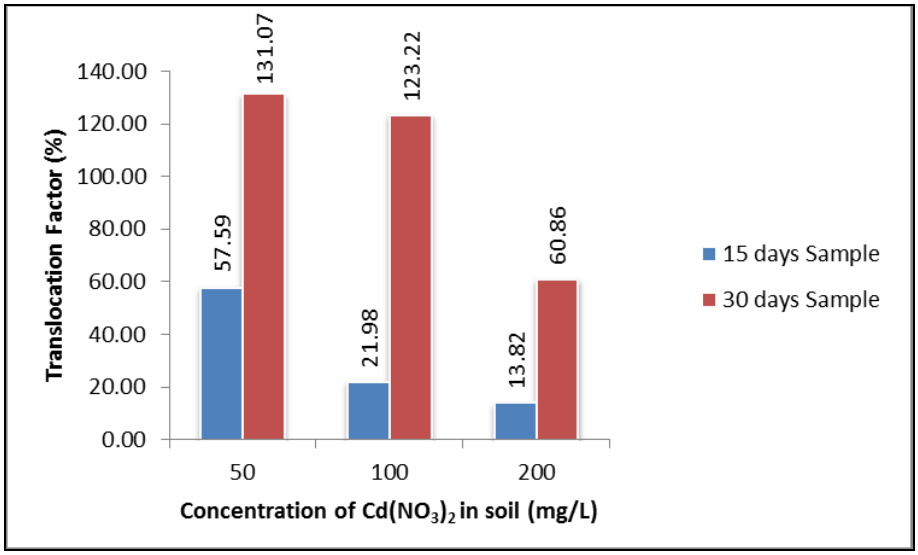

Figure 7: Translocation factor of 15 days and 30 days sample without EDTA

Table 3 shows that translocation factor in percentage for comparing with 30 days plant that being put 0.1 g/kg and 0.3 g/kg of EDTA according to their respective concentration of heavy metal. From the data, Figure 8 is plotted.

From the Figure 8, the translocation factor for plant with 0.1 g/kg EDTA is higher compare to the plant with 0.3 g/kg EDTA for all concentration of the heavy metal. The highest translocation factor for plant with 0.1 g/kg EDTA is about 145.3 percent while for the plant with 0.3 g/kg EDTA is 141.43 percent and both are from the 50 mg/L of $Cd(NO_3)_2$. The 200 mg/L of $Cd(NO_3)_2$ shows the lowest translocation factor for both plant with 0.1 g/kg and 0.3 g/kg EDTA which are 111.26 and 56.03 percent. The translocation factor for the plant with 0.1 g/kg and 0.3 g/kg EDTA is decreasing in percentage when the concentration of $Cd(NO_3)_2$ is going up.

Table 3: Translocation factor of sample with 0.1 g/kg and 0.3 g/kg EDTA

Concentration of heavy metal (mg/L)	Translocation Factor (%)	
	Sample with 0.1g/kg EDTA	Sample with 0.3g/kg EDTA
50	145.30	141.43
100	135.27	129.49
200	111.26	56.03

Based on this result, plant with the 0.1 g/kg EDTA enhances the translocation process of heavy metal of the plant more compare to the plant with the 0.3 g/kg EDTA. The plant also can translocate more amount of heavy metal when the concentration is low. The translocation process going slow when it is at 200 mg/L of $Cd(NO_3)_2$. So 0.1 g/kg EDTA is enough and preferable to enhance the translocation of heavy metal for the plant.

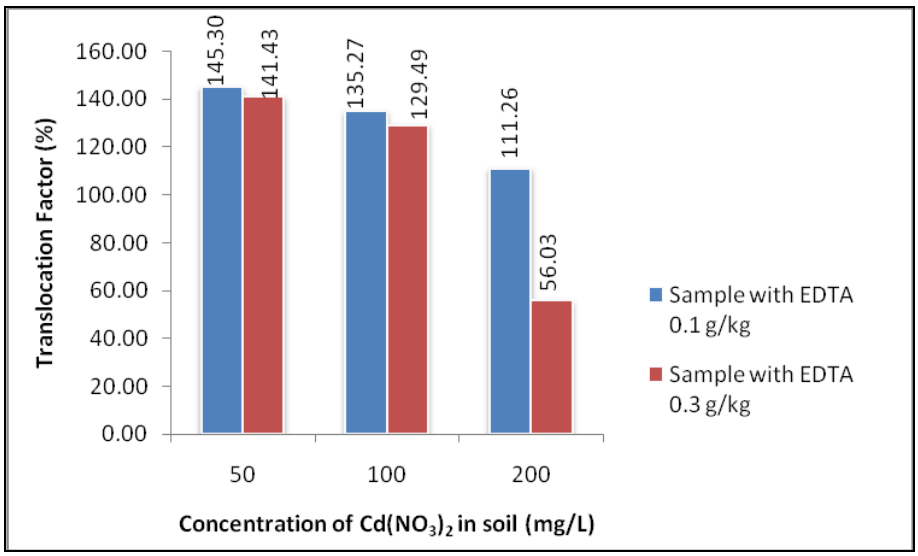

Figure 8: Translocation factor of sample with 0.1 g/kg and 0.3 g/kg EDTA

Table 4 shows that translocation factor in percentage for comparing with 30 days plant which were plant without EDTA, plant with 0.1 g/kg and 0.3 g/kg of EDTA according to their respective concentration of heavy metal. From the data, Figure 9 was plotted.

Table 4: Translocation factor for 30 days sample

Concentration of heavy metal (mg/L)	Translocation Factor (%)		
	30 days sample without EDTA	Sample with 0.1g/kg EDTA	Sample with 0.3g/kg EDTA
50	131.07	145.30	141.43
100	123.22	135.27	129.49
200	60.86	111.26	56.03

From the Figure 9, the translocation factor for plant with 0.1 g/kg EDTA is higher compare to the plant with 0.3 g/kg EDTA and plant without EDTA for all concentration of the heavy metal. The highest translocation factor for plant with 0.1 g/kg EDTA is about 145.3 percent, Plant with for 0.3 g/kg EDTA is 141.43 percent and 131.07 percent for plant without EDTA. All are from the 50 mg/L concentration of $Cd(NO_3)_2$. The 200 mg/L of $Cd(NO_3)_2$ shows the lowest translocation factor

for plant without EDTA, plant with 0.1 g/kg and 0.3 g/kg EDTA which are 60.86, 111.26 and 56.03 percent. The translocation factor for the plant without EDTA, plant with 0.1 g/kg and 0.3 g/kg EDTA is decreasing in percentage when the concentration of $Cd(NO_3)_2$ is going up.

Based on this result, the plant with 0.1 g/kg EDTA enhances the translocation process of heavy metal of the plant more compare to the plant with the 0.3 g/kg EDTA. The plant also can translocate more amount of heavy metal when the concentration is low. The translocation process going slow when it is at 200 mg/L of $Cd(NO_3)_2$. Presence of EDTA for 30 days plant enhance the translocation factor compare to the plant without EDTA except for the plant with 200 mg/L concentration of $Cd(NO_3)_2$. This is maybe due to the higher concentration of $Cd(NO_3)_2$ with the longer period of experiment for the plant.

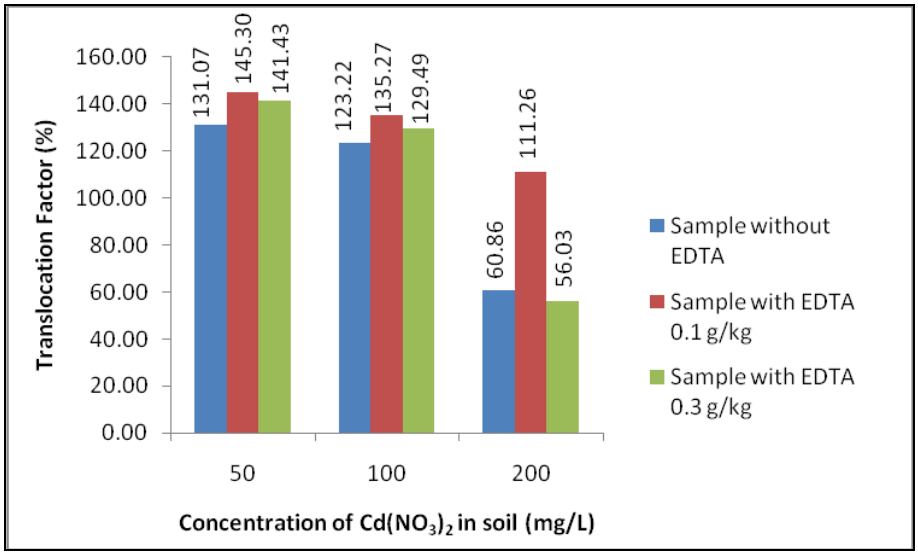

Figure 9: Translocation factor for 30 days sample

Table 5 shows the bio concentration factor in percentage for 15 days plant and 30 days plant without EDTA according to their respective concentration of heavy metal. From the data, Figure 10 is plotted.

From the Figure 10, the bio concentration factor for 15 days plant is lower than 30 days plant without EDTA for all concentration of the heavy metal. The highest bio concentration factor for 15 days plant is about 95.86 percent on the 50 mg/L $Cd(NO_3)_2$ while the highest bio concentration factor for 30 days plant is 239.52 percent also on the 50 mg/L $Cd(NO_3)_2$. The lowest bio concentration factor is on the 200 mg/L $Cd(NO_3)_2$ for both 15 and 30 days plant which are 73.33 and 175.34 percent. The bio concentration factor is decreasing when the concentration is double for each group of plant.

Based on this result, it shows that bio concentration of 30 days plants is higher compare with the 15 days plant. The bio concentration on the lower concentration of heavy metal is higher compare to higher concentration of $Cd(NO_3)_2$. So longer period of time of experiment and lower concentration of $Cd(NO_3)_2$ will increase the bio concentration of the plant.

Table 5: Bio concentration factor of 15 days and 30 days sample without EDTA

Concentration of heavy metal (mg/L)	**Bio concentration Factor (%)**	
	15 days sample	**30 days sample without EDTA**
50	95.86	239.52
100	83.46	196.19
200	76.33	175.34

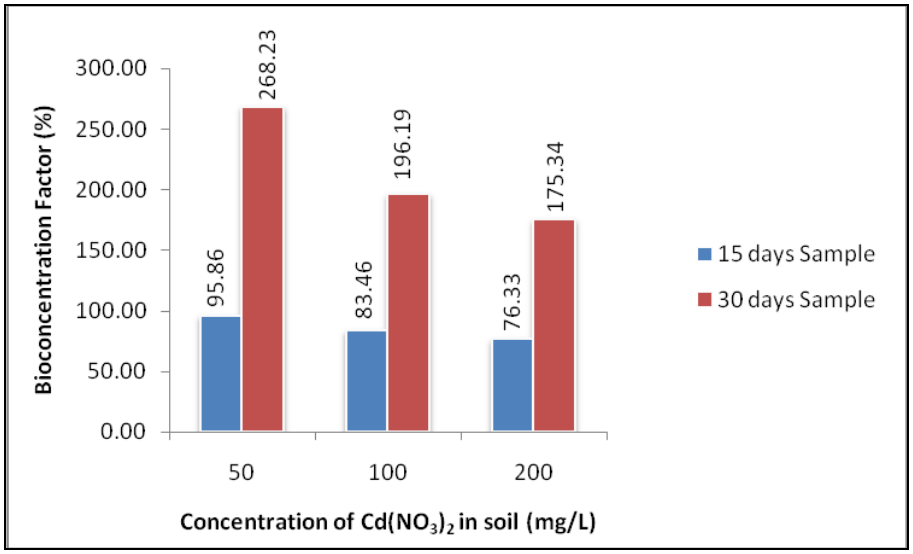

Figure 10 : Bioconcentration factor of 15 days and 30 days sample without EDTA

Table 6 shows that translocation factor in percentage for comparing with 30 days plant that being put 0.1 g/kg and 0.3 g/kg of EDTA according to their respective concentration of heavy metal. From the data, Figure 11 is plotted.

Table 6 : Bio concentration factor of sample with 0.1g/kg and 0.3g/kg EDTA

Concentration of heavy metal (mg/L)	Bio concentration Factor (%)	
	Sample with 0.1g/kg EDTA	Sample with 0.3g/kg EDTA
50	240.24	228.77
100	209.93	198.92
200	177.10	107.42

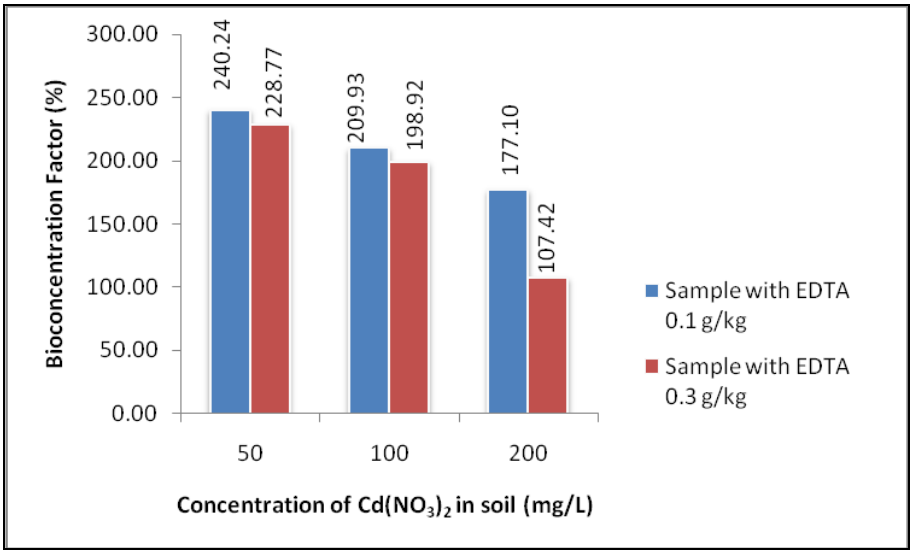

Figure 11: Bio concentration factor of sample with 0.1g/kg and 0.3g/kg EDTA

From the Figure 11, the bio concentration factor for plant with 0.1 g/kg EDTA is higher compare to the plant with 0.3 g/kg EDTA for all concentration of the heavy metal. The highest bio concentration factor for plant with 0.1 g/kg EDTA is about 240.24 percent while for plant with 0.3 g/kg EDTA is 228.77 percent and both are from the 50 mg/L of $Cd(NO_3)_2$. The 200 mg/L of

$Cd(NO_3)_2$ shows the lowest bio concentration factor for both plant with 0.1 g/kg and 0.3 g/kg EDTA which are 177.10 and 107.42 percent. The bio concentration factor for the plant with 0.1 g/kg and 0.3 g/kg EDTA is decreasing in percentage when the concentration of $Cd(NO_3)_2$ is going up.

Based on this result, the plant with 0.1 g/kg EDTA enhances the bio concentration of heavy metal of the plant more compare to the plant with the 0.3 g/kg EDTA. When the concentration of $Cd(NO_3)_2$ is low, the bio concentration tend to be higher compare when it is high concentration of $Cd(NO_3)_2$. So the 0.1 g/kg EDTA and lower concentration of $Cd(NO_3)_2$ will make the bio concentration of the plant will increase.

Table 7 shows that bio concentration factor in percentage for comparing with 30 days plant which were plant without EDTA, plant with 0.1 g/kg EDTA and 0.3 g/kg of EDTA according to their respective concentration of heavy metal. From the data, Figure 12 is plotted.

Table 7: Bio concentration factor for 30 days sample

Concentration of heavy metal (mg/L)	Bio concentration Factor (%)		
	30 days sample without EDTA	Sample with 0.1g/kg EDTA	Sample with 0.3g/kg EDTA
50	239.52	240.24	228.77
100	196.19	209.93	198.92
200	175.34	177.10	107.42

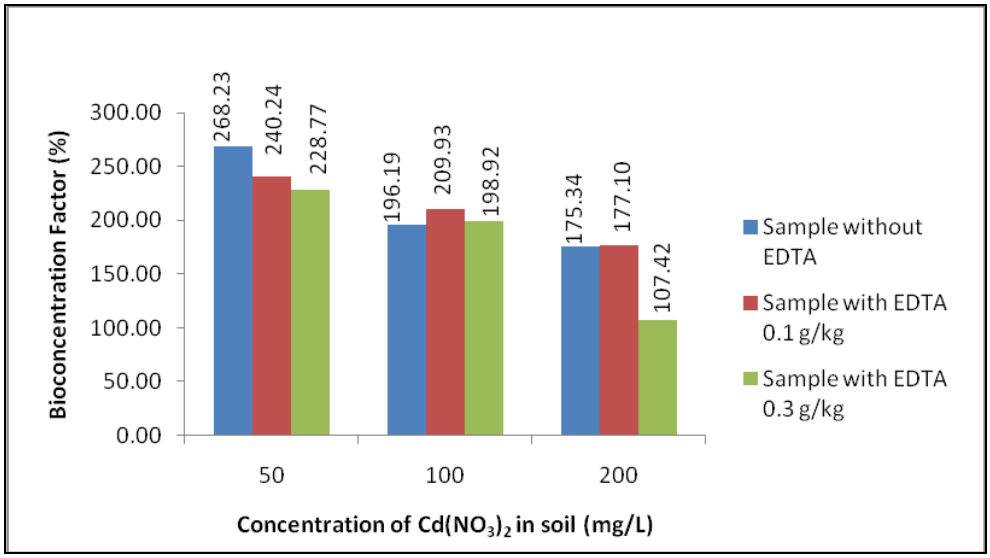

Figure 12: Bio concentration factor for 30 days sample

From the Figure 12, the bio concentration factor for plant with 0.1 g/kg EDTA is higher to compared with 0.3 g/kg EDTA and plant without EDTA for all concentration of the heavy metal. The highest bio concentration factor for plant without EDTA is 239.52 percent, 240.24 percent for plant with 0.1 g/kg EDTA and 228.77 percent for plant with 0.3 g/kg EDTA. All are from the 50 mg/L concentration of $Cd(NO_3)_2$. The 200 mg/L of $Cd(NO_3)_2$ shows the lowest bio concentration factor for plant without EDTA, plant with 0.1 g/kg and 0.3 g/kg EDTA plants which are 175.34, 177.10 and 107.42 percent. The bio concentration factor for the plant without EDTA, plant with 0.1 g/kg and 0.3 g/kg EDTA is decreasing in percentage when the concentration of $Cd(NO_3)_2$ is going up.

Based on this result, the plant with 0.1 g/kg EDTA enhances the bio concentration process of heavy metal of the plant more compared with the plant with 0.3 g/kg EDTA and plant without EDTA. The plant also can translocate more amount of heavy metal when the concentration is low. The bio concentration process going slow when it is at 200 mg/L of $Cd(NO_3)_2$. Presence of EDTA

for 30 days plant enhance the bio concentration factor compare to the plant without EDTA except for the 200 mg/L of $Cd(NO_3)_2$.

Table 8 is the average length of root and shoot for controlled plants and plant with variance concentration of heavy metal. From the data, Figure 13 is plotted.

Table 8: Average section length of plants by group

Section Length	Group of Plants			
	Controlled	50 mg/L	100 mg/L	200 mg/L
Root (cm)	30	23	25	25
Shoot (cm)	115	110	110	110

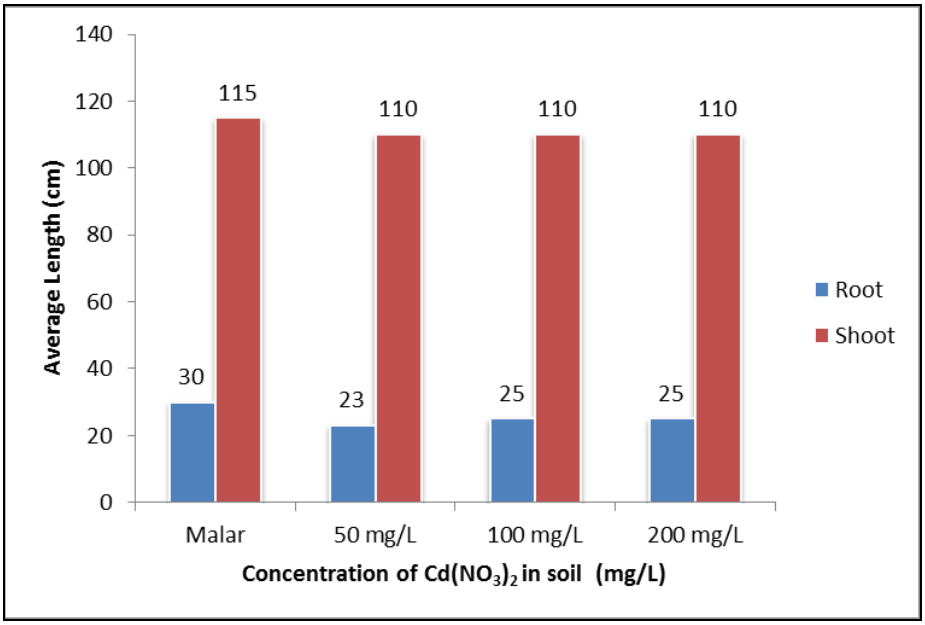

Figure 13 : Average section length of plants by group

From Figure 13, the root and shoot for controlled plants were the longest compared to the other plants which were 30 cm and 115 cm. Plants with 50 mg/L of $Cd(NO_3)_2$ had the shortest roots that was 23 cm and the shoots for the rest of plants were about the same which were 110 cm. The length of roots and shoots for the plants with $Cd(NO_3)_2$ were around the same number but a little bit shorter compared to the controlled plants. This shows that presence of the heavy metal at any concentration slightly will give effect on the plants growth compare to the controlled plants. However, the amount of concentration of $Cd(NO_3)_2$ in the plants not really give differences between the plants. Table 9 is the average fresh and dry weight of the plants with variance concentration of $Cd(NO_3)_2$. From the table shown above, Figure 14 is plotted.

Table 9: Average of fresh and dry weight of plants by group

Weight	Group of Plants			
	Controlled	50 mg/L	100 mg/L	200 mg/L
Fresh Weight (g)	55	45	41	42
Dry Weight (g)	14	12	13	13

From Figure 14, the heaviest fresh and dry weights were controlled plants that give the value of 55 g and 14 g. The lightest fresh weight was about 41 g for the plants at 100 mg/L of $Cd(NO_3)_2$ while the dry weight was about 12 g for the plants at 50 mg/L of $Cd(NO_3)_2$. The fresh and dry weight of plants with $Cd(NO_3)_2$ were around the same value but slightly much more lighter compared to the controlled plants. This shows that presence of the heavy metal at any concentration

slightly will give effect on the plants growth compared to the controlled plants. However, the amount of concentration of $Cd(NO_3)_2$ in the plants did not really give differences between the plants. The higher amount of fresh weight shows that the controlled plants were healthier compared to the plants with heavy metal.

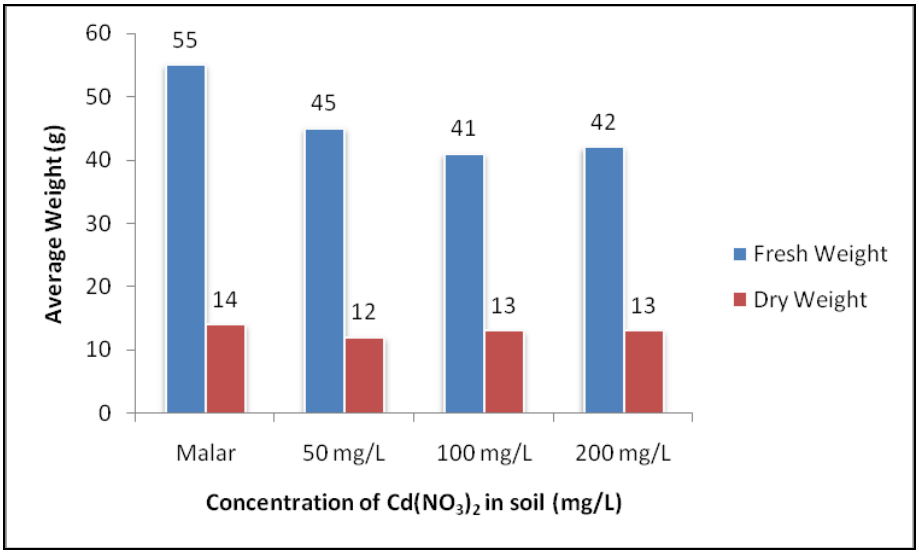

Figure 14: Average of fresh and dry weight of plants by group

Conclusion

Based on the results, it can be concluded that vetiver plant can be use in the phytoremediation technique to remove cadmium as a hyperaccumulator plant. The plant has the ability to translocate the heavy metal from the soil to the shoot either in a short period of time or in the presence of the EDTA. The uses of EDTA at 0.1 g/kg soil in this experiment is the optimum amount in order to enhance the plant to uptake the heavy metal from the soil.

Basically the vetiver plant plant can be use to uptake the heavy metal in the soil either at high or low concentration because the plant can endure the toxicity of heavy metal cadmium even at high concentration. However, the efficiency of the plant to translocate the heavy metal will decrease at high concentration.

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