Removal of Heavy Metal Lead Using Vetiver Grass

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Abstract: The importance of this study is to prove that phytoremediation method can overcome soil pollution of heavy metal other than using conventional method. This study was conducted to identify the efficiency of vetiver grass as hyper accumulator plant in order to remove Pb. This study also focused on the performance of vetiver grass in removal of Pb when EDTA is added in the soil. 50 vetiver grass has been planted at Environmental Engineering Laboratory, Faculty of Civil Engineering, UTM, Skudai, Johor. All of the vetiver grass were planted in soil which already mixed with organic fertilizer. The ratio for the mixing of soil and organic fertilizer is 2 to 1 ratio. All of the vetiver grass has been planted in the polybag with dimension 15 cm height and 10 cm diameter. After 2 months of seedling the vetiver, 42 vetiver grass has been chosen with the criteria of uniform height and size. The vetiver grass were watered for 15 days and 30 days alternately with Pb and tap water. Plants in set A were watered only with Pb solution for 15 days. Plants in set B were watered with Pb solution and EDTA (0.1 and 0.3 g/kg) for 30 days. 6 vetiver plants act as controlled plant which only watered with tap water for 15 and 30 days. The sample has been prepared and analyse using Atomic Absorption Spectrophotometer (AAS). The technique for this AAS is using flaming method. The result from this experiment is to obtain the translocation factor (TF) and bio concentration factor (BCF) of vetiver grass. In conclusion, the overall result showed that vetiver grass act as a hyper accumulator plant and it can remove Pb from the contaminated soil. Other than that, by adding EDTA can enhance the uptake of Pb from the soil by vetiver grass at 0.1 g EDTA / 1 kg soil.

Introduction

Nowadays, heavy metals contamination at river or soil is become worst. One of the reason because of the industrial sector or agricultural was in huge development. This followed by no control system or no proper treatment in the industrial and agricultural activities. Therefore it cause the heavy metals been released into the environment without proper treatment which is bad impact for the environment. To solve the problem, several methods has been used. One of the methods is phytoremediation technique. Using this technique the contaminated soil which from heavy metals will be removed from the soil. The objective for this study is to determine the effectiveness of vetiver grass as hyper accumulator plant. The second objective is to identify the effect of EDTA can enhance the ability for the vetiver grass in removal heavy metals. Scope for this study is about to identify the nature of vetiver grass either it have features as hyper accumulator plant in removal heavy metals using phytoremediation technique. This study is using vetiver grass scientifically names Chrysopogon zizanioides. Besides that, for the heavy metals used in this study is lead nitrate solution, $Pb(NO_3)_2$.

Previous Study

Phytoremediation

Phytoremediation is a process which using plants to remove, transfer, stabilize and destroyed the contamination which is organic or non-organic in soil or underground water. This technique use different plants in biological process and the physical features of the plants in order to help removing the contamination of the soil. Different plants usually have different biological features in their system. This means that different plants will have different level of effectiveness of clearing the contaminated soil. Phytoremediation can be dividing into several classes. Determination of this class is important in order to have a good outcome. The classes are phyto-accumulation, phytostabilization, rhizofiltration, phytovolatization, phytodegradation, rhizodegradation and hydraulic control [1].

Hyper Accumulator Plant

Hyper accumulator plants means plants that have ability to grow in the contaminated soil with heavy metals. Not just able to grow, that plant also can uptake the heavy metals without show any symptoms of phytotoxicity. The basic types for hyper accumulator plants is actively take up exceedingly large amounts of one or more heavy metals from the soil. Moreover, the heavy metals are not retained in the roots but are translocated to the shoot and accumulated in above ground organs, especially leaves. Although a distinct feature, hyper accumulation also relies on hyper tolerance, an essential key property allowing plants to avoid heavy metal poisoning. About 450 angiosperm species have been identified so far as heavy metal hyper accumulators [2].

Vetiver Grass. Vetiver grass is a perennial grass belonging to the Poaceae family. It has short rhizomes and a massive, finely structured root system. The deep root system makes the vetiver grass extremely drought tolerant and very difficult to dislodge when exposed to a strong water flow. Likewise, the vetiver grass is also highly resistant to pests, diseases, fire. It is known to be tolerant to heavy metals. There are reports on the use of this plant for phytoremediation of soils contaminated with heavy metals, phenol, radionuclides and nuclear waste. However, there was no report about using vetiver grass for phytoremediation of soil contaminated with trichloroethylene (TCE) [3].

Types of heavy metal

Lead. Lead is naturally occurring, bluish-grey metal usually found as a mineral combined with other elements, such as sulphur PbS or oxygen $PbCO_3$, and ranges from 10 to 30 mg kg^{-1} in the earth's crust. Typical mean Pb concentration for surface soils worldwide averages 32 mg kg^{-1} and ranges from 10 to 67 mg kg^{-1}. Uses of lead include solders, bearings, cable covers, ammunition, plumbing, pigments and caulking. Metals commonly alloyed with Pb are antimony (in storage batteries), calcium and tin (in maintenance-free storage batteries), silver (for solder and anodes), strontium and tin (as anodes in electrowinnig processes) and tellurium (pipe and sheet in chemical installations and nuclear shielding). Two routes of exposure to lead are by inhalation and ingestion has the same effects. Pb accumulates in the body organs (brain), which may lead to poisoning (plumbism) or even death. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and metal deterioration, with children under the age of six being at more substantial risk.

Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead. Lead can cause serious injury to the brain, nervous system, red blood cells and kidneys. Exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing young and infants being more sensitive than adults. Lead poisoning, which is so severe as to cause evident illness, is now very rare. Lead performs no known essential function in the human body, it can merely do harm after uptake from food, air, or water. Lead is a particularly dangerous chemical, as it can accumulate in individual organisms, but also in entire food chains. The most serious source of exposure to soil lead is through direct ingestion (eating) of contaminated soil or dust. In general, plants do not absorb or accumulate lead [4].

EDTA. EDTA, a member of the polyamino carboxylic acid family, is a hexahydric acid which forms strong complexes with metals through its two amines and four carboxylates groups. The introduction of EDTA as a chelator is considered an important breakthrough in chelation therapy. The EDTA molecule is widely used in domestic products and industrial processes owing to its useful physico-chemical properties. In agriculture, EDTA is used to enhance the bioavailability and uptake of essential nutrients. For example, Fe forms insoluble salts at near-neutral pH and becomes unavailable for plants. EDTA causes the solubilisation of Fe near-neutral pH and this property is used in hydroponic studies and in calcareous soils. EDTA can form a complex with almost all heavy metal ions. In the 1990s, EDTA was reported as a chelating agent for improvement in phytoextraction processes. Later on, this ligand is widely used in several pot and hydroponic experiments for enhanced heavy metal accumulation by plants. Several studies showed EDTA as the most efficient and effective chelating agents for increasing the solubility of heavy metals in soils and it has been widely used for the extraction of heavy metals from contaminated soils. The estimated annual consumption of EDTA is 55.6 metric tons in the United States and Western Europe [5].

Methodology

Seedling Process for Vetiver Grass

Total of 50 vetiver grass has been planted at Environmental Engineering Laboratory, Faculty of Civil Engineering, UTM, Skudai, Johor. All of the vetiver grass has been seedling almost for 2 months to get uniform size and heights. Vetiver grass planted in the polybag which consists of soil mixed with organic fertilizer. The vetiver grass were watered for 15 days and 30 days alternately with Pb solution. Plants in set A were watered only with Pb solution for 15 days. Plants in set B were watered with Pb solution and EDTA (0.1 and 0.3 g/kg) for 30 days. 6 vetiver plants act as controlled plant which only watered with tap water for 15 and 30 days.

Figure 1: Vetiver Grass been plant inside the polybag

Preparation Process for Vetiver Grass

The vetiver grass has been divided for set A and set B. From 50 vetiver grass, only 42 plants were chosen. 6 of them become as controlled plants, which only watered with tap water for 15 and 30 days. Set A is for 15 days and watered with Pb solution only at three concentrations (50, 100 and 200 mg/L). Whereas for set B is for 30 days and watered with Pb solution at three concentrations and EDTA at two concentrations (0.1 and 0.3 g/kg).

Digestion of the sample

After the 3 months period, all vetiver grass were harvested for the analysis and to test the effectiveness of vetiver grass in removal of heavy metals. First, the vetiver grass were washed with tap water then rinsed with distilled water. After that, each vetiver grass and its soil were weighted before placed it in the oven for 3 days at 70 degree Celsius. After 3 days the vetiver grass and the soil were weight again to get the moisture content of the soil and dry weight of vetiver grass. Each vetiver grass including soil were divided into 3 sample consist of soil, root and shoot. Only 1 gram was taken for each sample. For the root and shoot sample, it was cut into small pieces for easy digestion. After all samples were weight and prepared, the digestion process takes place. For the digestion process, nitric acid (HNO^{3-}), hydrogen peroxide (H_2O_2) and distilled water with ratio 6:2:2 were used as solution. During the digestion process, the sample was heated on hot plate inside the fume chamber for 2 hours and each hour 10 ml of the solution were added because the sample will dried out. After digestion process finished, samples were cooled down before mixed with 25 ml of distilled water.

Figure 2: Sample been digested on hot plate

Filtering Process and Analyse sample using AAS

In filtering process, all samples been filtered 2 times for each samples. The first filtration is using filter paper to remove large particles or foreign objects whereas the second filter by using syringe filter which size at 0.25 micrometers to remove fines and smaller particles. Before samples was analyse using AAS, all samples must be stored in cold room to prevent damage of the samples. The sample been prepared and analyse using Atomic Absorption Spectrophotometer. The technique for this AAS is using flaming method. Before starting the analysis, a set of standard solution for Pb must be prepared first which consist of 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1.0 ppm. The result from this experiment is to see the translocation factor of vetiver grass can act as hyper accumulator plant or not. The second result is to analyse either vetiver grass can remove Pb effectively or not.

Result and Discussion

In this topic we discuss about result get from the analysis of vetiver grass as a sample. The result from this study is about translocation factor and bio concentration factor. The formulas are as in Eq. (1) and Eq. (2).

Other than that, optimum concentration of EDTA can also be identified. As known EDTA can enhances the uptake of Pb for vetiver grass but too much EDTA does not means more uptake of Pb. From the study, concentration EDTA of 0.1 g/kg of soil is an optimum value for enhances the uptake of Pb compare to the concentration EDTA of 0.3 g/kg of soil.

$$\text{Translocation Factor}=\frac{\text{Shoot Concentration}}{\text{Root Concentration}}\times 100\% > 100\% \quad (1)$$

$$\text{Bio Concentration Factor}=\frac{\text{Plant Tissue Concentration}}{\text{Soil Concentration}}\times 100\% > 100\% \quad (2)$$

Translocation Factor

Translocation factor means the efficiency of hyper accumulator's plant uptake the Pb from the contaminated soil. If the percentage is greater than 100% it shown that the plants can act as hyper accumulator plant because it is one of the characteristic for hyper accumulator plants. What is means when have higher percentage of translocation factor is the uptake of Pb from the soil goes into the root and then into the shoot. This means that the plant can be harvest instead of removing it in order to removing the Pb. This makes the phytoremediation process become more economically because the plants can always be harvest and continue growing while uptake the Pb. Compare to low percentage of translocation factor, means that the uptake of Pb from the soil goes into the root only not for the shoot. In this case, phytoremediation is not economically because after plants uptake the Pb it will stick at their root. So it will need to be remove entire plants for clearing the Pb.

Translocation factor with EDTA at 30 days

Table 1: Percentage of Translocation Factor

Concentration of Pb	**0.1 g/kg EDTA**	**0.3 g/kg EDTA**
50 mg/L	215.4%	120.0%
100 mg/L	193.8%	103.0%
200 mg/L	122.2%	91.1%

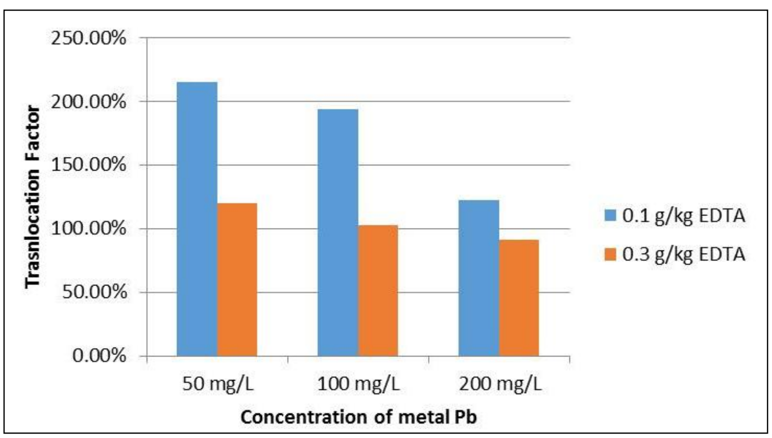

Figure 3: Translocation factor with adding EDTA

From the Figure 3 and Table 1 showed the percentage of translocation of metal Pb from root to shoot at 30 days. Two types concentration of EDTA which consists of 0.1 g/kg and 0.3 g/kg showed different value and different result for translocation of Pb. Three types of concentration $Pb(NO_3)_2$ which is 50 mg/L, 100 mg/L and 200 mg/L also give different value and result for translocation of Pb. From figure 4, the concentration of $Pb(NO_3)_2$ at 50 mg/L showed the highest translocation factor for both concentration of EDTA which is 0.1 g/kg and 0.3 g/kg.

The highest percentage of translocation factor is 215.4% for 0.1 g/kg EDTA and 120.0% for 0.3 g/kg EDTA. Whereas for the concentration of $Pb(NO_3)_2$ at 200 mg/L it showed the lowest translocation factor in both concentration of EDTA 0.1 g/kg and 0.3 g/kg . The lowest percentage of translocation factor in 200 mg/L of $Pb(NO_3)_2$ is 122.2% for EDTA 0.1 g/kg and 91.1% for EDTA 0.3 g/kg. In 100 mg/L of concentration $Pb(NO_3)_2$ it showed the average percentage of translocation factor for Pb. The percentage for concentration 100 mg/L of $Pb(NO_3)_2$ is 193.8% for 0.1 g/kg of EDTA and 103.0% for 0.3 g/kg of EDTA.

From the result of translocation factor, it can be concluded that the concentration of EDTA and solution of $Pb(NO_3)_2$ in soil affect the translocation of Pb from root to shoot. From the result, it showed that EDTA 0.1 g/kg was the optimum value to be used. This is because when at 0.1 g/kg of EDTA it helps the translocation of Pb to the highest compare to the 0.3 g/kg of EDTA. Other than concentration of EDTA, concentration of $Pb(NO_3)_2$ also play a role in helps the translocation of Pb. Translocation of Pb at vetiver grass only effective at 50 mg/L and 100 mg/L of $Pb(NO_3)_2$.

Translocation factor without EDTA

Table 2: Percentage of Translocation Factor

Concentration of Pb	15 Days	30 Days
0	0.0%	90.6%
50 mg/L	90.1%	160.5%
100 mg/L	123.4%	233.9%
200 mg/L	147.5%	219.4%

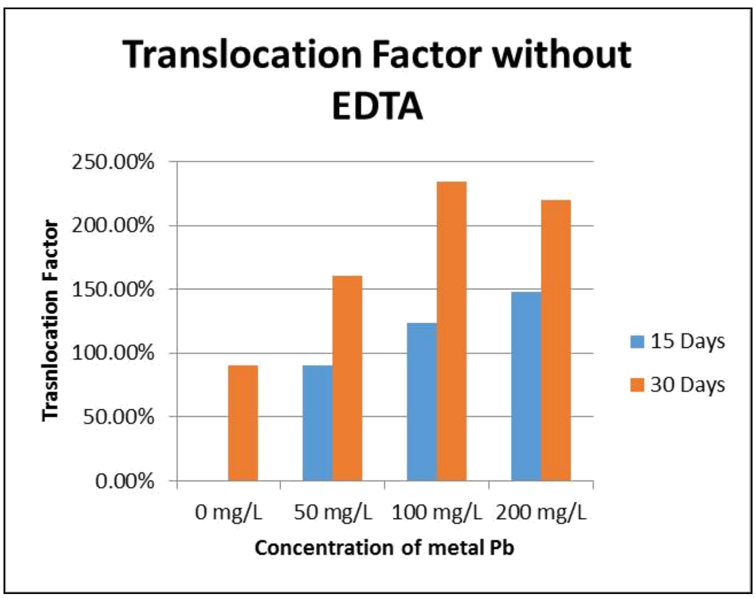

Figure 4: Translocation factor without adding EDTA

From the Figure 4 and Table 2 showed the percentage of translocation of metal Pb from root to shoot at 15 days and 30 days without concentration of EDTA. The result show that plants that act as controlled and plants that has been watered with three concentration $Pb(NO_3)_2$ which is 50 mg/L, 100 mg/L and 200 mg/L give different value and result for translocation of Pb. Based on Figure 4, the concentration of $Pb(NO_3)_2$ at 100 mg/L and 200 mg/L showed the high translocation factor value at 15 days and 30 days. The highest percentage of translocation factor is 233.9% for 100 mg/L concentration of $Pb(NO_3)_2$ and the second highest percentage of translocation factor is 219.4% for 200 mg/L of $Pb(NO_3)_2$ at 30 days. Whereas for 15 days, the highest percentage of translocation factor is 147.5% for 200 mg/L of $Pb(NO_3)_2$ and the second highest percentage of translocation factor is 123.4% for 100 mg/L of $Pb(NO_3)_2$. In 50 mg/L concentration of $Pb(NO_3)_2$, the translocation percentage for 15 days is 90.1% and 160.5% for 30 days. For the controlled sample, which is 0 mg/L of $Pb(NO_3)_2$ the percentage for translocation factor is 0% at 15 days and 90.6% at 30 days. From all the percentage of translocation factor, it can comes to a conclusion that concentration of $Pb(NO_3)_2$ and number of days for the vetiver grass to uptake the Pb can affect the percentage of translocation of Pb.

From the result, the uptakes of Pb by vetiver grass are increasing from 15 days to 30 days. This means that vetiver grass will uptake the Pb slowly and continuously for long period of time. It also means that vetiver grass is hyper accumulator plant (greater than 100%) because of the high percentage of translocation from root to shoot. When this happen, vetiver grass just need to be harvest instead of removing it from the soil for phytoremediation process.

Translocation factor by comparing with or without EDTA at 30 days

Table 3: Percentage of Translocation Factor

Concentration of Pb	0.1 g/kg EDTA	0.3 g/kg EDTA	No EDTA
50 mg/L	215.4%	105.9%	160.5%
100 mg/L	248.0%	146.3%	233.9%
200 mg/L	122.2%	91.1%	123.4%

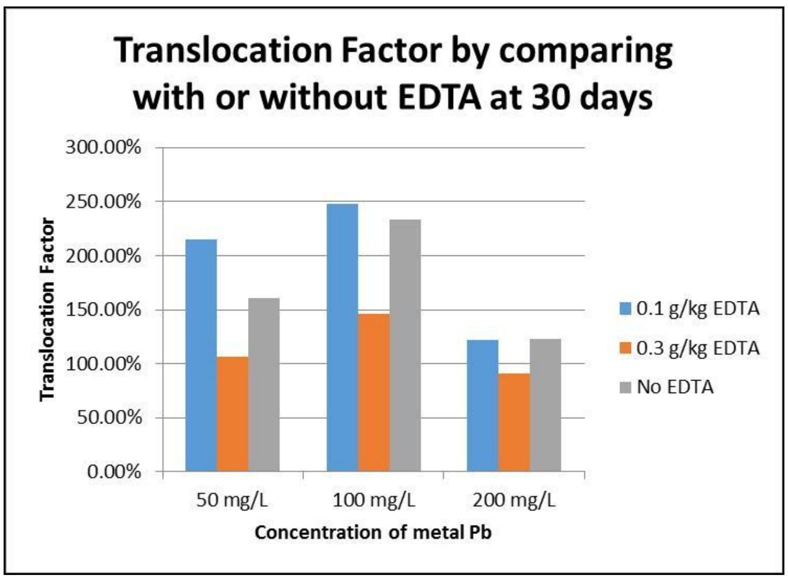

Figure 5: Translocation factor by comparing with adding EDTA and without adding EDTA

Based on Figure 5 and Table 3 showed the percentage of translocation of metal Pb from root to shoot. The graph is about comparing the translocation factor when adding 0.1 g/kg EDTA, 0.3 g/kg EDTA and without adding EDTA. Two types concentration of EDTA and without adding EDTA showed different value and different result for translocation of Pb. Three types of concentration $Pb(NO_3)_2$ which is 50 mg/L, 100 mg/L and 200 mg/L also give different value and result for translocation of Pb. From Figure 5, the concentration of $Pb(NO_3)_2$ at 50 mg/L and 100 mg/L showed the high translocation factor for both concentration of EDTA which is 0.1 g/kg and 0.3 g/kg. The highest percentage of translocation factor is 248.0% for 0.1 g/kg EDTA, 146.3% for 0.3 g/kg EDTA and 233.9% for no adding of EDTA. All of the highest translocation factor is at concentration 100 mg/L of $Pb(NO_3)_2$. Whereas for the concentration of $Pb(NO_3)_2$ at 200 mg/L it showed the lowest translocation factor in concentration of EDTA 0.1 g/kg, 0.3 g/kg and no EDTA.

The lowest percentage of translocation factor in 200 mg/L of $Pb(NO_3)_2$ is 122.2% for EDTA 0.1 g/kg, 91.1% for EDTA 0.3 g/kg and 123.4% for no EDTA. In 50 mg/L of concentration $Pb(NO_3)_2$ it showed the average percentage of translocation factor for Pb. The percentage for concentration 50 mg/L of $Pb(NO_3)_2$ is 215.4% for 0.1 g/kg of EDTA, 105.9% for 0.3 g/kg of EDTA and 160.5% for no EDTA. From the results of translocation factor, it can be concluded that the concentration of EDTA and solution of $Pb(NO_3)_2$ in soil affect the translocation of Pb from root to shoot. From the result, it showed that EDTA 0.1 g/kg was the optimum value to be used and the uses of EDTA is best at concentration of $Pb(NO_3)_2$ at 100 mg/L.

This is because when at 0.1 g/kg of EDTA it helps the translocation of Pb to the highest compare to the 0.3 g/kg of EDTA due to the high concentration of EDTA will give negative effects to the vetiver grass. By comparing with no adding EDTA it showed that the percentage of translocation of Pb is much higher than adding EDTA 0.3 g/kg in three concentration of of $Pb(NO_3)_2$. Thus it means that high concentration of EDTA does not make better translocation of Pb but just enough to use the optimum EDTA value. Other than concentration of EDTA, concentration of $Pb(NO_3)_2$ also play a role in helps the translocation of Pb. Translocation of Pb at vetiver grass only effective at 50 mg/L and 100 mg/L of $Pb(NO_3)_2$ with or without adding EDTA.

Bio Concentration Factor

Bio Concentration factor means the efficiency for the plants to uptake the Pb from the contaminated soil. If the percentage is greater than 100% it shown that the plants can uptake high value of Pb from contaminated soil but still cannot act as hyper accumulator plant because it need to see the translocation factor first. If the translocation factor is also greater than 100% so the plants can be recognized as hyper accumulators plants because one of the characteristic for hyper accumulator plants is to have percentage of translocation more than 100%. What is means when have higher percentage of bio concentration factor is the uptake of Pb from the soil goes into the root and shoot. This means that the plant cannot be harvest because the uptake of Pb is at root and shoot. For the plants be a hyper accumulator's plant it needs to have more uptakes at the shoot compare at the root for easy harvesting the plants which make phytoremediation more economically. Compare to low percentage of bio concentration factor, means that the uptake of Pb from the soil goes into the root and shoot just with small quantities. In this case, phytoremediation process will failed due to the plants that cannot uptake the Pb in contaminated soil in large quantities.

Bio Concentration factor with EDTA at 30 days

From the Figure 6 and Table 4 showed the percentage of bio concentration of vetiver grass in uptake metal Pb from soil to root and shoot at 30 days. Two types concentration of EDTA which consists of 0.1 g/kg and 0.3 g/kg showed different value and different result for bio concentration percentage of vetiver grass in uptake Pb. Three types of concentration $Pb(NO_3)_2$ which is 50 mg/L, 100 mg/L and 200 mg/L also give different value and result for bio concentration of vetiver grass in uptake Pb. As can see from the Figure 6, the concentration of $Pb(NO_3)_2$ at 50 mg/L showed the lowest bio concentration factor for both concentration of EDTA which is 0.1 g/kg and 0.3 g/kg. The lowest percentage of bio concentration factor is 238.2% for 0.1 g/kg EDTA and 225.7% for 0.3 g/kg EDTA. Whereas for the concentration of $Pb(NO_3)_2$ at 200 mg/L it showed the highest bio concentration factor in both concentration of EDTA 0.1 g/kg and 0.3 g/kg . The highest percentage of bio concentration factor in 200 mg/L of $Pb(NO_3)_2$ is 433.2% for EDTA 0.1 g/kg and 312.3% for EDTA 0.3 g/kg. In 100 mg/L of concentration $Pb(NO_3)_2$ it showed the average percentage of bio concentration factor for Pb. The percentage for concentration 100 mg/L of $Pb(NO_3)_2$ is 268.8% for 0.1 g/kg of EDTA and 243.8% for 0.3 g/kg of EDTA.

Table 4: Percentage of Bio Concentration Factor

Concentration of Pb	0.1 g/kg EDTA	0.3 g/kg EDTA
50 mg/L	238.2%	225.7%
100 mg/L	268.8%	243.8%
200 mg/L	433.2%	312.3%

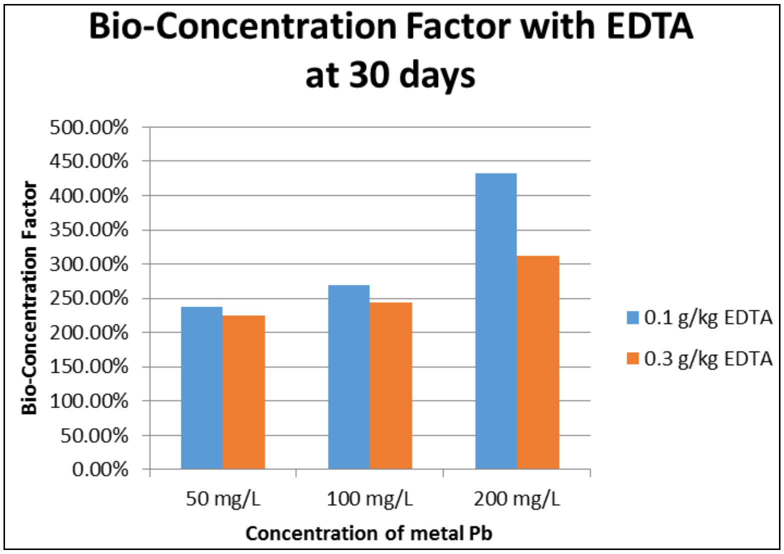

Figure 6: Bio Concentration factor with adding EDTA

From the results of bio concentration factor, it can be concluded that the concentrations of EDTA and solution of $Pb(NO_3)_2$ in soil can affect the bio concentration of vetiver grass in uptake metal Pb from soil to root and shoot. From the result, it showed that EDTA 0.1 g/kg was the optimum value to be used due to the percentage of bio concentration of vetiver grass in uptake Pb is high in all three state of concentration $Pb(NO_3)_2$. This is because when at 0.1 g/kg of EDTA it helps the bio concentration of vetiver grass in uptake Pb to the highest compare to the 0.3 g/kg of EDTA. Other than concentration of EDTA, concentration of $Pb(NO_3)_2$ also play a role in helps the bio concentration of vetiver grass in uptake Pb. Bio concentration of vetiver grass in uptake Pb only effective at 100 mg/L and 200 mg/L of $Pb(NO_3)_2$.

Bio Concentration without EDTA

Figure 7 and Table 5 showed the percentage of bio concentration of vetiver grass in uptake metal Pb from soil to root and shoot at 15 days and 30 days without concentration of EDTA. The results showed that plants that acs as controlled and plants that has been watered with three concentration $Pb(NO_3)_2$ which is 50 mg/L, 100 mg/L and 200 mg/L give different value and result for bio concentration of vetiver grass in uptake metal Pb. As can see in Figure 7, the concentration of $Pb(NO_3)_2$ at 50 mg/L and 100 mg/L showed the high bio concentration factor value at 30 days. The highest percentage of bio concentration factor is 355.0% for 100 mg/L concentration of $Pb(NO_3)_2$ and the second highest percentage of bio concentration factor is 240.5% for 50 mg/L of $Pb(NO_3)_2$ at 30 days. For concentration of $Pb(NO_3)_2$ at 200 mg/L, the bio concentration percentage is 188.18 % at 30 days. Other than that, for 15 days the highest percentage of bio concentration factor is 188.2% for 200 mg/L of $Pb(NO_3)_2$ whereas for the 15 days result it showed almost identical of bio concentration factor for 0 mg/L, 50 mg/L and 100 mg/L concentration of $Pb(NO_3)_2$. In 50 mg/L concentration of $Pb(NO_3)_2$, the bio concentration percentage for 15 days is 95.3% and in 100 mg/L concentration of $Pb(NO_3)_2$ the bio concentration percentage for 15 days is 84.7%. For the controlled sample, the percentage for bio concentration factor is 82.5% at 15 days and 98.7% at 30 days.

Table 5: Percentage of Bio Concentration Factor

Concentration of Pb	15 Days	30 Days
0	82.5%	98.7%
50 mg/L	95.3%	240.9%
100 mg/L	84.7%	355.0%
200 mg/L	155.6%	188.2%

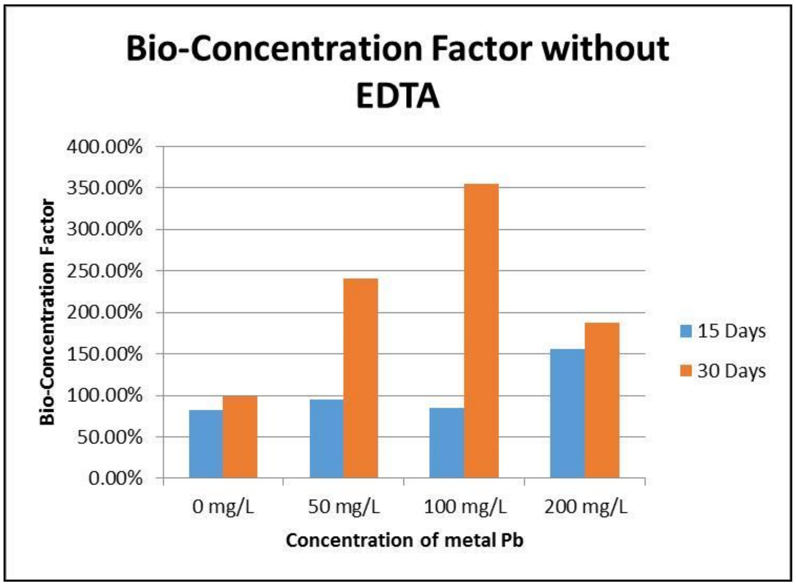

Figure 7: Bio Concentration factor without adding EDTA

From the results of bio concentration factor, it can be concluded that solution of $Pb(NO_3)_2$ in soil and number of days for the vetiver grass to uptake the Pb can affect the percentage of bio concentration for vetiver grass. From the result, the uptakes of Pb by vetiver grass are increasing from 15 days to 30 days but it only suitable at low concentration of $Pb(NO_3)_2$. During high concentration of $Pb(NO_3)_2$, the bio concentration of vetiver grass in uptake Pb is gradually decreasing. This means that vetiver grass will uptake the Pb slowly and continuously for long period of time but at suitable concentration of $Pb(NO_3)_2$. But this does not means that vetiver grass is hyper accumulator plant because of the high percentage bio concentration is not the characteristic for hyper accumulator plant. To see either vetiver grass is hyper accumulator plants must refer to result of translocation factor and the translocation factor graph must exceed more than 100%.

Bio Concentration factor by comparing with or without EDTA at 30 days

Figure 8 and Table 6 showed the percentage of bio concentration of vetiver grass in uptake metal Pb from soil to root and shoot. The graph is about comparing the bio concentration factor when adding 0.1 g/kg EDTA, 0.3 g/kg EDTA and without adding EDTA. Two types concentration of EDTA and without adding EDTA showed different value and different result for bio concentration of vetiver grass in uptake metal Pb.

Table 6: Percentage of Bio Concentration Factor

Concentration of Pb	0.1 g/kg EDTA	0.3 g/kg EDTA	No EDTA
50 mg/L	272.8%	225.7%	240.9%
100 mg/L	433.2%	243.8%	355.0%
200 mg/L	433.2%	312.3%	261.1%

Three types of concentration $Pb(NO_3)_2$ which is 50 mg/L, 100 mg/L and 200 mg/L also give different value and result for bio concentration of vetiver grass in uptake Pb. From Figure 8, the concentration of $Pb(NO_3)_2$ at 100 mg/L and 200 mg/L showed the high bio concentration factor for the concentration 0.1 g/kg EDTA. The highest percentage of bio concentration factor is 433.2% for 0.1 g/kg EDTA in both concentration of $Pb(NO_3)_2$ at 100 mg/L and 200 mg/L. For concentration 100 mg/L $Pb(NO_3)_2$ the percentage of bio concentration at 0.3 g/kg EDTA is 243.8% and 355.0% without EDTA. Whereas for concentration 200 mg/L $Pb(NO_3)_2$ the percentage of bio concentration at 0.3 g/kg EDTA is 312.3% and 261.1% without EDTA. In concentration 50 mg/L $Pb(NO_3)_2$ has the lowest value for percentage of bio concentration which is 272.8% for 0.1 g/kg EDTA, 225.7% for 0.3 g/kg EDTA and 240.9% for no adding of EDTA.

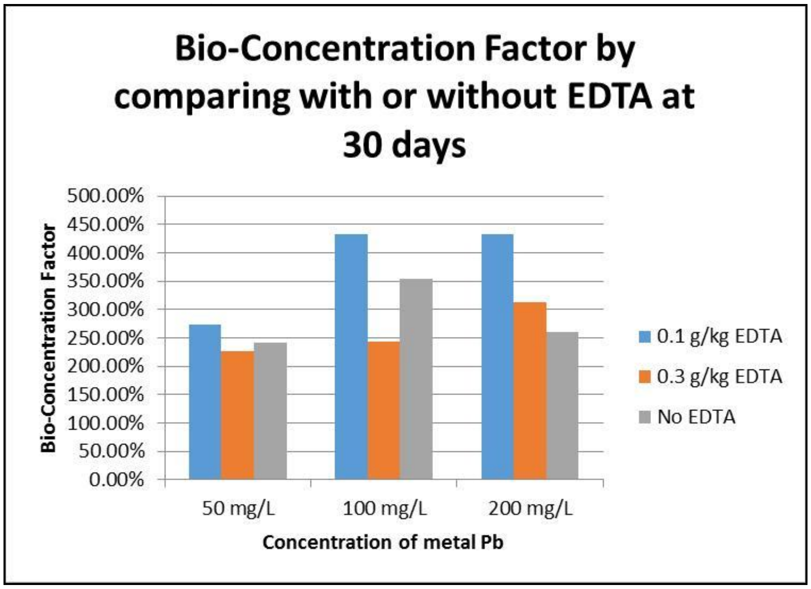

Figure 8: Bio Concentration factor with adding EDTA and without adding EDTA

From the results of bio concentration factor, it can be concluded that the concentration of EDTA and solution of $Pb(NO_3)_2$ in soil can affect the bio concentration of vetiver grass in uptake metal Pb from soil to root and shoot. From the result, it showed that EDTA 0.1 g/kg was the optimum value to be used and the uses of EDTA is best at concentration of $Pb(NO_3)_2$ at 100 mg/L and 200 mg/L. This is because when at 0.1 g/kg of EDTA it helps the bio concentration of vetiver grass in uptake metal Pb to the highest compare to the 0.3 g/kg of EDTA due to the high concentration of EDTA will give negative effects to the vetiver grass.

By comparing with no adding EDTA it showed that the percentage of bio concentration of vetiver grass in uptake metal Pb is much higher than adding EDTA 0.3 g/kg in three concentration of of $Pb(NO_3)_2$. Thus it means that high concentration of EDTA does not make better bio concentration of vetiver grass in uptake metal Pb but just enough to use the optimum EDTA value. Other than concentration of EDTA, concentration of $Pb(NO_3)_2$ also play a role in helps the bio concentration of vetiver grass in uptake metal Pb. Bio concentration of vetiver grass in uptake metal Pb only effective at 100 mg/L and 200 mg/L of $Pb(NO_3)_2$ with or without adding EDTA.

Conclusion

Objective for this study is about to determine the level effectiveness of vetiver grass as hyper accumulator plant and the second objective is to identify the effect of EDTA can enhance the ability for the vetiver grass in removal heavy metals. In conclusion, vetiver grass showed the effectiveness in uptake metal Pb and with the adding of EDTA it also enhances the uptake of Pb but only with the optimum concentration of EDTA. Throughout 30 days of the experiment, the results for the translocation factor and bio concentration factor showed enhancement from day to day. Even though the uptake of Pb is low at the beginning of the experimental, however the uptake is gradually increasing when the plants already can adapt with the condition of the soil. These prove that the uses of vetiver grass as hyper accumulator plant is effective in removal of Pb from contaminated soil. In addition, the use of EDTA also will help the vetiver grass in uptake the Pb. It enhances the vetiver grass in uptake the Pb but only if with the optimum amount of EDTA (0.1 g/kg). Too much of concentration EDTA does not make the plant uptake more Pb but it will give bad impact to the plants and environment. The overall experiment showed that the optimum concentration for EDTA is 0.1 g/kg of soil. This is because with the three concentration of

$Pb(NO_3)_2$ which is 50 mg/L, 100mg/L and 200 mg/L, it showed that concentration of EDTA 0.1 g/kg of soil can still enhances the percentages of translocation and bio concentration for 30 days of experiment.

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