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Research Article

Phytoremediation : Vetiver Grass in Remediation of Soil Contaminated with Trichloroethylene

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Abstract

Trichloroethylene (TCE) is chlorinated hydrocarbon which used in degreasing oil and grease from process products. It was found that this chemical was contaminated in environmental, soil and water around industrial area. Soil was collected from Pratum Thani province which had TCE higher than the standard set by Ministry of Industry in Thailand. Four ecotypes of vetiver grass (*Vetiveria zizanioides*) were used for phytoremediation including Songkla3, Sri Lanka, Kamphaeng Phet2 and Surat Thani. All ecotypes grew up and the survival rate was 100% after planting for 1 month. Surat Thani had the most number of leaves (6.67±0.58). Songkhlar3 had the longest shoots followed by Sri Lanka (6.67±0.29, 5.33±0.76 cm). Songkla3 and Sri Lanka had the longest leaves (40.57±1.39 and 39.30±5.88 cm). However, there were no statistical differences ($p > 0.05$) in sprouts quantity and leaves width among the four ecotypes. Songkhlar3 and Sri Lanka were selected for further experiment. Vetiver grass was planted in contaminated soil mixed with soil conditioners including coconut residue : soil : manure in ratio 3 : 2 : 1 by weight. TCE was higher accumulated in leaves than shoots and roots. The removal of TCE from contaminated soil was about 98% for two ecotypes. However, in the field trial more parameters would be put to concern than laboratory scale.

Keywords: Phytoremediation, Trichloroethylene, Vetiver grass.

1. Introduction

Phytoremediation is an emerging green technology that uses plants to remediate soil, sediment, surface water, and groundwater environments contaminated with toxic metals, organics, and radionucides [4]. This method has the benefit of contributing to site restoration when remedial action is ongoing. The action of plants can include the degradation, adsorption, accumulation and volatilization of compounds or the enhancement of soil rhizosphere activity. Many different compounds and classes of compounds can be removed from the environment by this method, including solvents in groundwater, petroleum and aromatic compounds in soils, and volatile compounds in the air [6]. Phytoremediation is more cost-effective than alternative mechanical or chemical methods of removing hazardous compounds from the soil [4].

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Trichloroethylene (TCE) is one of the most common environmental pollutants in the industrialized world. It has been used for decades in military, industrial, medical and household settings, in a wide range of applications including uses as a metal degreaser, dry cleaning agent, and anaesthetic. TCE is very stable, and in some aquifers it has persisted for decades. It is known to be a hepatotoxin, and there is also growing evidence that it may be a carcinogen [13]. In Thailand, TCE was found contaminated in soil and groundwater beside industries [9]. Many recent researches have shown that TCE can be taken up by plants in both lab scale experiments and field-site-scale studies [15] for example, tobacco, poplar [7,13], carrots, spinach, tomatoes [14], cattail, eastern cottonwood [5], apple and peach tree [2]. To find another plant and can grow in a TCE contaminated site that has the efficiency to take up TCE from soil, we selected vetiver grass (*Vetiveria zizanioides*) to determine for the capability to remediate contaminated soil. by phytoremediation.

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 [8]. It is known to be tolerant to heavy metals [10]. There are reports on the use of this plant for phytoremediation of soils contaminated with heavy metals [3,16], phenol [11], radionuclides and nuclear waste [12]. However, there was no report about using vetiver grass for phytoremediation of soil contaminated with TCE.

The goal of this report is to examine four ecotypes of vetiver grass including, Songkla3, Sri Lanka, Kamphaeng Phet2 and Surat Thani for their ability to grow in soil contaminated with TCE and to investigate phytoremediation efficiency of Songkla3 and Sri Lanka. The result was used to consider the potential to use vetiver grass to remediate TCE contaminated area.

2. Materials and Methods

All of the vetiver grass plantlets were received from Office of The Royal Development Projects and the research site was located in the Department of Environmental Science, Faculty of Science, Kasetsart University.

Plant Material. The plantlets of vetiver grass (*Vetiveria zizanioides*) for four ecotypes including Songkla3, Sri Lanka, Kamphaeng Phet2 and Surat Thani were used for the experiments. To compare the growth capability of vetiver grass in TCE contaminated soil, we used four ecotypes of them. The age of the plants was between 1-1.5 months and all of them were planted in planting material to develop root system for 3 weeks. They were maintained and cut for 25 cm length before planted in soil contaminated with TCE (1 plant/pot).

Another experiment analyzed for the accumulation of TCE in plant parts, two ecotypes were selected. The plantlets were prepared as same as the first experiment but they were planted in soil mixed with soil conditioners. The plant pots were put in clear-roof, opened building. Water was applied every 2 days.

Soil Preparation. Soil was collected from Pratum Thani province in Thailand. The soil was collected from a lagoon. The characteristic of the soil were clay. The soil was air-dried, crushed to pass through a 1 cm diameter sieve, and mixed thoroughly. Analyzed for texture, moisture after air dried, pH, organic matter (OM), carbon exchange capacity (CEC) and TCE concentration.

The prepared soil was separated into 2 conditions. First, put into pots (5 kg/pot) for testing the growth capability of vetiver grass in this soil. Second, mixed with soil conditioners including coconut residue : soil : animal manure in a ratio 3 : 2 : 1 by weight as a common ratio for planting material. One kilogram of mixture was added in each pot.

Samples Analysis. Soil samples were collected and 5 g of the soil was put into a 25 ml amber vial. All samples were extracted with 5 ml methanol and 25 ml deionized water, closed immediately with Teflon line rubber and aluminium cap and kept at room temperature for 24 h.

Plant samples were prepared by cutting and dividing into root, shoots and leaves, 5 g of samples were extracted with 5 ml conc. H_2SO_4 and 15 ml deionized water, closed immediately with Teflon line rubber and aluminium cap. After that, heated with a hot plate at 90°C for 30 min and kept at room temperature for 48 h.

All soil and plant samples were shaken with incubator shaker at 25°C, 60 rpm. Head space sampler HP 7694 was used for shaking for 10 min. TCE was analyzed using a Gas chromatography electron capture detector (GC-ECD) HP 6890 series using a capillary (HP-5) 30 m x ID 0.32 mm, film thickness 0.25 µm coated with 5% phenyl-methylpoly-siloxane. Column inlet temperature 210°C, oven temperature 90°C, carrier gas flow 1.50 ml/min with velocity 34 cm/sec.

Translocation in the plant parts from shoot to root was measured using Translocation factor (TF) which is given below Eq. (1) [1].

$$TF = C_s/C_r \quad (1)$$

Where, C_s and C_r are TCE concentrations (mg/kg) in the shoot and root, respectively. Wherein, TF > 1 indicates that the plant has an efficiency to translocate TCE from root to shoot.

Experimental Design. All four ecotypes of vetiver grass were cut to 15 cm height and planted in the soil collected from Pratum Thani province in Thailand (5 kg/pot). The lay out plan was completely randomized design with 3 replications, outdoors grown with no chemical fertilizers. After 1 month, the number of leaves, length of leaves, length of shoot, width of leaves and quantity of sprouts were measured. Two ecotypes that had the best efficiency growth were selected for the further experiment.

Selected vetivier grasses (2 ecotypes) were used in planting in soil mixed with soil conditioners. They were planted for 1 month and the TCE was measured in plant parts and planting materials. This experiment was set up to check the TCE accumulation in plants. SPSS program was used with 0.05 significant difference.

3. Results and Discussion

Characteristic of Soil. The soil was extremely acidic (pH3.8), organic matter (OM) 2.11%. It was high in carbon exchange capacity (CEC) 22.37 mol/kg and the texture was clay (clay = 55.60%). Because of the high value of clay content, made it difficult for water to pass through the soil. Moisture after air drying was 5.29%. TCE concentration was 549 mg/kg, which was higher than the standard set by Ministry of Industry in Thailand; 28 mg/kg (Table 1).

Table 1. Characteristic of soil.

Parameter	Value
Texture	Clay
Soil moisture	5.29%
pH	3.8
OM	2.11%
CEC	22.37 mol/kg
TCE	549 mg/kg

Growth Capability of Vetiver Grass. It was found that all ecotypes of vetiver grass can grow in TCE contaminated soil and had a 100% survival. The statistical result of growth capability at significance 0.05 levels showed that Surat Thani had the largest number of leaves (6.67±0.58) different from the other ecotypes (Figure 1). Sri Lanka, Songkhlar3 and Kamphaeng Phet had similar number of leaves, 5.33±0.58, 5.00±0.00, and 5.00±0.00, respectively.

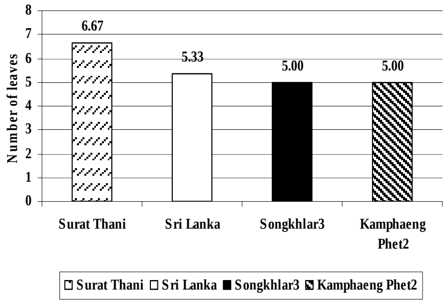

Figure 1. Number of leaves, planting in TCE contaminated soil.

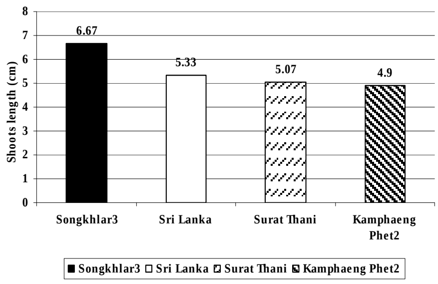

Figure 2. Shoots length, planting in TCE contaminated soil.

Songkhlar3 had higher shoot length followed by Sri Lanka and Surat Thani (6.67±0.29, 5.33±0.76 and 5.07±1.21 cm). The statistical result at significance 0.05 levels showed Kamphaeng Phet2 had the shortest of shoot length (4.90±0.96 cm) different from other ecotypes (Figure 2). The result of shoot length was in the same trend of leaves length. Songkhlar3 had the most leaves length followed by Sri Lanka, Kamphaeng Phet2 and Surat Thani (40.57±1.39, 39.30±5.88, 32.48±2.67 and 26.21±4.64 cm, respectively) (Figure 3). However, there was no statistical differences ($p > 0.05$) in sprouts quantity and leaves width among the 4 ecotypes (Table 2).

Kamphaeng Phet2, Songkhlar3, Surat Thani and Sri Lanka had similarly number of sprouts 4.00±1.00, 2.67±1.53, 2.67±0.58 and 2.00±1.00 cm and the leaves width were 0.76±0.09, 0.77±0.05, 0.77±0.02 and 0.74±0.99 cm, respectively. From this experiment, two ecotypes that gave the best result in growth capability were selected. Thus, we selected Songkhlar3 and Sri Lanka for further experiment. The difference in the growth of four ecotypes may due to both of the growing nature of the plants and the efficiency to tolerate TCE in soil.

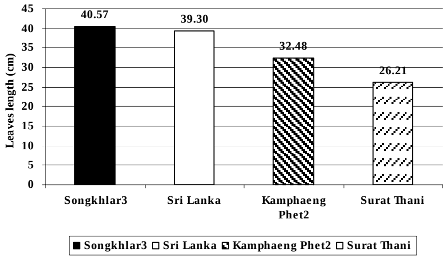

Figure 3. Leaves length, planting in TCE contaminated soil.

TCE Accumulation in Plant Parts. The result showed that TCE was accumulated in leaves more than root and shoots (Table 3). Sri Lanka had more TCE accumulated in leaves than Songkhlar3 (4.48±1.07 and 4.06±2.18 mg/kg) and accumulation in shoots were 4.04±2.44 and 3.71±1.04 mg/kg. However, in root Songkhlar3 had more TCE accumulation than Sri Lanka (2.73±1.36 and 1.74±0.42). It is the advantage of vetiver grass that it's plant parts above ground level had capability to accumulated TCE. 1) TCE was taken out from the soil via harvesting of the above ground level parts. 2) While vetiver grass was growing, TCE was translocated from soil to root to new shoots and leaves. That left the soil cleaner.

TF of TCE in two ecotypes of vetiver grass was higher than 1 (Table 4). It was indicated that vetiver grass had an efficiency to translocate TCE from root to shoot. TCE was analyzed from soil left in the pots. The result showed that TCE removal was very high (Table 4). About 98% of TCE was lost after growing vetiver grass for 1 month, Songkhlar3 and Sri Lanka showed the same trend (98.39% and 98.36%, respectively).

Table 2. Growth capability of vetiver grass.

Ecotypes	Number of Leaves	Shoot Lngth (cm)	Leaves Length (cm)	Number of Sprouts	Leaves Width (cm)
K2	5.00±0.00 b	4.90±0.96 b	32.48±2.67 bc	4.00±1.00 a	0.76±0.09 a
Si	5.33±0.58 b	5.33±0.76 ab	39.30±5.88 ab	2.00±1.00 a	0.74±0.09 a
So3	5.00±0.00 b	6.67±0.29 a	40.57±1.39 a	2.67±1.53 a	0.77±0.05 a
Su	6.67±0.58 a	5.07±1.21 ab	26.21±4.64 ab	2.67±0.58 a	0.77±0.02 a

Note: Mean and standard deviation (n=3); K2=Kamphaeng Phet; Si=Sri Lanka; So3=Songkhlar3; Su=Surat Thani; small letters stand for significance at 0.05 levels

Table 3. TCE concentration in plant parts (mg/kg).

Plant Parts	TCE concentration (mg/kg)	
	Sri Lanka	Songkhlar3
Root	1.74±0.42 b	2.73±1.36 a
Shoots	4.04±2.44 ab	3.71±1.04 a
Leaves	4.48±1.07 a	4.06±2.18 a

Note: Mean and standard deviation (n=4); small letters stand for significance at 0.05 levels

Table 4. Translocation factor (TF) and %removal of TCE in soil mixed with soil conditioners.

Treatment	TF	%Removal
Sri Lanka	2.32	98.36
Songkhlar3	1.36	98.39

4. Conclusions

Four ecotypes of vetiver grass including, Songkla3, Sri Lanka, Kamphaeng Phet2 and Surat Thani, were planted in soil contaminated with TCE for 1 month. All of them had 100% of survival rate. Songkhlar3 and Sri Lanka showed the best growth capability. These two ecotypes were selected to plant in soil mixed with soil conditioners including coconut residue : soil : animal manure at ratio 3 : 2 : 1 by weight to determine for the TCE accumulation in plant parts. The results showed that TCE was removed from this mixture materials and accumulated mostly in leaves followed by shoots and root. Wherein, TF > 1 indicated that TCE had an efficiency to translocate from root to shoots. TCE removal from soil was very high about 98% of TCE was lost from soil mixed with soil conditioners. This is an advantage for this plant for phytoremediation. While, TCE was accumulated in leaves, cutting leaves could remove TCE from contaminated soil. Vetiver grass was then left to grow in site to continuous removing TCE. However, more parameters would be put in considerations in the field site compared to laboratory scale study.

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