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Proceeding Paper

Phytoremediation Efficiency of Weathered Petroleum-Contaminated Soils by *Vetiveria zizanioides* and *Cymbopogon nardus itle* †

Pei-Cheng Cheng [1,2], Yuan-Chung Lin [1,2], Min-Siou Lin [3], Sun-Long Lin [4], Yin-Hsiu Hsiao [4], Chin-Yuan Huang [5], Pei-Chun Tu [6,*] and Shu-Fen Cheng [5,*]

1 Institute of Environmental Engineering, National Sun Yat-Sen University, Kaohsiung 804, Taiwan; eric62795@gmail.com (P.-C.C.); yclin@faculty.nsysu.edu.tw (Y.-C.L.)
2 Center for Emerging Contaminants Research, National Sun Yat-Sen University, Kaohsiung 804, Taiwan
3 Institute of Biochemical Technology, Chaoyang University of Technology, Taichung 413, Taiwan; minsiou.lin@gmail.com
4 CPC Exploration & Development Research Institute, Miaoli City 360, Taiwan; 155381@cpc.com.tw (S.-L.L.); 965090@cpc.com.tw (Y.-H.H.)
5 Department of Environmental Engineering and Management, Chaoyang University of Technology, Taichung 413, Taiwan; sanada.r88686@msa.hinet.net
6 Department of Horticulture and Landscape Architecture, National Taiwan University, Taipei 106, Taiwan
* Correspondence: topage2@gmail.com (P.-C.T.); shufen@cyut.edu.tw (S.-F.C.)
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Abstract: Weathered petroleum-contaminated soil was treated with *Vetiveria zizanioides* (Vetiver) and *Cymbopogon nardus* (Lemongrasss) to investigate the efficiency of phytoremediation. The initial total petroleum hydrocarbon (TPH) concentration of soil was 3000−8000 mg/kg, and after 6 months, the TPH concentrations were degraded by 50−75% under the action of soil native microbial. Planting vetiver and lemongrass stabilized soil pH and electrical conductivity, and it accelerated the decomposition of TPH in soil. Planting vetiver showed a better effect. After 6 months of planting, the TPH decomposition efficiency reached about 90%, and most of the easily decomposed TPH has been decomposed. The results of rhizosphere soil microbiota analysis also showed that planting vetiver increased the abundance of soil microbiota.

Keywords: TPH; phytoremediation; vetiver; lemongrass

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1. Introduction

Petroleum is the main fuel for human life. Thus, petroleum contamination of soil is often caused by accidental spills of petroleum from manufacturing, storage, and transportation [1–3]. The main components of petroleum are alkane hydrocarbons, which are therefore referred to as total petroleum hydrocarbon (TPH). A lot of the components in petroleum have adverse effects on human health. Therefore, petroleum is an important pollutant for soil in many countries. Remediation technologies for TPH-contaminated soil at present include biological remediation, soil vapor extraction, thermal desorption, and chemical oxidation. Compared with physical and chemical remediation technologies, the bioremediation method using microorganisms is more economical with low energy consumption and is environmentally friendly [3–5]. However, for TPH-contaminated soil with high carbon numbers and weathering, the decomposition efficiency of bioremediation was often not high.

Phytoremediation is a remediation technology that uses plants to remove pollutants [6,7]. It is used to remediate various pollutants, including heavy metals, inorganic salts, and organic pollutants. The mechanism of phytoremediation for the removal of organic pollutants includes phytoextraction, phytodegradation, phytovolatilization, and rhizosphere bioremediation [8–12]. Phytoextraction is to uptake and accumulate pollutants in plants through the absorption of water and nutrients by plants in order to remove pollutants from the soil. The degradation of pollutants through the metabolism of plants is called phytodegradation or phytotransformation. The evaporation of pollutants into the atmosphere through the evapotranspiration of plant leaves is called phytovolatilization [8–10]. In addition, plant roots release exudates, such as low-molecular-weight organic acids, to the soil to stimulate the degradation of organic chemicals, the growth of new species, and/or increase soluble substrate concentrations for all microorganisms, which is known as phytostimulation or plant-assisted bioremediation [11,12].

In addition to the advantages of less energy consumption, low cost, and less impact on soil properties, phytoremediation greens landscapes, and it is the most accepted remediation technology by the public. Corresponding to the international goal of promoting net zero by 2050, phytoremediation reduces the energy required for remediation and achieves the effect of carbon reduction. Thus, it needs to be used more. Therefore, with vetiver and lemongrass that have economic values and strong growth ability, we carried out the phytoremediation experiment of petroleum-contaminated soil to study the efficiency of remediation of the two plants on weathered petroleum-contaminated soil. Vetiver is an evergreen perennial herb and is propagated by divisions. It grows in an environment with an annual rainfall of 300−6,000 mm and survives for 8 months in a water-soaked environment and for 5 months in the arid desert edge. It can survive in harsh soil environments such as acidic, alkaline, saline land, sandy land, gravel land, and mining spoil, and in a temperature of −15 to 55 °C. Vetiver has strong ecological adaptability and resistance to adversity and has the advantages of fast growth, a well-developed root system, and easy cultivation. Lemongrass is a perennial herb and is propagated by seeds or divisions. Lemongrass prefers a sunny and warm climate with strong drought tolerance. It does not restrict a type of soil with a well-developed root system. The leaves contain lemon fragrance, which can be distilled to extract essential oils and which have economic value.

2. Methods

The soil in this study was collected from the site of the Taichung oil supply center of China Petroleum Corporation in Taiwan in the coordinates of 120.5432 E and 24.2993 N The soil was classified into three soils according to the TPH concentrations. The average concentration of the low TPH concentration was about 3000 mg/kg, the middle concentration was about 4500 mg/kg, and the high concentration was about 8000 mg/kg. The experiment was carried out with potted plants, each of which was filled with about 30 kg of soil. Three experimental soils were tested: control, vetiver, and lemongrass groups. Each experimental group had three soils. The control group did not have any plants, and the same method was applied with vetiver and lemongrass planted, with the same growth conditions of water and sunshine. The rhizosphere soil was collected every two months for the analysis of pH, electrical conductivity, and TPH concentration. For the measurement of soil pH, 20 g of air-dried soil was taken and added 20 mL of reagent water. After mixing and stirring, the solution was left to take the supernatant liquid to measure pH with a pH electrode. Soil conductivity was measured as the conductivity of the filtrate with a conductivity meter after taking 10 g of air-dried soil, to which was added 50 mL of deionized water and shaken at 140 rpm for 1 h and filtered with Whatman No.5 filter paper. For the analysis of soil TPH concentration, 2 g of soil was taken and 10 mL of n-hexane was added, which was extracted by ultrasonic wave, concentrated under reduced pressure, and then quantified by GC-FID. Before the test and 5 months after the test, the soil microbiological analysis was carried out by the Next Generation Sequencing method.

3. Results and Discussion

3.1. Soil Properties

The particle size analysis result of the soils with the three concentrations of TPH showed that the proportion of sand particles accounted for about 98.0% and silt for about 2.0%. Thus, the soil was classified to be sandy soil. The average concentration of TPH in the low-concentration soil was 3029 mg/kg, pH was 6.97, electrical conductivity was 171.2 μS/cm, and organic matter content was 7.43%. The average concentration of TPH in the medium-concentration soil was 4617 mg/kg, pH was 7.39, electrical conductivity was 169.8 μS/cm, and organic matter content was 7.90%. The average concentration of TPH in high-concentration soil was 7865 mg/kg, pH was 7.60, electrical conductivity was 167.7 μS/cm, and organic matter content was 8.67%.

The soil pH after 6 months of the test was shown in Figure 1. After 6 months of the test, the pH of the soil increased. However, the increase in pH in the soil planted with vetiver and lemongrass was lower than that in the control group. Figure 1b shows the measured results of soil electrical conductivity after 6 months of the test. The electrical conductivity of all soils increased significantly after 6 months of testing. The increase in the soil with vetiver was the smallest, followed by the soil with lemongrass, and the soil without plant showed the largest increase in electrical conductivity. Such results show that planting plants slowed the changes in soil pH and electrical conductivity.

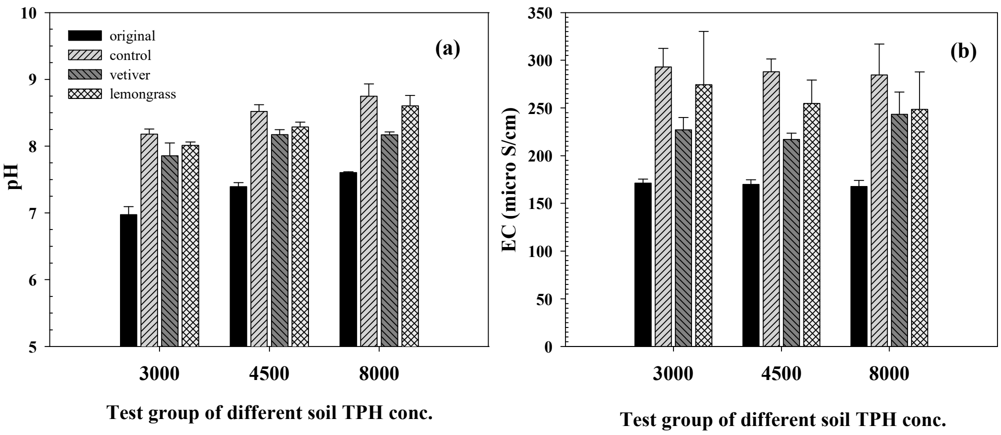

Figure 1. Properties of soil samples. (**a**) pH and (**b**) electrical conductivity.

3.2. TPH Concentration

Figure 2 shows the change in the TPH concentration in the control soil. The initial average concentration of TPH in low-concentration soil was 3029 mg/kg. After 2 months, the average concentration was 1455 mg/kg, and the degradation rate of TPH was about 52%. After 6 months, the average concentration of TPH was about 904 mg/kg, and the degradation rate of TPH was about 70%. During the period of 6−15 months, the residual TPH concentration in the soil did not change much. At 15 months, the average concentration of TPH was about 827 mg/kg, and the average removal rate was about 73%. The initial average concentration of TPH in the medium-concentration soil was 4617 mg/kg. After 2 months, the average concentration was 2533 mg/kg, and the TPH degradation rate was about 45%. After 6 months, the average TPH concentration was about 2000 mg/kg, and the TPH degradation rate was about 56%. At 15 months, the average concentration of TPH was about 1109 mg/kg, and the degradation rate of TPH was about 76%. The average initial TPH concentration of high-concentration soil was 7865 mg/kg, and after 2 months, the average soil TPH concentration was 5125 mg/kg, and the TPH degradation rate was about 35%. At 6 months, the average TPH concentration was about 3893 mg/kg, and the TPH degradation rate was about 50%/. After 15 months, the average TPH concentration was about 1285 mg/kg, and the TPH degradation rate was about 83%.

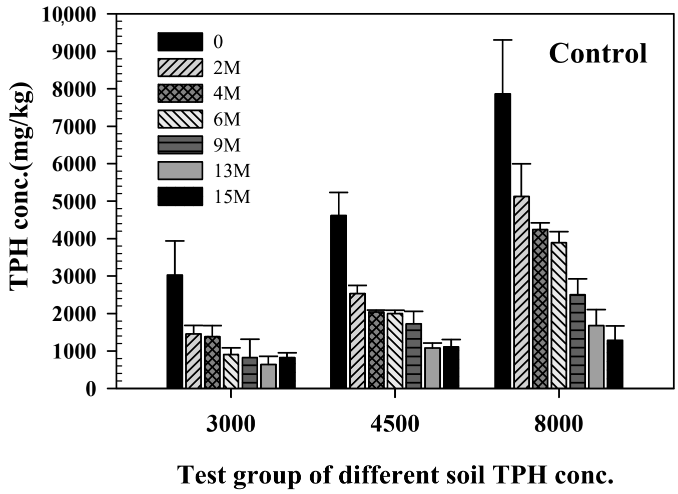

Figure 2. The change of soil TPH concentration in the control soil.

Figure 3 showed the change in the TPH concentration in the soil with vetiver. After 2 months in low-concentration soil, the average concentration of TPH in the soil was 897 mg/kg, and the degradation rate of TPH was about 70%. After 6 months, the average concentration of TPH was about 278 mg/kg, and the degradation rate of TPH was about 90%. During 6−15 months, the soil TPH concentration did not change much, indicating that most of the easily decomposed TPH had been decomposed at 6 months. In medium-concentration soil, after 2 months, the average concentration of TPH in the soil was 1431 mg/kg, and the TPH degradation rate was about 70%. After 6 months, the average concentration of TPH was about 578 mg/kg, and the TPH degradation rate was 88%. After 15 months, the average TPH concentration was about 528 mg/kg, and the TPH degradation rate increased to 89%. For high-concentration soil, after 2 months, the average concentration of TPH in the soil was 4478 mg/kg, and the degradation rate of TPH was about 43%. After 6 months, the average concentration of TPH was about 1616 mg/kg, and the degradation rate of TPH was about 80%. After 15 months, the average concentration of TPH was about 927 mg/kg, and the degradation rate of TPH was close to 90%.

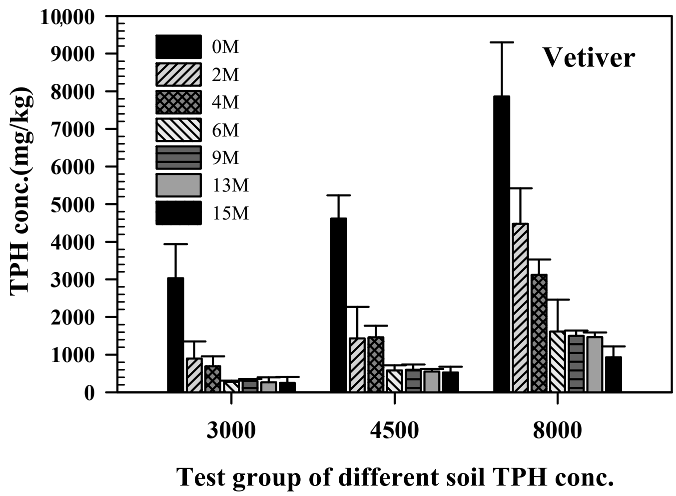

Figure 3. The change of soil TPH concentration with vetiver.

Figure 4 showed the variation of TPH concentration in the soil with lemongrass. For the low-concentration soil, after 2 months, the average concentration of TPH in the soil was 669 mg/kg, and the degradation rate of TPH was about 78%. After 6 months, the average concentration of TPH was about 263 mg/kg, and the degradation rate of TPH was about

91%. From 6 to 15 months, the residual TPH concentration in the soil did not change much. In the medium-concentration soil, after 2 months, the average concentration of TPH in the soil was 1015 mg/kg, and the degradation rate of TPH was about 78%. After 6 months, the average concentration of TPH was about 587 mg/kg, and the degradation rate of TPH was about 87%, and little change in TPH concentration was observed up to 15 months. In the high-concentration soil, after 2 months, the average concentration of TPH in the soil was 3669 mg/kg, and the TPH degradation rate was about 53%. After 6 months, the average concentration of TPH was about 2245 mg/kg, and the TPH degradation rate was about 71%. After 15 months, the TPH average concentration was about 1126 mg/kg, and the degradation rate of TPH was about 86%.

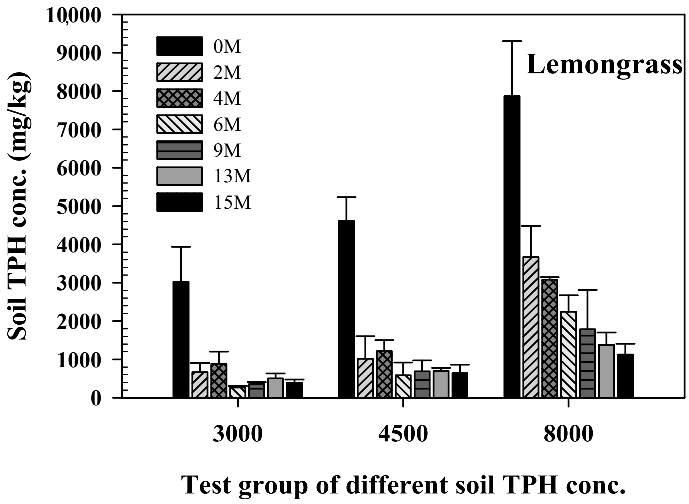

Figure 4. The change of soil TPH concentration in the soil with lemongrass.

In the control soil without planting plants, the soil TPH slowly degraded. During the 15-month test period, the soil removal rate of the low-concentration soil was about 73%, that of the medium-concentration soil was about 76%, and that of the high-concentration soil was about 83%. The TPH degradation efficiency of the high-, medium-, and low-concentration soil with vetiver reached about 90% in 6 months, and the TPH degradation rate remained almost similar during 6 to 15 months. It showed that TPH was easily degraded by up to 90%. With lemongrass, the degradation rate of TPH was close to 90% in 6 months for the medium-and low-concentration soils. The degradation rate during 6 to 15 months was similar to that with vetiver without a significant increase. However, the degradation efficiency of TPH in the high-concentration soil was relatively slow, showing a degradation rate of about 71% in 6 months and 86% in 15 months. For the high-concentration soil, the TPH degradation efficiency with lemongrass was less than that with vetiver.

3.3. Soil Bacteria

Table 1 showed the distribution of bacteriophage in the soils before the test and after 5 months of the test. For the low-concentration soil (3000 mg/kg), before the test, the bacteria in the soil included 715 genera and 1092 species. After 5 months, 604 genera and 772 species remained. There were 865 genera and 1123 species in the soil with vetiver while 612 genera and 768 species in that with lemongrass. After 5 months of experimentation, except for the soil with vetiver, the bacteriophage in the soils with lemongrass and without plant decreased. For the medium-concentration soil (4500 mg/kg), before the test, the soil bacteria included 735 genera and 1148 species. After 5 months, the control soil had 640 genera and 832 species, 823 genera, and 1086 species with vetiver, and 706 genera and 909 species with lemongrass. For the high-concentration soil (8000 mg/kg), before the test, the soil bacteria included 695 genera and 1078 species. After 5 months of testing, the control soil had 627 genera and 827 species, 721 genera and 947 species in the soil with vetiver, and 670 genera and 877 species in the soil with lemongrass. The three TPH concentration soils showed that the bacteriophage with vetiver was the most abundant.

Table 1. Changes in soil bacterial phase abundance before and after 5 months of the experiment.

Test Group	TPH 3000 mg/kg		TPH 4500 mg/kg		TPH 8000 mg/kg	
	Genus	**Species**	**Genus**	**Species**	**Genus**	**Species**
Before test	715	1092	735	1148	695	1078
Control	604	772	640	832	627	827
Vetiver	865	1123	823	1086	721	947
Lemongrass	612	768	706	909	670	877

The abundance of bacteria in the control soil decreased after 5 months of the test. It is speculated that the longer the test time, the fewer available nutrients in the soil, resulting in a decrease in the abundance of bacteria. The bacterial abundance of the soil with lemongrass also decreased after 5 months. However, the soils with plants had more abundant bacteria than the control soil. The soil bacterial abundance with lemongrass did not increase, which may be related to the poor growth of the lemongrass. After 5 months of the experiment, bacteria abundance in the soil with vetiver increased compared with other soils, which showed that vetiver helped increase the richness of the microbiota in the soil.

4. Conclusions and Suggestion

The results of this study showed that the remediation of the petroleum-contaminated soil by the native microorganisms was slow, and the degradation rate of TPH was about 50−70% in 6 months. Planting vetiver and lemongrass stabilized soil pH and electrical conductivity and accelerated the degradation of TPH in soil with vetiver having a better effect. In this study, phytoremediation by vetiver was more effective with a TPH degradation rate of about 90% in 6 months. The bacteriophage in the rhizosphere of the soils also showed that planting vetiver increased the abundance of soil microbiota. Phytoremediation with vetiver was helpful to remediate petroleum-contaminated soil. Other than the remediation effect, vetiver has economic value as it is used to extract aromatic oil and used to produce handicraft materials, papers, and fuels. Thus, it is recommended to use the vetiver for the phytoremediation of petroleum-contaminated soils with multiple benefits.

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