Postprint: Comparative Phytoremediation Effects of Alfalfa (Medicago sativa) and Coreopsis lanceolata in Petroleum-Contaminated Soils in the Longdong Region

Authors: Jing Mingbo

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Abstract

The phytoremediation effects of alfalfa (Medicago#1;sativa) and Coreopsis (Coreopsis#1;lanceolata) on oil-contaminated soil in the Longdong Loess Plateau region were analyzed and compared. After a 5-month field remediation experiment, analysis results of relevant plant physiological, soil physicochemical, and microbial indicators showed that: (1) The highest removal rates of total petroleum hydrocarbons (Total Petroleum Hydrocarbons, TPH) in the rhizosphere soil of Coreopsis and alfalfa reached 75.33% and 69.88%, respectively; (2) 7% and 9% oil-contaminated soil had relatively small inhibitory effects on the physiological characteristics of Coreopsis, and its rhizosphere soil pH, urease, and alkaline phosphatase activities were significantly higher than those of alfalfa ($P<0.05$); (3) The relative abundances of Alcanivorax, Halomonas, and Nocardioides in the rhizosphere soil of Coreopsis were significantly higher than those of alfalfa ($P<0.05$); (4) Increased oil contamination concentration inhibited alfalfa physiology, thereby limiting its rhizosphere TPH removal rate, while the combined effects of soil physicochemical properties and microorganisms resulted in higher rhizosphere soil TPH removal rates in Coreopsis than in alfalfa.

Full Text

Comparison of Phytoremediation Effects of Medicago sativa and Coreopsis lanceolata on Oil-Contaminated Soil in Eastern Gansu Province

JING Mingbo[1,2], WANG Jincheng[1,2,3], ZHANG Wei[3], ZHOU Lihui[4], ZHANG Shaopeng[5]

[1]College of Life Science and Technology, Longdong University, Qingyang 745000, Gansu, China
[2]Gansu Key Laboratory of Protection and Utilization for Biological Resources and Ecological Restoration, Longdong University, Qingyang 745000, Gansu, China
[3]Key Laboratory of Extreme Environmental Microbial Resources and Engineering of Gansu Province, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, Gansu, China
[4]Institute of Safety and Environmental Protection Technology, Oil and Gas Technology Research Institute of Changqing Oilfield Company, Xi' an 710021, Shaanxi, China
[5]No. 11 Oil Production Plant, Changqing Oilfield Company, CNPC, Qingyang 745000, Gansu, China

Abstract

A five-month field remediation experiment was conducted to analyze and compare the phytoremediation effects of Medicago sativa and Coreopsis lanceolata on oil-contaminated soil in the Loess Plateau region of eastern Gansu. Analysis of relevant plant physiological, soil physicochemical, and microbial indicators revealed the following key findings: (1) The maximum total petroleum hydrocarbons (TPH) removal rates in the root-zone soil of Coreopsis lanceolata and Medicago sativa reached 75.33% and 69.88%, respectively; (2) Oil contamination exhibited relatively weaker inhibition on the physiological characteristics of Coreopsis lanceolata, and the pH, urease, and alkaline phosphatase activities in its root-zone soil were significantly higher than those of Medicago sativa ($P < 0.05$); (3) The relative abundances of crude oil-degrading bacteria including Alcanivorax, Halomonas, and Nocardioides in the root-zone soil of Coreopsis lanceolata were significantly higher than those in Medicago sativa ($P < 0.05$); (4) Increased oil concentration inhibited the physiological processes of Medicago sativa, thereby limiting its root-zone TPH removal rate, whereas the combined effects of soil physicochemical and microbial properties resulted in higher TPH removal rates in the root-zone soil of Coreopsis lanceolata compared to Medicago sativa.

Keywords: crude-oil-contaminated soil; Medicago sativa; Coreopsis lanceolata; phytoremediation; eastern Gansu Province

Introduction

Phytoremediation technology for crude oil-contaminated soil represents a major focus and challenge in contemporary environmental remediation research. This approach is particularly favored due to its low cost, high effectiveness, absence of secondary pollution, sustainability for large-scale in-situ remediation, and environmental beautification benefits. Currently, various plant species including Medicago sativa, Axonopus compressus, Spartina anglica, Lolium perenne, Impatiens balsamina, and Hylotelephium spectabile have been successfully applied in phytoremediation of oil-contaminated soils. However, the phytoremediation process is susceptible to multiple influencing factors, among which climatic conditions, topography, and soil properties are critical determinants of remediation efficacy. Therefore, screening native oil-tolerant plants for oil-contaminated soil phytoremediation not only ensures remediation effectiveness but also improves plant colonization rates and prevents disease outbreaks, thereby promoting oil removal in the root zone.

The Loess Plateau region in eastern Gansu, as a major oil-producing area, is characterized by arid climate, low rainfall, poor soil fertility, and high salinization, resulting in distinct regional vegetation distribution patterns. Our research team has conducted long-term ecological restoration of oil-contaminated soils in this region, during which we observed that Compositae family plants not only exhibit effective remediation capabilities but also possess strong oil tolerance, cold resistance, and non-palatability to livestock. Previous studies have reported on the phytoremediation effects of Calendula officinalis and Gerbera jamesonii on oil-contaminated soil in the Loess Plateau, but comparative studies with other plant species remain lacking.

Consequently, this study selected the leguminous plant Medicago sativa as a reference species and the Compositae plant Coreopsis lanceolata as the test species to conduct a five-month field remediation experiment in an operational area of Changqing Oilfield. By comparing the responses of plant physiological characteristics, soil physicochemical properties, and soil microbial characteristics of both species under different oil concentration stresses, we aimed to identify the key microecological factors influencing the phytoremediation effects of Medicago sativa and Coreopsis lanceolata in eastern Gansu, thereby providing alternative plant varieties and baseline reference data for oil-contaminated soil remediation in this region.

Materials and Methods

1.1 Study Site and Experimental Design

The study area was located in Qingcheng County, Qingyang City, Gansu Province (107°16 32 -108°05 49 E, 35°42 29 -36°17 22 N), with a soil type classified as loessal soil. To maximize the evaluation of in-situ phytoremediation effectiveness, oil-contaminated soil samples were collected from different concentrations (0.72%-36.37%) within an operational area of Changqing Oilfield, with crude oil pollutants consisting of a mixture of spilled oil and tank-cleaning oil. Background nutrient values of the contaminated soil were: total nitrogen 0.31-1.81 $g \cdot kg^{-1}$, total phosphorus 1.49-1.81 $g \cdot kg^{-1}$, available phosphorus 22.69-26.37 $mg \cdot kg^{-1}$, alkali-hydrolyzable nitrogen 16.15-18.71 $mg \cdot kg^{-1}$, organic matter 10.51-13.19 $g \cdot kg^{-1}$, and pH 7.78-7.94.

The experiment utilized oil-contaminated soils with total petroleum hydrocarbons (TPH) contents of 3.07%, 6.93%, 9.12%, and 11.43%, designated as 3%, 7%, 9%, and 11% TPH treatments, respectively. The field remediation experiment employed plots measuring 3 m × 3 m × 0.5 m. Based on preliminary experiments showing that Coreopsis lanceolata root systems naturally extend to 17.5–22.3 cm in non-contaminated soil, the oil-contaminated soil layer was set at 30 cm thickness to ensure root penetration. Each concentration treatment included three replicate plots, plus a control group (0% TPH), totaling 15 plots. Seeds were sown on May 15, 2019, with 500 seeds per plot and covered with approximately 2 cm of soil. Manual irrigation maintained maximum field water capacity at 60–70% for germination. On June 15, seedlings were thinned to a height of approximately 10 cm, with final densities maintained at 30 plants per m^2 for both Coreopsis lanceolata and Medicago sativa to ensure root-zone remediation effects. The remediation experiment ran from May 15 to October 15, 2019.

1.2 Plant Physiological Indicators and Soil Physicochemical and Enzyme Activity Measurements

After remediation, five representative plants of each species were harvested using the diagonal five-point method. Root-zone soil samples were collected from each plant using the root-shaking method, mixed thoroughly, and divided into two portions: one for soil microbial (bacterial) high-throughput sequencing and the other air-dried and sieved for physicochemical and enzyme activity analyses. Soil alkali-hydrolyzable nitrogen, available phosphorus, urease, and polyphenol oxidase were measured using the alkaline hydrolysis diffusion method, 0.5 $mol \cdot L^{-1}$ $NaHCO_3$ molybdenum-antimony anti-colorimetry, indophenol blue colorimetry, and pyrogallol colorimetry, respectively. Plant root vitality, leaf chlorophyll content, above- and below-ground biomass, root-shoot ratio, and emergence rate were determined according to established protocols. Root-zone soil TPH residues were measured using ultrasonic extraction, with the removal rate calculated as:

$$\text{Removal rate }(\%) = \frac{\text{Initial TPH }(\text{mg}\cdot\text{kg}^{-1}) - \text{Residual TPH }(\text{mg}\cdot\text{kg}^{-1})}{\text{Initial TPH }(\text{mg}\cdot\text{kg}^{-1})} \times 100\%$$

1.3 Soil Microbial Diversity Sequencing

High-throughput sequencing of soil microbial (bacterial) communities was performed by a commercial company using the Illumina Miseq PE250 platform. Amplification primers were 338F (5 -ACTCCTACGGGAGGCAGCAG-3) and 805R (5 -GACTACHVGGGTATCTAATCC-3). The complete Illumina sequencing workflow included library preparation, sequencing, and preliminary analysis of raw data.

1.4 Data Analysis

Statistical analyses were performed using SPSS 25.0, R 3.3.1, and GraphPad Prism 5.0. Soil microbial community structure (at the genus level) was analyzed using the online platform (www.bio-cloud.org). Variance partitioning analysis (VPA) was conducted using the "Vegan" package in R 3.3.1 to quantify the contributions of plant physiological traits, soil physicochemical properties, and microbial characteristics to soil TPH removal rates under different oil concentrations. All data were Hellinger-transformed prior to analysis.

Results

2.1 Changes in Root-Zone Soil TPH Removal Rates Under Different Oil Concentrations

F-test and multiple comparison (Duncan, $\alpha = 0.05$) results showed that TPH removal rates in the root-zone soil of both plant species decreased with increasing oil concentration ($P < 0.05$). Compared with the 3% oil concentration group, the 7%, 9%, and 11% treatments showed significant reductions of 39.06%, 72.52%, and 97.12% for Medicago sativa, respectively, while Coreopsis lanceolata showed reductions of 43.62%, 97.07%, and 97.12%, respectively. Independent samples t-test results revealed that at oil concentrations of 3% and 7%, the TPH removal rates in the root-zone soil of Coreopsis lanceolata were significantly higher than those of Medicago sativa, exceeding them by 75.81% and 121.21%, respectively ($P < 0.05$). However, at oil concentrations of 9% and 11%, neither species demonstrated significant root-zone remediation effects. Research indicates that increasing oil concentrations inhibit root-zone remediation effects differently across plant species, as phytoremediation of oil-contaminated soil depends on microbial degradation in the root zone. The types and quantities of crude oil-degrading functional bacteria in plant root-zone soil are key factors determining TPH removal rates, but different plant species host distinct microbial community responses, ultimately leading to differential TPH removal efficiencies.

2.2 Changes in Plant Physiological Indicators Under Different Oil Concentrations

Since neither test plant exhibited significant root-zone remediation effects at 9%-11% oil concentrations, subsequent analyses focused on the 3%-7% range. Compared with the non-contaminated control, the emergence rate and chlorophyll a/b ratio of Medicago sativa decreased by 43.08%-106.92% and 31.42%-87.15%, respectively, under 3%-7% oil concentrations, while Coreopsis lanceolata showed decreases of 20.00%-68.15% and 28.70%-70.48%, respectively, with no significant differences between species at any concentration ($P > 0.05$). However, compared with non-contaminated soil, the root-shoot ratio, aboveground dry weight, and underground dry weight of Medicago sativa at 3% oil concentration increased by 8.40%, 16.48%, and 26.43%, respectively ($P < 0.05$), whereas Coreopsis lanceolata showed no significant changes. When oil concentration increased to 7%-9%, the reduction in these parameters for Medicago sativa was significantly greater than for Coreopsis lanceolata ($P < 0.05$). Additionally, root vitality of Coreopsis lanceolata increased by 164.64%-216.95% compared with the non-contaminated control across 3%-9% oil concentrations ($P < 0.05$), while Medicago sativa showed increases of only 57.68%-150.94%. These results demonstrate that oil concentrations of 3%-7% had relatively minor effects on both test plants, whereas concentrations of 7%-9% significantly inhibited the emergence rate, chlorophyll ratio, aboveground dry weight, underground dry weight, and root-shoot ratio of Medicago sativa to a greater extent than Coreopsis lanceolata.

2.3 Changes in Soil Physicochemical Indicators in Root-Zone Soil

Soil pH increased with oil concentration, while alkali-hydrolyzable nitrogen, available phosphorus, urease, and alkaline phosphatase activities decreased ($P < 0.05$). T-test results showed that at 3% oil concentration, urease activity in the root-zone soil of Coreopsis lanceolata was 54.42% higher than that of Medicago sativa ($P < 0.05$), while alkaline phosphatase activity was 65.85% higher. At 7%-9% oil concentrations, urease and alkaline phosphatase activities in the root-zone soil of Coreopsis lanceolata were significantly higher than those of Medicago sativa, with increases of 68.39% and 188.80%, respectively ($P < 0.05$). These findings indicate that oil concentrations of 3%-7% had relatively minor inhibitory effects on soil available phosphorus content in the root-zone soil of Medicago sativa ($P < 0.05$), whereas soil pH, urease, and alkaline phosphatase activities in the root zone of Coreopsis lanceolata were significantly elevated compared to Medicago sativa at 7%-9% concentrations ($P < 0.05$).

2.4 Changes in Soil Microbial Communities Under Different Oil Concentrations

High-throughput sequencing results showed that compared with non-contaminated soil, the Chao1 and Shannon indices of root-zone soil microbial communities decreased with increasing oil concentration. The Chao1 index for Medicago sativa decreased by 14.21%-72.20%, while for Coreopsis lanceolata it decreased by 28.86%-67.10%. The Shannon index for Medicago sativa decreased by 30.96%-57.54%, while for Coreopsis lanceolata it decreased by 6.35%-61.79%. These results demonstrate that oil toxicity effects intensify with concentration, leading to reduced microbial richness and diversity. However, at oil concentrations of 3%-7%, the Chao1 and Shannon indices in the root-zone soil of Coreopsis lanceolata were significantly higher than those of Medicago sativa ($P < 0.05$), indicating that increasing oil concentrations had relatively weaker inhibitory effects on the richness and diversity of microbial communities in the root zone of Coreopsis lanceolata.

Dominant bacterial genera (relative abundance $\geq 0.1\%$) included Alcanivorax, Gracilimonas, Marinobacter, Halanaerobium, Halomonas, Sphingomonas, Pseudoxanthomonas, Lutibacter, Fodinicurvata, Proteiniphilum, Pseudomonas, Nocardioides, Mycobacterium, Marinobacterium, and Thalassolituus. Cluster analysis (Euclidean distance method) grouped soil samples into three clusters: Cluster I comprised non-contaminated soils, Cluster II included Medicago sativa treatments, and Cluster III included Coreopsis lanceolata treatments, reflecting that plant species differences are key determinants of microbial community structure in oil-contaminated soil. Notably, the relative abundances of Alcanivorax, Marinobacter, Halanaerobium, Halomonas, and Nocardioides in the root-zone soil of Coreopsis lanceolata were significantly higher than in Medicago sativa ($P < 0.05$). These genera, along with Sphingomonas, Pseudoxanthomonas, Pseudomonas, Mycobacterium, Marinobacterium, and Thalassolituus, contain known crude oil-degrading functional bacteria, providing the biological basis for root-zone remediation by both plant species.

2.5 Variance Decomposition of Plant Physiological, Soil Physicochemical, and Microbial Effects on Soil TPH Removal

Variance partitioning analysis (VPA) was performed to quantify the contributions of plant physiological traits, soil physicochemical properties, and microbial characteristics to soil TPH removal rates under oil concentrations of 3%, 7%, and 9%. Results showed that the pure effects of plant physiological traits on TPH removal rates decreased from 41.7% to 7.8% for Medicago sativa and from 38.9% to 9.2% for Coreopsis lanceolata as oil concentration increased, indicating that oil concentration inhibition on plant physiology limited root-zone TPH removal. Conversely, the combined effects of soil physicochemical and microbial characteristics were the primary environmental factors determining the higher TPH removal rates in Coreopsis lanceolata compared to Medicago sativa. At 3%-7% oil concentrations, the combined soil physicochemical $\times$ microbial effects contributed 31.2%-45.6% to TPH removal in Coreopsis lanceolata, significantly higher than the 18.4%-22.1% contribution in Medicago sativa. LEfSe analysis revealed that at 7% oil concentration, differential indicator bacteria in the Coreopsis lanceolata treatment group were predominantly crude oil-degrading functional genera, including Alcanivorax, Halomonas, and Nocardioides, with LDA scores > 4.0. In contrast, Medicago sativa treatments showed fewer indicator genera, primarily Sphingomonas, Pseudomonas, and Mycobacterium, suggesting that Coreopsis lanceolata more effectively enriches crude oil-degrading functional bacteria in its root zone.

Conclusion

As oil concentration increased from 3% to 9%, the root-zone soil TPH removal rates of Coreopsis lanceolata consistently exceeded those of Medicago sativa. Oil concentrations of 3%-7% had relatively minor effects on the root-shoot ratio, root vitality index, chlorophyll ratio, soil pH, and enzyme activities of Coreopsis lanceolata, whereas concentrations of 7%-9% significantly inhibited these parameters in Medicago sativa. The combined effects of soil physicochemical and microbial properties were the key environmental factors determining the superior TPH removal efficiency of Coreopsis lanceolata. These findings demonstrate that Coreopsis lanceolata is more suitable than Medicago sativa for phytoremediation of crude-oil-contaminated soils in the Loess Plateau region of eastern

Gansu.

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Note: Figure translations are in progress. See original paper for figures.