

Effects of Crude Oil, Oil Components, and Bioremediation on Plant Growth

Kyung-Hwa Baek,^{1,2} Hee-Sik Kim,² Hee-Mock Oh,² Byung-Dae Yoon,²
Jaisoo Kim,¹ and In-Sook Lee^{1,*}

¹Department of Life Science, Ewha Womans University, Seoul, Korea

²Environmental Biotechnology Laboratory, Korea Research Institute of
Biotechnology and Bioscience, Daejeon, Korea

ABSTRACT

The phytotoxic effects of crude oil and oil components on the growth of red beans (*Phaseolus nipponesis* OWH1) and corn (*Zea mays*) was investigated. In addition, the beneficial effects of bioremediation with the oil-degrading microorganism, *Nocardia* sp. H17-1, on corn and red bean growth in oil-contaminated soil was also determined. It was found that crude oil-contaminated soil (10,000 mg/kg) was phytotoxic to corn and red beans. In contrast, obvious phytotoxicity was not observed in soils contaminated with 0–1000 mg/kg of aliphatic hydrocarbons such as decane (C₁₀) and eicosane (C₂₀). Phytotoxicity was observed in soils contaminated with 10–1000 mg/kg of the poly aromatic hydrocarbons (PAHs) naphthalene, phenanthrene, and pyrene. It was observed that phytotoxicity increased with the number of aromatic rings, and that corn was more sensitive than red beans to PAH-contaminated soil. Bioremediation with *Nocardia* sp. H17-1 reduced phytotoxicity more in corn than in red bean, suggesting that this microbial species might degrade PAHs to some degree.

*Correspondence: In-Sook Lee, Department of Life Science, Ewha Womans University, Seoul 120-750, Korea; E-mail: islee@ewha.ac.kr.

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INTRODUCTION

Crude oil is a complex mixture of thousands of hydrocarbons and non-hydrocarbon compounds, including heavy metals. Although the toxicity of each individual component is known, the toxicity of complex mixtures such as crude oils and refined products is extremely difficult to assess,^[1] because researchers know little about the additive, synergistic, or antagonistic effects of the various mixtures. In addition, the chemical composition of each crude oil and petroleum product varies significantly, and can have diverse effects on different organisms within the same ecosystem.^[1] These differences in toxic effects are due to qualitative compositional differences in the various products, as well as concentration differences of the chemical constituents.^[2,3]

Bioassays such as measurements of seed germination and early seedling growth have been used to monitor treatment effects and restoration of oil-contaminated sites.^[3,4] Dorn and Salanitro^[5] found that seed germination and plant growth using corn, wheat, and oats differed from different soils and oil combinations before, during, and after bioremediation. Sayles et al.^[4] showed that oil-contaminated soil treated with aerobic biodegradation was less toxic to lettuce and oat root elongation. Hanson et al.^[6] reported that *Acinetobacter* sp. A3-treated soil permitted better germination and growth of mung beans, as evidenced by better plant length, weight, and leaf chlorophyll content. This indicated that *Acinetobacter* sp. A3 was capable of reducing crude oil phytotoxicity through biodegradation.

The objectives of this study were to: (i) evaluate the comparative phytotoxicity response of corn and red beans to crude oil and its components; and (ii) investigate the reduction in phytotoxicity following bioremediation with the crude oil-degrading bacterium, *Nocardia* sp. H17-1, which can convert approximately 83% of oil into nonextractable forms.^[7]

MATERIALS AND METHODS

Crude Oil and Soils

Arabian light crude oil was obtained from a petroleum company (Yu-Gong Petrol, Korea). The oil had an API (American Petroleum Institute) gravity of 33.4, sulfur and nitrogen contents of 1.8% (w/w) and 1.7% (w/w), respectively, and consisted of 13.4% asphaltene, 54.9% aliphatic hydrocarbons, 10.5% aromatic hydrocarbons, and 21.2% polar materials. Hexadecane (99% purity), eicosane (99% purity), naphthalene (99% purity), phenanthrene (98% purity), and pyrene (98% purity) were all purchased from Sigma-Aldrich.

For biodegradation of the crude oil, a sandy soil (0.9% organic matter, pH 6.7) with no history of hydrocarbon contamination was used. Soil was passed through



a 2 mm sieve and air-dried. For each experiment, aliquots (1 kg each in 1.5 L plastic cylinders) of soil were artificially contaminated with 30 g of the Arabian light oil.

Biodegradation of Crude Oil

The oil-degrading bacterium *Nocardia* sp. H17-1, which was isolated from oil-contaminated soil and previously reported by Lee et al.,^[7] was grown to the late exponential phase in Luria-Bertani liquid medium. Cells were centrifuged at $10,000 \times g$ for 20 min and collected cell pellets were washed twice in 20 mL of sterile water. 10^6 – 10^7 cells were suspended in 1 mL medium, and 5 mL was applied to oil-contaminated soil samples, which were thoroughly mixed. Control samples, treated with oil only, were used for comparison purposes to measure the degradation of crude oil by indigenous microorganisms. The water content of the soil samples was adjusted with sterile water to 50% of the maximum water holding capacity, and samples were kept in the dark at room temperature for 120 days.

Analysis of Total Petroleum Hydrocarbons (TPH)

At various intervals (0, 25, 50, 85, and 120 days), soil samples were transferred to 25 mL vials and mixed with 2 g anhydrous sodium sulfate and 20 mL of dichloromethane. The vials were tightly capped, thoroughly mixed for 5 min with a vortex mixer and sonicated for 30 min in a water bath. The supernatant was passed through a $0.45 \mu\text{m}$ Teflon filter, and $1 \mu\text{L}$ of extract was analyzed on a gas chromatograph (Varian 3400CX, USA) equipped with a flame ionization detector. A DB-1 capillary column ($30 \text{ m} \times 0.32 \text{ mm}$ with $0.25 \mu\text{m}$ film thickness, J & W Scientific, USA) was used, and purified N_2 was employed as the carrier gas at a flow rate of 50 mL min^{-1} . The operating temperature program was started at 40°C for 5 min, increased at a rate of 4°C min^{-1} to 170°C for 3 min and then increased again at a rate of 5°C min^{-1} to 300°C for 10 min. The injector and detector were maintained at 250 and 300°C , respectively. The hydrocarbon components in the crude oil were quantified by integration, normalized to the squalene peak from each sample, and expressed as percent degraded relative to the corresponding peak in the appropriate abiotic control sample.

Plants Assay

Seed germination and root elongation toxicity tests were performed according to the U.S. EPA protocol.^[8] One species of monocotyledon, corn (*Zea mays*), and one species of dicotyledon, red bean (*Phaseolus nipponesis* OWH1) were used for testing. For pure hydrocarbons tests, each soil sample was spiked with pure CH_4Cl_2 hydrocarbon solution, which was thoroughly mixed and then allowed to volatilize for 2 h under a hood. For crude oil tests, the appropriate amount of crude oil was mixed into the soil then volatilized for three days. Test pots containing 300 g of contaminated soil were planted with 5 seeds each, then placed in darkness at 23°C



for 14 days. At the end of the test period, seeds were scored as germinated if a shoot was visible, the shoot and root lengths were measured to the nearest centimeter, their weights were estimated, and then biomasses were calculated. Each test was conducted two to four times before and after bioremediation. Data for each parameter were analyzed separately using one-way ANOVA followed by Duncan's multiple comparisons test.

RESULTS AND DISCUSSION

Phytotoxicity of Crude Oil and Various Hydrocarbons

The phytotoxicity of crude oil was tested on two plants, red bean and corn (Table 1). The growth of corn was more sensitive to crude oil than that of red bean, especially in root development, which was acutely reduced in soil contaminated with as little as 1% (w/w) crude oil. Indeed, corn was entirely unable to germinate in 5% (w/w) oil-contaminated soil.

The growth of both plants appeared normal in C₁₀- and C₂₀-contaminated soils (Fig. 1), although corn root growth was slightly reduced in soil contaminated with high concentrations of C₂₀. In contrast, contamination with individual PAHs such as naphthalene, phenanthrene, and pyrene allowed the germination of corn and red beans, but inhibited their growth in a concentration-dependent manner (Fig. 2). This is in contrast to previous reports indicating that plant germination and growth were strongly inhibited by low molecular weight hydrocarbons such as benzene, toluene, xylene (BTX), styrene, and naphthalene but not by PAHs (3–5 rings) of high molecular weight.^[9] Indeed, it was observed that PAHs increased in growth inhibition effect according to their concentration and number of aromatic rings.^[2,10]

In comparing corn and red beans, it was found that corn was slightly more sensitive to crude oil and aliphatic hydrocarbons, and much more sensitive to PAHs (Table 1, and Figs. 1 and 2). This is consistent with previous reports of interspecies differences in sensitivity to petroleum hydrocarbons, and may be related to differences in systemic uptake of oil compounds, nutrient availability, and cell wall structural differences.^[2,10]

Table 1. Effects of crude oil concentration on the initial growth of plants ($n = 5$).

Crude oil (%, w/w)	Red bean		Corn	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
0	23.84 ± 0.76	15.30 ± 2.01	18.30 ± 1.63	17.78 ± 1.23
1	21.24 ± 1.51	10.86 ± 2.07	13.16 ± 0.89	8.42 ± 1.10
3	12.56 ± 2.80	5.80 ± 1.58	7.12 ± 1.38	5.78 ± 1.45
5	1.84 ± 1.54	4.18 ± 0.67	—	—
10	—	—	—	—



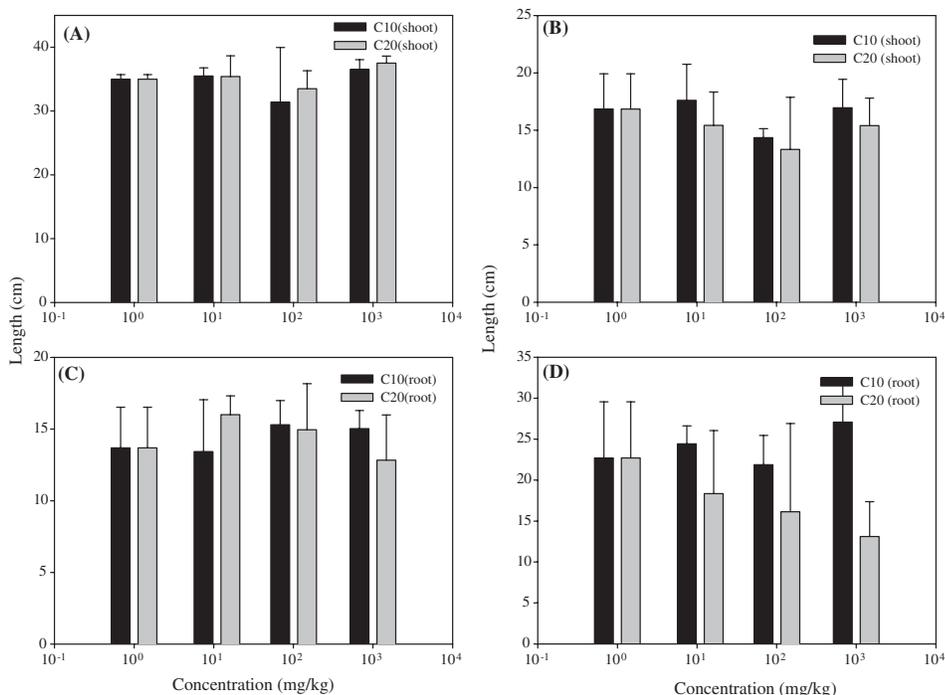


Figure 1. Effect of aliphatic hydrocarbon concentration on the initial growth (14 days) of *Phaseolus nipponesis* OWH1 (A and C) and *Zea mays* (B and D). The data for each parameter were analyzed separately using one-way ANOVA followed by Duncan's multiple comparisons test.

Phytotoxicity of Bioremediated Soils

The concentrations of TPH in each treated soil sample were determined at different time periods during the course of the study (Table 2). At the beginning of the study, the concentration of TPH in H17-1-treated soil was 10,116 mg/kg of soil, and that in the control soil (containing only indigenous microorganisms) was 8380 mg/kg. Within the first 25 days, 72.65% of this TPH was removed from the H17-1-treated samples, in comparison to 21% in the control samples ($P < 0.05$). After 85 days, the removal rates were similar in *Nocardia* sp. H17-1-treated and control soil samples (2607 mg/kg and 2380 mg/kg, respectively), and after 120 days, 86.5% of TPH was removed from H17-1-treated soil, in comparison to 81.0% in control samples.

The effect of bioremediation was examined with *Nocardia* sp. H17-1 on phytotoxicity, as measured by corn and red bean growth. Seed germination did not significantly differ in H17-1-treated- and untreated-bioremediation soils, but root and shoot elongation showed significantly less toxicity in treated-remediation soil (Fig. 3). In soil treated with *Nocardia* sp. H17-1, the lengths of corn and red bean plants were 77 and 56%, respectively, of control plants (grown in uncontaminated



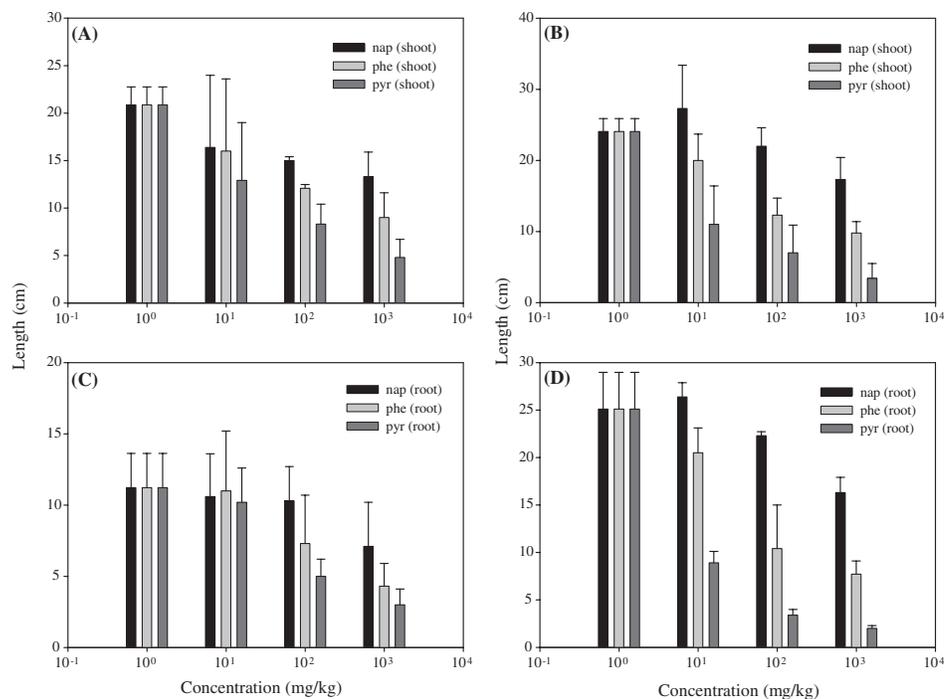


Figure 2. Effect of PAH concentration on the initial growth (14 days) of *Phaseolus nipponesis* OWH1 (A and C) and *Zea mays* (B and D). Data for each parameter were analyzed separately using one-way ANOVA followed by Duncan's multiple comparisons test.

Table 2. Degradation of total petroleum hydrocarbons (TPH) by *Nocardia* sp. H17-1 in crude oil-contaminated soil ($n = 3$).

Incubation time (day)	TPH (mg/kg)	
	H17-1-treated	Untreated
0	10,111 ± 1,243	8,378 ± 803
25	2,765 ± 1,834	6,646 ± 74
50	2,079 ± 153	4,123 ± 1,078
85	2,607 ± 891	2,380 ± 162
120	1,362 ± 214	1,608 ± 13

soil), while lengths in untreated soil were 16 and 49%, respectively. Although TPH was reduced to 80% in both treated and untreated soil after 120 days, a high degree of phytotoxicity still remained in the untreated soil as the result of Salanitro et al.^[11] This indicates that most of the components degraded by the indigenous microorganisms were aliphatic components that did not significantly affect plant growth. The decreased corn-specific phytotoxicity in H17-1-treated soil indicates



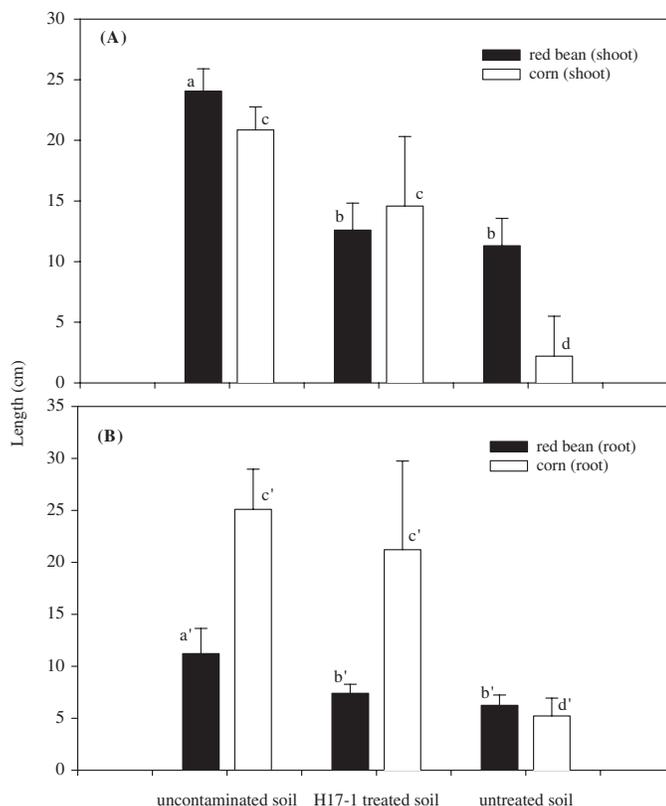


Figure 3. Plant growth in oil-contaminated sand after bioremediation. Data for each parameter were analyzed separately using one-way ANOVA following by Turkey's HSD test. Values designated A (shoot length) and B (root length) differ significantly ($P < 0.05$). Means and SD are shown ($n = 5$).

that more PAHs were degraded than in the untreated soil, since corn proved more sensitive to PAHs than red beans. This is consistent with previous reports of improved germination and plant growth after bioremediation^[12,13] and PAH-preferential bioremediation by H17-1.^[14,15] Although this study had not been tested, other bioassays such as earthworm survivals and reproductions, the Microtox assay, and Ames assay demonstrated, overall, that addition of *Nocardia* sp. H17-1 enhanced crude oil degradation more effectively than did indigenous microorganisms, and that bioremediation with H17-1 reduced oil phytotoxicity in soil.

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