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PHYTOSTIMULATION OF HYDROCARBON BIODEGRADATION BY ARROYO WILLOWS IN LABORATORY MICROCOSMS

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ABSTRACT: Controlled laboratory microcosms with and without Arroyo Willows (*Salix lasiolepis*) were used to elucidate potential mechanisms of phytoremediation of hydrocarbon-contaminated groundwater at a contaminated oil field near Guadalupe, CA. Laboratory control allows us to examine the synergistic effects between the plants themselves and the rhizobial bacteria associated with them. Laboratory microcosms were set up in triplicate with (1) sodium azide-inhibited soil, (2) soil with active bacteria, and (3) soil with active bacteria and willows. Hydrocarbon-contaminated groundwater was recirculated through the root zone for 105 days. Biodegradation rates were estimated by measuring total petroleum hydrocarbon (TPH) concentration and monitoring chemical oxygen demand (COD). TPH results showed a decrease in all chambers, the smallest decrease was for the sodium azide control chambers and the largest was for the willow chambers. For an initial TPH concentration of 3.6 ± 0.61 mg/L, the soil only chambers dropped to 0.40 ± 0.036 mg/L, and the soil and willow chambers dropped to 0.26± 0.080 mg/L. These results show a statistically significant effect of the willow trees compared to soil alone, suggesting the trees did contribute to bioremediation under these conditions, either directly via phytodegradation or indirectly via phytostimulation of bacterial biodegradation.

INTRODUCTION

The Guadalupe Oil Field is located on the coast of California near Santa Maria. The viscous crude from this site was thinned with a diluent from a nearby oil refinery to facilitate pumping. The diluent used at the site was transported through pipes to the oil field and stored in tanks. Many of these pipes, tanks and fittings leaked diluent into the sand dunes at 90 cataloged leakage sites with approximately 4 to 8 million gallons of leaked diluent. The diluent contamination is a mixture of hydrocarbons in the range of C_{10}-C_{30}. The site is comprised of more than 3000 acres of homogeneous sand adjacent to a river channel. The depth to groundwater fluctuates depending on the height of the sand dunes, ranging from 0 – 130 feet. Twenty feet below the water table there is a confining clay layer. Endangered species such as the red legged frog and snowy plover thrive at the site, and thus it is important that the dune ecosystems be kept intact as much as possible during remediation. For this reason phytoremediation would be particularly attractive.

Due to the extensive cleanup efforts at the site, many traditional and novel remediation methods have been demonstrated. Biosparging, steam extraction, excavation, land treatment, and phytoremediation are some of the methods being evaluated at the site. Two phytoremediation field test sites have been established, one with a monoculture of arroyo willows, and another with a variety of native California species, including arroyo willows. It is hoped that by planting native species at the site bioremediation and ecological restoration can be achieved simultaneously-a technique we refer to as "ecoremediation." However, it has been difficult to evaluate the efficacy of these plantings for phytoremediation due to the non-uniformity of the site. Variability of soil contamination levels, water flow, and sampling locations all contribute to the difficulty of evaluating phytoremediation in the field. Due to this difficulty, bench scale laboratory experiments were conducted to control these variables while investigating phytoremediation with arroyo willows. These experiments were designed primarily to determine if the willows stimulate biodegradation by soil microbes.

Phytoremediation is a remediation technology that has been successful for the remediation of heavy metals and some volatile organic compounds. Phytoremediation research has been described as having five primary goals "(1) understand the mechanisms by which these technologies work, (2) develop appropriate testing protocols and methodologies that illustrate their utility, (3) improve predictive capabilities, (4) facilitate validation of the effectiveness and persistence of the technique, and (5) prepare guidelines for its implementation." (Cunningham et. al 1997). Though each of these goals deserves attention, the first and fourth goals are the focus of this paper. This research is being conducted to evaluate the use of willow trees to degrade the aqueous hydrocarbon plumes under conditions mimicking those at the Guadalupe site.

Growth chambers used in these experiments were based on a design previously used to evaluate degradation of trichloroethylene (TCE) by hybrid poplar trees (Orchard et. al 1999). The original design included both soil and foliar chambers so that volatile emissions from the soil and leaves could also be monitored. However, a companion study in our laboratory showed no volatilization of diluent hydrocarbons under these conditions (Elliot, 2002). Thus, due to the non-volatile nature of diluent, the top chamber from this model was removed and degradation was only measured from change in concentration of the water. Arroyo willows were grown in glass containers in sand collected from the field site. Diluent contaminated groundwater was recirculated through the root zone of the plants and the biodegradation rates of contaminant were measured. TPH was measured at the start and end of the experiment, chemical oxygen demand (COD) was also measured as an indicator of TPH to see degradation rates. Controls were run to check that there was no loss of contaminant to the chamber apparatus. The influence of the willows on biodegradation rate was examined by comparing biodegradation rates with and without willow trees in the soil.

METHODS

Biodegradation rates were measured with 3 sets of 3 chambers. The first set of chambers had autoclaved soil from the Guadalupe site inhibited with sodium azide with no willows. The second set of chambers has only soil from the Guadalupe site with no willows and the third set of chambers contained an Arroyo Willow grown in soil from the Guadalupe site (Figure 1.) Each chamber has an individual water reservoir, which contained groundwater from the Guadalupe site, collected up-gradient from the current field phytoremediation experimental site. This water was recirculated at a rate of 1 liter per day, to mimic site hydraulic conditions, using a peristaltic pump with silicone tubing (Figure 2.) Each chamber consists of a 4-liter glass jar, a drip tube, and a well tube. The drip tube is made of Viton® tubing which has approximately 0.5 mm diameter holes cut in the sides of it. This drip tube is placed at the bottom of the jar so that the tube encircles the bottom of the jar. The well tube is made from six inches of Purflex® tubing with a ¾ inch inside diameter and a piece of stainless steel woven wire cloth zip-tied to the end of it to be used as a screen. The mesh has a width opening of 0.0029 inches and

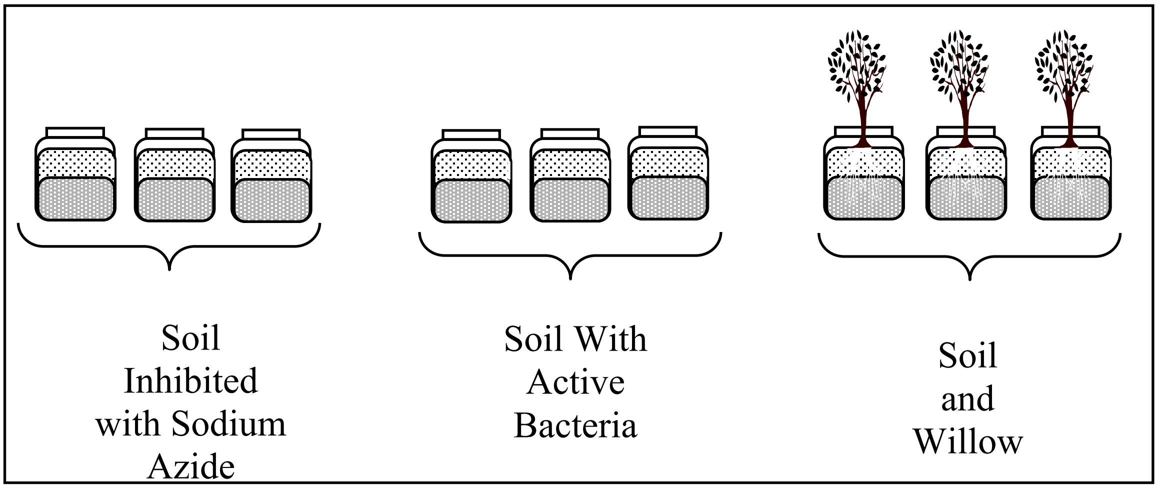

FIGURE 1. Experimental design: Triplicate chambers for (1) Azide-inhibited soil, (2) Soil with active bacteria, and (3) Soil with active bacteria and willows.

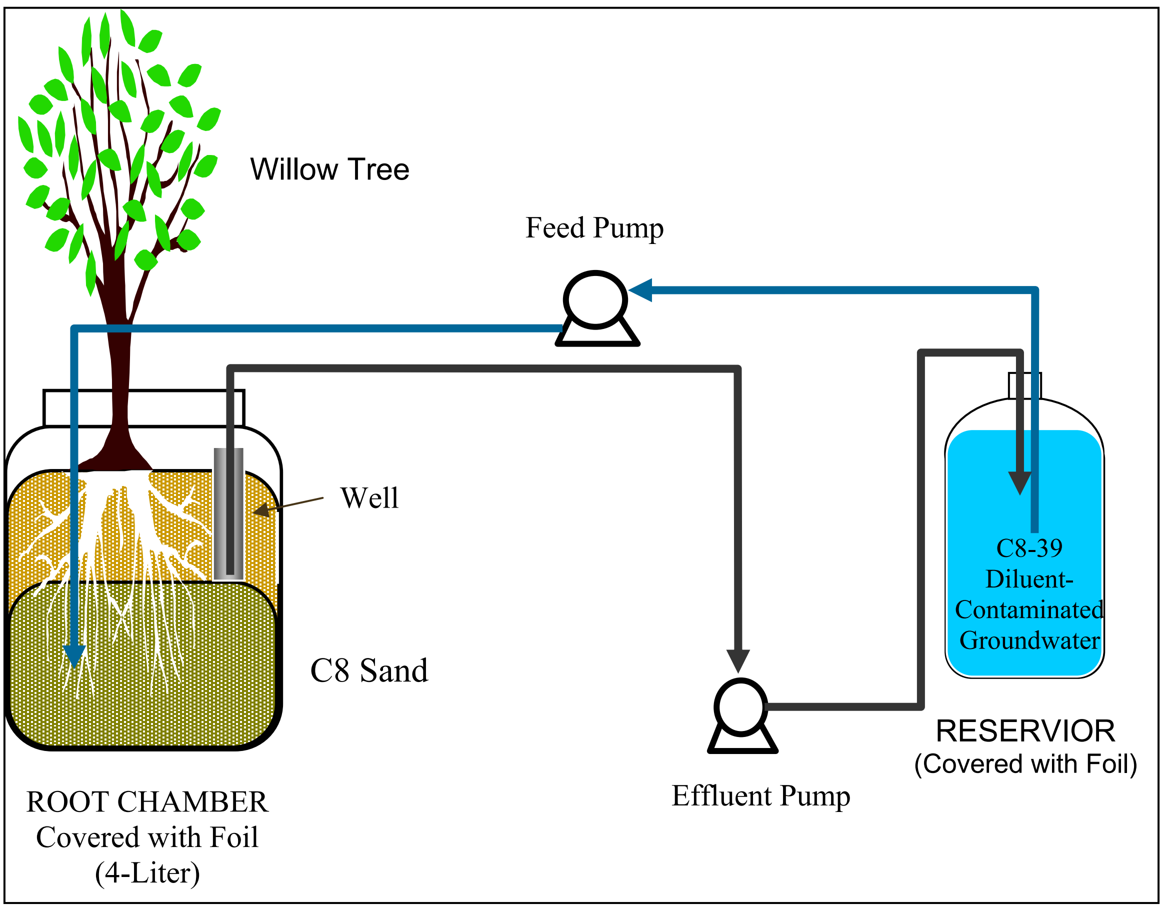

FIGURE 2. Plant apparatus setup.

33.6% open area. The mesh screens out all sands larger than the very fine sands. This well tube is placed at the top of the jar within one inch of the mouth with the mesh side pointed down and the water outlet tube is placed inside it. This outlet tube can then be used to maintain a water table level inside the chamber. The jars are filled with Guadalupe site sand to within two inches of the top of the mouth of the jar. Two Masterflex® peristaltic pumps (Cole Parmer, Vernon Hills, IL) with two eight-channel heads each were used to provide the nine chambers with water recirculation. The effluent pump tubing is larger than the influent pump tubing so that the water level will always be maintained because the effluent pump tubing is always pumping faster. This causes the creation of bubbles in the reservoir chamber due to over pumping of the effluent tubing. The reservoir chamber is a two-liter Pyrex sample jar with a narrow mouth, containing 2 L of Guadalupe groundwater. The loss of water due to evapotranspiration and evaporation is compensated for by the addition of de-ionized water to the reservoir to keep the liquid level at 2 L.

Arroyo Willows (*Salix lasiolepis*) cutting used for the experiment were placed in 10 inches of tap water for one month to induce rooting. The cuttings were then transferred to the growth chambers and allowed to grow in site water, which was replace at least monthly, for six months to be certain that sufficient time was given that plants could root and that microbial communities could be established in the root zone. For two days before the first sampling, contaminated groundwater was passed through each chamber by running the effluent tube directly to a waste container instead of the water reservoir. Two liters of groundwater were pumped through each chamber over these two days to flush the chamber of old water and replace it with fresh site water. After flushing, the reservoirs were refilled and the effluent tube was returned to the reservoir. At no time during this experiment were any nutrients added to any water or chamber nor were the concentration of any nutrients measured. It should be noted that dissolved oxygen levels were not measured during the experiment.

Initial total petroleum hydrocarbon (TPH) concentrations were measured by an independent analytical laboratory with gas chromatography (GC). Three samples were measured at the beginning and it was assumed that the sample pool was homogeneous. Chemical oxygen demand (COD) was measured for each chamber with premixed and packaged COD vials from Bioscience® (Bethlehem, PA) on days: 0, 2, 3, 5, 8, 12, 19, 25, 39, 59, 80 and 105. At day 105, 1 Liter of sample was taken from each chamber and analyzed for TPH.

RESULTS

Results of the 105-day phytoremediation experiment are shown in Tables 1 and 2 and Figure 3 below. The aqueous TPH concentration of triplicate initial samples ranged from 2.90 to 3.9 mg/L (Table 1), for an average initial TPH concentration of 3.6 ± 0.61 mg/L (Table 2). For the three chambers for which the bacteria were inhibited with sodium azide, the TPH concentration decreased to 1.97 ± 0.12 mg/L (Tables 1 and 2). In comparison, the TPH concentration for chambers with active soil bacteria (but no plant) decreased to 0.40 ± 0.036 mg/L (Tables 1 and 2), indicating that biodegradation was 89% complete. For chambers with soil and willow trees, the final TPH concentration was only 0.26 ± 0.08 mg/L. This indicates that the presence of the willow trees resulted in a measurable enhancement of biodegradation (Figure 3). Although the final TPH

TABLE 1. TPH concentrations of initial and final groundwater samples from the experimental chambers.

Chamber Type	TPH Conc. (mg/L)	PQL (mg/L)	HYDROCARBON RANGE
Initial Concentration	2.90	0.050	C10-C32
	4.00	0.050	C10-C32
	3.90	0.050	C10-C32
Inhibited with Sodium Azide	1.90	0.050	C12-C30
	1.90	0.050	C12-C30
	2.10	0.050	C12-C30
Soil Only	0.39	0.050	C14-C30
	0.44	0.050	C14-C30
	0.37	0.050	C14-C30
Willows & soil	0.25	0.050	C14-C30
	0.34	0.050	C14-C30
	0.18	0.050	C14-C30

TABLE 2. Summary of initial and final TPH concentrations.

Chamber Type	Avg. TPH Conc. (mg/L)	Std Dev. (mg/L)
Initial Concentration	3.60	0.608
Inhibited with Sodium Azide	1.97	0.115
Soil Only	0.40	0.036
Willows & soil	0.26	0.080

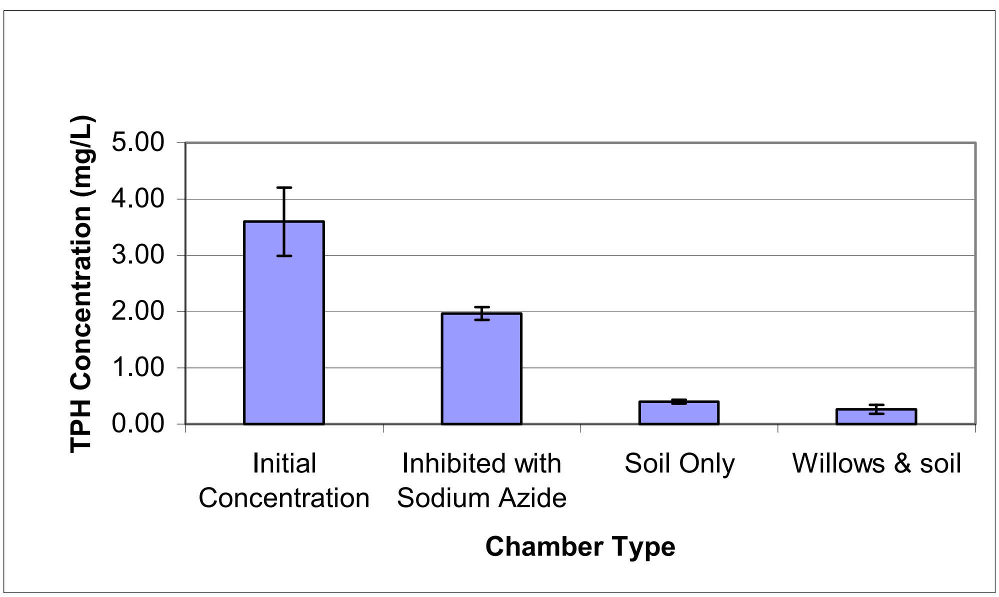

FIGURE 3. Phytostimulation of hydrocarbon biodegradation by willow trees.

concentration was only slightly lower for chambers with willow trees compared to chambers with soil only (Figure 3), the difference was statistically significant. A student's *t*-test indicates that there is a 95% probability that the final TPH concentrations with and without willow trees are statistically distinguishable.

COD concentrations during TPH biodegradation in each chamber are shown in Table 3 with the corresponding day the sample was taken. The COD concentrations for the autoclaved soil with azide have a COD concentration two times higher than that of the other chambers, most likely because sodium azide exerts its own additional COD. COD concentrations decreased with time for the water in each chamber (Figure 4). Results only show a small difference between tree chambers and soil only chambers.

TABLE 3. Measured COD (mg O_2/L) values.

Day / Chamber type	0	2	3	5	8	12	19	25	39	59	80	105
Willows & soil	89	77	73	69	56	63	43	44	40	41	33	35
Willows & soil	87	85	76	70	68	55	46	44	35	40	30	35
Willows & soil	88	73	74	74	73	62	52	53	35	38	35	49
Soil Only	93	81	78	73	68	56	44	46	40	50	33	41
Soil Only	94	80	78	74	63	57	41	58	43	54	32	ns
Soil Only	95	82	77	70	62	53	46	45	43	43	45	42
Inhibited with Sodium Azide	209	206	212	203	203	193	181	ns	ns	ns	190	171
Inhibited with Sodium Azide	206	197	197	187	193	187	171	ns	ns	ns	184	161
Inhibited with Sodium Azide	209	197	197	193	187	187	168	ns	ns	ns	178	161

Note: Ns = note sampled

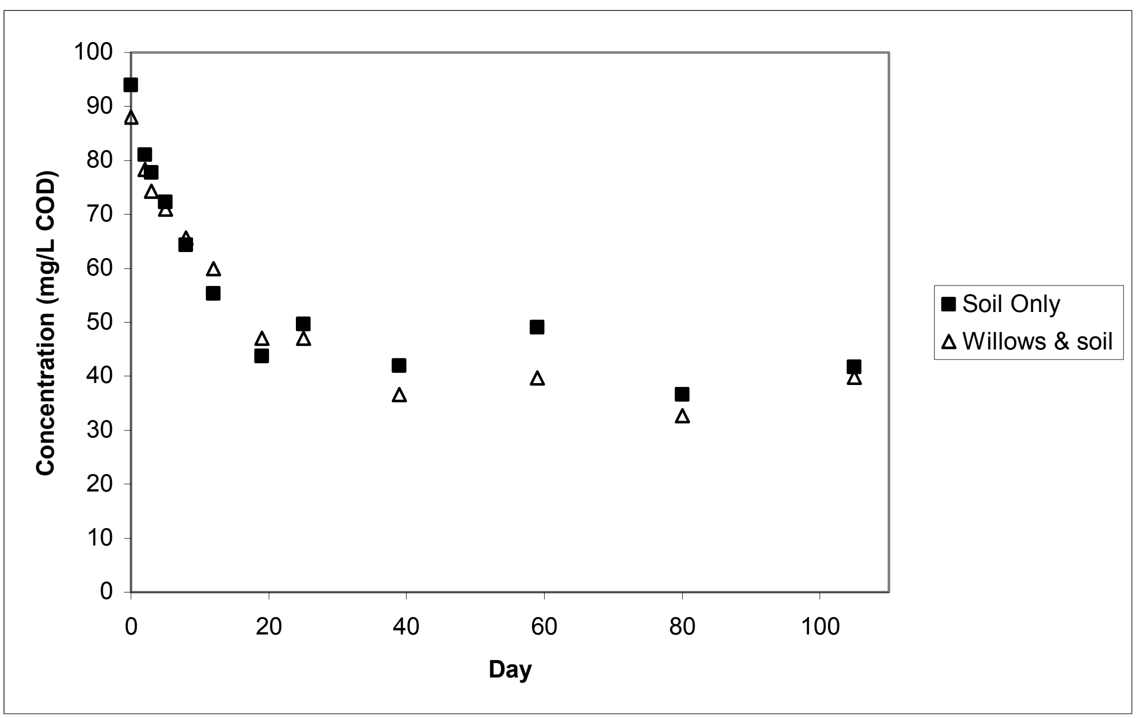

FIGURE 4. COD concentration averaged from triplicates by type.

Actual TPH degradation rates were only measured at the beginning and end of the 105-day experiment due to the sample size required for analysis. Therefore COD was measured more frequently as an indicator of TPH concentration so that kinetics of the degradation could be observed. Based on COD data, the degradation rate appeared to follow first order kinetics during the first 20 days as indicated by R^2 values greater than 0.98 for the semi-log plot in Figure 5. After this initial first order degradation, the degradation rates leveled off significantly between 20 and 105 days (Figure 5). The first order rate constants for the soil only and willows and soil chambers for the linear sections of the data (Day 0 to 20) are 0.0386 day^{-1} and 0.0305 day^{-1}, respectively. This shows a slightly larger initial degradation rate for the first 19 days of the experiment for the soil only chamber. However, this is not a conclusive rate constant because it is derived from the COD indicator data and not true TPH data. During the slow degradation period (after 20 days), the chambers with willows appear to exhibit slightly lower COD concentrations (Figure 5), in keeping with TPH observations (Figure 3). Thus the willows do not appear to increase initial degradation rate but instead result in lower final TPH concentrations, indicating some benefit to microbial TPH degradation during the slow degradation period at the end of the degradation process.

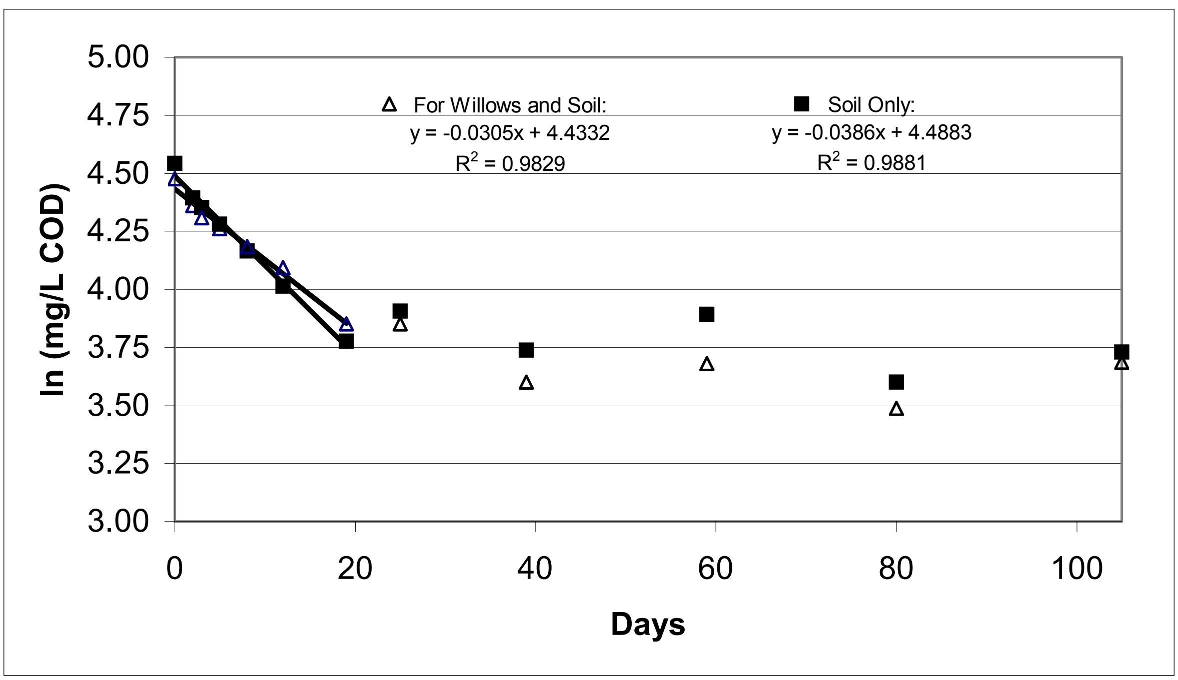

FIGURE 5. Natural log of COD Concentrations with and without willow trees.

CONCLUSIONS

These results indicate that willow trees enhanced TPH biodegradation under the conditions of these laboratory experiments. Because of the large molecular weight of the hydrocarbon contaminants, it is unlikely that the hydrocarbons were taken up directly into plant tissue. Indeed, companion volatilization experiments indicated that no hydrocarbons are transpired by the willow trees grown under these conditions. (Elliot, 2002) Thus it is our hypothesis that the willows enhance microbial activity in the rhizosphere, either through the release of root exudates or through improvement of physical soil properties. It is important to note that dissolved oxygen concentrations were likely to be high during these experiments because of the aeration created by recirculation of the groundwater. Additional benefits may be created by vegetation in the field via improved soil aeration, and this effect is not likely to have been observed in the laboratory experiments. Similarly, vegetation may influence soil moisture content in the field which could further enhance microbial hydrocarbon biodegradation.

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