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Evaluation of Phosphorus Sources Promoting Bioremediation of Diesel Fuel in Soil

S. A. Mills, W. T. Frankenberger, Jr.

Department of Soil and Environmental Sciences, University of California, Riverside, California 92521, USA

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Microorganisms require phosphorus (P) in the biosynthesis of nucleic acids (DNA, RNA), ATP, and other cellular components (e.g., teichoic acids and phospholipids). Generally, P is added in petroleum hydrocarbon-contaminated soil to bring the C:P close to the bacterial biomass. The proposed C:P ratio for mixed microbial biomass in soil is 100:1 (Thompson et al., 1954). However, complete assimilation of petroleum hydrocarbons into biomass is not achievable under natural conditions. Some components of petroleum hydrocarbons are recalcitrant to degradation and may be metabolized over long periods of time. Under most conditions, about half of the C available for growth is eventually converted into cellular biomass. Petroleum hydrocarbons readily degraded and mineralized into CO_2.

Several studies have shown that the addition of P can stimulate the biodegradation of petroleum hydrocarbons (Dibble and Bartha, 1972; Pritchard and Costa, 1991; Frankenberger, 1992). However, the addition of inorganic P sources can result in precipitation of calcium and iron phosphates making the P unavailable to the microbes (U.S. Environmental Protection Agency, 1985). In this study, we wanted to compare the addition of organic vs. inorganic P fertilizers on the mineralization of diesel fuel. Organic P fertilizers are much more mobile in soil than inorganic P sources since they do not precipitate with alkaline earth cations. However, they must be mineralized to be available as a P source in the oxidation of hydrocarbons. In this experiment, the effectiveness of diethylphosphate, methylphosphonic acid, and potassium phosphate (K_2HPO_4) were compared as P fertilizers to stimulate the biodegradation of diesel fuel in soil.

Correspondence to: W. T. Frankenberger, Jr.

MATERIALS AND METHODS

Borosilicate glass vials (41 mL) were filled with 10 g of soil contaminated with 11,733 mg kg^{-1} of total petroleum hydrocarbons (TPH) as determined by EPA 418.1 (EPA, 1983). This soil was characterized in terms of: pH, 8.30; organic C, 0.84%; total N, 0.078%; CEC, 16.8 meq/100 g; clay, 29%; silt, 28%; and sand, 43%. Duplicate vials of both contaminated and non-contaminated soil were treated with P additives.

The optimum concentration of K_2HPO_4 in promoting the biodegradation of diesel fuel was evaluated. Concentrations of P varied from 0, 100, 250, 500, 750 and 1,000 mg kg^{-1} on an elemental basis. The soils were maintained under field moist (-33 kPa) conditions. The vials were fitted with a gas-tight Mininert valve (Alltech, Deerfield, IL) and incubated at room temperature (23°C ± 2°C) for 46 days. The headspace was sampled for CO_2-C three days after initiation of the experiment, and subsequently at 7-day intervals thereafter.

Carbon dioxide was analyzed by gas chromatography (GC) by withdrawing a 1-mL headspace sample with a gas-tight series pressure Lok® gas syringe (Alltech, Deerfield, IL). The GC analysis was performed on a Varian Model 3700 connected to a Hewlett-Packard 3390A integrator. The samples were separated on an Alltech Porapak-Q column and the peaks were detected by thermal conductivity (TC). The carrier gas was helium at a flow rate of 30 mL min^{-1}. The amount of CO_2-C released was determined by use of standards. The detection limit for CO_2-C was 0.5 mg kg^{-1} using GC-TCD. Immediately after sampling the vials were opened to ambient air. The headspace was flushed with air for 5 min to ensure that aeration would not be a limiting factor with long-term incubation. After aeration, water was added to the soil to maintain the proper moisture level at -33 kPa. The amount of CO_2-C released upon each treatment was expressed as cumulative mg CO_2-C kg^{-1} soil over the specified incubation period.

An additional experiment was conducted to determine the most effective P source to biodegrade the diesel fuel. Four treatments consisted of deionized water alone, 500 mg kg^{-1} as K_2HPO_4-P, 500 mg kg^{-1} of diethylphosphate-P, and 500 mg kg^{-1} as methylphosphonic acid-P. Mineralization of the diesel fuel in the presence of these P additives was monitored up to 49 days of incubation.

RESULTS AND DISCUSSION

Biodegradation of diesel fuel was significantly enhanced with the addition of K_2HPO_4 from concentrations of 100 to 500 mg kg^{-1} (Fig. 1). The most optimum treatment was at 500 mg P kg^{-1} as K_2HPO_4 promoting

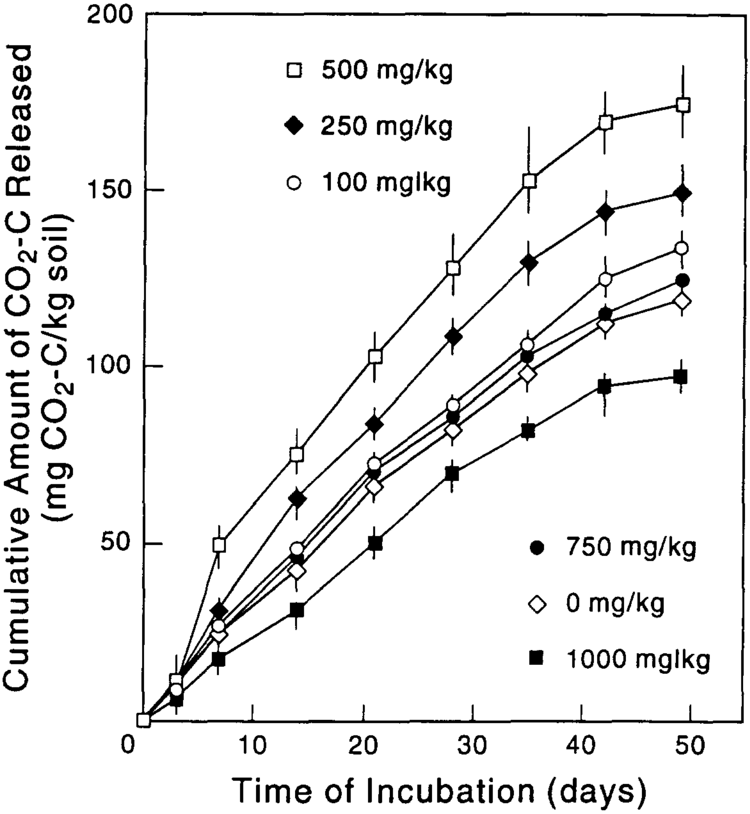

Figure 1. Mineralization of diesel fuel upon the addition of potassium phosphate added to soil.

biodegradation of diesel fuel by 1.46-fold over the non-amended treatment. Other effective concentrations of K_2HPO_4 which promoted biodegradation of diesel fuel included 250 mg kg^{-1} (1.25-fold) and 100 mg kg^{-1} (1.13-fold). The highest concentration of K_2HPO_4 (1,000 mg P kg^{-1}) applied inhibited the biodegradation of diesel fuel by 18%, most likely as a result of osmotic pressure due to the high concentration of soluble salts. With the optimum concentration of K_2HPO_4 being 500 mg P kg^{-1} and the TPH content at 11,700 mg TPH kg^{-1}, this calculates to a C:P ratio of 20:1 (assuming 85% C of the TPH content). This ratio is considerably lower than that reported to be required for microbial biomass (100:1) (Thompson et al., 1954). This may be due to the low bioavailability of P when added to soils as a result of sorption and precipitation. Often it is necessary to add 3 to 5 times more P fertilizers to polluted and agricultural soils because much of it will be precipitated in soil with Ca, Mg, Al, and Fe (A. H. Goldstein, personal communication).

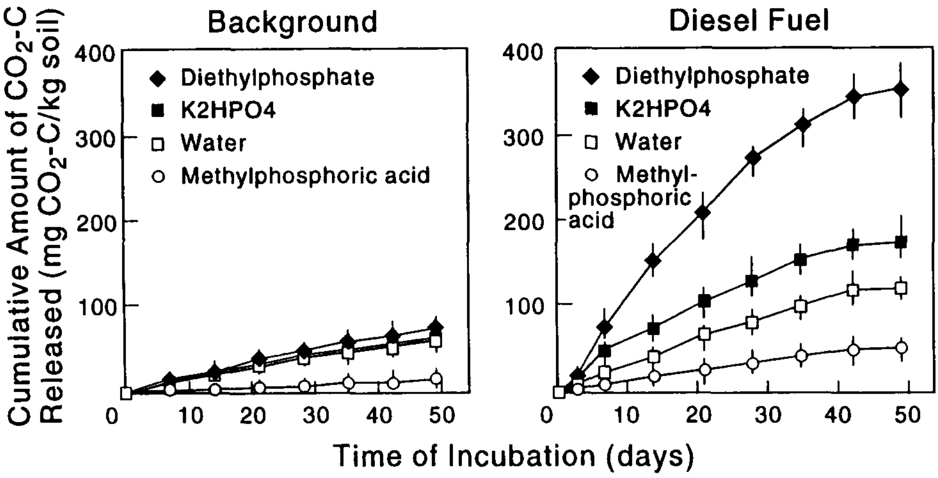

Figure 2. Influence of potassium phosphate, diethylphosphate and methylphosphoric acid on the mineralization of diesel fuel in soil.

An evaluation of various P sources and their effects on biodegradation of diesel fuel is illustrated in Fig. 2. Diethylphosphate showed the most stimulatory effect with a 2.92-fold enhancement in degradation of diesel fuel. The addition of K_2HPO_4 enhanced diesel fuel degradation by 1.48-fold when compared with the treatment of distilled water alone. There was little effect of K_2HPO_4 and diethylphosphate on the mineralization of the native organic matter in the background sample. Methylphosphonic acid inhibited mineralization of the native soil organic matter and diesel fuel. Carbon dioxide evolution was inhibited by 75% in the background non-contaminated soil and 56% in the contaminated sample with this treatment.

This study indicates that various P sources can have different effects on petroleum hydrocarbon degradation. These effects may be dependent on the solubility of the P sources and their toxicity. For example, K_2HPO_4 and dimethylphospohate are readily soluble in water and nontoxic. Methylphosphonic acid, on the other hand, is extremely toxic (lipophilic in nature, causing destruction to viscid membranes) and showed no beneficial effects as a P source in the degradation of diesel fuel.

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