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Increased microbial catabolic activity in diesel contaminated soil following addition of earthworms (*Dendrobaena veneta*) and compost

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ABSTRACT

This study sought to assess the influence of compost and earthworms (*Dendrobaena veneta*) upon the level of hydrocarbon catabolism in petroleum contaminated forecourt soil (extractable petroleum hydrocarbons (EPH) 10 + 1.8 g kg^{-1} and total 16 United States Environment Protection Agency (USEPA) polycyclic aromatic hydrocarbons (PAH) 1.62 ± 0.5 g kg^{-1}). The catabolic activity of the indigenous microorganisms within uncombined materials (soil and compost) and within the combined treatments (soil plus compost; either with or without earthworms) was assessed by ^{14}C-radiorespirometry (^{14}C-hexadecane, ^{14}C-toluene and ^{14}C-phenanthrene). Maximum levels of catabolic activity were observed (at the end of the incubation period; 84 d) for all three compounds in the combined contaminated soil, compost and earthworm mixtures. Significant ($p < 0.05$) enhancement factors (relative to the soil only control) in catabolic activity in the combined treatments (soil:compost (1:0.5)) of 3.6 times, 1.5 times and 3.5 times were observed for ^{14}C-hexadecane, ^{14}C-phenanthrene and ^{14}C-toluene, respectively; with maximum levels of catabolic activity for these substrates being 68.6 ± 1.7%, 37.9 ± 5.3% and 85.9 ± 1.3%.

1. Introduction

Catabolically competent microorganisms are often present within soils contaminated with organic compounds. However, biodegradation may be limited on account of: low compound availability/accessibility, oxygen limitations, pH, nutrient limitations, suboptimal C:N:P ratio, temperature, inappropriate moisture conditions, toxic levels of contaminants (or co-contaminants), and/or inadequate concentrations of terminal electron acceptors (Atlas, 1981; Alexander, 2000; Boopathy, 2000; Reid et al., 2000; Romantschuck et al., 2000; Semple et al., 2003). Thus, bioremediation methodologies often require the optimisation or enhancement of such environmental and biological conditions.

It is widely recognised that earthworms can significantly and positively affect the soil environment in terms of soil organic matter dynamics and turnover, improved soil structure, improved soil fertility (Edwards and Bohlen, 1996; Lavelle et al., 2004; Kersante et al., 2006), breakdown of soil particles, substrate aeration and moisture retention and drainage (Edwards and Bohlen, 1996). Many of these actions and factors, such as the excretion of protein rich mucous, reworking and fragmentation of carbon and deposition of cast excreta are stimulators for soil microorganisms (Edwards and Bohlen, 1996; Brown and Doube, 2004). Thus, within the arena of bioremediation, the use of earthworms to improve soil conditions and to subsequently promote microbial numbers, diversity and activity should realise benefits for levels of catabolic activity and subsequent enhancement of organic contaminant biodegradation. Indeed, recent work has shown that earthworms, through their biological, chemical and physical actions upon soils, can assist in increased losses of PAHs (Contreras-Ramos et al., 2006), crude oils (Schaefer and Filser, 2007) and PCBs (Tharaken et al., 2006). A recent article (Hickman and Reid, 2008) provides a review of these manuscripts and the wider literature with respect to how earthworms might assist bioremediation.

Beyond the isolated application of earthworms for the bioremediation of hydrocarbon contaminated soil, the co-application of both compost and earthworms is putatively advantageous. Whilst the compost provides additional microbial numbers and diversity, nutrients, pH buffering, and improved moisture retention (Semple et al., 2001), earthworm digestive actions result in greater soil particle surface areas, which could theoretically improve accessibility of bound or sequestered contaminants to degrader microorganisms (Verma and Pillai, 1991; Gevao et al., 2001). Significantly, the release of previously bound contaminants (Gevao et al., 2001), providing they are subsequently degraded, would facilitate attainment of lower bioremediation end points.

This work relates the influence of treatment type (namely, contaminated soil only, compost only, and soil:compost mixtures (in ratios 1:0.5 and 1:2 wt/wt), in the presence and absence of earthworms) to observed levels of catabolic activity assessed by

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^{14}C-hydrocarbon respirometry. Ultimately, this work sought to establish if earthworms and compost, as individual additions and in combination with each other, enhanced hydrocarbon catabolic activity. Catabolic potential was assessed by ^{14}C-radiorespirometry using three representative hydrocarbons, namely, ^{14}C-hexadecane, ^{14}C-toluene and ^{14}C-phenanthrene. These hydrocarbons are commonly found in petroleum impacted soils and have previously been used in other studies (Foght et al., 1990; Robinson et al., 1990; Geerdink et al., 1996; Prenafeta-Boldu et al., 2002; Reid et al., 2002; Ostberg et al., 2007).

2. Materials and methods

Treatments included soil and compost mixtures at ratios of 1:0.5 and 1:2 (wt/wt), in addition to contaminated soil only and compost only control treatments. For 84 d, half of the treatments were incubated with earthworms (W) (ten earthworms per kg material), and half without earthworms (NW) ($n = 5$ of each treatment). Samples were taken at 0 d and 84 d for ^{14}C-respirometer assays.

2.1. Experimental set-up

Mature compost (C:N ratio of 19:1; moisture content of 70% of maximum water holding capacity (WHC); pH of 7.2) was produced from shredded leaves, grass and other green material (50%) and stalks and woodier materials (50%) that underwent rapid composting utilising an enclosed force aerated system. The composted material continued to 'mature' outdoors for approximately 4 months prior to use. Aged petroleum contaminated soil (EPH 10 ± 1.8 g kg^{-1}; total 16 USEPA PAH 1.62 + 0.5 g kg^{-1} (hereafter referred to as ΣPAH); sand 0.8%, silt 42.7% and clay 56.4%; organic matter content of 0.8% (mass loss on ignition); C:N ratio of 8:1; pH of 6.2) was used in this experimental study.

Plastic vessels (10 L capacity) measuring 35 cm in height and 40 cm in diameter were used to contain the treatments. The vessels had plastic piping (7.5 mm diameter) inserted and sealed into three holes in the base, which were attached to an air compressor, and aerated for 15 min every 24 h. No further manual mixing was undertaken throughout the study.

Silvaperl washed gravel (obtained from a building supplies store) was washed in water and laid to a thickness of 3 cm in the base of each vessel (to promote more uniform air dissipation). The gravel was then covered with a perforated plastic sheet that allowed air to percolate up from the base and disallowed the migration of earthworms into the gravel.

Soil and/or compost was rehydrated and manually mixed into the experimental vessel. The mass of the contaminated soil (at 70% of maximum WHC) was kept constant at 2 kg (wet weight), equivalent to 1.82 kg dry weight. The compost substrate (at 70% of maximum WHC) was added as wet weight at the ratios of: 0.5 (1 kg) and 2 (4 kg). This equated to 0.78 kg and 3.16 kg dry weight, respectively. The contaminated soil and the compost only treatments contained only 2 kg (wet weight) of either contaminated soil or compost, respectively.

Adult earthworms (*Dendrobaena veneta*) were depurated (laid on damp filter paper in an enclosed plastic petri dish) for 24 h prior to use. Ten earthworms per kg of material (each depurated earthworm weighing 1.0 ± 0.2 g) were added to each of the required treatments and replicates. The earthworms were combined with the material (resulting in 1:100 biomass:material w/w ratio) and perforated lids were sealed around the vessels. Water was added periodically to maintain levels at 70% of maximum WHC. It was noted from prior research (data not shown) that *D. veneta*, when compared to other widely used earthworm species (in this instance *Lumbricus rubellus* and *Eisenia fetida*), outperformed them in terms of survival and tolerance of hydrocarbon contaminated soils (up to 3 g kg^{-1} total PAH). It was thus felt justifiable to utilise *D. veneta* within this study to ensure survival for the incubation period.

2.2. Hydrocarbon and ∑PAH analysis

Extractable Petroleum Hydrocarbons (EPH – C_{10}–C_{40}) were quantified by GC-FID and ∑PAH by GC–MS. Initially, samples (5 g; $n = 3$ of all treatments) were extracted using an end over end shake extraction method with 50:50 (Hexane:Acetone) at a soil:solvent ratio of 1:10 for 1 h. GC–FID analysis was undertaken using a Hewlett Packard 5890/6890 FID with a Zebron ZB-1 (Phenomenex, UK) fused silica capillary column (15 m × 0.32 mm × 1 μm) using helium at a constant pressure of 15 psi at an initial flow of 1.5 ml min^{-1} with nitrogen at 30 ml min^{-1}, hydrogen at 30 ml min^{-1} and air at 300 ml min^{-1} and a split injection (2:1 ratio) of 2.0 μl at a flow of 7.1 ml min^{-1}. The column oven was set at 60 °C for 1.0 min, 60–325 °C programmed at 15 °C min^{-1} and 325 °C for 2.0 min.

GC–MS was undertaken using a Hewlett Packard 6890 Gas Chromatograph with HP7683 series injector and HP7683 series autosampler and a Hewlett Packard 5973 MSD Mass Selective Detector. A fused silica capillary column was used with measurements of 15 m × 0.25 mm × 0.1 μm using helium at a constant flow of 1 ml min^{-1} and a pulsed splitless injection of 1.0 μl at 20 ml min^{-1}. The column oven was set at 49 °C for 1.25 min, 49–250 °C at 25 °C min^{-1}, 250–300 °C at 35 °C min^{-1} and 300–340 °C at 60 °C min^{-1}.

2.3. Assessment of catabolic activity by ^{14}C-respirometry

Schott bottles (250 ml) were adapted such that a glass vial (7 ml) could be suspended from the lid. This vial contained GF/A filter paper (20 mm × 20 mm) and 1 M sodium hydroxide (1 ml; supplied by Merck, UK) to 'trap' mineralised $^{14}CO_2$ (Allan et al., 2007). Samples (10 g) to be screened for catabolic activity (contaminated soil, compost, 1:0.5 and 1:2 (soil:compost (wt/wt)) were slurried in respirometers with sterile distilled water (30 ml)). ^{14}C-9-Phenanthrene, ^{14}C-1-hexadecane and ^{14}C-UL-toluene (added as individual compounds) (all supplied by Sigma, UK; radioactive and chemical purity >95%) were spiked into the slurried respirometers, using toluene as the carrier solvent, such that 100 μl of spike delivered 200 Bq per respirometer. Respirometers were continuously shaken at 100 rpm using an IKA Labortechnik KS 501 digital flatbed shaker and sampled periodically until 648 h (27 d) assay time had elapsed. Spiking efficiency was determined to be >94 ± 1.0%. Ultima Gold (6 ml) was added to changed vials, which were stored in darkness for a minimum of 48 h prior to analysis. Trapped $^{14}CO_2$ was determined by liquid scintillation counting, using a Canberra Packard Tri-Carb 2900TR liquid scintillation counter for 10 min per sample.

3. Results

3.1. Earthworm survival

Following the initial incubation period, prior to the catabolism study, it was noted that earthworm survival was highest in the compost treatments (70 ± 12%) and lowest in the contaminated soil control (15 ± 13.2%). Following addition of compost to the soil, the treatments showed significantly higher survival with increasing compost amounts ($p < 0.05$). Survival in the 1:0.5 and 1:2 treatments were 23 ± 8.5% and 64 ± 15%, respectively. Thus, as the contaminated soil content increased, earthworm mortality increased, which was attributed to soil toxicity and the possibility of inappropriate food quality and availability, as previously noted as detrimental factors (Edwards and Bohlen, 1996; Schaefer and Filser, 2007).