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Effects of hydrocarbon contamination on soil microbial community and enzyme activity

Sulaiman A. Alrumman [a,*], Dominic B. Standing [b], Graeme I. Paton [b]

[a] King Khalid University, Faculty of Science, Biological Department, P.O. Box 3100, Abha 61471, Saudi Arabia
[b] Department of Plant and Soil Science, Cruickshank Building, University of Aberdeen, Aberdeen AB24 3UU, UK

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Abstract Soil enzymatic activities and microbial biomass carbon (C_{mic}) are considered to be two important soil biological activities influenced by oil contamination occurring in the soil ecosystem. This study focused on changes in the soil microbial community enzymatic activities as a result of the potential inhibitory effects of hydrocarbon contamination. The relationship between hydrocarbons (kerosene and diesel), C_{mic} and enzymatic activity (dehydrogenase and phosphatase) was evaluated in three amended soil types collected from different areas (Fresh Boyndie, Insch and Brechin) in Aberdeenshire (UK). Results showed that hydrocarbon contamination inhibited enzymatic activities in all the amended soil samples. The extent of inhibition increased significantly with increasing levels of hydrocarbons, and varied with the incubation period. Insch soil had high C_{mic} values and high numbers of heterotrophic bacteria CFU, but it had the lowest dehydrogenase and phosphatase activities of all three soils. Brechin soils had the highest phosphatase activity. Results also showed that both Insch and Brechin soils had similar numbers of culturable hydrocarbon degrader bacteria across all soil treatments with the exception of kerosene treatments, while Brechin soils had the highest culturable numbers of hydrocarbon degrading fungi across all three soil treatments with the exception of incubated control and kerosene treatments. There were generally strong positive relationships in non-treated samples between bacterial heterotrophs and hydrocarbon degrading bacteria in all three soils. Both incubated Insch and Brechin soil treatments exhibited a strong correlation between fungal heterotrophs and hydrocarbon degraders. However, non-incubated Insch and Brechin soils had a weak relationship between fungal heterotrophs and degrading fungi. Hydrocarbons in soils provide a source of carbon for microbial growth and this helps to explain the high variation in fungal data between soils which may be associated with different microbial communities in each soil.

* Corresponding author.
E-mail address: salrumman@kku.edu.sa (S.A. Alrumman).
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1. Introduction

Petroleum oils are used in large quantities as fuels (Bierkens and Geerts, 2014). Petroleum hydrocarbons are becoming a global problem for the environment. They are highly persistent in the environment, toxic and present significant health risks to

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human (Hentati et al., 2013). The use of indigenous microorganisms in bioremediation processes can reduce the risks associated with hydrocarbon contaminated soils (Suja et al., 2014).

Soil biological activity, including soil microbial biomass and enzymatic activity, is influenced by a range of physico-chemical, environmental parameters and perturbations (Labud et al., 2007). Therefore, soil microbial activity is commonly used to assess disturbed soil.

Consideration of soil C_{mic} was largely neglected until the mid 1970s. Several methods have been developed to quantify soil C_{mic} and these methods can be broadly divided into direct or indirect methods (Winding et al., 2005; Kaschuk et al., 2010). Examples of direct methods are microscopy or use of culture media and enumeration of cultures. Indirect methods comprise fumigation extraction (Jenkinson and Powlson, 1976) and substrate induced respiration (Anderson and Domsch, 1978). Indirect methods are more rapid, cost effective and easier to apply than direct methods.

Chloroform (ethanol free) fumigation is the most commonly used indirect method (Winding et al., 2005). Chloroform vapour lyses cells of living soil microorganisms (Jenkinson and Powlson, 1976; Vance et al., 1987; Winding et al., 2005), without affecting the non-living fraction of organic matter. Non-fumigated and fumigated soils are subsequently compared to estimate the size of the freshly lysed biomass. The carbon released by the chloroform is immediately measured either as respired CO_2 over a specified period of incubation or by direct extraction of soil with saline solution (i.e. 0.5 M K_2SO_4). These techniques are known as the chloroform fumigation incubation method (CFI) and the chloroform fumigation direct extraction method (CFE), respectively (Jenkinson and Powlson, 1976; Vance et al., 1987; Winding et al., 2005).

A consideration of the k_{ec}-factor (the extractable component of C_{mic} after fumigation) is required in the fumigation extraction method to convert the flush of microbial biomass carbon mineralised to CO_2 over an incubation period. This factor can be estimated, for example, by the addition of a known amount of microbial C to the soil and measuring the amount mineralised after incubation. The use of K_{ec}-factor is controversial, however, common values of k_{ec} are 0.45 (Vance et al., 1987), 0.41 (Anderson and Domsch, 1978) and 0.33 depending on intrinsic soil properties such as pH and organic matter content.

If soil quality is to be defined according to the presence and activity of soil microbial populations, then an appropriate technique must be selected. Plate count methods have been used to monitor bioremediation processes (Margesin et al., 2000; Alamri, 2006). It has been reported that the plate count method is a reliable and sensitive technique compared to other methods (e.g., Most Probable Number) used for assessing the potential of biodegradation (Cassidy et al., 2000; Alamri, 2006). Number and species of total microbial population present in soil can be quantified by colony enumeration on selective media. More specific information can be collected by using mineral salt medium (MSM) with a suitable hydrocarbon as a carbon source (Balba et al., 1998).

Soil enzyme activities have been considered as parameters to provide a biological assessment of soil function. Several of the soil enzyme activities have been proposed for evaluating and monitoring remediation of hydrocarbon contaminated soil. In this study, enzyme activity of dehydrogenase and phosphatase will be considered as indicators for assessing recovery of hydrocarbon impacted soils.

Dehydrogenase activity (DHA) has been proposed as a sensitive indicator for evaluating microbial oxidative activity in soils (Turgay et al., 2010; Serrano et al., 2009; Dawson et al., 2007). Quantification of DHA in soil is made by measuring the amount of an artificial electron acceptor reduced by the microbial activity. Compounds such as soluble tetrazolium salts are reduced to red coloured formazans, which can be extracted and measured colorimetrically (Camiña et al., 1998; Shaw and Burns, 2006).

Phosphatase activity (PA) is important to soil P cycling. PA is sensitive to environmental perturbations, thus may be a suitable selection for inclusion in a soil quality index (Amador et al., 1997; Turgay et al., 2010). PA is frequently measured by quantifying the transformation of *p*-nitrophenyl phosphate. The yellow product of PA (*p*-nitrophenol) can be quantified colorimetrically (Tabatabai and Bremner, 1969; Shaw and Burns, 2006). Soil quality can be determined according to the presence and activity of soil microbial populations whereas, the enzyme activities have been considered as parameters for evaluating and monitoring remediation of hydrocarbon contaminated soil.

The conceptual model of this study hypothesised different trajectories of response variables to a given time point. This point in time represents soil recovery. Soil recovery itself is a composite of variables and processes, which individually may follow different trajectories: (1) Functions in control soils remain steady. (2) The addition of hydrocarbons causes an increase in a given response variable against control soil, or the addition of hydrocarbons may cause a decrease in a given response variable initially. However, the given response variable coincides with control at the end of incubation. (3) The addition of hydrocarbons causes a decrease in a given response variable against control soil. The main objective therefore of this study is to evaluate the effect of hydrocarbon (diesel and kerosene) contamination on soil microbial activity, in freshly contaminated and incubated soils. Assays that constitute this comprise soil C_{mic}, culturable counts and soil enzyme activities.

2. Materials and methods

2.1. Determination of soil characteristics

Fresh Boyndie, Insch and Brechin soils were collected in Aberdeenshire (UK) and sieved (2 mm) to remove stones and large root fragments.

The physical–chemical properties of the soils studied were determined by standard methods generally used in chemical-soil laboratories. The following parameters were also determined for each soil: pH, Total N%, Total C% and EC (μS) (Table 1), and hydrocarbon concentrations (Table 2).

2.2. Experimental design

Each individual soil sample was then subdivided into three main treatment groups: an unamended control soil (C), kerosene (1% v/w) amended treatment (K), and diesel (1% v/w) amended treatment (D). Kerosene and diesel were added to the soils by nebulisation and the soils thoroughly mixed to