

# Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil

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## Abstract

Mesocosm studies using sub-Antarctic soil artificially contaminated with diesel or crude oil were conducted in Kerguelen Archipelago (49°21' S, 70°13' E) in an attempt to evaluate the potential of a bioremediation approach in high latitude environments. All mesocosms were sampled on a regular basis over six months period. Soils responded positively to temperature increase from 4°C to 20°C, and to the addition of a commercial oleophilic fertilizer containing N and P. Both factors increased the hydrocarbon-degrading microbial abundance and total petroleum hydrocarbons (TPH) degradation. In general, alkanes were faster degraded than polyaromatic hydrocarbons (PAHs). After 180 days, total alkane losses of both oils reached 77–95% whereas total PAHs never exceeded 80% with optimal conditions at 10°C and fertilizer added. Detailed analysis of naphthalenes, dibenzothiophenes, phenanthrenes, and pyrenes showed a clear decrease of their degradation rate as a function of the size of the PAH molecules. During the experiment there was only a slight decrease in the toxicity, whereas the concentration of TPH decreased significantly during the same time. The most significant reduction in toxicity occurred at 4°C. Therefore, bioremediation of hydrocarbon-contaminated sub-Antarctic soil appears to be feasible, and various engineering strategies, such as heating or amending the soil can accelerate hydrocarbon degradation. However, the residual toxicity of contaminated soil remained drastically high before the desired cleanup is complete and it can represent a limiting factor in the bioremediation of sub-Antarctic soil. © 2004 Elsevier Ltd. All rights reserved.

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## 1. Introduction

Under cold climates, an efficient bioremediation of sites contaminated with petroleum hydrocarbons has been reported although biodegradation is hampered by

low ambient temperature for much of the year (reviewed by Margesin and Schinner, 1999). Bioremediation experiments have been conducted on Antarctic soils (Kerry, 1993; Aislabie et al., 1998; Delille, 2000; Delille et al., 2003; Ferguson et al., 2003) but, to our knowledge, only bioattenuation observations have been carried out in sub-Antarctic soils (Delille and Pelletier, 2002).

Among the environmental factors known to limit biodegradation of soil containing petroleum hydrocarbons,

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temperature fluctuation and nutrient availability are among the most important ones, particularly in high latitude regions (Walworth and Reynolds, 1995; Mohn and Stewart, 2000).

Temperature plays a significant role in controlling the nature and extent of microbial hydrocarbon metabolism (Nedwell, 1999). Temperature affects the rate of biodegradation, as well as the physical nature and chemical composition of hydrocarbons (Whyte et al., 1998; Rowland et al., 2000). Although microbial activity is generally reduced at low temperatures, many of the components in crude oil and diesel can actually be degraded by psychrophilic and psychrotrophic microorganisms (Leahy and Colwell, 1990; Whyte et al., 1998; Margesin and Schinner, 1999; Delille, 2000; Gibb et al., 2001; Baraniecki et al., 2002; Eckford et al., 2002). The bioavailability of soluble hydrophobic substances, such as aliphatic and polyaromatic hydrocarbons, is temperature dependent. A temperature increase leads to an increase in diffusion rates of organic compounds notably by a decrease of their viscosity (Northcott and Jones, 2000). Thus, higher molecular reaction rates due to smaller boundary layers are expected at elevated temperatures. In counterpart, the increased volatilisation and solubility of some hydrocarbons at elevated temperature may enhance their toxicity (Whyte et al., 1998; Niehaus et al., 1999). Such an increase in toxicity may delay the onset of degradation (Leahy and Colwell, 1990; Itävaara et al., 2000).

Additionally, soil hydrocarbon degradation can be increased by the addition of supplemental nutrients, particularly nitrogen and, to a lesser degree, phosphorus (Walworth and Reynolds, 1995; Braddock et al., 1997). The addition of an oleophilic fertilizer, such as the commercially available Inipol EAP 22®, can overcome the nutrient limitations in contaminated soils.

In order to estimate the potential of bioremediation of contaminated sub-Antarctic soils a mesocosm study was initiated in January 2001 in a sub-Antarctic island (Grande Terre, Kerguelen Archipelago, 49°21' S, 70°13' E). The goal of the present paper is to examine the effects of nutrient addition and temperature increase on biological degradation of petroleum hydrocarbons and determine the residual toxicity of oiled soils. This is the first experimental study characterizing hydrocarbon biodegradation in sub-Antarctic soils and incorporating nutrients and temperature effects.

## 2. Materials and methods

### 2.1. Study site

Ocean dominates the area between the latitude 40° South and the coast of Antarctica. However, scattered throughout this vastness are a number of islands that

are affected by their latitude and their position in relation to the polar, sub-Antarctic and subtropical fronts. The boundary of these fronts has changed throughout time and is subject to continuous fluctuations. The most southern islands, the Antarctic coastal islands such as Ross Island, are mainly ice-covered and influenced by the cold, dry air flowing from the Antarctic continent. Moving further north, the maritime islands, such as Elephant Island are influenced by the oceanic climate of the Southern Ocean and can be surrounded by pack ice in winter. South Georgia is one of another group of sub-Antarctic islands, along with Heard and MacDonald islands that lie near to, but south, of the Polar front. Although they are glaciated, they are not usually surrounded by pack ice in winter. The last group of sub-Antarctic islands are those lying between the polar and subtropical fronts, including the Crozet Islands and Kerguelen Archipelago. These islands all have ice-free coastlines and a relatively rich vegetation with tussock grass and treeless peaty uplands. From a strictly oceanographic point of view Kerguelen Archipelago is located in the northern part of the Southern Ocean, in the Antarctic Polar Frontal Zone (between Polar and sub-Antarctic fronts). The climate is cold, oceanic, usually with very strong winds, mostly from the southwest. Surface soils of the Archipelago experience large temperature fluctuations with surface temperatures frequently falling to 0°C overnight and reaching over 20°C during summer sunny afternoons.

### 2.2. Soil

Selected soil supported an abundant vegetal cover (*Acacia magellanica*). It was collected from the surface to a depth of about 0.2 m in an approximately 20 m<sup>2</sup> area located near the “Port aux Français” scientific French station in the Kerguelen Archipelago. This area has no known history of hydrocarbon contamination. Water content of 40% (w/w) was determined gravimetrically after heating a sub-sample at 105°C for 24 h. The soil had a pH of 6.4 (determined after mixing 1 part of soil with 2.5 parts of deionized water). The total organic carbon and total natural nitrogen concentrations determined by CHN analysis were 158 g kg<sup>-1</sup> and 14 g kg<sup>-1</sup>, respectively. The C/N ratio was 13.6 (on molecular basis). Soil was sandy loam structure with grain size analysis of 2% fine ground (<40–63 µm), 23% medium ground (63–250 µm), 36% coarse ground (250–800 µm) and 22% very coarse ground material (800–2000 µm), as well as 17% gravel and plant residues (2000–3150 µm).

### 2.3. Incubation experiment

Mesocosm experiments were conducted in polyethylene containers of dimensions 27 × 24 × 13 cm. After removal of plant residues and soil aeration, 5 kg (w/w) of

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