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The application of bioassays as indicators of petroleum-contaminated soil remediation

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Abstract

Bioremediation has proven successful in numerous applications to petroleum contaminated soils. However, questions remain as to the efficiency of bioremediation in lowering long-term soil toxicity. In the present study, the bioassays Spirotox, Microtox®, Ostracodtoxkit F™, umu-test with S-9 activation, and plant assays were applied, and compared to evaluate bioremediation processes in heavily petroleum contaminated soils. Six higher plant species (*Secale cereale* L., *Lactuca sativa* L., *Zea mays* L., *Lepidium sativum* L., *Triticum vulgare* L., *Brassica oleracea* L.) were used for bioassay tests based on seed germination and root elongation. The ecotoxicological analyses were made in DMSO/H_2O and DCM/DMSO soil extracts. Soils were tested from two biopiles at the Czechowice oil refinery, Poland, that have been subjected to different bioremediation applications. In biopile 1 the active or engineered bioremediation process lasted four years, while biopile 2 was treated passively or non-engineered for eight months. The test species demonstrated varying sensitivity to soils from both biopiles. The effects on test organisms exposed to biopile 2 soils were several times higher compared to those in biopile 1 soils, which correlated with the soil contaminants concentration. Soil hydrocarbon concentrations indeed decreased an average of 81% in biopile 1, whereas in biopile 2 TPH/TPOC concentrations only decreased by 30% after eight months of bioremediation. The bioassays were presented to be sensitive indicators of soil quality and can be used to evaluate the quality of bioremediated soil. The study encourages the need to combine the bioassays with chemical monitoring for evaluation of the bioremediation effectiveness and assessing of the contaminated/remediated soils.

Keywords: Bioassays; Biopiles; Petroleum hydrocarbons; Bioremediation

1. Introduction

Until recently the assessment of environmental hazards of remediated sites was mostly based on chemical analyses. In these investigations the analyses are typically based on-site specific contaminants of concern (COCs). However, not all COCs may be known, and undetected

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metabolites or compounds may be formed during biogeochemical processes. There is increasing interest for incorporation of toxicity tests (with a battery of different assays) for ecological assessment at hazardous waste sites and for supporting management decisions for remediation (Renoux et al., 1995; Salanitro et al., 1997; Saterbak et al., 1999). The use of biological endpoints can help to appropriately define acceptable cleanup standards (Braud-Grasset et al., 1993; Debus and Hund, 1997; Dorn et al., 1998; Dorn and Salanitro, 2000). Chemical data alone are not sufficient to evaluate the biological effects, because it is impossible to analyze all the compounds and synergistic effects contributing to toxicity.

In recent years ecotoxicological tests have been used as supplementary tools to monitor bioremediation of hydrocarbons, both in laboratory and field studies (Thomas and Cline, 1985; Wang and Bartha, 1990; Salanitro et al., 1997; Saterbak et al., 1999). Various bioassays representing different trophic levels have been used for screening soil toxicity (Greene et al., 1988; Hund and Traunspurger, 1994; Keddy et al., 1995; Dorn et al., 1998; Juvonen et al., 2000). Mostly, soil quality tests using bacteria, plants and on invertebrates are promising tools for risk-based corrective action. The effects-based assessment provides more realistic targets for cleanup criteria than non-specific measurements such as total petroleum hydrocarbons (TPH). Recently, the available information of soil quality assessment endpoints has been compiled. There is no agreed method for assessing contaminated and remediated soils.

The aim of the present study was: (1) to apply some bioassays for evaluation of toxicity of petroleum contaminated and bioremediated soils, and (2) to encourage the need to combine bioassays with chemical data for soil quality assessment.

2. Materials and methods

2.1. Field characterization and sampling

More than a century of continuous use of a sulphuric acid-based oil refining method by the Czechowice Oil Refinery in Poland produced an estimated 120 000 tons of acidic, highly weathered, petroleum sludge (IETU, 1999). This waste was deposited into three open waste lagoons of 3 m depth and of a total surface of 3.8 ha. One of the waste lagoons (0.3 ha) was chosen for a demonstration study on an aerobic biopile, based on physico-chemical characterization. The waste from the lagoon was removed, and 5000 tons of heavily petroleum-contaminated soil was treated in the bioremediation process. Numerous COCs were present at this site but the petroleum hydrocarbons were the main concern. The biopile with actively and passively aerated sections, referred to further as biopile 1 or engineered biopile, was constructed in 1997 in the smallest lagoon at the Czechowice Oil Refinery (Fig. 1). The purpose of this study, the results of which are reported in Altman et al. (1997) and IETU (1999) was to evaluate novel technologies and applications for environmental restoration of soils heavily contaminated with petroleum waste. This was accomplished by comparing bioremediation processes under active vs. passive aeration and the removal rates of both easily biodegradable and recalcitrant petroleum hydrocarbons. The project focused on the application of cost-effective amendments for biostimulation, including NPK fertilizers, the surfactant Rokafenol N8, and employing an indigenous microbial consortium for an enhanced hydrocarbon biodegradation.

In 2001, another biopile was constructed to cleanup soil mixed with petroleum hydrocarbon waste (Worsztynowicz et al., 2001). This biopile, referred to as biopile 2 or non-engineered biopile, is situated within the second (middle) lagoon at the refinery (Fig. 1). In this biopile, contaminated soil was first mixed with mineral fertilizers and wood chips, and then covered with a dolomite layer. Subsequently, the dolomite layer was covered with unpolluted soil and grass seeds sown over the biopile area. A simple drainage system was also built to allow natural soil aeration. The total number of sampling locations at each biopile was 23. Soil samples (≈1 kg) were taken from shallow (≈30–40 cm) and deep (≈80–100 cm) layers, and put into sterilized containers. The samples were stored at 4 °C before analysis.

2.2. Characterization of soil samples

Each sample was examined for the following physicochemical parameters: TPH = total petroleum hydrocarbons (non-polar aliphatic hydrocarbons); TPOC = total petroleum organic carbon (polar and non-polar ali-

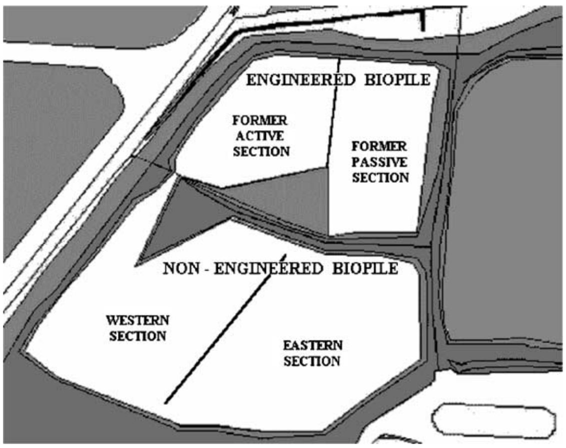

Fig. 1. Localisation and division in sampling sections of biopiles at the oil refinery in Czechowice-Dziedzice.