International Biodeterioration & Biodegradation 126 (2018) 57–68

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Effect of remediation strategies on biological activity of oil-contaminated soil - A field study

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ARTICLE INFO

Keywords:
Hydrocarbons
Pollution
Podzolic soil
Enzymatic activity
Basal respiration

ABSTRACT

The effects of oil contamination and different remediation strategies (natural attenuation, biostimulation, and bioaugmentation) on physico-chemical and biological parameters of podzolic soil were studied. The relationships between petroleum hydrocarbons, total organic carbon, nutrients, basal respiration and enzymatic activity (dehydrogenase, catalase and urease) were evaluated in soil over a 9-year period. The principal component analysis indicated that hydrocarbons were mainly responsible for changing metabolic activity for all treatments. Dehydrogenase activity was the most sensitive biological indicator with greater levels in unpolluted soil than those recorded in contaminated soil under all remediation strategies. The activity of urease was not directly correlated with oil degradation, while the relationships of catalase and respiration rate with petroleum hydrocarbons were dependent on method of remediation. Although both biostimulation and bioaugmentation had a positive influence on the biological activity of soil and its physicochemical properties, the considerable part of decontamination could be attributed to degradation activities of indigenous microorganisms. The addition of oil-degrading bacteria (bioaugmentation) enhanced biodegradation rates only temporarily indicating that biostimulation is a better remediation strategy for podzolic soil in the field.

1. Introduction

Oil pollution has become one of the acute global environmental problems. Huge quantities of crude and refined oil that are produced and transported over long distances are associated with increased contamination by petroleum and its derivate products (Wolińska et al., 2016). Oil spills in particular affect large areas of land, rivers, lakes and seas (Kimes et al., 2014; Riveroll-Larios et al., 2015). Petroleum oil enters the environment through different routes including leakages from wellheads, pipelines, and underground storage tanks, incorrect disposal of petroleum wastes, drilling operations and many other ways.

Oil spills on land affect whole ecosystems, changing vegetation, wildlife, microbial processes, soil characteristics and overall soil health. Ecological impact of oil on the functioning of soils is most clearly seen through the change in the activity of soil microorganisms and enzymes (Li et al., 2007; Silva-Castro et al., 2015), thus making soil microbial activity a sensitive biological and biochemical indicator of soil quality (Margesin et al., 2000; Kaczyńska et al., 2015). At the same time, the ability of microorganisms to metabolize petroleum hydrocarbons has successfully been used for the decontamination of oil-polluted areas as one of the most environmentally friendly and versatile approaches (Bento et al., 2005; Silva-Castro et al., 2013).

Numerous studies have demonstrated high efficiency of bioremediation in the purification of oil polluted soils (Zhao et al., 2011; Dias et al., 2012; Souza et al., 2014). Since remediation technologies affect not just the oil concentration, but the enzymatic response (Margesin et al., 1999; Gong, 2012), enzyme activity is a sensitive indicator used for evaluation and monitoring of bioremediation process (Dawson et al., 2007; Medvedeva et al., 2010). Enzymes that were found useful for monitoring hydrocarbon removal include soil dehydrogenases, catalases and ureases (Maila and Cloete, 2005; Polyak and Bakina, 2015; Wu et al., 2016).

The success of bioremediation depends on the conditions that influence oil biodegradation: soil type and characteristics, microbial activity, humidity, temperature, pH, availability of oxygen, nutrients and oil concentration (Suja et al., 2014; Varjani and Upasani, 2017). However, these data come mostly from laboratory experiments, performed under controlled conditions, while long-term field

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http://dx.doi.org/10.1016/j.ibiod.2017.10.004
Received 7 June 2017; Received in revised form 13 September 2017; Accepted 9 October 2017

bioremediation experiments are rare (Ouyang et al., 2005; Couto et al., 2010; Das and Chandran, 2010).

There are inherent limitations, associated with laboratory studies. The duration of experiment is among the main differences between studies carried out under laboratory and field conditions. Mostly, the former are focused on the short-term effects, seen after a few weeks or even days of contamination, while the study period of several years is uncommon (Li et al., 2007; Colla et al., 2014). The properties of the soil change dramatically during long-term incubation in the laboratory, thus complicating interpretation of any observed effects. Additionally, laboratory experiments cannot reproduce the changes which take place under natural conditions with time. Overall progress in developing effective bioremediation strategies depends on achieving as good results in the field as in the laboratory, which in itself is a complicated problem (Bento et al., 2005).

In this study, a long-term field experiment was performed to evaluate the ecological effects of petroleum contamination on soil biological activity over nine years and to compare two remediation methods. We tested bioremediation by bioaugmentation (introduction of hydrocarbon-degrading microorganisms) and biostimulation (introduction of additional nutrients into the soil, in order to enhance the activity of indigenous degraders), and compared them with natural attenuation. In the present study, we analyzed the interplay of environmental and biological factors involved in decontamination to better understand the response of the soil microbiota to various remediation strategies.

2. Materials and methods

2.1. Study site

The experiment was started in June 2006 and continued until October 2015 at the trial field near St. Petersburg, Russia (59°44′34″N, 30°22′49″E). This area has a humid continental climate with the average annual temperature, precipitation and air humidity of 5.8 °C, 660 mm and 78%, respectively. The soil moisture in the area is mostly high, since evapotranspiration is low due to the cool climate. The soil itself is a loamy podzol (48.1% sand, 30.3% clay, 21.6% silt) with pH_{H2O} of 6.81, 2.9% organic carbon, and 175 and 250 mg kg^{-1} available P and K, respectively.

2.2. Experimental design

Four square plots (8 m^2 each) were established separately in the flattened part of the study area. The first plot served as an uncontaminated control (1), and three were treated with crude oil (10 L m^{-2}) to test: natural attenuation (2), biostimulation by adding nutrients (3) and bioaugmentation in the presence of added nutrients (4). For bioaugmentation we used a commercial bacterial product. For biostimulation, and to supplement bioaugmentation, we added mineral amendments (30 g m^{-2} K_2O, 30 g m^{-2} P_2O_5 и 25 g m^{-2} N_20). Biopreparation and nutrients were added to a depth of 0–20 cm.

Soil samples were collected one week and three month after the contamination, and later on annually at the end of September. Each plot was divided into 4 subplots of 2 m^2 and 10 coring sites were chosen within the subplot with a method of randomized repetitions (Dospehov, 1985). We took samples with a soil corer (20 cm diameter), pooled and sieved (< 2 mm) all ten soil cores, collected from each subplot. This combined sample represented one replicate, so that there were four replications from each plot. The total weight of a combined sample was 900 g.

2.3. Physical and chemical analyses

Chemical and physical characteristics of the collected samples were determined according to Arinushkina (1970). Total petroleum hydrocarbons (TPH) were extracted from soil samples with hexane and measured according to PND F 16.1:2.21–98 (2012). TPH were determined by UV fluorescence spectroscopy by using a FLUORAT-02-3M spectrofluorometer (Nordinkraft-Sensor, Russia). The fluorescence of the samples was measured at 270 nm excitation and at 320 nm emission wavelengths.

2.4. Soil biological activity

We measured soil basal respiration (BR) using alkali absorption method. Moist samples (50 g dry weight equivalent) were adjusted to 60% of field holding capacity and pre-incubated at 25 °C for 14 days. Following incubation each soil sample was spread on the bottom of a 500-ml glass jar and reaction container with 20 ml NaOH (0.02 N) solution was suspended inside the jar, above the soil. After incubation at 25 °C for 24 h 2 drops of phenolphthalein indicator were added into the reaction containers, and then titrated with 0.02 N H_2SO_4. The jars without soil served as the controls. The difference in the consumed volume of H_2SO_4 between the treatment and control was used to calculate the quantity of CO_2 emission from soil microorganisms. The quantity of CO_2 (the ratio of respiration) was calculated as μg CO_2-C g^{-1} h^{-1}.

We determined three types of soil enzymatic activity (dehydrogenase, catalase and urease) using the methods described by Haziev (2005) with little modification. Dehydrogenase activity (DA) was analyzed via the reduction of the 2,3,5-triphenyltetrazolium chloride (TTC) to a red-colored 2,3,5-triphenyl formazan (TPF) with 1% (w/v) glucose solution added as a source of organic carbon. After incubation period of 24 h at 30 °C, we extracted TPF with methanol and measured the optical density of solution at 484 nm (spectrophotometer Genesys 10 UV, Thermo Spectronic, USA). The latter values served to determine the TPF concentration. Dehydrogenase activity was expressed as mg TPF g^{-1} day^{-1}.

The activity of catalase (CA) was determined following Johnson and Temple method (1964) by back-titrating residual H_2O_2 with $KMnO_4$. The mixture of 2 g of dry soil and 0.3% hydrogen peroxide solution was shaken at 120 rpm for 20 min and then 1.5M H_2SO_4 was added. Afterwards the solution we filtered and titrated the liquid using 0.1M $KMnO_4$. The result was expressed as ml $KMnO_4$ g^{-1}.

To measure urease activity (UA) we followed method described by Haziev (2005) with urea used as a substrate. We mixed 5 g of dry soil and 1 ml of methylbenzene in a 150-ml conical flask. After 15 min, 10 ml of 10% urea and 20 ml of citrate buffer (pH = 6.7) were added to the sample. The mixture was incubated at 37 °C for 24 h and filtered. One ml of filtrate was transferred into a volumetric flask, mixed with 4 ml of sodium phenoxide, 3 ml of sodium hypochlorite and diluted to 50 ml. Absorbance was determined at 578 nm using a spectrophotometer Genesys 10 UV (Thermo Spectronic, USA). The urease activity was expressed as μg NH_3-N g^{-1} h^{-1} at 37 °C.

2.5. Data analyses

We compared variables describing soil conditions through the duration of the experiment, as well as across the treatment types. Since empirical distributions of the measured variables were non-normal and displayed pronounced heteroscedasticity, we employed non-parametric methods of statistical testing. Wilcoxon signed rank test was used to compare central tendencies of paired data. For all variable pairs we calcualted Spearman rank order correlation coefficients with Tukey's HSD correction. To study the relationship between biological parameters and soil physicochemical properties, we used principal component analysis (PCA). Differences were considered significant at $p < 0.05$. Statistical analyses were performed using Statistica software (version 10; Statsoft).