Journal of Environmental Management 153 (2015) 121–131

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: A comparative study

Marco Andreolli [a], Silvia Lampis [a, *], Pierlorenzo Brignoli [b], Giovanni Vallini [a, *]

[a] Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy
[b] Eurovix SpA, Viale Europa 10, 25046 Cazzago San Martino, BS, Italy

ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form
13 January 2015
Accepted 8 February 2015
Available online 15 February 2015

Keywords:
Bioaugmentation
Bioremediation
Biostimulation
Natural attenuation
Toxic hydrocarbons
Trichoderma sp.
Wildfire-impacted forest soil

ABSTRACT

In this work, the natural attenuation strategy (no soil amendments done) was compared with two different bioremediation approaches, namely bioaugmentation through soil inoculation with a suspension of *Trichoderma* sp. mycelium and biostimulation by soil addition with a microbial growth promoting formulation, in order to verify the effectiveness of these methods in terms of degradation efficiency towards toxic hydrocarbons, with particular attention to the high molecular weight (HMW) fraction, in a forest area impacted by recent wildfire in Northern Italy. The area under investigation, divided into three parcels, was monitored to figure out the dynamics of decay in soil concentration of C_{12-40} hydrocarbons (including isoalkanes, cycloalkanes, alkyl-benzenes and alkyl-naphthalenes besides PAHs) and low molecular weight (LMW) PAHs, following the adoption of the foregoing different remediation strategies. Soil hydrocarbonoclastic potential was even checked by characterizing the autochthonous microbial cenoses.

Field experiments proved that the best performance in the abatement of HMW hydrocarbons was reached 60 days after soil treatment through the biostimulation protocol, when about 70% of the initial concentration of HMW hydrocarbons was depleted. Within the same time, about 55% degradation was obtained with the bioaugmentation protocol, whilst natural attenuation allowed only a 45% removal of the starting C_{12-40} hydrocarbon fraction. Therefore, biostimulation seems to significantly reduce the time required for the remediation, most likely because of the enhancement of microbial degradation through the improvement of nutrient balance in the burned soil.

1. Introduction

The Mediterranean Basin is heavily affected by forest fires. Every year, more than 50,000 wildfires destroy about 0.6–0.8 M Ha in the European Union (EU) context, with a significant number of them occurring in the southern countries of the EU (EEA, 2008; FAO, 2013). Unlike other parts of the world, where a large percentage of fires is of natural origin (especially lightning), the Mediterranean Basin is characterized by a prevalence of human-induced fires, with natural causes representing only a small percentage (1–5%) (Leone et al., 2009). In the recent decades – more precisely from the '60s of the last century – socio-economic changes such as rural exodus and industrialization have led to the abandonment of large portions of land (Moreira et al., 2011). These transformations, followed by encouraged forestation plans with highly flammable eucalyptus and pine species (Grove and Rackham, 2001) along with a general warming and drying trend (Harding et al., 2009; Pausas and Fernández-Muñoz, 2012; Stott et al., 2004), have tremendously increased wildfire activity (Pausas et al., 2008; Pausas and Keeley, 2009).

An important consequence resulting from the impact of wildfires on forest lands is – among the effects on the physical and chemical soil properties (Bento Gonçalves and Vieira, 2011) as well as on the biota (Verma and Jayakumar 2012) – the formation of hazardous organic compounds such as polycyclic aromatic

* Corresponding authors.
E-mail addresses: silvia.lampis@univr.it (S. Lampis), giovanni.vallini@univr.it (G. Vallini).

http://dx.doi.org/10.1016/j.jenvman.2015.02.007
0301-4797/

hydrocarbons (PAHs) and – to a minor extent – polychlorinated biphenyls (PCBs) and dioxins, as a result of combustion processes of the original plant biomass (Gabos et al., 2001; Kim et al., 2003; Martínez et al., 2000). For instance, in soils subjected to fires in coniferous forests, PAHs with 3–4 benzene rings such as anthracene, phenanthrene, pyrene, chrysene and tetraphene, along with variable concentrations of LMW PAHs such as naphthalene and fluorene (Gennadiev and Tsibart, 2013; Kim et al., 2011a) were detected. These substances not only represent an actual toxicological risk for people who may possibly be exposed to such contaminants while crossing burnt forests, but can also act as an obstacle to the biodiversity resettlement due to their strong toxicity to both plant and animal organisms (Delistraty, 1997). Nevertheless, soil microorganisms, including those capable of degrading recalcitrant polyaromatic compounds, are in turn inhibited by high concentrations of these organic contaminants (García-Falcón et al., 2006; Sikkema et al., 1995). Therefore, since the microbial communities in wildfire-affected soils normally undergo a drastic reduction and/or inhibition, resulting in a lower biological reactivity, biostimulation or even bioaugmentation measures on these areas by means of appropriate land farming procedures or hydrocarbonoclastic microbial (either bacterial or fungal) inocula – within the immediate after-fire stages – represent possible valuable tools to increase rate and extent of biodegradation of such pollutants, thus stimulating a more rapid restoration of the preexisting ecosystems (Bamforth and Singleton, 2005; Mrozik and Piotrowska-Seget, 2010; Straube et al., 2003).

Two different bioremediation strategies, namely soil bioaugmentation by means of the addition of a mycelial suspension of a *Trichoderma* sp. strain and soil biostimulation through the dispersion of a commercial formulation to enhance the native soil hydrocarbonoclastic microbial community by simply adjusting soil nutrients, were compared in this study to verify the bio-treatability of wildfire generated toxic hydrocarbons (BTEX, LMW PAHs and C_{12-40} hydrocarbon fraction). Efficacy of the aforementioned approaches was evaluated with respect to the natural attenuation (no soil treatments) in three contiguous parcels cordoned off within a woodland located in Northern Italy (Pezzaze municipality, Brescia, Lombardy) and recently impacted by wildfire. The experimental plots were then monitored throughout the further nine months to figure out the dynamics of decay in soil concentration of toxic hydrocarbon compounds, due to the adoption of the different remediation strategies.

2. Materials and methods

2.1. Experimental site

The forest area investigated in the present study (named P; GPS coordinates: 45°47′07″N; 10°13′19″E, Castegnacolo di Sopra) is located in the municipality of Pezzaze (Brescia, Lombardy, Italy) at an elevation of about 1300 m above sea level. It is dominated by a hardwood forest, whose the prominent tree genera are represented by *Fagus*, *Castanea* and *Quercus*. The area was impacted by wildfire on April 2nd, 2012. Unburned control soil was collected in a land parcel not impacted by fire nearby P area.

2.2. Chemicals

Coal tar solution was purchased from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). Antibiotics, Dibenzothiophene, Malachite Green, Congo Red and Methylene Blue were furnished by Sigma–Aldrich Srl (Milan, Italy). All the compounds were analytical grade. Diesel fuel (Shell V-Power Diesel) was bought at a petrol station.

2.3. Bioremediation treatments

2.3.1. Experimental set-up

Experimental trials started four months after fire had occurred. Two different *in-situ* bioremediation protocols were carried out at the P area. This latter (homogenous for slope, soil characteristics and vegetation) was divided in three parcels of 75 m^2 each. Parcel P1 was used as untreated control only left to the natural attenuation (Na). Bioaugmentation protocol (Ba) was applied in parcel P2 while biostimulation (Bs) was carried out in parcel P3. For the different bioremediation treatments, bioaugmentation and biostimulation respectively, two commercial products were used: the fungal strain *Trichoderma* sp. Evx1 and the Micropan™ formulation both provided by Eurovix SpA (Brescia, Italy). The experimental trials lasted 270 days.

Bioaugmentation protocol: *Trichoderma* sp. Evx1 was maintained on Potato Dextrose Agar (PDA, Oxoid). Fungal growth was obtained through batch fermentation in Czapek minimal medium (Smith, 1960) and a lignocellulosic matrix as sources of carbon and energy. Bioaugmentation was performed by distributing 2 l fungal spore suspension in water/m^2 soil, corresponding to about 5×10^{11} spores per square meter. This formulation was sprayed on parcel P2 at the beginning (t_0) of the bioremediation trial.

Biostimulation protocol: the commercial product Micropan™ was used consisting in a mixture of nutrients derived from vegetable sources and containing 40% organic carbon (even in form of carbohydrates), 1% organic nitrogen (including free amino acids), and 1% phosphorus. Trace elements and growth factors represent also additional components of this product. Biostimulation was performed by spreading 2 l aqueous suspension containing 50 g of Micropan™/m^2 soil. The product was dispensed on parcel P3 by means of a mechanical sprayer pump at the beginning (t_0) of the experimental trial.

2.3.2. Soil sampling and physico-chemical analyses

The soil samples were collected before the experimentation started to assess the chemical and physical characteristics of the soil and during the experimentation. In particular samples collected at the beginning (t_0) and after 20 (t_{20}), 60 (t_{60}) and 270 (t_{270}) days were analyzed. Topsoil aliquots were sampled according to the quartering procedure: 5 kg of mixture from 5 different spots in each area were divided into quarters and each quarter was mixed individually. Two quarters were then mixed to form halves. Afterwards, the two halves were blended together to form a uniform matrix. This procedure was repeated twice until the final sample was adequately homogenized. Three replicas for each analysis were considered. The chemical analyses were carried out by Dolomiti Energia Laboratories (Trento, Italy) according to the Official Standard Methods procedure. Total Organic Carbon (TOC) and pH was determined by means of UNI-EN 13137 (2002) and UNI 10501 (1996) methods respectively. BTEX and styrene was analyzed by US-EPA 5030C (2003) + US-EPA 8260C (2006), PAHs quantification was carried out using US-EPA 3546 (2007) + US-EPA 8270D (2007) and C_{12-40} hydrocarbons where quantified through UNI-EN ISO 16703 (2011) methods.

2.3.3. Enumeration of heterotrophic bacteria and fungi and recovery of Trichoderma sp. Evx1 from the P soil

Quantification of total cultivable microorganisms in the soils was carried out as follows: 5 g of soil was stirred in 45 ml of physiologic solution (0.9% NaCl) for 1 h. Thus, serial dilutions were inoculated on Oxoid R_2A-agar and Malt Yeast Extract Agar (MYEA: malt extract 20 g l^{-1}, yeast extract 5 g l^{-1} and 1.5% agar, pH: 7.0) plates added with 15 mg l^{-1} of rifampicin for the count of bacteria and fungi, respectively. These media were selected among those