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Dissipation of 3–6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass

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Abstract

Plants may contribute to the biodegradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils. Different mechanisms have been proposed, such as an increase in microbial numbers, but are not clearly elucidated. This study investigates the dissipation of a mixture of eight PAHs, ranging from 3 to 6 rings, in the rhizosphere of ryegrass (*Lolium perenne* L.). Two pot experiments were conducted with or without plants using soil spiked with 1 g kg^{-1} of PAHs in a growth chamber. The first experiment was carried out shortly after spiking and the second after 6 months of ageing. At the end of both experiments, the extractable concentrations of all PAHs were lower in rhizospheric than non-rhizospheric soil. PAH dissipation was lower after soil ageing than before, but was still significantly higher in the rhizospheric soil, even for three of the high molecular weight PAHs. Total culturable microflora were higher in the rhizospheric than non-rhizospheric soil, but was at the same level in spiked and non-spiked soil. The number of PAHs degraders, estimated by a modified MPN procedure, was not significantly different in the freshly spiked rhizospheric and non-rhizospheric soils, but was significantly higher in the rhizosphere of the aged spiked soil.

Keywords: Dissipation; Microflora; Polycyclic aromatic hydrocarbons; Quinones; Rhizosphere; Soil

1. Introduction

The soil environment influenced by plant roots, or rhizosphere, represents a complex ecosystem with the potential to accelerate biodegradation of organic contaminants, including polycyclic aromatic hydrocarbons (PAHs) (Aprill and Sims, 1990; Anderson et al., 1993; Walton et al., 1994a). Although a few studies indicated that the plant rhizosphere is able to enhance degradation of 4-ring PAHs such as pyrene (Schwab and Banks, 1994; Reilley et al., 1996), they were performed with a limited number (2–4 compounds) of PAHs. To our knowledge, only Goodin and Webber (1995) studied the degradation of a 5 ring PAH (benzo(a)pyrene) in the rhizosphere. They reported inconsistent degradation of benzo(a)pyrene in the rhizosphere. The studies of Schwab and Banks (1994) and Reilley et al. (1996) measured the disappearance with time of PAHs in freshly spiked soils, where the availability of PAHs may be higher than in industrial soils, where the contamination has a greater residence time.

Plants may contribute to the dissipation of PAHs by an increase in microbial numbers, improvement of physical and chemical soil conditions, increased humification and adsorption of pollutants in the rhizosphere, but the impact of each process has not been clearly elucidated. Several studies, based on the hypothesis that root exudates increase the rhizosphere microbial community, investigated the significance of plant microbial interactions for the degradation of PAHs. Walton et al. (1994b) speculated that when a chemical stress is present in soil, a plant may respond by increasing or changing exudation to the rhizosphere which modifies rhizospheric microflora composition or activity. As a result, the microbial community might increase the transformation rates of the toxicant. Günther et al. (1996) noted that in a soil polluted with PAHs and aliphatic hydrocarbons, microbial plate counts and soil respiration rates were higher in the rhizosphere of ryegrass than in the bulk soil. Reilley et al. (1996) showed that degradation of pyrene increased in rhizosphere soil and that the highest pyrene mineralisation rate was found when organic acids, typically found in root exudates, were added to the soil. Nichols et al. (1997) showed a selective enrichment of the bacterial populations that were organic compound degraders in the rhizosphere of alfalfa and bluegrass, in a soil amended with organic compounds, including phenanthrene and pyrene.

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However, bioremediation of the compounds was not estimated. Chaineau et al. (1995) showed a rapid adaptation of the soil microbial community to degradation of hydrocarbons in an agricultural field plot amended with drill cuttings, and a specific diversity of the degraders, but did not compare rhizospheric and non-rhizospheric soils. From these data, it is not clear whether increased dissipation of PAHs in the rhizosphere is due to a specific stimulation of PAH degraders in the rhizosphere.

Field experiments should rather be used than pot experiments with spiked soils to study the feasibility of PAH phytoremediation. However, soils contaminated with PAHs rarely contain only PAHs, but contain also other organic pollutants, possibly heavy metals, and are very heterogeneous. Such complex systems cannot be used to study the mechanisms involved in the biodegradation of PAHs in the rhizosphere. Pot experiments with spiked soils, especially using radio-labelled (^{14}C or ^{13}C) are useful to follow the fate of known amounts of PAHs added to a soil. With single compounds such as benzo-a-pyrene, it has been performed (Goodin and Webber, 1995) and inconsistent degradation was reported. However, it is not realistic for long-term experiments and for experiments with many PAHs due to cost of the compounds (especially to have uniform labelling). Further, the fate of a single (radio-labelled) PAH in the rhizosphere may not reflect the fate of the same compound in a mixture of PAHs. A major disadvantage of pot experiments with spiked soil is that the availability of the PAHs may be different from PAH contaminated field sites. But the effect of ageing on the dissipation of PAHs in the rhizosphere was never investigated.

We investigated the dissipation of eight PAHs, including 3–6-ring PAHs, in ryegrass rhizosphere in pot experiments with soil freshly spiked and after 6 months of ageing. To estimate rhizosphere, spiking and ageing effects on PAH degraders, total culturable microflora and PAH degraders were estimated, using a modified MPN procedure.

2. Materials and methods

2.1. Experimental design

Pregerminated ryegrass (*Lolium perenne* L., cv. Barclay) seedlings were grown in pots containing 250 g of an agricultural soil, either spiked or un-spiked with PAHs. The soil was a gleyic luvisol, with a pH of 6.6 and 15 g kg^{-1} C, and has no previous history of exposure to PAHs or other contaminants (Leyval and Binet, 1998). The agricultural soil was spiked with a mixture of eight PAHs (1 g kg^{-1}) as described in Leyval and Binet (1998). The concentrations of PAHs in the soil were, respectively, 200 mg kg^{-1} for anthracene, phenanthrene, fluoranthene and chrysene, and 50 mg kg^{-1} for benzo(a)anthracene, benzo(k)fluoranthene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene. Seeds of ryegrass were surface sterilised with 30% H_2O_2 and pre grown for 15 days in vermiculite. Two seedlings were then transplanted to dark plastic pots containing 250 g of soil. Seedlings were thinned to one after one week and the soil was covered with a layer of coarse sand to minimize PAH volatilisation and leaching. Three treatments were carried out: vegetated pots with un-spiked and spiked soil and un-vegetated pots with spiked soil. There were five pots per treatment randomly arranged in a growth chamber (Conviron, 24/20°C day/night, 16 h day, 80% RH, 200–300 mmol s^{-1} m^{-2} PAR). Plants were harvested 40 days after transplanting and dry weights estimated after drying at 105°C. The first experiment was performed 12 h after soil spiking and the second one after ageing the same spiked soil for 180 days (from June to December). During ageing, the spiked soil was kept outdoors, in dark condition, at temperatures ranging from 5 to 25°C and was maintained at 60% of water holding capacity.

2.2. PAHs analysis

All the soil from the vegetated pots was considered as rhizospheric soil. The soil from vegetated (rhizospheric soil) and non-vegetated pots (non-rhizospheric soil) was carefully collected, homogenised and crushed. PAHs and a few metabolites (anthraquinone, naphthoic acid and benzo(a)anthraquinone) were extracted from soil using Soxlhet method (50 g dry soil with 200 ml chloroform for 4 h). Soil extracts were filtered through a cellulose filter and analysed using a 3400 CX Varian gas chromatograph coupled to a mass spectrometer (ION TRAP Saturn III, Varian GC–MS). Compounds were separated with a He flow on a 30 m DB5 MS column, 0.25 mm internal diameter and 0.25 μm film thickness. The column oven temperature was: 70–150°C at 10°C min^{-1} and 150–300°C at 6°C min^{-1}. The Programmable Sample Injector (PSI) temperature was set between 25 and 300°C at 180°C min^{-1}. The mass spectrometer was operated at 70 eV in impact electronic mode. Detection and quantification of the eight PAHs and of the metabolites were carried out by Single Ion Monitoring (Table 1). The ion trap temperature was set to 220°C. The concentrations are expressed per unit soil dry weight. The initial extractable concentration of PAHs (T_0) was measured within 1 h after spiking.

2.3. Enumeration of culturable PAHs degraders and total microflora

PAH degraders were enumerated using the most-probable-number (MPN) procedure (Wrenn and Venosa, 1995) modified for our study. A PAH mixture consisting of phenanthrene (10 g l^{-1}), anthracene (1 g l^{-1}), fluorene (1 g l^{-1}) and fluoranthene (1 g l^{-1}) was added to 96-well microtiter plates (10 μl/well) as a solution in hexane before the plates were filled with the growth medium. Hexane was