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Low effect of phenanthrene bioaccessibility on its biodegradation in diffusely contaminated soil☆

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ABSTRACT

This study focused on the role of bioaccessibility in the phenanthrene (PHE) biodegradation in diffusely contaminated soil, by combining chemical and microbiological approaches. First, we determined PHE dissipation rates and PHE sorption/desorption isotherms for two soils (PPY and Pv) presenting similar chronic PAH contamination, but different physico-chemical properties. Our results revealed that the PHE dissipation rate was significantly higher in the Pv soil compared to the PPY soil, while PHE sorption/desorption isotherms were similar. Interestingly, increases of PHE desorption and potentially of PHE bioaccessibility were observed for both soils when adding rhamnolipids (biosurfactants produced by *Pseudomonas aeruginosa*). Second, using ^{13}C-PHE incubated in the same soils, we analyzed the PHE degrading bacterial communities. The combination of stable isotope probing (DNA-SIP) and 16S rRNA gene pyrosequencing revealed that *Betaproteobacteria* were the main PHE degraders in the Pv soil, while a higher bacterial diversity (*Alpha-*, *Beta-*, *Gammaproteobacteria* and *Actinobacteria*) was involved in PHE degradation in the PPY soil. The amendment of biosurfactants commonly used in biostimulation methods (*i.e.* rhamnolipids) to the two soils clearly modified the PHE sorption/desorption isotherms, but had no significant impact on PHE degradation rates and PHE-degraders identity. These results demonstrated that increasing the bioaccessibility of PHE has a low impact on its degradation and on the functional populations involved in this degradation.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous carcinogenic organic contaminants, originating from multiple sources (*i.e.* biogenic, petrogenic and pyrogenic). Among them, human activities are responsible for the major releases into the environment, particularly through atmospheric deposition (Tobiszewski and Namieśnik, 2012). PAHs physico-chemical properties (high chemical stability, low vapor pressure, low aqueous solubility, high water-organic carbon K_{oc} partition coefficient) make them persistent in soils. However, this persistence is also strongly dependent on soil physico-chemical properties and soil microbial activities (Doyle et al., 2008).

In the case of diffuse contamination, soils are continuously exposed to low and repeated deposits of PAHs. In these soils, PAHs can be found in different soil compartments, depending on the age and history of the contamination. A small fraction of these PAHs is usually present in the soil aqueous phase, and is considered as readily bioaccessible to microorganisms for degradation. Indeed, the bioaccessible fraction is the one available to cross cellular membranes if the organism is present and has access to it, even after a period of time or through spatial rearrangements, whereas the bioavailable fraction is the one that is freely available to cross the organism's cellular membrane at a given time (Riding et al., 2013). The PAHs recently adsorbed on soil constituents are also

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generally readily bioaccessible to microorganisms, while PAHs in contact with soil constituents for a longer time become poorly bioaccessible. A last fraction of PAHs can be strongly bound to soils, by slow diffusion into soil micropores. The strong sorption/sequestration mechanisms of PAHs to organo-mineral complexes eventually creates non-extractable bound-residues (Ahangar, 2010), considered as not bioaccessible (Semple et al., 2004).

After adaptation and selection processes, the soil bacterial communities acquire the potential to biodegrade the bioaccessible PAH fraction (Haritash and Kaushik, 2009). Indeed, different bacterial strains isolated from various contaminated soils have been described as capable of degrading PAHs and of growing with PAHs as sole carbon source. These bacteria belong to different phyla or classes such as α-, β- and γ-*Proteobacteria* (Haritash and Kaushik, 2009; Jeon et al., 2006; Kim and Jeon, 2009; Niepceron et al., 2013), *Bacteroidetes*, *Actinobacteria* (Timmis et al., 2010) or *Firmicutes* (Wu et al., 2008).

The bioaccessibility factor depends on the physico-chemical properties of PAH compounds and on the soil properties (clays and organic matter contents and nature) (Brandli et al., 2008; Jonker et al., 2005; Mechlińska et al., 2009; Xia et al., 2010). Besides that, the bioavailability factor depends on the physiological and catabolic potential of soil microorganisms (Crampon et al., 2014). The combination of these complex processes determines the fate of PAHs in soils (Semple et al., 2003, 2004). Enhancement of PAH bioaccessibility in contaminated soils through addition of surfactants has already been reported (Ahn et al., 2008; Paria, 2008; Zhou et al., 2007; Zhou and Zhu, 2008). Biosurfactants (*e.g.* surfactants naturally produced by (micro)organisms) are more environmental friendly and interesting due to their low critical micellar concentration (CMC) and their lower toxicity for soil microorganisms than synthetic ones (Bustamante et al., 2012). Rhamnolipids, produced by *Pseudomonas aeruginosa* (Bordas et al., 2005), have been described for their ability to remobilize sorbed PAHs and to potentially enhance their biodegradation in slurry batches or column experiments (Bordas et al., 2005; Congiu and Ortega-Calvo, 2014). However, the real impact of these rhamnolipids on PAHs accessibility after direct treatment of contaminated soils remains to be determined.

In this context, the aim of this study was to determine the parameters mostly governing phenanthrene (PHE) degradation rates in diffusely contaminated soils. PHE was chosen as a model compound representative of low molecular weight PAHs (2–3 fused aromatic rings). To address such an objective, two soil types (PPY and Pv) characterized by similar chronic PAH contamination, but different physico-chemical characteristics, were analyzed and compared. PPY is a **P**ermanent **P**asture located nead **Y**vetot and Pv is a soil located near **P**etit**v**ille, in France. In each soil type, chemical and molecular biological methods were combined to investigate the fate of phenanthrene (PHE) by focusing on: (i) PHE accessibility, (ii) PHE degradation kinetics, and (iii) PHE-degrading bacterial diversity in relation to the amendment or not of rhamnolipids. PHE degradation was monitored using a microcosm approach during two months. In microcosm, PHE sorption isotherms with or without rhamnolipids were determined to estimate PHE accessibility. PHE degradation kinetics were determined using ^{13}C-PHE and PHE-degrading bacterial populations were identified through DNA-Stable Isotope Probing (DNA-SIP) and 16S rRNA gene amplicon pyrosequencing.

2. Materials and methods

2.1. Soil sampling and soil analyses

Soil samples were collected in the Seine river basin (France), which is a highly urbanized and industrialized location accounting for 40% of the French economic activity and including 33% of oil refining and 50% of river traffic (Niepceron et al., 2010). In spring 2014, approximately 15 kg of soil (0–15 cm depth) of two diffusely contaminated soil types (PPY and Pv) were sampled. PPY soil is an agricultural soil characterized by a permanent grassland and located near Yvetot (Seine-Maritime department, 49°36′40.6″N 0°44′10.7″E), at about 15 km from the Seine River. The PPY soil is classified as Luvisol according to the World Reference Base for soil resources. The second soil type sampled (Pv) is located near Petiville (Seine Maritime department, 49°25′54.0″N 0°36′25.4″E) in the Seine river estuary, and is partially covered by young willow. This soil, periodically flooded, is classified as fluvic Gleysol according to the World Reference Base for soil resources.

After sampling, field-moist soils were sieved through a 5 mm mesh and stored at room temperature for 8 days for acclimation. Aliquots were stored at −20 °C for molecular characterization of the initial microbial communities before PAH spiking. The natural background concentrations of PAHs in both soils were lower than 1 mg kg^{-1} for the sum of the 16 PAHs classified as priority by the US-Environmental Protection Agency (US-EPA) (Table 1).

Pv and PPY soil organic matter (OM) was analyzed on dried crushed soils by RockEval 6 pyrolysis (Vinci Technologies, France) and S2 peak deconvolution (Sebag et al., 2006) was obtained using peakfit software (SigmaPlot, Systat) and the “Bulk Rock” method, as described previously (Crampon et al., 2014) (Table 1). Nature of clays in soils was analyzed by X-Ray diffraction (XRD) (Philips PW3040 diffractometer, France), as described previously (Crampon et al., 2014).

Soil pH was determined according to the French NF ISO 10–390 standard. Granulometry was analyzed according to the NF X31-107 standard. CEC (Cationic Exchange Capacity) was obtained following the French NF ISO 31–130 standard.

2.2. Phenanthrene sorption isotherm determination

Sorption isotherm experiments were performed for both soils as previously described (Groboillot et al., 2011), in the presence or not of rhamnolipids. Briefly, sorption reactors consisted in 4 g of soil sieved at 355 μm and mixed with 40 mL deionized water in glass tubes under magnetic agitation. $HgCl_2$ (500 mg.L^{-1}) was added at the beginning of the experiment to avoid bacterial PAH biodegradation, which could decrease PHE concentration in water after many hours. PHE was then introduced in the reactors with a known volume of PHE stock solution (100 mg.L^{-1} in acetonitrile), to obtain a range of concentrations from 0.1 to 1 mg.L^{-1}. No additives were added into the reactors to aid PHE solubilisation, as already done in other studies (*e.g.* Cobas et al., 2014), because PHE was introduced under constant stirring below its limit of solubility in water (i.e. 1.6 mg.L^{-1}). The volume of organic solvent injected (<1% of the total volume) was low enough to have no impact on PHE sorption, as previously studied (Gaboriau and Saada, 2001; Blanc et al., 2006; Groboillot et al., 2011). To evaluate the remobilization capacity of rhamnolipids, the biosurfactant (AGAE Technologies (USA), 90% purity) was added in the reactors 5 h after the introduction of PHE, to a final concentration of 0.55 g.L^{-1} (concentration ten times higher than its CMC of 0.05 g.L^{-1}) to ensure the formation of micelles in the aqueous phase. Five hours was chosen as the time to add the biosurfactant after PHE spiking because it was observed that a “quasi” plateau was reached for PHE sorption after this time (data not shown). Mixtures treated or not with rhamnolipids were stirred during 24 h at 25 °C to be sure to reach thermodynamic equilibrium (time evaluated during preliminary tests) (Barnier et al., 2014). Thereafter, reactors were centrifuged at 7000 rpm (10,213 g) (Sigma 4K15, thermo scientific) during 20 min to recover