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Phytoavailability and mechanism of bound PAH residues in filed contaminated soils☆

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ABSTRACT

Understanding the phytoavailability of bound residues of polycyclic aromatic hydrocarbons (PAHs) in soils is essential to assessing their environmental fate and risks. This study investigated the release and plant uptake of bound PAH residues (reference to parent compounds) in field contaminated soils after the removal of extractable PAH fractions. Plant pot experiments were performed in a greenhouse using ryegrass (*Lolium multiflorum* Lam.) to examine the phytoavailablility of bound PAH residues, and microcosm incubation experiments with and without the addition of artificial root exudates (AREs) or oxalic acid were conducted to examine the effect of root exudates on the release of bound PAH residues. PAH accumulation in the ryegrass after a 50-day growth period indicated that bound PAH residues were significantly phytoavailable. The extractable fractions, including the desorbing and non-desorbing fractions, dominated the total PAH concentrations in vegetated soils after 50 days, indicating the transfer of bound PAH residues to the extractable fractions. This transfer was facilitated by root exudates. The addition of AREs and oxalic acid to test soils enhanced the release of bound PAH residues into their extractable fractions, resulting in enhanced phytoavailability of bound PAH residues in soils. This study provided important information regarding environmental fate and risks of bound PAH residues in soils.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are recognized as important class of persistent organic pollutants because of their ubiquitous presence in the environment, their resistance to degradation, and their potential toxicity and carcinogenicity to humans and many other organisms (Man et al., 2013; Nisbet and LaGoy, 1992; Reeves et al., 2001; Ruby et al., 2016). PAHs can originate from a wide range of natural and anthropogenic sources such as wildfires, volcanic eruptions, oil seeps, fossil fuel combustion, petroleum refinery, metal smelter and foundries, and open burning or incineration of wastes (Ruby et al., 2016; Wilson and Jones, 1993). PAHs are widespread in waters, soils, sediments, and the atmosphere, and can even reach the most remote and pristine areas of the Earth such as the Arctic, Antarctic, and Tibetan Plateau (Friedman and Selin, 2012; Ma et al., 2013; Montone et al., 2016; Wang et al., 2014; Wilcke, 2007). Soils are critical reservoirs of PAHs with concentrations up to hundreds or thousands of mg/kg in many regions of the world (Man et al., 2013; Thavamani et al., 2011; Wilcke, 2007). Therefore, there are urgent needs worldwide to remediate urban and agricultural soils that have been heavily contaminated with PAHs (de Boer and Wagelmans, 2016; Lamichhane et al., 2016; Wilson and Jones, 1993).

Among soil remediation strategies used in the last two decades, chemical treatment and bioremediation have been most often used (de Boer and Wagelmans, 2016; El Amrani et al., 2015; Lamichhane et al., 2016; Wilson and Jones, 1993). Remediation schemes are generally aimed to decrease organic solvent-extractable PAH concentrations in soils, which is often used to assess the outcome of remediation operations (Barraclough et al., 2005; Ranc et al., 2016). PAHs remaining in soils after exhaustive organic solvent extraction (e.g., Soxhlet extraction) are considered non-extractable bound residues (BRs) (Gao et al., 2013a; Nieman et al., 1999; Richnow et al., 1999). The BRs can include parent compounds and non-naturally occurring metabolites (Führ et al., 1998; Gevao et al., 2000) that become tightly bound with soil organic matter (SOM) (Gao et al., 2015; Nieman et al., 1999; Richnow et al., 1999). If binding

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between PAHs and soil matrix is permanent and irreversible, enhanced formation of BRs would in principle reduce the bioavailability of PAHs in soils (Boesten, 2016; Poirier et al., 2014). The term ‘availability’ is defined as the ability for a given compound to desorb from the soil and react in the liquid phase, and ‘bioavailability’ refers to the fraction that is freely available to cross an organism's cellular membrane (Barnier et al., 2014; Ouvrard et al., 2013). Indeed, binding of PAHs with SOM could occur due to enzymatic activities or condensation reactions during natural soil humification (Richnow et al., 1999). Certain condensation and polymerisation reactions could also be mediated by soil clays and metal oxides (Gao et al., 2015). In laboratory soil incubation studies, the BRs were found as major fractions of soil PAHs (Kästner et al., 1999; Nieman et al., 1999). Because BRs are believed to be sequestered in soil matrix, the BRs of PAHs and other organic contaminants such as pesticides were once thought no longer bioaccessible or bioavailable to living organisms, thus posing little risks to human and ecosystem health (Barraclough et al., 2005; Berry and Boyd, 1985; Liu et al., 2013; Northcott and Jones, 2000; Richnow et al., 1995).

However, it has been increasingly recognized that the BRs of organic contaminants can be released and become bioavailable under certain conditions. Thus, there are growing concerns over whether the release of BRs could pose a long-term threat to the environment (Barraclough et al., 2005; Fenlon et al., 2011). Previous studies have extensively focused on the bioavailability of pesticide BRs. The BRs of atrazine, isoproturon, and dicamba in soils could be taken up by earthworms (Gevao et al., 2001); the BRs of methabenzthiazuron, trifuralin, cypermethrin, and hydroxymonolinuron could be accumulated in plants (Führ and Mittelstaedt, 1980; Haque et al., 1982; Mostafa et al., 1982; Roberts and Standen, 1981). Relatively less work has been devoted to the bioavailability of bound PAH residues. Our recent work demonstrated significant phytoavailability of bound phenanthrene and pyrene in artificially-spiked soils to ryegrass (*Lolium multiflorum Lam.*) (Gao et al., 2013a), and the release of bound PAH residues in contaminated soils was facilitated by low molecular weight organic acids (LMWOAs) commonly found in plant root exudates (Gao et al., 2015). The term ‘phytoavailability’ refers to the fraction for a given compound that is freely available to be absorbed by plant. Because the binding strength and extent of bound PAH residues in soils increase over time (Nieman et al., 1999), and PAH availability is also strongly reduced by aging (Ouvrard et al., 2013), further investigation is needed on the phytoavailability of bound PAH residues in field contaminated soils with PAHs aged for years.

Therefore, this study investigated the release and phytoavailability of bound PAH residues (reference to parent compounds) in field contaminated soils collected nearby a petrochemical plant as influenced by ryegrass growth. It was hypothesized that ryegrass root exudates would enhance the release of bound PAH residues and subsequently their bioavailability and plant uptake. Ryegrass was selected because of its high growth rate, well-developed root system, and resistant PAH contamination in previous investigations. Ryegrass is widely vegetated all over the world and has been proven to be effective in phytoremediation for soils contaminated with organic chemicals (Gao et al., 2011a,b, 2013a). This work provides important information needed for assessing environmental risks and developing remediation strategies for PAH-contaminated sites.

2. Materials and methods

2.1. Chemicals

Hydroxypropyl-beta-cyclodextrin (HPCD; >99% purity) was purchased from Qianhui Fine Chemical Co., Ltd (Zibo, China). A mild extraction solution was prepared by dissolving 70 mM/L HPCD and 50 g/L NaN_3 in Milli-Q water. Oxalic acid (analytically pure) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Organic solvents used for extraction procedures included analytical-grade dichloromethane, acetone, and *n*-butanol. Aqueous solution of artificial root exudates (AREs) was prepared as previously described (Joner et al., 2002; Sun et al., 2013), consisting citric acid, oxalic acid, malic acid, serine, alanine and fructose in molar concentration ratio of 2:2:2:1:1:4. Total mass concentration of ARE solution was calculated as the sum of the mass concentration of all ARE components.

2.2. Soil preparation

Field soils contaminated with PAHs were collected from the A horizon (0–20 cm) nearby a petrochemical plant located in Nanjing, China. This plant has been operating for more than 30 years. Five soil samples were collected within 0–1000 m from the plant. Soil samples were labelled as Soil 1–Soil 5. Soil 1 was closest, and Soil 5 was farthest to the plant. Soil samples (one kg for each sample) were air-dried and sieved several times through a 2-mm sieve to homogenize the soil samples before use. The soil was a Typic Paleudalf, and a typical zonal soil in Eastern China. Soil pH was 5.87, and soil organic carbon content was 13.6 g/kg. The soil was composed of 26.3% of clay, 13.0% of sand, and 60.7% of silt, and was thus as silt loam according to the USDA classification. The soil water holding capacity was 24.5% (w/w). The initial total concentrations of 16 USEPA priority PAHs in Soil 1, Soil 2, Soil 3, Soil 4 and Soil 5 were 3.88, 3.12, 2.67, 1.85 and 1.28 mg/kg, respectively. The soil samples containing only bound PAH residues were generated as follows (Gao et al., 2015; Sabaté et al., 2006). Briefly, dichloromethane:acetone mixed solvent (1:1, vol/vol) was added to soils at a ratio of 5 mL solvent: 1 g soil. Then the soil was extracted by ultrasonication for 10 min. Afterwards the solvent was decanted, and the soil was extracted again with fresh solvent. The extraction process was repeated six times, and then the soils were air-dried to obtain the test soils containing only bound PAH residues. BRs of 11 PAHs in test soils were detectable according to the method described below, and their general properties are shown in Table 1. PAH free soil was also collected from Nanjing, China with soil pH of 6.02 and soil organic carbon content of 14.3 g/kg. It was composed of 26.3% of clay, 13.0% of sand, and 60.7% of silt.

2.3. Fractionation of PAH residues in soils

The sequential extraction approach previously described (Gao et al., 2009; Sabaté et al., 2006) was employed to fractionate PAHs in soils. PAH residues in soil include three fractions, i.e., desorbing fraction (DF), non-desorbing fraction (NDF), and BR fraction. A mild extraction technique was utilized to extract the DF of PAHs from soils. Three grams of soil sample were placed in a 25-mL glass centrifuge tube, and 15 mL mild extraction solution was added. The tubes were capped with a Teflon-liner cap, shielded from light, and shaken horizontally at 150 r/min and 25 °C. At 60, 120, and 240 h, the soil was separated from aqueous solution by centrifugation for 25 min at 2000 r/min. The supernatant was collected, followed by replenishing with fresh mild extraction solution and a second extraction. The supernatants were combined and then liquid–liquid extracted three times using 10 mL dichloromethane. Dichloromethane solvent was dehydrated by percolation through Na_2SO_4 anhydride, combined, concentrated first by rotary evaporation, then evaporated under a gentle N_2 flow, and finally diluted into 2 mL methanol. After filtration through a 0.22-μm filter, PAHs were detected by a HPLC. The HPLC was

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