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Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene

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ABSTRACT

An available remediation technique — arbuscular mycorrhizal phytoremediation (AMPR) — is further proposed for soils contaminated with phenanthrene and pyrene as representative polycyclic aromatic hydrocarbons (PAHs) utilizing a greenhouse pot experiment. The initial concentrations of phenanthrene and/or pyrene in soils were 103 mg kg^{-1} and 74 mg kg^{-1}, respectively. The host plant was alfalfa (*Medicago sativa* L.), and the experimental arbuscular mycorrhizal fungi (AMF) were *Glomus mosseae* and *G. etunicatum*. More than 98.6% and 88.1% of phenanthrene and pyrene were degraded after 70 days in soils with AMPR. Use of multiple mycorrhizal species significantly promoted degradation of PAHs in soils. The co-contaminant (pyrene) present clearly inhibited the degradation of a single PAH (phenanthrene) in soil. Mycorrhizal colonization caused increased accumulation of PAHs in plant roots but a decrease in shoot. However, plant uptake contributed negligibly to PAH dissipation in AMPR, and plant accumulated PAHs amounted to less than 3.24% of total PAH degradation in mycorrhizal soils. In contrast, the optimized microbiota in mycorrhizal association was responsible for PAH degradation in AMPR. The high rate of PAH dissipation in mycorrhizal soils, the evident promotion of PAH degradation by AM colonization, and the healthy plant growth suggest encouraging opportunities for AMPR of PAH-contaminated soils.

1. Introduction

Soil is a primary natural resource for humans. However, organic pollutants frequently find their way into soils as a result of air deposition, sewage irrigation, and industrial accidents. Soil contamination by organics poses a great threat worldwide to agricultural food quality, the food chain, and eventually to human health. Polycyclic aromatic hydrocarbons (PAHs) are by-products of the incomplete combustion or pyrolysis of organic materials, and are of the main pollutants of concerns in the environment due to their recalcitrance and strong mutagenic/carcinogenic properties [1,2]. The hydrophobic characteristics and persistence of PAHs result in their accumulation and enrichment in soils [3–6]. They are widespread and present at high concentrations of hundreds of mg kg^{-1} in soils of many countries [7,8]. Hence, immediate action is required to remediate such contaminated sites at risk.

Phytoremediation is recognized as one of the most cost-effective, reliable, and promising technology for decontamination of polluted soils [6,9]. The enhanced degradation of organic pollutants is due to plant-stimulated microbial degradation in the rhizosphere [10]. The surface area of root–soil contact and rhizosphere microbial activity are major limiting factors in the phytoremediation process, and are theoretically met by mycorrhizal associations.

Plant–microbe symbioses are ubiquitous in the environment. As the most widespread type of mycorrhizae, arbuscular mycorrhizae (AM) are starting to receive attention in the context of phytoremediation of organic pollutants [10–13]. Recent studies have shown that AM have positive effects on plant establishment and survival in contaminated soils [13,14], suggesting the potential of AM in phytoremediation schemes. To our knowledge, Binet et al. [15] and Joner et al. [10] first reported the effects of inoculating herbaceous plants with AM on degradation of organics in soils. Positive effects of AM inoculation were subsequently observed on degradation of PAHs including phenanthrene, anthracene, chrysene, dibenz(a,h)anthracene, pyrene, and benzopyrene, and the mycorrhiza-associated microbiota was considered to be responsible for the PAH reduction in soil [10,16–18]. Enhanced dissipation of other organic contaminants in soil by the presence of AM has also been reported in literature [19].

However, there are about 170 species of AM and more than 1000 suspected organic pollutants [20]. Only a very limited number of AM and organics have been investigated. More experimental data on arbuscular mycorrhizal phytoremediation (AMPR) are urgently needed to support this technique. In addition, there are still important challenges to be considered in AMPR. Many results reported so

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far are generally concerned with only a single pollutant and/or a single AM. This is not the case in the natural environment. The effects of co-existing AM on phytoremediation of soil containing multiple pollutants require further study. Variation of the microbial community and enzyme in mycorrhizal soils is not well defined. As a result, the mechanisms involved in AMPR still need to be further evaluated and more experimental data are required. Moreover, although plant uptake of organics is negligible in the removal of organics by phytoremediation [3,6], information about PAH distribution in plants is essential to guide the screening of plants used in phytoremediation and to ensure the security of agricultural products grown on contaminated sites. However, little experimental data are hitherto available on the impacts of AM on plant uptake of organics such as PAHs in soil.

Thus the objectives of this work were to investigate the effects of co-existing AM on AMPR for soils with single/multiple PAH pollutants,and the impacts of AM on plant uptake and accumulation of PAHs in soils. The mechanisms of AMPR for PAH-contaminated soils were elucidated.

2. Materials and methods

2.1. Solutes

Phenanthrene and pyrene (purity >98%) were obtained from Aldrich Chemical Co. The molecular weight (M_w, g mol^{-1}), solubility in water at 25 °C (S_w, mg L^{-1}), and log K_{ow} (where K_{ow} denotes the octanol–water partition coefficient) of phenanthrene and pyrene are 178.23 and 202.26 g mol^{-1}, 1.18 and 0.12 mg L^{-1}, 4.46 and 4.88, respectively [21].

2.2. Greenhouse pot experiment

Soil samples were collected from the A (0–20 cm) horizon in Nanjing, China, with a pH of 6.02, 2.41% soil organic matter, and originally free of PAHs. The soil type is Typic Paleudalfs. The clay, silt, and sand contents of the tested soil were 24.7%, 61.9%, and 13.4%, respectively. Soil samples were air-dried and sieved through a 2-mm mesh.

Original inoculum of the AM fungi *Glomus mosseae* (AMF1; BGC GD01A) and *G. etunicatum* (AMF2; BGC HUN02C) was propagated in pot culture on sorghum for 10 weeks in a zeolite-sand mixture in a greenhouse. Then inoculum, a mixture of spores, mycelium, sand and root fragments, was air-dried and sieved (<2 mm).

The plant growth medium was a 3:1 (w/w) mixture of soil and sand (sieved through a 2-mm mesh). The soil mix (henceforth referred to as soil) was sterilized by γ-radiation (10 kGy, 10 MeV γ-rays) to inactivate the native AM fungi. Soil samples were then spiked with a solution of phenanthrene and/or pyrene in acetone (10% of soil to be spiked). After the acetone had evaporated, the spiked soils were progressively mixed with clean soil and homogenized. To ensure homogeneity of the treatments, soils were then sieved again through a 2-mm mesh [22]. The final concentrations of phenanthrene and/or pyrene in treated soils, chosen according to the general concentrations observed in contaminated soil, were 103 mg kg^{-1} and 74 mg kg^{-1} (on a dry weight basis), respectively. The treated soils were then packed into pots (350 g dry weight soil per pot). Mycorrhizal pots were inoculated with 20 g AMF1 and/or AMF2. The non-mycorrhizal controls received an equivalent amount of γ-radiation-sterilized inoculum to provide similar conditions, except for the absence of the active mycorrhizal fungus. All pots were equilibrated in a glass greenhouse to 50% water-holding capacity.

Pre-germinated seeds of alfalfa (*Medicago sativa* L.) were sown in each pot. The seedlings grew in greenhouse at 25–30 °C during daytime and at 20–25 °C during night, and were thinned 7–10 days after emergence, to leave six plants per pot, giving spiked soils with phenanthrene and/or pyrene (a) without AM inoculation, (b) with AMF1 inoculation, (c) with AMF2 inoculation, and (d) with AMF1 and AMF2 inoculation. Each treatment was replicated in triplicate, and the treated pots were arranged randomly in the greenhouse and re-randomized every 4 days. Soils and plants were destructively sampled after 30, 45, 60 and 70 days since sowing. Plant shoots and roots separated from soils were washed with distilled water and then dried with filter paper.

2.3. Sorption experiment

The same pot cultivation system was used to obtain alfalfa roots for sorption experiment without AMF inoculation. The sterilized soils packed in pots were un-spiked and free of PAHs. Roots of alfalfa were collected 60 days after cultivation. A modified glass bead compartment cultivation system was utilized to obtain AM hyphae according to Chen et al. [23] and Gao et al. [20].

A batch experiment was conducted to determine PAH sorption by roots and hyphae according to literatures [20,24]. We mixed 20 ml PAH solution in water containing 0.05% NaN_3 with roots or AM hyphae in 30-ml glass centrifuge tubes sealed with screw caps. The tubes were shaken in the dark for 24 h at 250 rpm on a gyratory shaker to reach the equilibrium state. An aliquot of supernatant was removed and analyzed for PAH concentrations. All equilibrium concentrations of PAH measured in solution were below their aqueous solubilities.

2.4. Determination of root colonization

Root colonization by AM was determined according to Phillips and Hayman [25] and Huang et al. [19]. A 1-g subsample of fresh roots was randomly taken and cut into approximately 1-cm pieces to estimate the proportion of total root length colonized by AM. Root segments were immersed in 10% (v/v) KOH for 10 min at 90 °C in a water bath, rinsed with water, and then stained with 0.1% trypan blue for 3–5 min at 90 °C in a water bath. Mycorrhizal colonization was determined by the grid line intersect method [19,25]. Briefly, the stained root segments were arranged lengthwise on a thin layer of PVA mounted on a microscope slide. A hairline graticule inserted into the eyepiece of a compound microscope acted as a line of intersection with the roots. Fungal structures at each intersection were calculated by observation at 200× magnification.

2.5. PAH analysis of soil, plant, and water samples

The soil from pots was carefully collected, homogenized, and passed through a 20-mesh standard sieve. Sample preparation included mixing the sample with anhydrous Na_2SO_4 to remove moisture and ultrasonicating in 10 ml dichloromethane for 1 h, followed by centrifugation at 3000 × *g* for 20 min. Then, 3 ml of supernatant were filtered through a 2-g silica gel column with a 12 ml 1:1 (v/v) elution of hexane and dichloromethane. The solvent fractions were then evaporated off, and exchanged for methanol, to a final volume of 2 ml [8,24]. The samples were then rotary-evaporated and exchanged for methanol to a final volume of 2 ml for HPLC analysis. Recoveries of known amounts of phenanthrene and pyrene through the complete analytical process amounted to 91.3–95.6% with a relative standard deviation (RSD) of less than 3.1%.

Plant samples were freezing-dried, ground, and homogenized, and extracted by ultrasonication for 1 h in a 1:1 (v/v) solution of acetone and hexane. The solvent was then decanted, collected, and replenished. This process was repeated three times. The solvents were then evaporated and exchanged for 2 ml hexane, followed