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# Effects of dissolved organic matter derived from forest leaf litter on biodegradation of phenanthrene in aqueous phase

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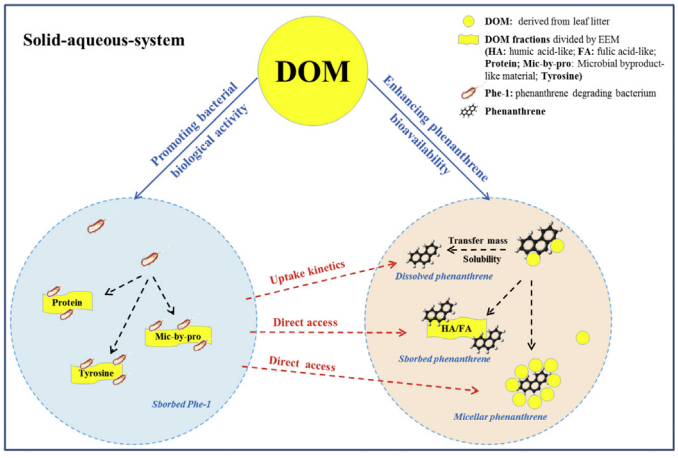
## HIGHLIGHTS

- Phenanthrene was degraded by Phe-1 coexisting with DOM in solid-aqueous system.
- DOM decomposed up to 12 months best enhanced phenanthrene degradation.
- Protein and tyrosine in DOM stimulated biological activity of degrading strain.
- Fulvic acid and humic acid in DOM enhanced the bioavailability of phenanthrene.
- Humic acid co-metabolized by Phe-1 further stimulated phenanthrene degradation.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Dissolved organic matter (DOM) released from forest leaf litter is potentially effective for the degradation of polycyclic aromatic hydrocarbons (PAHs), yet the inherent mechanism remains insufficiently elucidated. In this study, we investigated the effects of DOM derived from *Pinus elliottii* and *Schima superba* leaf litter on the degradation of phenanthrene by the phenanthrene degrading bacterium *Sphingobium* sp. Phe-1. DOM from different origins and at a large range of concentrations enhanced the degradation rate of phenanthrene. DOM derived from *P. elliottii* leaf litter decomposed for 12 months used at a concentration of 100 mg/L yielded the highest degradation rate (16.9% in 36 h) and shortened the degradation time from 48 h to 24 h. Changes in the composition of DOM during degradation as measured by EEMs-FRI showed that proteins and tyrosine in the DOM supplied readily available nutrients that stimulated biological activity of Phe-1, increasing its growth rate and catechol 2,3-dioxygenase activity. Simultaneously, fulvic

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acid and humic acid in the DOM enhanced phenanthrene bioavailability by increasing the solubility and mass transfer of phenanthrene, enhancing the uptake kinetics of Phe-1, and increasing the bacteria's direct access to DOM-associated phenanthrene. Humic acid was co-metabolized by Phe-1, resulting in further stimulation of phenanthrene degradation.



## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants with stable ring structures, poor water solubility, and high octanol-water partition coefficients. PAHs can persist in soils and sediments, where they pose a serious threat to the environment and human health [1,2]. The forest canopy can remove PAHs from the atmosphere by interception, adsorption, and absorption, and subsequently the PAHs can return to the soil via wet or dry deposition (including litter fall) [3,4]. Hence, the soil is the main reservoir for POPs(Persistent Organic Pollutants) including PAHs. It was reported that the yearly fluxes of the PAHs are about 53.3–80 mg/year/$m^2$, of which litterfall and throughfall contribute to only 40 μg/year/$m^2$ and 213 μg/year/$m^2$, respectively [5]. Among the various PAHs, phenanthrene is the dominant one [6], and its concentration in the forest soil is the highest as well [7,8]. Although microbial degradation is the primary way that PAHs are removed from forest soils [2,9,10], this biodegradation occurs to a limited extent in natural environments because of the low bioavailability of PAHs (they have low water solubility and are inclined to adsorb to the soil solid phase) and the low adaptability or activity of microbes [11–13]. Many studies have reported that degradation of PAHs could be enhanced by stimulation of microbial growth through addition of a supplemental carbon source or other microbial nutrients as well as by addition of humic acid (HA), fulvic acid (FA), or a surfactant/biosurfactant to promote the bioavailability of PAHs by enhancing their apparent solubility and diffusive mass transfer [11,12,14,15]. However, much research in this area focus on adding a single material (such as fulvic acid, humic acid, glucose or peptone) to promote microbial growth or enhance the bioavailability of PAHs, which is, more or less, insufficient to elucidate the potentially complex function of exogenous amendments in the natural environment.

Nevertheless, though the effect of the dissolved organic matters (DOM) on PAHs degradation has been studied in some other researches, the interacting mechanisms remain unclear [16–20]. Up to date, the effect of DOM on the degradation of PAHs was mainly explicated by the multiple actions of DOM against both PAHs and their degrading microorganisms [21–23]. On the one hand, DOM can form complexes or micelle with PAHs because of its special structure or performance similar to surfactant, thereby affecting their bioavailability and toxicity [24]. Some authors suggested that addition of DOM decreases the solubility and consequently the bioavailability of PAHs, which seems like a drawback in their degradation [25,26]. However, other researchers concluded that the solubility decrease will not influence the degradation of PAHs because the DOM could bind PAHs and hence increase the diffusive flux of PAHs, making them much more available to bacteria [21–23,27]. By this way, exemplary cases reported by Ortega-Calvo et al. and Haderlein et al. showed that in the presence of HA-clay in liquid culture, phenanthrene was more rapidly mineralized [21,28]. On the other hand, to acquire sufficient nutrients, the bacteria will preferentially adsorb to DOM rather than PAHs, which could favor the growth of microbial strains active in biodegradation, and subsequently induce the expression of intermediate degradation enzymes by these strains [29–31]. But it should be noted, though reported in limited literature, that the DOM will also inhibit the cell adhesion to the pollutant source and inhibit the degradation of PAHs [22]. Nevertheless some other studies proved that contaminants adsorbed to mineral-HA are bioavailable, and sorption of both the bacteria and the PAHs to HA stimulates PAHs degradation [23,28]. From the foregoing, it could be concluded that the influence of DOM on the degradation of PAHs is strongly compound dependent, which is closely related to the binding sites and functioning groups embedded in different compounds of the DOM.

DOM in soil is a mixture or continuum of organic molecules with different structures and molecular weights. In view of its diverse composition, DOM was regarded as part of bio-stimulation strategies to accelerate the biodegradation of PAHs [9,32,33] through its various compounds by influencing either the PAHs bioavailability or the bacterial process. Toyama [34] proved that the phenolic compounds of *Phragmites australis* root exudates acted as a carbon source supporting growth and induced benzo[a]pyrene-degrading activity of the a *Mycobacterium* strain. Yi [35] suggested that linoleic acid was the major substance that stimulated PAHs degradation based on comparison of the chemical compositions of the effective plants. Meng [36] proved that lipophilic extract was the major rhizodeposit enhancing pyrene biodegradation, while water-soluble extract stimulated microbial growth most efficiently. The DOM released from forest leaf litter is an important source of organic carbon in the forest system. However, the mechanisms responsible for these effects are not very clear. For example, which fraction of DOM contributes to the bioavailability of PAHs and/or promote the functioning of the degrading strain, which DOM fractions act on PAHs, and which process come first? How to enhance both the bioavailability of PAHs and the biological activity of degrading bacteria through the use of DOM are questions addressed by the present study, because the best biological stimulation strategy will exploit the synergy between these factors [37].

In the current study, phenanthrene was chosen as a representative PAH (the concentration of phenanthrene in the forest soil is highest among the 16 PAHs in south China [7], our previous study also showed similar result (Fig. S12)), and a bacterial strain (*Sphingobium* sp. Phe-1) with high degradation efficiency was selected by screening bacteria collected from a site highly contaminated by PAHs. DOM was derived from *Pinus elliottii* and *Schima superba* leaf litter that had decomposed in the wild for 0, 2, 5, 8, 12 months. Experiments were conducted to study the effect of DOM on the degradation of phenanthrene by the Phe-1 strain and to better understand the interacting mechanisms in the DOM-PAH-microorganism system. Excitation-emission matrix fluorescence spectroscopy coupled with fluorescence regional integration (EEMs-FRI) was used to determine the composition of DOM before and after degradation of phenanthrene based on analysis of five excitation-emission regions [38].

## 2. Materials and methods

### 2.1. Chemicals

Phenanthrene (TGI, Japan, purity >97%) was obtained from Aldrich Chemical Co. Phenanthrene-d10 and M-terphenyl (O2Si,