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Fate of di (2 ethylhexyl) phthalate in different soils and associated bacterial community changes

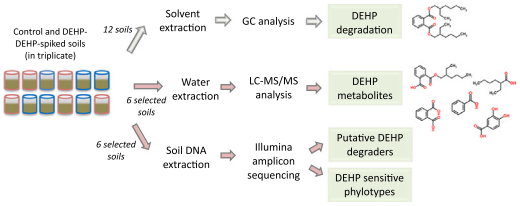
Fengxiao Zhu [a], Changyin Zhu [a,b], Evelyn Doyle [c], Hailong Liu [a,b], Dongmei Zhou [a], Juan Gao [a,*]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Environmental Microbiology Group, School of Biology and Environmental Science and Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland

HIGHLIGHTS

- DEHP degradation positively correlated with bacterial numbers in 12 agricultural soils.
- DEHP metabolites, including mono (2 ethylhexyl) phthalate, 2 ethylhexanoic acid, phthalic acid, protocatechuic acid and benzoic acid, were quantified.
- Similar bacterial groups were enriched in the two soils displaying the greatest DEHP degradation.
- *Tumebacillus* was particularly sensitive to DEHP.

GRAPHICAL ABSTRACT



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ABSTRACT

Di (2 ethylhexyl) phthalate (DEHP) is a ubiquitous organic pollutant, which has caused considerable pollution in arable soils. In this study, the relationship between DEHP degradation potential and soil properties in 12 agricultural soils (S1-S12) was examined in a microcosm based experiment. Six of these soils were then selected to monitor patterns in bacterial community responses. It was found that DEHP degradation was positively correlated with bacterial counts in the original soils, suggesting a key role for bacteria in degradation. However, DEHP metabolism did not always lead to complete degradation. Its monoester metabolite, mono (2 ethylhexyl) phthalate (MEHP), was present at appreciable levels in the two acidic soils (S1 and S2) during the incubation period of 35 days. Based on high-throughput sequencing data, we observed a greater impact of DEHP contamination on bacterial community structure in acidic soils than in the other soils. *Nocardioides*, *Ramlibacter* and unclassified *Sphingomonadaceae* were enriched in the two near-neutral soils where degradation was highest (S4 and S7), suggesting that these organisms might be efficient degraders. The relative abundance of *Tumibacillus* was greatly reduced in 50% of the six soils examined, demonstrating a high sensitivity to DEHP contamination. Furthermore, putative organic-matter decomposing bacteria (including *Tumebacillus* and other bacteria taxa such as members from *Micromonosporaceae*) were greatly reduced in the two acidic soils (S1 and S2), possibly due to the accumulation of MEHP. These results suggest a crucial role of soil acidity in determining the fate and impact of DEHP in soil ecosystems, which deserves further investigation. This work contributes to a better understanding of the environmental behavior of DEHP in soil and should facilitate the development of appropriate remediation technologies.

* Corresponding author.
E-mail address: juangao@issas.ac.cn (J. Gao).

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1. Introduction

The occurrence of phthalate esters (PAEs) in the environment has received much attention in recent years. PAEs are primarily manufactured and used as plasticizers to improve the flexibility and durability of plastic products. Phthalate plasticizers are by far the most commonly used plasticizer, accounting for 70% of world consumption in 2014 (Malveda, 2015). PAEs are also used as solvents, lubricants or fixatives in a large variety of commodities, such as pesticides, detergents, cosmetics and pharmaceuticals (Lyche et al., 2009). Due to their broad use, PAE contamination has been detected in almost all environmental media, including lake water, marine water, sediment, soil, food and human beings. PAEs are of great concern because exposure to PAEs may cause adverse health effects on humans and the wildlife, by interfering with male and female reproductive systems or by inducing various cancers (Caldwell, 2012; Foster et al., 2001; López-Carrillo et al., 2010). PAEs have also been reported to impact crop quality, soil enzyme activities, and soil invertebrates such as earthworms (Chen et al., 2004; Yin et al., 2003).

Di (2 ethylhexyl) phthalate (DEHP) is the most commonly used phthalate plasticizer because of its low cost and high performance, with some plastics containing as much as 40% of this compound (Cao, 2010). Consequently, DEHP is a widely distributed contaminant in soil. Chen et al. (2017) reported that DEHP was detected in all 111 soil and 128 vegetable samples collected from greenhouses and open fields of 10 cities in China. Greenhouses with a longer period use of plastic films tended to contain higher levels of soil DEHP. In another study, DEHP was found to be the most abundant PAE component in 123 arable soils across Mainland China, with soil concentrations ranging from 0.82 to 6.22 mg kg^{-1} (Niu et al., 2014). Much higher levels of contamination have also been documented. For example, Zeng et al. (2008) reported that DEHP concentrations in the peri-urban agricultural fields of Guangzhou city (China), ranged from 0.107 to 29.37 mg kg^{-1}. Guo and Wu (2011) reported that DEHP concentrations in the cotton soils of Xinjiang (China) reached 149.0 mg kg^{-1}. In these studies, the application of agricultural plastic films and activities for soil fertility were proposed to be major sources of PAE (Chen et al., 2017). The particularly high content of PAEs in cotton soils was possibly due to heavy utilization of pesticides. In addition, soils from phthalate manufacturing factories are likely to contain high levels of PAEs (although relevant data is scarce). DEHP concentrations as high as 1458 mg kg^{-1} have been reported for a factory in Mexico (Ferreira and Morita, 2012). Overall, soil contamination by DEHP is ubiquitous and more efforts are needed to evaluate the environmental fate of this compound and accelerate its dissipation in contaminated lands.

Microbial metabolism has frequently been reported to be the main mechanism of PAE degradation in natural soils or sediments (Peterson and Staples, 2003), whereas acid or alkaline hydrolysis occurs very slowly (Wolfe et al., 1980). Research has shown that microbial metabolism of DEHP in soil is generally much slower than other PAEs containing shorter alkyl-chains; with only 10–40% removal after an incubation time over 30 d (Cartwright et al., 2000; Chen et al., 1997; Zhu et al., 2018). However, higher levels of degradation (>60%) have also been observed (Shanker et al., 1985; Wang et al., 2009).

Although soil characteristics such as soil acidity, nutrition status, microbial biomass and/or composition and even history of prior exposure, are known to affect the degradation of organic compounds (Anderson, 1984; Dyson et al., 2002; García-Delgado et al., 2015; Mauffret et al., 2017), it is not clear which of these impact degradation of DEHP to the greatest extent. Furthermore, the degradation of DEHP may generate metabolites more toxic than their parent compound (Horn et al., 2004). For example, the DEHP metabolite, 2 ethylhexanoic acid (2-EHA) has been shown to be more teratogenic than DEHP (Ritter et al., 1987) and a sludge obtained from a wastewater treatment plant was reported to contain significant concentrations of both DEHP (31.4 mg kg^{-1}) and 2-EHA (28.8 mg kg^{-1}) (Pham et al., 2011). Few studies to-date have quantified the metabolic intermediates generated in DEHP-contaminated soils (Shanker et al., 1985; Schmitzer et al., 1988) and there is limited information about which microbial groups have a higher capacity for DEHP degradation under environmental conditions, and which one are more susceptible to DEHP exposure (Wang et al., 2017). Such information is crucial for assessing the overall risk of DEHP to soil ecosystems and for designing remediation strategies.

The present study aimed to address these knowledge gaps by investigating the degradation of DEHP in a range of soils. The specific objectives were: 1) to identify the correlation between DEHP degradation capacity and key soil properties; 2) to examine the metabolites generated at early and late stages of degradation in different soils; 3) to identify specific microbial groups associated with DEHP degradation in different soils.

2. Materials and methods

2.1. Chemicals and soils

Analytical grade DEHP (purity ≥99.5%) and its metabolites MEHP (97%), 2-EHA (≥99%) and phthalic acid (PA, ≥99.5%) were purchased from Sigma-Aldrich (USA), while other metabolites including protocatechuic acid (PCA, 97%) and benzoic acid (BA, ≥99.5%) were purchased from Sinopharm Group Co., Ltd. (China). The chemical structure and physico-chemical properties of these compounds are shown in Table S1 in Supplementary data. Organic solvents used in this study including acetone, hexane and methanol were at analytical or HPLC grade.

Twelve top-20 cm soil samples (S1-S12) were obtained from different agricultural fields in China. Soils were selected mainly on their acidity so that the effect of a range of pHs from 5.0–9.0 on DEHP degradation could be assessed. All soils had low (<1 mg kg^{-1}) background levels of DEHP. Soil samples were air-dried, passed through a 2 mm sieve after removing root, plant residue and stones. Table 1 shows the selected physicochemical and microbiological properties of these soils. Soil pH was determined in 1:2.5 soil/deionized water suspensions. Soil organic carbon (SOC) was determined by wet digestion with a mixture of $K_2Cr_2O_7$ and concentrated H_2SO_4 (Nelson and Sommers, 1996). Total soil nitrogen (TN) was measured by the semi-micro Kjeldahl method and total soil phosphorus (TP) was determined colorimetrically after digestion with acids H_2SO_4 and $HClO_4$ (Fu et al., 1999). Enumeration of bacterial and fungal populations was carried out by spreading soil dilutions on nutrient agar and potato dextrose agar plates, respectively (Vargas Gil et al., 2009).

2.2. Experimental setup

Microcosm experiments were conducted in triplicate in 40 mL brown glass bottles containing 20 g of each of the 12 soils (dry weight). Soils were amended with DEHP (using acetone as solvent carrier) to give an initial concentration of 200 mg kg^{-1} to allow the impact of high levels of the pollutant be assessed. Microcosms containing each of the 12 soils to which the same amount of acetone but no DEHP was added were included as controls to take account of any effects of the solvent. In addition, abiotic controls containing soils that had been autoclaved at 121 °C for 30 min for three consecutive days and DEHP (200 mg kg^{-1}) were included to assess the role of microorganisms in DEHP degradation. After homogenously mixing the samples, all soils were left in the fume hood for 2 h to evaporate the solvent. The water content was then adjusted to 55% water-holding capacity using deionized water, and the top of the bottles covered with a piece of aluminum foil with five tiny holes.

The microcosms were incubated in an incubator at a temperature of 25 ± 1 °C in the dark. Soil moisture content was kept constant by adding deionized water every 5 days. Samples were sacrificed after 0, 7 and 35 days and then freeze-dried and stored at −20 °C until analysis.