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A short-term study on the interaction of bacteria, fungi and endosulfan in soil microcosm

HuiJun Xie [a], FuWei Gao [b], Wei Tan [a], Shu-Guang Wang [b,*]

[a] Environment Research Institute, Shandong University, Jinan 250100, China
[b] Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan 250100, China

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ABSTRACT

Endosulfan is one of the few organic chlorine insecticides still in use today in many developing countries. It has medium toxicity for fish and aquatic invertebrates. In this study, we added different concentrations of endosulfan to a series of soil samples collected from Baihua Park in Jinan, Shandong Province, China. Interactions of exogenous endosulfan, bacteria and fungi were analyzed by monitoring the changes in microbe-specific phospholipid fatty acids (PLFA), residual endosulfan and its metabolites which include; endosulfan sulfate, endosulfan lactone and endosulfan diol during a 9 days incubation period. Our results showed that endosulfan reduced fungi biomass by 47% on average after 9 days, while bacteria biomass increased 76% on average. In addition, we found that endosulfan degraded 8.62% in natural soil (NE), 5.51% in strepolin soil (SSE) and 2.47% in sterile soil (SE). Further analysis of the endosulfan metabolites in NE and SSE, revealed that the amount of endosulfan sulfate (ES) significantly increased and that of endosulfan lactone (EL) slightly decreased in both samples after 9 days. However, that of endosulfan diol (ED) increased in NE and decreased in SSE. After collective analysis our data demonstrated that fungi and bacteria responded differently to exogeous endosulfan, in a way that could promote the formation of endosulfan diol during endosulfan degradation.

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1. Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) is a water-insoluble organochlorine-based pesticide widely used as a broad spectrum insecticide worldwide on cotton, tea, sugarcane, vegetables, and fruit crops (U.S. EPA, 2002). China exported over 2400 tons of endosulfan during 2007–2008, preceded only by India and Germany (UNEP/POPS/POPRC.5/3, 2009). Although endosulfan is a potent pesticide in crop protection systems, it is a potential contaminate of soil, waterways and air (Jia et al., 2010; Chakraborty et al., 2010).

Endosulfan in soil can be degraded either by photodegradation or biodegradation, but it has been demonstrated that the latter is the primary pathway for removal of endosulfan from natural soil. Many kinds of microbes, such as, *Pseudomonas sp.* KS-2P, *Aspergillus* sp. (ElZorgani and Omer, 1974; Katayama and Matsumura, 1993; Kim et al., 2001) *Trichoderma harzianum* (Kullman and Matsumura, 1996), *Phanerochaete chrysosporium* (Kumar and Philip, 2006; Lee et al., 1995) and *Cladosporium oxysporum* (Mukherjee and Mittal, 2005) contribute to the process (Ang et al., 2005). Endosulfan is first metabolized to endosulfan sulfate or endosulfan diol, followed by further degradation from endosulfan diol to endosulfan ether (EE) and endosulfan lactone (UNEP/POPS/POPRC.5/3, 2009). Endosulfan diol is described as a non-toxic metabolite formed through the hydrolytic pathway of biodegradation (Hussain et al., 2007) whereas endosulfan sulfate is a toxic formed by an oxidative pathway by biodegradation (Kim et al., 2001; Hussain et al., 2007). It was reported that some bacterial species could completely utilize the hydrolytic pathway (Hussain et al., 2007), and some fungal species could partly utilize the pathway (Verma et al., 2006).

Fungi and bacteria are the drivers of many major soil processes such as carbon and nutrient cycling (Milne and Haynes, 2004), therefore, the diversity and activity of fungi and bacteria reflect the health condition of the soil. Although a number of reports have shown little or insignificant effects of pesticides and insecticides on microbial activities in planted soil (Jha and Mishra, 2005), there have also been investigations reporting an inhibitory effects (Jha and Mishra, 2005). Intriguingly, Joseph et al. (2010) observed that endosulfan had a short-lived inhibitory effect on fungi but increased the growth of bacteria. The reason for this might lie in the fact that endosulfan, bacteria and fungi in soil could reach a balance in a short time and then coexist for a long time.

In this study, we determine if the microbial populations could be affected by endosulfan in different soil types, and the roles of different microbes in endosulfan degradation were categorized and broken down.

* Corresponding author. Tel.: +86 53188362802; fax: +86 531 88364513.
E-mail address: xiehuij@sdu.edu.cn (S.-G. Wang).

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2. Materials and methods

2.1. Soil samples

Soil from Baihua Park in Jinan, Shandong Province, China, was collected at a depth of 5–15 cm, a region containing the greatest microbial activity in breaking down pesticides (Jha and Mishra, 2005). The samples were mixed, crushed and homogenized by sieving them through a 2 mm mesh screen to remove roots and large particles. The mixed soil was acclimated at 28 °C for 2 days prior to physicochemical analysis. As a form of typical east Chinese earth, the basic characteristics of the soil were: a silt loam texture, pH = 8.0, total carbon content 2.43 g kg^{-1} dry soil, total nitrogen content 0.69 g kg^{-1} dry soil, total phosphate content 0.22 g kg^{-1} dry soil, and cation exchange capacity (CEC) 21.03 cmol kg^{-1} dry soil.

2.2. Microcosm set up

The design included three treatments; high pressure steam sterilization (SE), streptomycin sulfate-treated (SSE) and natural (NE) soil samples with nine replications of each treatment. Homogenized soil was separated into 1000 g, 27 lots and loaded into a 5-liter glass column with the moisture content adjusted to 45%. In addition, all the SE, SSE and NE groups were supplemented with four different concentrations of endosulfan (0, 3, 6, 12 mg kg^{-1} soil) with the α/β isomers ratio of 7:3 (n = 3). Sterile groups were used to evaluate the effects of abiotic degradation of endosulfan, whereas streptomycin sulfate-added groups (SSE) were used to evaluate endosulfan degradation by fungi. The microcosms were kept in the dark at 27 °C and the moisture content was kept at 45% by supplying water every two days for a month. Every 3 days 50 g of soil was collected from each group and stored at −20 °C for further analysis.

2.3. Chemicals

All pesticide reference standards and metabolites were purchased from Sigma (USA). All chemicals including anhydrous sodium sulfate, hexane, acetone and methanol were nanograde or HPLC grade.

2.4. Phospholipid fatty acid (PLFA) analyses

The 0, 3, 6 and 9 days samples were used for PLFA analysis. Total lipid fractions were extracted according to the procedure previously described by Zhang (Zhang et al., 2008). In short, the microbial pellet was transferred to a glass tube with a Teflon-lined screw cap. Lipids were then extracted in triplicate from samples by a one-phase chloroform, methanol and 0.15 M citrate buffer extractant solution. The total lipid extract was then fractioned into neutral, glyco- and polar (phospho-) lipids by silicic acid chromatography. Polar phospholipids were then converted to fatty acid methyl esters using a mild alkaline methanolysis reaction. An internal standard, methyl ester of nonadecanoic acid (20:0) was added and these were dissolved in hexane for chromatographic analysis. Fatty acid methyl esters (FAMEs) were analyzed by a GC-MS system (Hewlett Packard HP 6890) equipped with a HP-5 capillary column (60 m × 0.32 mm). The total quantity (nmol g^{-1}) was assessed using the methyl ester of nonadecanoic acid as an internal standard.

In this study, we chose the fatty acid nomenclature as previously described by Frostegård et al. (1993). Fatty acids with carbon lengths of 14 to 22 were used to analyze microbial community structure. The isomers commence with an “i”, cycle structures are marked “cy”, and the ω after the colon stands for a double bond. The fatty acids a15:0, i15:0, i16:0, i17:0, a17:0, 16:1ω, 17:0, cy17:0, and 18:1ω were used to represent our bacterial PLFAs (Chinalia and Killham, 2006) where a15:0, i15:0, i16:0, i17:0 and a17:0 were used to represent Gram-positive bacteria (Ratledge et al., 1988), 16:1ω, cy17:0 and 18:1ω to represent Gram-negative bacteria (Zogg et al., 1997), and unsaturated PLFA 18:2ω6,9 to represent fungi (Johansen and Olsson, 2005).

We analyzed fatty acids of 14 to 22 carbons to monitor microbial population change. Principal-component analyses (PCA) were performed using SPSS 11.5 software (version 13.0, SPSS Inc.) to compare the PLFA profiles among the samples.

2.5. Quantification of endosulfan

20 g examples were weighted exactly and endosulfan and its metabolites were extracted by Soxhlet extraction. A Hewlett Packard 5890 series gas chromatograph equipped with Ni (550 MBq) ECD electron capture detector was used to analyze them. The auto injector injected one microlitre volume of each hexane extract. ADB 17 fused silica capillary column with 30 m in length, 0.32 mm in internal diameter and 0.25 μm in film thickness was fitted and a temperature program (55 °C for one min 180 °C at 40 min 240 °C at 20 min final time) was used. Nitrogen was used as the carrier gas at a column head pressure of 24 kPa giving a linear carrier flow of 4 cm with nitrogen as the detector makeup gas (2 mL min^{-1}). The injector's temperature was 250 °C and detector temperature was 280 °C. The α endosulfan eluted at 18.05 min, β endosulfan at 22.77 min endosulfan diol at 12.5 min, endosulfan lactone at 21.3 min and endosulfan sulfate at 28.50 min. All the metabolites were detected within 45 min. The chromatograms were recorded and integrated using a Hewlett Packard computer and Chemstation software. External standards were used to quantify the concentrations of the metabolites. All the data analyzed with statistical analysis was calculated using Microsoft Excel, and $p<0.05$ was taken as no significant difference.

3. Result

3.1. Effects of endosulfan on microbial microcosm

As shown in Fig. 1, we found that the total amount of PLFA in NE groups showed minor change over time. Without endosulfan (NE-0), the concentration of total PLFA slightly increased from 153.71 to 162.92 mg kg^{-1} after 9 d. In contrast with NE-12, the high endosulfan concentration, PLFA decreased from 182 to 139 mg kg^{-1} after 9 d.

Further analysis showed that PLFA in fungal NE groups decreased in 9 days. Without endosulfan NE-0, PLFA production dropped from 5.78 to 3.31 mg kg^{-1}. With the increasing concentrations of endosulfan, fungal PLFA proportionally decreased, with the lowest level (2.17 mg kg^{-1}) seen in the NE-12 group.

On the other hand, the bacterial PLFA showed an apparent increase after 9 days. In the bacteria NE-0 group, the PLFA nearly doubled in concentration rising from 12.74 to 25.11 mg kg^{-1}. Interestingly, low concentration of endosulfan (bacteria NE-3 group) promoted the growth of bacteria where PLFA reached its highest level at 28.45 mg kg^{-1}. However, when more endosulfan was supplemented (bacteria NE-6 and bacteria NE-12), such promotion begun to reverse, indicating an inhibitory effect of bacterial growth by high concentrations of endosulfan.

The diversity index for SSE and NE in various endosulfan conditions is shown in Fig. 2. The diversity index at 0, 3, 6 and 9 days ranged from 1.52×10^{-4} (0 day for SSE-0) to 39.43×10^{-4} (6 days for NE-12). Generally the diversity index of NE group was a little higher than that of SSE group. However, there was no obvious trend over time or endosulfan concentration.

3.2. The degradation of endosulfan

As shown in Fig. 3, after 9-day, we found that the percentage of endosulfan degradation in NE were 8.4% on average, which was better than 6.4% in SE and 2.7% in SSE, and these percentages wavered little among different concentration groups.

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