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Microbial degradation of alpha-cypermethrin in soil by compound-specific stable isotope analysis

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HIGHLIGHTS

- Alpha-cypermethrin (α-CP) can be degraded by microorganisms in soil.
- Biodegradation of α-CP resulted in carbon isotope fractionation.
- A relationship was found between carbon isotope ratios and concentrations of α-CP.
- An enrichment factor ϵ of α-CP was determined as −1.87‰.
- CSIA is applicable to assess biodegradation of α-CP.

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ABSTRACT

To assess microbial degradation of alpha-cypermethrin in soil, attenuation of alpha-cypermethrin was investigated by compound-specific stable isotope analysis. The variations of the residual concentrations and stable carbon isotope ratios of alpha-cypermethrin were detected in unsterilized and sterilized soils spiked with alpha-cypermethrin. After an 80 days' incubation, the concentrations of alpha-cypermethrin decreased to 0.47 and 3.41 mg/kg in the unsterilized soils spiked with 2 and 10 mg/kg, while those decreased to 1.43 and 6.61 mg/kg in the sterilized soils. Meanwhile, the carbon isotope ratios shifted to -29.14 ± 0.22‰ and -29.86 ± 0.33‰ in the unsterilized soils spiked with 2 and 10 mg/kg, respectively. The results revealed that microbial degradation contributed to the attenuation of alpha-cypermethrin and induced the carbon isotope fractionation. In order to quantitatively assess microbial degradation, a relationship between carbon isotope ratios and residual concentrations of alpha-cypermethrin was established according to Rayleigh equation. An enrichment factor, $\epsilon = -1.87$‰ was obtained, which can be employed to assess microbial degradation of alpha-cypermethrin. The significant carbon isotope fractionation during microbial degradation suggests that CSIA is a proper approach to qualitatively detect and quantitatively assess the biodegradation during attenuation process of alpha-cypermethrin in the field.

1. Introduction

With the phasing out of organochlorine pesticides, synthetic pyrethroids have taken over a large share of the market as an ideal substitute with low persistence and mammalian toxicity [1]. Cypermethrin, a broad-spectrum pyrethroid insecticide, has been extensively used to control insects and diseases for both agricultural and domestic purposes [2]. After agricultural application, however, cypermethrin is adsorbed on the surface of soils and then flushed into rivers and lakes [3,4]. Cypermethrin residue has been detected in vegetables and fruits [5,6] as well as various environmental media, such as soils, waters, and sediments [7,8].

Cypermethrin has raised increasing concern for its adverse effects to non-targeted organisms, especially to aquatic organisms due to their weak abilities to metabolize and eliminate

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cypermethrin [9]. Acute toxicity of cypermethrin to fishes [3,10] and other aquatic organisms [11,12] has been reported in a considerable amount of papers. In addition, studies also indicated that cypermethrin could lead to adverse outcomes on mammals, such as neurotoxicity, endocrine disruption, reproductive toxicity as well as genotoxicity [13–17].

The attenuation of cypermethrin and its isomers has been well studied in previous researches [18–21]. The half-lives were reported to vary from 4.1 days to as long as 12 weeks in different kinds of soils [22–24]. In natural soils, hydrolysis at the ester linkage is a major degradative route of cypermethrin, which leads to the formation of 3-phenoxy phenyl hydroxyacetonitrile and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate [25,26]. The intermediate metabolite 3-phenoxy phenyl hydroxyacetonitrile is then oxidized to 3-phenoxybenzaldehyde and further to 3-phenoxybenzoic acid due to its unstability [27,28]. Additionally, microbial degradation is also believed to be a main and most effective way contributing to the attenuation of cypermethrin in natural environment [20,21]. In previous studies, biological metabolisms by single strain [21,27], cooperation of two strains [20,29], and soil microorganisms as a mixture [24] were reported to significantly accelerate the degradation of cypermethrin. So, microbial degradation is usually employed to indicate the attenuation of cypermethrin in soils. However, traditional approach to quantify the biodegradation of organic pollutants is conventionally based on concentration data. With the existence of abiotic processes including photolysis and hydrolysis as well as some physical processes, such as violation and dilution, biodegradation is inevitably overestimated.

Compound-specific stable isotope analysis (CSIA) has been developed to qualify and quantify biodegradation of organic compounds since the late 1990s [30–32]. It is now well known that, in most of cases, biodegradation of organic compounds leads to enrichment of heavier isotopes (e.g., ^{13}C) rather than abiotic degradation or physical processes [31,33]. Biodegradation assessment by CSIA is considered to be more in accordance with the reality. Therefore, CSIA is gradually employed for in situ biodegradation assessment of organic pollutants [34–36]. In recent years, CSIA has also been successfully applied to some pesticides, such as atrazine [37], isoproturon [38], lindane [39], phenoxy acids [40], and organophosphorus pesticides [41]. However, to our best knowledge, application of CSIA to assess biodegradation of cypermethrin has not been reported yet.

In this study, we explored the microbial degradation of alpha-cypermethrin which is a widely-used category of pyrethroid in soil by CISA. Our study aims to reveal the stable carbon isotope fractionation during biodegradation of alpha-cypermethrin, and for further purpose, to give a perspective into the application of CSIA for biodegradation assessment of pyrethroids.

2. Materials and methods

2.1. Reagents and materials

Alpha-cypermethrin standard (99.7% purity) was purchased from Sigma–Aldrich Inc., USA. Hexane (P.R.A. Grade, 99.9%) and acetone (ultra res-analyzed, ≥99.4%) were obtained from Mreda technology Inc., USA and Mallinckrodt Baker Inc., USA, respectively. Florisil (150–250 μm, Sigma–Aldrich Inc., USA), silica gel (60–200 μm, Acros Inc., Belgium), and anhydrous Na_2SO_4 (Enox Inc., China) were used for extract clean-up and activated prior to use. Alpha-cypermethrin was dissolved in hexane at 100 mg/L as stock solution and 10 and 50 mg/L as work solution.

Soil samples (0–10 cm deep) were collected from a farmland in Quzhou city, Zhejiang Province, China. The pH, water content, and organic matter (by dry weight) were determined as 4.77, 72%, and 1.12%, respectively. The soils were air-dried and passed through a 2-mm sieve before use.

2.2. Experimental control settings

Five gram dried soil was added into each flask (50 mL) of a batch of flasks. Distilled water was added into the flask to reach to 60% of moisture. To activate the soil microorganisms, these flasks were incubated in an incubator at 30 °C for 2 weeks. Another batch of flasks with the same soil sample were sterilized by high pressure steam sterilization at 121 °C for three times (each for 30 min) to inactive the soil microorganisms. After that, 1 mL of 10 or 50 mg/L of alpha-cypermethrin was added into the unsterilized or sterilized soil samples to reach a concentration of 2 or 10 mg/kg, respectively. The flasks were shook while adding of work solution so as to mix better and evaporate hexane to minimize the effects of solvent. After adjusted to 60% of moisture with distilled water, all of the flasks were sealed with rubber plugs and placed in an incubator out of direct sunlight at 30 °C. Three flasks for each concentration (including control) of unsterilized and sterilized soils were taken out to analyze the residual concentration and $\delta^{13}C$ composition of alpha-cypermethrin every 10 days.

2.3. Extraction and cleanup

The soil sample in each flask was mixed with anhydrous sodium sulfate and extracted with 160 mL hexane/acetone (v/v, 7:1) by Soxhlet extractor for 24 h. After concentrated to about 2 mL on a rotary evaporator, the extract was passed through a column packed with (from bottom to top) 2 g anhydrous sodium sulfate, 5 g florisil, 3.5 g silica gel, and 2 g anhydrous sodium sulfate for purification. The column was eluted with 100 mL hexane/acetone (v/v, 9:1). The eluent was then concentrated to nearly dryness and redissolved in 1 mL hexane for GC–MS analysis. After GC–MS analysis, the extract was reconcentrated to nearly dryness and redissolved in 50 μL nonane for GC–C–IRMS analysis.

2.4. GC–MS analysis

Concentration analysis of alpha-cypermethrin was performed on an Agilent 7890A gas chromatography coupled with an Agilent 5975C mass detector operated in positive electron ionization mode (70 eV). An HP-5ms column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent J&W GC columns) was used for the separation. Helium (purity ≥99.999%) was employed as a carrier gas with a flow rate of 1.2 mL/min. A volume of 1 μL extract was injected in splitless mode at 260 °C. The oven temperature program was initially 100 °C, held for 4 min, ramped to 200 °C at 25 °C/min, held for 10 min, then to 270 °C at 10 °C/min, held for 5 min, and finally to 280 °C at 20 °C/min, held for 5 min. The MS was operated in selected ion monitoring (SIM) mode, using the characteristic fragment ions (m/z = 163, 181, and 209) for alpha-cypermethrin analysis.

2.5. GC–C–IRMS analysis

Analysis of carbon isotope composition was performed with a GC–C–IRMS system which consisted of an Agilent 7890A gas chromatography coupled to a GV Isoprime IRMS via a modified GC5 combustion interface. An HP-5ms column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent J&W GC columns) was equipped in the gas chromatography. Helium (purity ≥99.999%) was used as a carrier gas with a flow rate of 1.2 mL/min. An aliquot of 2 μL of sample was injected in splitless mode at 260 °C. The oven temperature program was identical to that used in GC–MS analysis (see above). All samples were measured in three replicates and