Non-extractable residues formed during biodegradation of 2,4-D in soil are biogenic

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Abstract

During degradation of organic pollutants, non-extractable residues (NER) are formed. NER are believed to be composed of parent compounds or primary metabolites with hazardous potential. However, some part of NER may be biogenic and contain harmless microbial biomass components, which are stabilised in soil organic matter. We investigated the formation of biogenic residues from biodegradation of $^{13}C_6$-2,4-dichlorophenoxyacetic acid in soil during a 64-day incubation. The results showed that at the end biogenic components accounted for virtually all the NER. Therefore, in the case of biodegradable organic contaminants, the environmental and toxicological risks related to NER formation may be overestimated.

Introduction

Biodegradation of organic contaminants in soils results in the formation of mineralisation products (CO_2 + H_2O), metabolites, microbial biomass and non-extractable residues (NER). Studies on NER formation are limited to simple systems containing the target compound and humic acids [1] and to quantitative analyses using ^{14}C tracers after extraction with polar and/or non-polar solvents in soils [2]. The chemical composition of NER thus remains unclear. Due to the lack of this knowledge, it is assumed that NER consist of largely unaltered molecules and are formed by various physical and chemical interactions between the compound and soil organic matter (SOM, [2]). They may be remobilised and thus pose an environmental and toxicological risk [3].

During biodegradation of natural organic compounds in soil biogenic residues are formed [4]. The carbon from organic compounds is used by the microorganisms for synthesis of their biomass compounds such as fatty acids (FA) and amino acids (AA). After cell lysis, these components are incorporated into non-living SOM and stabilised ultimately forming biogenic residues. However, biogenic residue formation from biodegradation of organic contaminants in soil has not been considered yet. Biogenic residues thus could be included in the NER fraction in radio-isotope mass balance studies. This may result in an overestimation of pollutant-derived NER, and thus of the environmental risk associated with them. We investigated biogenic residue formation during biodegradation of the most commonly used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in soil and traced their fate during 64 days of incubation.

Materials and Methods

A Haplic Chernozem was taken from the agricultural long-term experiment “Statischer Düngungsversuch” located in Bad Lauchstädt (Germany). Labelled $^{13}C_6$-2,4-D (Alsachim, Illkirch, France) was added to the soil yielding a final concentration of 20 mg/kg soil [5]. Incubataion experiments were performed according to the OECD guideline 307 [6] in the dark and at constant temperature (20°C) for 64 days. The soil systems were sampled after 2, 4, 8, 16, 32 and 64 days of incubation and analysed for the amount and the isotopic composition of AA in two fractions: in the living biomass fraction (bioAA) and in the total fraction of SOM (tAA).

AA from proteins in the total SOM were hydrolysed with 6 M HCl for 22 hours at 110°C according to Miltner et al. [7]. After hydrolysis, samples were filtered through glass-fibre membrane filters, evaporated and purified over cation exchange resin [8]. After purification, the carboxyl groups of AA were esterified with a mixture of isopropanol/acetyl chloride and the amino groups were trifluoroacetylated with a mixture of dichloromethane/trifluoroacetic acid anhydride [7]. After derivatisation, the samples were dissolved in chloroform and the remaining impurities extracted into phosphate buffer [9]. For the determination of the AA in the living biomass, the biomass was first extracted from the soil with 1 g Chelex 100 and sodium deoxycholate/polyethylenglycol solution [7]. Biomass pellets containing AA were further hydrolysed, purified and derivatised as AA in the total SOM.

The AA in both fractions were identified and quantified after separation on a BPX-5 column by means of gas chromatography-mass spectrometry; their isotopic composition was determined by gas chromatography combustion-isotope ratio mass spectrometry [5].

Results and Discussion

This study provides the first evidence for a significant contribution of microbial residues to NER formation from $^{13}C_6$-2,4-D. The ^{13}C-label was detected in AA in both the living (bioAA) and the total SOM (tAA) which clearly indicated a

biogenic origin of NER [5]. The difference between the total and the biomass AA fractions represents the refractory AA in the non-living SOM and thus provides information about the stabilisation of biogenic residues.

Since $^{13}C_6$-2,4-D was biodegraded rapidly, incorporation of the ^{13}C-label into AA in the living fraction was fast (after 4 days). The amounts of ^{13}C-AA in the living SOM were highest on days 4 and 8 (2.2 and 2.5% of $^{13}C_6$-2,4-D equivalents, respectively; see Figure 1).

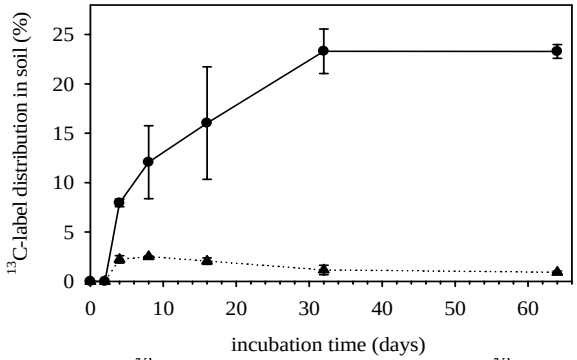

Figure 1. ^{13}C-label incorporation during $^{13}C_6$-2,4-D biodegradation in soil in tAA (●) and bioAA (▲) [5]

Thereafter, their contents decreased continuously until the end of the incubation, and the label was incorporated to the non-living SOM pool. From day 32 to 64, ^{13}C-AA in the living and in the non-living SOM were surprisingly stable, demonstrating a rapid stabilisation of the proteins from the decaying microbial biomass [5]. At the end, the amount of ^{13}C in total AA (nonliving SOM + bioAA fraction) was high, reaching 22% of the initially added $^{13}C_6$-2,4-D equivalents. The proteins containing AA are the most abundant components (~ 50% of dry mass) of bacterial cells [5]. Therefore, the total content of biogenic residues derived from this ^{13}C-labelled pesticide could be determined by correcting the amount of 13C found in total AA in SOM (22% of the initially added 13C6-2,4-D equivalents) considering the general abundance of proteins in microbial cells (conversion factor of two). At the end of the incubation nearly all of the $^{13}C_6$-2,4-D-derived NER (44% of $^{13}C_6$-2,4-D equivalents) could be explained by microbial compounds (Figure 2).

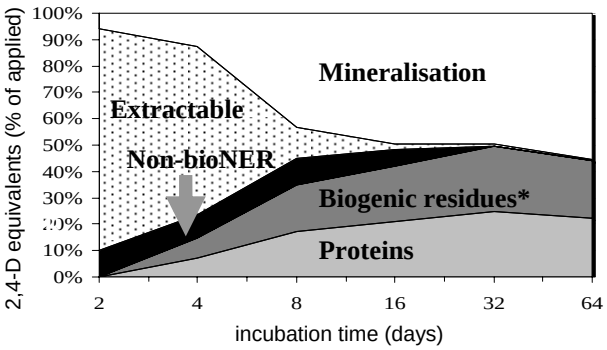

Figure 2. Mass balance including biogenic residue formation during microbial degradation of $^{13}C_6$-2,4 D in soil [5]. *The biogenic residues were estimated from AA by using a conversion factor of 2.

Conclusions

The results show that microbial biomass is a source for NER formation during biodegradation of $^{13}C_6$-2,4-D in soil: cellular components (e.g. AA) carrying the isotope label are incorporated into SOM. The fact that the amount of NER can be almost completely explained by biogenic components contradicts the generally accepted view that NER mainly consist of the parent compounds or their metabolites [2]. Biogenic residue in soil contain only microbial components, therefore, the toxicological risk related to NER formation from readily biodegradable pollutants may be overestimated. The estimation of a proper mass balance and proper risk assessment of a contaminant in soil thus requires considering a possible biogenic origin of NER.

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