Science of the Total Environment xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transformation of metamitron in water-sediment systems: Detailed insight into the biodegradation processes

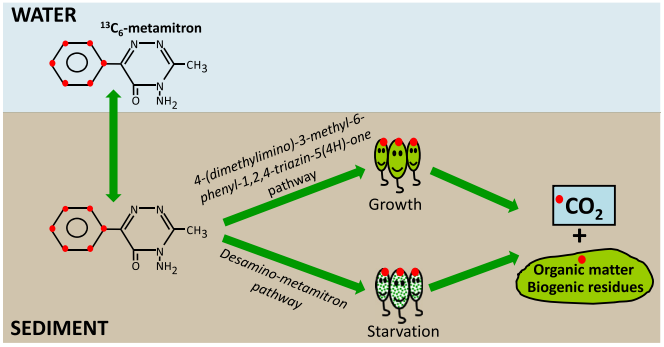
Shizong Wang [a,b], Anja Miltner [a], Matthias Kästner [a], Andreas Schäffer [c], Karolina M. Nowak [a,c,*]

[a] Helmholtz-Centre for Environmental Research – UFZ, Department of Environmental Biotechnology, Permoserstr, 15, 04318 Leipzig, Germany
[b] School of Water Resources and Environment, China University of Geosciences, Beijing, Beijing 100083, PR China
[c] RWTH Aachen University, Institute for Environmental Research, Worringerweg 1, 52074 Aachen, Germany

HIGHLIGHTS

- First study on $^{13}C_6$-metamitron turnover mass balance in water-sediment
- Biogenic residues make a great contribution to non-extractable residues formation.
- Small portion of $^{13}C_6$-metamitron is assigned to xenobiotic non-extractable residues.
- Metamitron is biodegraded via two pathways.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form 16 October 2016
Accepted 17 October 2016
Available online xxxx

Keywords:
$^{13}C_6$-metamitron
Water-sediment
Mass balance
Metabolites
Biodegradation pathways
Biogenic residues

ABSTRACT

Metamitron and its main metabolite desamino-metamitron are frequently detected in surface waters. To date, there are no studies targeting metamitron degradation in water-sediment systems. Therefore, the aim of this study was to trace the fate of metamitron in a water-sediment system using ^{13}C-isotope labeling. Mineralization of metamitron was high and accounted for 49% of $^{13}C_6$-metamitron equivalents at the end. In contrast, only 8.7% of $^{13}C_6$-metamitron equivalents were mineralized in the water only system demonstrating the key role of sediment for biodegradation. Metamitron disappeared from the water on day 40 and was completely removed from the sediment on day 80. This agrochemical was utilized as carbon source by microorganisms as shown by the incorporation of the ^{13}C label into microbial amino acids and finally into biogenic residues. The latter amounted to 24% of $^{13}C_6$-metamitron equivalents at the end. However, 17% of $^{13}C_6$-metamitron equivalents were detected in xenobiotic non-extractable residues (NER) with a release potential and delayed risk for the environment. Metamitron was degraded via two pathways, initially via 4-(dimethylimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one, which might be related to growth, and later via desamino-metamitron, which can be attributed to starvation.

1. Introduction

Metamitron, a selective pre- and post-emergence herbicide is commonly used in Europe for weed control in sugar beet crops (Coyette et

* Corresponding author at: Helmholtz-Centre for Environmental Research – UFZ, Department of Environmental Biotechnology, Permoserstr, 15, 04318 Leipzig, Germany.
E-mail address: karolina.nowak@ufz.de (K.M. Nowak).

http://dx.doi.org/10.1016/j.scitotenv.2016.10.125
0048-9697/

al., 2002). Due to its high water solubility (1.7 g L^{-1}) and weak sorption to minerals and organic matter (OM) of soils (Cox et al., 1996a; Autio et al., 2004; Mamy and Barriuso, 2007), this herbicide has a high leaching potential to surface and ground waters (Autio et al., 2004; Carafa et al., 2007; Schipper et al., 2008). Metamitron and its main metabolite desamino-metamitron are thus frequently detected in river waters (Battaglin et al., 2003; Reemtsma et al., 2013), with concentrations in the range of 48–1500 ng L^{-1} for metamitron and 8.4–680 ng L^{-1} for desamino-metamitron (Moschet et al., 2015). To date, little is known about metamitron behavior in the aquatic environment. Few studies demonstrated that metamitron can be photodegraded to desamino-metamitron in aqueous solutions (Cox et al., 1996b; Palm et al., 1997; Mijin et al., 2009). However, only sediments can lead to substantial elimination of contaminants from the aquatic environment, either via sorption or via biodegradation as demonstrated for $^{13}C_3^{15}N$-glyphosate (Wang et al., 2016). Therefore, detailed studies on the fate of metamitron with a particular focus on its biodegradation potential in water-sediment system are needed.

Metamitron is biodegraded quickly in soils which have been exposed to this compound for many years (Allen and Walker, 1987; Vischetti et al., 1999; Mamy et al., 2005). This herbicide is utilized as carbon source by pure cultures of *Arthrobacter* sp. (Engelhardt et al., 1982) and *Rhodococcus* sp. (Parekh et al., 1994). Recent experiments revealed that soil microorganisms use ^{13}C from $^{13}C_6$-metamitron for synthesis of their proteinaceous cell components which were ultimately incorporated into the OM of soil and stabilized as non-toxic biogenic residues (Wang et al., in press). Carbon dioxide and biogenic residues are the ultimate transformation products of $^{13}C_6$-metamitron in soil microcosms suggesting its complete detoxification (Wang et al., in press). In analogy to soil, similar degradation pattern of $^{13}C_6$-metamitron may also be relevant for the water-sediment system.

The objective of this study was therefore to investigate the turnover of metamitron in the water-sediment systems with a particular focus on its biodegradation pattern and biogenic residue formation using the ^{13}C-labeling approach.

2. Material and methods

2.1. Chemicals, sediment and water

$^{13}C_6$-metamitron (labeled in the phenyl moiety) was custom synthesized by Delta Bio Research Chemicals Ltd., Vaughan, Ontario, Canada. The isotopic purity of $^{13}C_6$-metamitron was 99 atom%. Cation exchange resin (DOWEX 50W-X8, 50–100 mesh) for purification of amino acids was purchased from VWR/Merck (Darmstadt, Germany). All other chemicals used in this study were obtained from Carl Roth Company, Karlsruhe, Germany.

The sediment and water used for water-sediment microcosms were collected from the upper layer of Getel creek (51°45′25.02°N, 11°17′ 50.25°E) located in the North-Eastern rim of the Harz Mountains in Saxony-Anhalt, Germany. The physicochemical properties of the sediment and the associated water had been described previously by Wang et al. (2016). Although no metamitron has been detected in the sediment and in the creek water, its previous application into the nearby agricultural lowlands cannot be excluded.

2.2. Experimental setup

The biodegradation experiments were based on OECD guideline 308 (OECD, 2002). Six treatments were prepared: (I) water-sediment without metamitron application (blank), (II) water-sediment with 55 mg L^{-1} unlabeled metamitron (control), (III) water-sediment with 55 mg L^{-1} labeled metamitron (^{13}C-metamitron), (IV) sterilized water-sediment with 55 mg L^{-1} labeled metamitron (abiotic), (V) water only with 55 mg L^{-1} unlabeled metamitron (water only, unlabeled) and (VI) water without sediment with 55 mg L^{-1} labeled metamitron (water only, ^{13}C-metamitron). Blank and control samples were included to correct for the ^{13}C natural abundance in the respective systems. The contributions of the sediment to metamitron turnover and dissipation were tested by comparison of the metamitron turnover in water-sediment systems to the one in water systems without the sediment; the contribution of abiotic processes was tested by analysis of the sterilized systems. Although the concentration of 55 mg L^{-1} is far above the realistic environmental concentrations, it was required to obtain reliable results for the isotopic enrichment even after pronounced degradation and in fractions with high natural background and low incorporation of the label. Therefore, the possible effect of the initial concentration on the mineralization of metamitron was tested in water-sediment microcosms with 5 mg L^{-1} labeled metamitron (slightly above minimum ^{13}C label detection limit in bulk soil).

The sediment was separated from the creek water by filtration and was sieved through a 2 mm screen. Sediments and water for the abiotic systems were sterilized by autoclaving five times at 120 °C for 20 min prior to incubation. Creek water was spiked with either 5 mg L^{-1} or 55 mg L^{-1} of metamitron (labeled or unlabeled). Aliquot amounts of sediment and of creek water were placed into 250 mL biometer Duran bottles. The final volume ratio of water to sediment was set to 3:1. Biodegradation experiments were conducted for 80 days in the dark and at 20 °C. The systems were destructively sampled after 0, 4, 8, 20, 40 and 80 days (abiotic after 40 and 80 days, blank, water and 5 mg L^{-1} only after 80 days). The CO_2 evolved from the mineralized metamitron was trapped in 2 M NaOH solution. In addition, the content of CO_2 trapped in the water phase was also analyzed. Finally, the mineralization for metamitron includes both the $^{13}CO_2$ trapped in 2 M NaOH and the $^{13}CO_2$ dissolved in the water phase.

2.3. Turnover mass balance of $^{13}C_6$-metamitron

The turnover mass balance of $^{13}C_6$-metamitron aiming at determination of ^{13}C label in the CO_2, in the extracted and in the non-extracted fractions provides only basic information about the transformation of this herbicide. The ^{13}C label incorporation into the amino acids (AA) of microbial biomass and ultimately into the OM of sediment as biogenic residues gives an indication about the extent of the compound's metabolisation. Therefore, a combined database on the mass balance including the formation of both the primary metamitron metabolites and of AA enabled the first identification of degradation pathways and an improved estimate of the extent of metamitron biodegradation.

2.3.1. CO_2

The total concentration of CO_2 was determined by means of a Total Organic Carbon Analyzer (Shimadzu TOC-5050, Duisburg, Germany). The isotopic composition of CO_2 was measured using a Gas Chromatography-Combustion-isotope ratio Mass Spectrometry (GC-C-irMS; Finnigan MAT 252, Thermo Electron, Bremen, Germany, coupled to a Hewlett Packard 6890 GC, Agilent Technologies, Germany, equipped with a 50 m × 0.32 m × 5 μm Porabondt Q-HT Plot FS column, Chrompack, Middburg, Netherlands) as described previously by Girardi et al. (2013).

2.3.2. Extractable metamitron and primary metabolites

Metamitron and its metabolites were extracted from the sediment using methanol/water (v/v: 4/1). The sediment extracts or water samples were purified over CHROMABOND® EASY columns (200 mg, 5 mL). Impurities were removed by acetone and ultra-pure water; ultimately metamitron and its metabolites were co-eluted from the column using ethyl acetate/acetone (1:1 v/v).

The quantitative analyses of metamitron and its metabolites were based on the external standard of metamitron using Gas Chromatography-Mass Spectrometry (GC–MS) with a BPX-5 column (50 m × 0.32 m × 0.5 μm). The initial temperature was 50 °C (1.5 min); thereafter, it increased as follows to 120 °C (0 min) at

Please cite this article as: Wang, S., et al., Transformation of metamitron in water-sediment systems: Detailed insight into the biodegradation processes, Sci Total Environ (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.10.125