Distribution of a nonylphenol isomer and the herbicide MCPA in soil derived organo-clay complexes

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Abstract

Organo-clay complexes in soil play an important role in the immobilization and persistence of xenobiotics. In this study, the fate of ^{14}C-labeled 4-(3,5-dimethylhept-3-yl)phenol (NP) and 4-chloro-2-mehtylphenoxyacetic acid (MCPA) in soil was examined using incubation periods from 1d to 180d (NP) and 1d to 120d (MCPA). Special emphasis was on the distributions of the compounds and its degradation products among the soil sub-fractions sand, silt and clay, which were obtained by particle size fractionation. Additionally the soil organic matter complexed to the clay fraction was fractionated by a conventional procedure into an humic acids (HA), fulvic acids (FA) and humin (HU) in order to localize the site of binding of the xenobiotics within this important soil fraction.

During the experiments, which were performed with two concentrations of the xenobiotics, the microbial activity in the assays was examined by determination of the DMSO reductase activity. A considerable reduction of the microbial activity was observed with the high concentrations of MCPA and NP (1 mg /g) as compared to the low concentrations (MCPA 559 µg per 100 g, NP: 121 µg per 100 g of soil).

A different incorporation behavior was observed for NP and MCPA which resulted in distinctly different distributions of radioactivity within soil sub-fractions. MCPA exhibited a lower tendency to interact with soil particles, although the portions associated with soil particles were non-extractable with organic solvents. In contrast, the high affinity of NP to particles was accompanied by a lower formation of non-extractable residues. The total distribution of ^{14}C seemed to be influenced significantly by the physico-chemical properties of the contaminants and the microbial activity in soil.

Key Words

4-(3,5-dimethylhept-3-yl)phenol (NP), 4-chloro-2-methylphenoxyacetic acid (MCPA), organo-clay complexes, xenobiotics, microbial activity

Introduction

Xenobiotics in agricultural soils originate predominantly from the application of pesticides and fertilizers including sewage sludge. Organic contaminants released into soils exhibit a complex environmental behavior. Besides extractable and therefore, available portions, chemicals can form so-called non-extractable or bound residues. The processes leading to these kind of residues including aging have been investigated for decades, especially regarding pesticides (Dec *et al.* 1997; Barriuso *et al.* 2008; Mac Rae 1986). So far, however, the knowledge on soil bound residues of xenobiotics is prevailingly restricted to the determination and of amounts of free and bound fractions. In contrast, detailed information on the behavior of chemicals in particular regarding their distribution in sub-fractions of soils is limited (e.g. Nieman *et al.* 1999).

Therefore, the aim of the present study was to follow the behavior of the anthropogenic contaminants MCPA, 4-chloro-2-methylphenoxyacetic acid, and a defined nonylphenol isomer, 4-(3,5-dimethylhept-3-yl)phenol (NP), in soils with time concerning different soil sub-fractions. The herbicide MCPA was regarded as representative for pesticides, the nonylphenol isomer as representative for sewage sludge derived xenobiotics. Besides different pathways of pollution, MCPA and NP also exhibit different physico-chemical properties which might result in a different behavior in soils (Xie *et al.* 1997). Special focus was laid on organo-clay complexes since these are known to play an important role for the formation of bound residues (Wang and Xing 2005).

Methods

Chemicals - ^{14}C-labeled 4-(3,5-dimethylhept-3-yl)phenol (NP, 304.14 MBq/mmol) was synthesized via Friedel–Crafts alkylation using 3,5-dimethylheptan-3-ol and a mixture of unlabeled and [ring-U-^{14}C] phenol (2.220 MBq/mmol) according to (Russ *et al.* 2005). [*Ring*-U-^{14}C] 4-chloro-2-methylphenoxyacetic acid

(MCPA, 59.94 MBq/mmol) was provided by Prof. M. H. Gerzabek (University of Vienna) as a mixture of 92 % MCPA methylester and 8 % of the free acid.

Spiking experiments - Aliquots of 559 µg (0.167 MBq) of MCPA dissolved in 0.5 mL methanol were applied to 100 g of air-dried, homogenized and sieved ($\leq$ 2 mm) soil samples from Fuhrberg, Germany. In a second experiment, 121 µg (0.167 MBq) of NP dissolved in 0.5 mL petrolether was similarly applied to a soil sample from the same location. Directly after application, the solvent was evaporated and the flasks were shaken for 15 min in an overhead shaker. Thereafter, the water content of the samples was adjusted to 60% of maximum water holding capacity. The flasks were closed with an absorption device for $^{14}CO_2$ containing soda lime (approx. 15 g). After incubation periods of 1 d to 180 d (NP) and 1 d to 120 d (MCPA) at 20 °C in the dark, 20g of the soil samples (based on dry weight) were suspended in 100 mL of water and subjected to ultrasonic assisted disaggregation (total energy 22 kJ) as described by Morra *et al.* (1991). The soda lime was dissolved in conc. HCl and the liberated $^{14}CO_2$ was captured in scintillation cocktail Lumasafe^{TM} plus (Lumac), and was examined by a 2250CA TRI-CarB® scintillation counter (Canberra-Packard). A similar approach was applied in an experiment using 1 mg per g soil of non-labeled NP and MCPA, while the assays contained 50 g of soil.

Particle size separation and fractionation of humic substances - Sand, silt and clay fractions were obtained by manual wet sieving for separation of sand from silt and clay. Silt and clay were then separated by centrifugation according to (Stemmer *et al.* 1998). Subsequently, the clay fraction containing predominantly organo-clay complexes was extracted using a Soxhlet apparatus and methanol and dichloromethane consecutively. The remaining extracted clay was then suspended in 0.5 M NaOH for 24 h with shaking. The mixture was subsequently subjected to centrifugation in order to separate the insoluble humin fraction (HU) from the solubilized humic acids (HA) and fulvic acids (FA). The latter fraction was finally acidified with HCl (pH 1). Pricipitated HA were separated from the FA, which remained in solution, by centrifugation.

Radioanalysis – Radioactivity contained in solid samples (aliquots of sand, silt and clay fractions) were combusted using a Biological Oxidizer OX500 (R. J. Harvey Instrument Corp.) and emerging $^{14}CO_2$ was trapped in Oxysolve C-400 scintillation cocktail (Zinnser Analytic). Radioactivity contained in liquid samples was determined by dissolving aliquots in Lumasafe^{TM} plus (Lumac) scintillation cocktail.

Microbial activity – The reduction of dimethylsulfoxide to dimethylsulfide was utilized for the determination of microbial activity in soil samples (Alef and Kleiner 1989). For this purpose, 1 g soil was transferred to a 20 ml flask. After application of 200 µl of 5% DMSO in water (v/v) the flasks were closed tightly. After incubation for 24 h, 100 µl of the headspace of the flasks was analyzed for developed DMS using an Agilent 6890N gas chromatograph equipped with a flame ionization detector.

Results

Mineralization – Concerning mineralization in the samples with low concentration (MCPA: 559 µg per 100 g of soil; NP: 121 µg per 100 g of soil), both substances exhibited an initial lag phase for 7 days, which was followed by a linear increase of mineralization for subsequent 7 days in case of MCPA and subsequent 24 days in case of NP. The mineralization rate decreased with both substances after 14 days and 30 days of incubation, respectively. After 14 days incubation times we detected 3% and on day 90 14% mineralization for NP, and for MCPA (at days 30, 60 and 120) 34%, 46%, and 48%, respectively. Samples with 1 mg xenobiotic per g of soil produced 0,6% and 2,3 % 14C-CO2 for NP after 14 and 90 days and for MCPA 1,2%, 3,8% and 57% mineralization respectively at days 30, 60 and 120. At the higher concentrations of the xenobitoics the microbial (DMSO reductase) activity was reduced by 50% as compared to the samples with low concentrations.

Percentages of ^{14}C in soil particle fractions – In the assays incubated with low concentration of MCPA for up to 14 days, most of the radioactivity contained in the samples was extracted with the water used for particle separation. About 20% of applied radioactivity was localized in the clay and silt fractions. In the assays with 1 mg /g of MCPA, however, about 60% of applied ^{14}C were detected in the water used for particle separation, and only 8% to 12% were associated with the clay and silt fractions. In case of NP, on average 32% of the applied radioactivity was found each in the clay and silt fractions, whereas only 6-11% of applied ^{14}C were extracted with the water used for particle separation.

Percentages of ^{14}C in organic matter associated with clay fractions – A significant increase of the portions of non-extractable residues in all organic matter fractions examined were observed during the first 14 days of incubation in the assays with low concentrations of both MCPA and NP. Generally, smaller amounts of radioactivity were extracted (methanol and dichloromethane) in case of MCPA as compared to NP. With both compounds, the fulvic acids fractions contained the lowest amounts of ^{14}C-residues. 5% of applied ^{14}C-MCPA were bound to the FA, 6% to HU, and 8% to the HA after 120 days of incubation. A similar result was obtained for NP: after 180 days, 4% were associated with the FA, 9% with HU, and 14% with the HA fraction. In case of high MCPA concentrations in soil (1 mg /g), portions of ^{14}C in these organic matter fractions were 40% to 60% lower than those obtained in soils incubated with low concentrations.

Conclusions

Organo-clay complexes as biogeochemical interfaces as well as the characteristics of the applied substances were of great importance for their immobilization and persistence in soil. Additionally the microbial activity in the soil influenced the mineralization rate as well as the immobilization and persistence of xenobiotics in soil. The present findings suggest that the lowered microbial activity detected during the first 60 days of incubation with 1 mg /g of MCPA had a considerable effect on the formation of non-extracable residues of the compounds.

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