



Formation of “bound” residues from biomass during the biotic degradation of a herbicide in soil

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During degradation of organic pollutants in soil, metabolites, microbial biomass, CO₂ and “bound” residues (“non-extractable” residues in soil organic matter) are formed. Enhanced transformation of these contaminants into “bound” residues has been proposed as an alternative remediation method for polluted soils. However, this kind of residues may pose a potential risk for the environment due to their chemical structure and possible remobilization under different conditions. Therefore particular attention is given actually to “bound” residues. Part of these non-extractable residues may be “biogenic,” because microorganisms use the carbon from the pollutant to form their biomass components (fatty acids, amino acids, amino sugars), which subsequently may be incorporated into soil organic matter. Furthermore, the CO₂ originating from mineralization of xenobiotics, can be re-assimilated by microorganisms and also incorporated into “biogenic residue”. The hazard posed by “bound” residues may be overestimated because they are “biogenic” (contain microbial fatty acids and amino acids). The knowledge about the pathways of “biogenic residue” formation is necessary for a proper assessment of the fate of tested pollutants and their turnover in the soil environment. Moreover, these data are needed to establish the realistic degradation rates of the contaminants in soil. The main objectives of this study are: to quantify the extent of “biogenic residue” (fatty acids, amino acids, amino sugars) formation during the degradation of a model pollutant (2,4-dichlorophenoxyacetic acid = 2,4-D) and during CO₂ assimilation by microorganisms and to evaluate which components are mainly incorporated into “bound” residues. To investigate the extent of “biogenic residue” formation in soil during the degradation of 2,4-D, experiments with either ¹⁴C-U-ring and ¹³C₆-2,4-D or carboxyl-¹⁴C 2,4-D were performed. The incubation experiments were performed according to OECD test guideline 307, in the dark, at constant temp 20°C (+/-2°C) and with intermittent aeration. During incubation, the mineralization was quantified and soil samples were analyzed for the presence of both “biogenic residues” and remaining 2,4-D. Mineralization of 2,4-D in both experiments was very high. However, the ¹⁴CO₂ evolution was higher from carboxyl-¹⁴C 2,4-D than from ¹⁴C-ring 2,4-D. After 7 days of incubation, 30% of initial amount of ¹⁴C in soil contaminated with ¹⁴C-ring 2,4-D was mineralized, whereas 40% of total radioactivity was evolved as CO₂ after 4 days from soil incubated with ¹⁴C-carboxyl 2,4-D. The amount of extractable 2,4-D residues was very low in both experiments (¹⁴C-ring 2,4-D: 2% and ¹⁴C-carboxyl 2,4-D: 1%). The soil incubated with ¹⁴C-ring 2,4-D contained 60% of “non-extractable” residues of 2,4-D after 7 days, while the amount of these residues in soil contaminated with ¹⁴C-carboxyl 2,4-D reached 50% of the initial radioactivity in the tested system 4 days after application. More “biogenic residues” were formed in soil spiked with ¹⁴C-U-ring 2,4-D (10%) than in soil with carboxyl ¹⁴C 2,4-D (7%). Both 2,4-D and CO₂-derived C were incorporated mainly into microbial amino acids (9.5% at day 7 and 7.0% at day 4, respectively). After 7 days of incubation, 0.5% of initial applied radioactivity in system was found in microbial lipids in the soil contaminated with ¹⁴C-ring 2,4-D. Only 0.1% of the total radioactivity was incorporated into lipids in soil treated with ¹⁴C-carboxyl 2,4-D on day 4 after application. Thin Layer Chromatography identified the microbial lipids containing the radioactivity as phosphatidylethanolamine, a phospholipid typical for microorganisms. The amount of microbial lipids (which corresponds to phospholipids) in both cases decreased with time; this can be explained by the death of the microbial biomass. To the best of our knowledge, this is the first report on the formation of “bound” residues from biomass during the biotic degradation of herbicide in soil.