



Spectroscopic Evidence for Covalent Binding of Sulfadiazine to Natural Soils via 1,4-nucleophilic addition (Michael Type Addition) studied by Spin Labeling ESR

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Among different classes of veterinary pharmaceuticals, Sulfadiazine (SDZ) is widely used in animal husbandry. Its residues were detected in different environmental compartments. However, soil is a hot spot for SDZ as it receives a large portion of excreted compounds through the application of manure during soil fertilization. Ample studies on the fate of SDZ in soils showed that a large portion forms nonextractable residues (NER) along with transformation products and a low mineralization (Mueller et al., 2013). A common observation was an initially fast formation of NER up to 10% of the applied amount promptly after the application of SDZ to soil, and this portion increased up to 50% within a few days (Mueller et al., 2013; Nowak et al., 2011). A common finding for SDZ, as for other sulfonamides, was biphasic kinetics of the formation of NER, which was attributed to the occurrence of two reaction processes: a rapid, often reversible process and a slower, irreversible process (Weber et al., 1996). A single-phase reaction process was also established under anaerobic treatment (Gulkowska et al., 2014).

A major focus of this work is to elucidate a reaction mechanism of covalent binding of SDZ to soil that is currently required to estimate a risk of NER formed by SDZ in soils for human health. Taking into account a key role of the amine functional groups of SDZ on its reactivity in soil, nitroxide radicals with the sewed aromatic or aliphatic amines labeled soil samples and then, were investigated by means of ESR spectroscopy. 2,5,5-Trimethyl-2-(3-aminophenyl)pyrrolidin-1-yloxy and 4-amino-2,2,6,6-Tetramethylpiperidin-1-oxyl modeled decomposition products of SDZ with the aromatic and aliphatic amines, respectively. The application of the defined combination of both spin labels (SL) to different soils well simulated a change of a paramagnetic signal of soil organic radicals interacted with SDZ. After their application to soil, SL were found in soil sites characterized with different polarity. As shown by the spin labeling ESR experiment, molecules modeling SDZ were promptly bound to non-hydrolysable network of soil organic matter only via the aromatic amines that was accompanied by a prompt enlargement of humic particles binding aromatic amines, whereas binding of decomposition products of SDZ to humic acids of soil via the aliphatic amines was not observable. The ESR spectra obviously showed a single-phase process of covalent binding of the aromatic amines. Repeated washouts of labeled soil samples using distil water and ultrafiltration through the membrane of 5000 MWCO PES confirmed irreversible binding of the aromatic amines, and showed that via the aliphatic amines, binding of SDZ or decomposition products of SDZ to soil might also occur but reversibly and only to small soil molecules, which don't enter into the composition of non-hydrolysable part of soil organic matter. SL ESR experiments of different soils at the presence of Laccase highlighted that covalent binding of the aromatic amines to humic particles occurred in the specific hydrophobic areas of soil found as depleted in oxygen. All measured data evidenced that first, SDZ might be decomposed that allowed for measuring the same change of a paramagnetic signal of soil organic matter influenced by both aromatic and aliphatic amines as in the experiment of the interaction of soil with SDZ. Second, a decomposition product of SDZ with the aromatic amine might be bound to non-hydrolysable parts of soil organic matter under specific anaerobic conditions only via 1,4 – nucleophilic addition, Michael-type addition.

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