



## Spin Labeling ESR Investigation of Covalently Bound Residues in Soil

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Organic xenobiotic chemicals, such as pesticides, biocides and veterinary pharmaceuticals, interact with soil, which results in the simultaneous formations of metabolites, mineralization products, and bound or non-extractable residues (NER). Substances or metabolites with reactive functional groups, such as aniline or phenol, have a tendency to give a larger proportion of NER. Despite numerous studies on NER, the majority of their chemical structures is still unknown. Reversible sequestration and irreversible formation of NER were also observed for veterinary antibiotic pharmaceuticals, after their application to soil with and without manure.

For this purpose, we hypothesized a key role of specific functional groups of soil contaminants, via which contaminants are covalently bound to soil constituents, and advance a method of spin labeling ESR investigation of reaction products using a membrane method.

Spin labels (SL) represent chemically stable paramagnetic molecules used as molecular labels and molecular probes for testing the covalent binding, structural properties, and molecular mobility of different physical, chemical, and biological systems. In the case of covalent binding of SL, their ESR spectra become broadened. We used stable nitroxide radicals (NR) as SL. These radicals modeled organic chemical contaminants and differed only in one functional group. The paramagnetic SL 4-Amino Tempo (4-amino-2,2,6,6-tetramethyl-1-piperidinylox) differed from Tempo (2,2,6,6-Tetramethylpiperidinoxy) in a substituent at the para-position of the piperidine ring, whereas Aniline Tempo (1-Piperidinyloxy, 2,2,6,6-tetramethyl, 6-Aniline) differed from Tempo in an Aniline substituting one CH<sub>3</sub> functional group. Before experimental analysis, we tested temporal changes in the concentration of both NR incubated with soil and found that the life-times of them in soil exceeded 3 days.

We contaminated and labeled soil samples with NR, adding to soil the aqueous solution, which already contained the paramagnetic substance at a required concentration of 3 mM. For some hours, soil samples were incubated with NR and then washed, using the serial additions of some distilled water to each soil sample. Subsequently, each washed soil sample was centrifuged. The content of NR in the retentates and permeates of centrifuged washed samples of contaminated soil were analyzed for different volumes of percolate by means of ESR spectroscopy.

We received a gradually decreasing content of both NR in retentates with increasing volumes of percolates. However, 4-Amino Tempo can be completely washed out, whereas approximately 6% of the Aniline Tempo remained in soil after repeated washing. Thus, both SL are reversibly bound because they can be washed out from soil. In addition, Aniline Tempo is partly irreversibly bound. We concluded that the aromatic moiety of Aniline Tempo favours the covalent binding of the NH<sub>2</sub> moiety in contrast to the aliphatic NH<sub>2</sub> moiety of 4-Amino Tempo.