



## **Kinetics of rapid covalent bond formation of aniline with humic acid: ESR investigations with nitroxide spin labels**

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Sulfonamide antibiotics used in livestock farming are distributed to farmland by application of slurry as fertilizer. Previous work suggests rapid covalent binding of the aniline moiety to humic acids found in soil. In the current work, kinetics of this binding were measured in X-band EPR spectroscopy by incubating Leonardite humic acid (LHA) with a paramagnetic aniline spin label (anilino-NO (2,5,5-Trimethyl-2-(3-aminophenyl)pyrrolidin-1-oxyl)). Binding was detected by a pronounced broadening of the spectral lines after incubation of LHA with anilino-NO. The time evolution of the amplitude of this feature was used for determining the reaction kinetics. Single- and double-exponential models were fitted to the data obtained for modelling one or two first-order reactions. Reaction rates of 0.16 min<sup>-1</sup> and 0.012 min<sup>-1</sup>, were found respectively. Addition of laccase peroxidase did not change the kinetics but significantly enhanced the reacting fraction of anilino-NO. This EPR-based method provides a technically simple and effective method for following rapid binding processes of a xenobiotic substance to humic acids.