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Abstract

TITLE: Fractal behavior of Humic Substances evaluated by Confocal laser scanning microscopy (CLSM) and Fluorescence Lifetime Imaging
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ABSTRACT BODY:
Abstract Body: Humic substances (HS) are major components of the natural organic matter (NOM) in soil and water. Particularly, HS affect the chemistry, cycling and bioavailability of chemical elements in soil. Regarding their structure, they are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification). Plant lignin and its transformation products, as well as polysaccharides, proteins, lipids, nucleic acids, etc., are important components taking part in this process. HS are highly chemically reactive, yet recalcitrant with respect to biodegradation. Humic substances can interact with several nutrients and toxic metals, which can be more available to organisms or actually sequestered so as to reduce their toxicity or beneficial value. The mechanisms of many of these interactions are unclear. That is a result of our lack of knowledge of the structural components of humic substances. In addition, HS are efficient fluorescent materials and can be divided into three main fractions: humic acids (HA), fulvic acids (FA) and humin. Fulvic acids are soluble in water at all pH; HA are insoluble at low pH and Humin is insoluble at all pH values. Fractal theory has been considered an alternative tool to explain the conformation of molecular aggregates. The presence of fractal models indicates that the system may be decomposed in parts, each part being a copy of the whole. In the present work, a drop of different HS materials was dried on a glass surface and its suspended molecules were deposited in a typical dendritic-like fashion during the evaporation process. Confocal laser scanning microscopy (CLSM), equipped with spectral analysis, Fluorescence Lifetime Imaging (FLIM) and two-photon (2P) excitation, was employed to quantify the formation of dendritic deposits in real time and to access a new variety of HS fluorescence states related to structural changes and molecular aggregation. Image methodologies were developed to enhance the viewing of fractal patterns, and observe variations in HS concentration and organization along the area of the original drop, with features that cannot be perceived in a usual image analysis. The fluorescence spectral shape and position correlate well to HS concentration, to the HA or FA fraction and to the fractal morphology. The fluorescence decay time became longer with the decrease HS aggregates along the dendritic structures, which corresponds to HS emission and decay time features in a less concentrated environment. These results suggest that HS arrangement in such fractal structures is based on a well-organized environment that ensures different level of interaction between the molecules. In addition, the variety of HS fluorescence states accessed by two-photon excitation can allow an even broader optical characterization of soil materials and their fractal properties.