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Humeomics for a structure-bioactivity relationship of Humic Substances

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Humic Substances (HS) represent a class of naturally occurring organic compounds and are commonly found in soils, sediments and natural bodies of water[1]. They have an effect on physical-chemical and biological properties of such environments, and are fundamental for their quality and fertility[2]. The heterogeneous and complex nature of humic components represents the main difficulty against the chemical characterization and structure elucidation of them. Recent findings have provided significant experimental evidence supporting the hypothesis that HS are organized in supramolecular architecture[3]. Such structure is composed by relatively simple molecules held together by weak bonds and allows the single compounds to be selectively separated and quali-quantitatively characterized with appropriate analytical methods, such as NMR[4,5] and mass spectrometry (MS)[4,6]. A comprehensive description of all components of HS, defined as "Humeomics" would bring an important achievement for research in agrarian and environmental chemistry. In order to accomplish this goal it is required to obtain sub-fractions of HS as homogeneous as possible and characterize their content.

A humic acid (HA) from a volcanic forestal soil (Allic Fulvudand) was isolated, purified, and fractionated[1,7]. The first step of this method consisted in a liquid extraction with a mixture of dicholormethane and methanol 2:1. This yielded an organic extract not bound to the residue via covalent bonds. Such residue underwent two trans-esterification stages in methanol, the first using BF_3 12%, and the second using KOH 1M. The extracts thus obtained were further separated in water/chloroform liquid extraction. Such components were defined as weakly and strongly ester bound to the residue. This step yielded also a residue, which is thereafter hydrolyzed in aqueous HI 47% to separate the organic matter bound via ether bonds. The final product showed minimal chemical reactivity. Such residual matter was separated with HP-SEC chromatography in order to separate fractions according to their hydrodynamic volume. The fractions obtained were analyzed with the following techniques: 1. Elemental analysis 2. NMR mono and bidimensional spectroscopy, in both solid and solution state (Bruker AV300 e AV400) 3. GC-MS and pyrolysis-GC-MS (Perkin-Elmer Turbomass Gold) 4. HPSEC coupled to MS with Elettrospray source and Orbitrap detector (Thermo Finnigan Orbitrap, High resolution).

The organosoluble fractions show linear aliphatic and, to a lesser extent, aromatic compounds. In hydrosoluble fractions several compounds with a chemical formula similar to saccarides are detected. This is suggested by formulas with high content of oxygen and nitrogen atoms. Aliphatic compounds are also revealed to a lesser extent. In the final residue quantitative assessment shows aromatic (mostly quaternary)>aliphatic>carbonyl molecules in order of abundance. From NMR relaxation time measurements, a more rigid architecture is attributed to this material with respect to the starting HA. Mass spectrometry revealed formulas with extensive oxygen substitution and unsaturation index, which can be attributed to the NMR signals in sp^2 carbon region. The MS analysis of the final residue showed for fatty acids, from the first to the tenth HPSEC fraction, an increase in the ratio between 1) short and long chained homologues and 2) hydrophilic and hydrophobic homologues, suggesting a correlation between the chemical composition of aggregates and their hydrodynamic properties: stacking between longer, saturated carbon chains results in larger aggregates whereas smaller hydroxylated compounds result in weaker aggregation forces.

The humeomic approach to HS analysis represents a solid basis for a more reliable and detailed characterization of Natural Organic Matter.

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