

Interaction of Humic Substances with Inorganic Matter evaluated by Confocal Laser Scanning Microscopy and Fluorescence Lifetime Imaging

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Humic substances (HS) are major components of the natural organic matter (NOM) in soil and water. Regarding their structure, they are complex and heterogeneous mixtures of polydispersed materials formed during the humification process. Humic substances can be divided into three main fractions: humic acids (HA), fulvic acids (FA) and humin. Like lignin in plant cell wall, HA and FA are efficient fluorescent materials and form molecular aggregates at low pH, but they are soluble in water for pH higher than 8.

We have produced fractal patterns of a crystallized salt matrix to study specific interactions with humic substances (HS). This system provides a controlled aqueous environment and some adequate conditions that could be present in a much more complex fashion as those found in soil. The fractals were formed by drying aqueous drops containing HA and FA and a fixed amount of sodium bicarbonate. During the slow drying process on glass slides, salt seeds were formed at the drop edge, inducing nucleation of the fractal patterns that grows parallel to the surface from outwards to the centre of the drop. Conversely, capillarity carries the suspended molecules outwards to the drop edge where the interaction with the crystallized salt takes place.

Confocal laser scanning microscopy (CLSM), equipped with spectral analysis for image pixel, Fluorescence Lifetime Imaging (FLIM) and two-photon (2P) excitation, was employed to quantify the formation of the fractal and HS deposits in real time and to access a new variety of HS fluorescence states related to structural changes and molecular aggregation.

The drying process of a water droplet containing 100 ppm of HA at pH=8 (sodium bicarbonate) on a glass substrate could be followed by confocal time lapse imaging in the spectral mode for an area of 425 $\mu\text{m} \times 425 \mu\text{m}$ close to a drop edge. Figure 1 summarizes the most significant images of the drop drying process. The total drying time of the drop on the glass substrate lasted 44 min for an ambient temperature of 18 °C and for a controlled room relative humidity of 40 %. The focal height was so adjusted in order to obtain the confocal plane positioned very close to the substrate surface for this time series imaging. The green-yellowish color of drop region is associated to the HA emission and the violet area corresponds to a small collected fraction of excitation laser at the wavelength of 405 nm. The images of the drop drying process do not change much over the first 40 minutes as demonstrated by the pinned contact line at the intersection with the liquid-gas interface on the substrate surface. In addition, the drop still remains after the complete formation of the fractal patterns along the image area in the remaining one and a half minute of the time series measurement. Within this short time lapse, deposits first seeded at the original outermost air-liquid interface in contact with the substrate and grown further inwards into 2 dimensional patterns resembling mineral dendritic morphologies. We believe that crystallization in the form of fractals is associated to the supersaturation of the bicarbonate in this region and to the enhanced capillary transport of ionic material from the volume to the edges of the drop. In addition, HA is deposited after the formation of the fractals on the bicarbonate crystal surface.

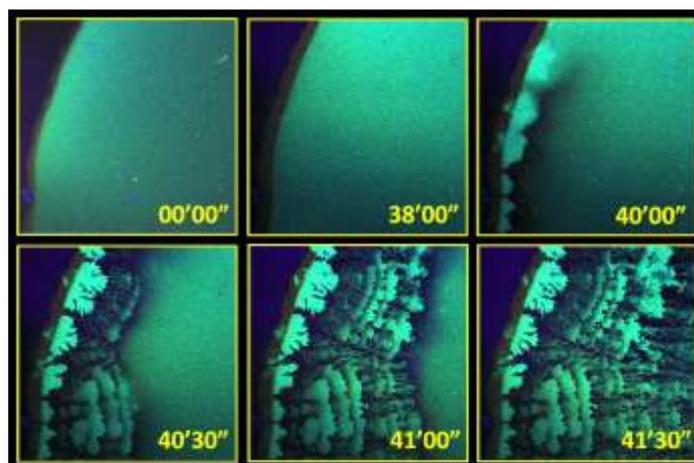


Figure 1: Confocal Time Lapse Imaging in the spectral mode following the drying process of drop of HS solution deposited on a glass surface for an area of $425\ \mu\text{m} \times 425\ \mu\text{m}$ close to a drop edge.

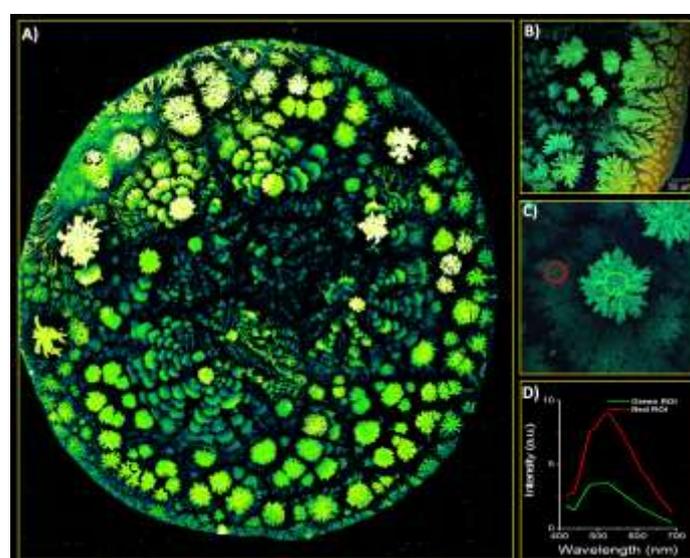


Figure 2: A) False-color confocal view (channel mode) of the full drop area after the drying process of an HA droplet. B) Detail of spectral image close the drop boarder. C) and D) Spectral features of two different regions of interest .

The depletion of the HS and bicarbonate during the drying process produce a large variety of HS-salt interactions and fractal shapes (Fig. 2). Confocal image of the full drop area can be seen in Figure 2A in order to emphasize the whole morphology evolution after the droplet has been completely dried. The wide variety of colors gives a notion of how the emission intensity and spectral shapes of HA varies throughout the observation area (Figs 2B, 2C and 2D), as a result of different interaction and aggregation processes. With this setup, the fractal patterns must necessarily have an almost two dimensional character since all them are 'in focus' over the observed substrate surface. In addition, the fractal morphology changes from dendrite at the outermost boarder of the drop to beautiful treelike form in the central area (Fig. 2B). The intensity of the emission of HA depends strongly on the shape of the fractal structure, which indicates different forms of incorporation or organization of HA molecules on the bicarbonate matrix during the drying process. The different interactions HS and with the salt matrix will be discussed in terms of the spectral analyses and lifetime imaging.