

Fluorescence properties of humic acids interaction products with S-Triazine and Bipyrilidium herbicides and their Cu complexes: A multivariate approach

Valeria D'Orazio and Nicola Senesi

Dip. Scienze del Suolo, della Pianta e degli Alimenti, Univ. degli Studi "Aldo Moro", Bari, Italy

The intrinsic fluorescence of soil organic components contains information related to structural components, functional groups, conformation and heterogeneity, as well as dynamic properties related to their intra- and inter-molecular interactions. Three-dimensional (3D) fluorescence spectroscopy (emission-excitation matrix, EEM) represents a powerful tool in the characterization of humic molecules, and its capability as qualitative and/or quantitative analysis can be strongly improved by the application of multivariate methods. In this work Principal component analysis (PCA) was used to evaluate the similarity among three different humic acids (HA) and their interaction products with the herbicides atrazine (A) and paraquat (P) in the presence or not of paramagnetic metal ion (Cu^{2+}).

Nine HAs have been isolated from soil samples collected in a citrus field located in Policoro (Basilicata, Southern Italy), under the plant rows (HAa), between the rows (HAb) and in an adjacent unexploited soil (control, HAc). Interaction products between each HA sample and the two herbicides atrazine and paraquat in the presence or not of copper were obtained according to a previously tested procedure. Fluorescence spectra were recorded on aqueous solutions using a Hitachi model F-4500 luminescence spectrophotometer. The EEM spectra were generated from TL spectral data by using Noesys 2.4 software. The fluorescence intensity (FI) values (in arbitrary units) were normalized using a quinine sulphate standard ($10\ \mu\text{mol}$ in $0.1\text{N H}_2\text{SO}_4$). PCA analysis was performed on fluorescence data by using the software STATGRAPHICS Centurion XVI.I.

The EEM spectra of the three untreated HAs are characterized by the presence of two distinct fluorophores, α and β , identified by the emission-excitation wavelengths pairs (EEX) $450\text{-}460_{\text{ex}}/530\text{-}540_{\text{em}}$ and $460_{\text{ex}}/580\text{-}585_{\text{em}}$, respectively, suggesting the presence of very complex structures, such as conjugated quinones and/or phenols with an elevated polycondensation degree. In particular HAb shows the highest FI values for both fluorophores, whereas similar and lower values are exhibited by HAa and HAc samples. The EEM spectra of the A-interaction products are featured by the same two fluorophores, with FI values decreased of about 50%, and a new fluorescent peak, γ , centered at EEX $390\text{-}410_{\text{ex}}/555\text{-}570_{\text{em}}$, ascribed to hydroxycoumarin-like structures. The P-interaction products show EEMs completely different, with a unique peak, δ , centered at $395\text{-}405_{\text{ex}}/510\text{-}525_{\text{em}}$ (aromatic moieties variously substituted) and characterized by very high FI values, especially in the HAb-P. Finally, the EEM spectra of HA-A- Cu^{2+} show, with respect to HA-A samples, a blue shift of the peak α with an additional decrease of FI values (about 60%) and the disappearance of the peaks β and γ , whereas those of HA-P- Cu^{2+} feature, in comparison with those of HA-P, a red-shift of the peak δ and FI values decreasing in the order $\text{HAc} > \text{HAb} > \text{HAa}$.

The results obtained indicate that PCA analysis of fluorescence EEM spectra could give additional information on the involvement of humic fluorophores in the adsorption mechanisms of HA and the two herbicides, and on the role of copper ion. The analysis of PCA yielded 2 PCs that account for 88% of the variance, i.e. PC1, characterized by α , β and δ peaks, and PC2, featured by γ peak. These results are confirmed by the score plot analysis which clearly shows that Cu does not appear to affect the interaction between HAs and P, whereas a completely different pattern, likely related to a different interaction mechanism, is shown by HA and A as a function of the presence of copper.