

# Assessment of aromaticity indices in soil humic acids based in spectroscopic, pyrolytic and wet chemical degradation data

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## Introduction and Experimental

In this study the structural characteristics of humic acids (HAs) from 16 soils have been approached by analytical pyrolysis, oxidative degradation with sodium perborate and spectroscopic techniques (FT-IR, UV-V, <sup>13</sup>C-NMR). The soils studied were considered as representative of the large variability found in continental Mediterranean ecosystems, including relictual forests, areas disturbed by wildfires or clearings followed by reforestation with conifers, bush encroachment, or cultivation. Up to 250 analytical descriptors were tabulated for each HA, and the total matrix was processed by bi- and multivariate data analyses. Amongst the starting hypotheses, the signal area between 110–160 ppm in the <sup>13</sup>C NMR spectrum was often *a priori* considered as an independent variable explaining most of the total variance of the aromaticity in HAs.

## Data management

Two assumptions were made: a) data fit with lineal model is useful to highlight analytical descriptors responsive for HA aromaticity and, b) HA aromaticity is a fuzzy concept that could be defined by a set of descriptors to be identified by multiple regression models after automatic backward variable selection.

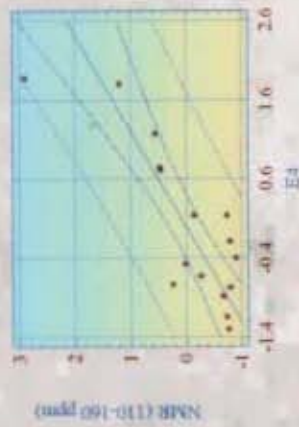


Fig. 1. Linear correlation between the amount of aromatic carbon as determined by <sup>13</sup>C NMR and the specific extinction (E<sub>4</sub>) of humic acid. Inner dashed lines indicate 95% confidence limits; dotted lines show 95% prediction limits for new observations.

## Results and Discussion

Very significant ( $P < 0.01$ ) linear regression coefficients were obtained between the optical density of the HA (i.e., at 465 nm, referred to as E<sub>4</sub>) and the <sup>13</sup>C NMR signal area in the 110–140 ppm or 140–160 ppm spectral regions. Negative correlations were also obtained between E<sub>4</sub> and the parameters related to the amounts of aliphatic constituents of the HAs: concerning the elementary composition, there was significant (negative) correlation between E<sub>4</sub> and the atomic H/C ratio, and positive correlation with the percentage of N. The latter could indicate a preferential accumulation of N in aromatic and diagenetically transformed HA structures. Parenthetically, the E<sub>4</sub> values were also negatively correlated with mineralization coefficients of the whole soils (as estimated by laboratory incubations), which emphasizes the importance of this spectroscopic parameter in forecasting the biogeochemical stability of the soil organic matter.

When the E<sub>4</sub> values were compared with the semiquantitative yields of the different degradation products from HAs (wet chemical degradation with sodium perborate) high significant correlations with the amounts of aromatic compounds were observed, mainly of high degree of oxidation (benzenecarboxylic- to-phenolic ratio). There were also high significant negative correlations with the percentage of aliphatic products as well as with the yields of lignin-derived methoxyphenols. This latter fact indicate that the extent of the lignin domain in HAs is not necessarily an index for aromaticity, but an indication of diagenetic alteration or selective preservation of plant-inherited macromolecules, as it is also found in comparatively immature HAs.

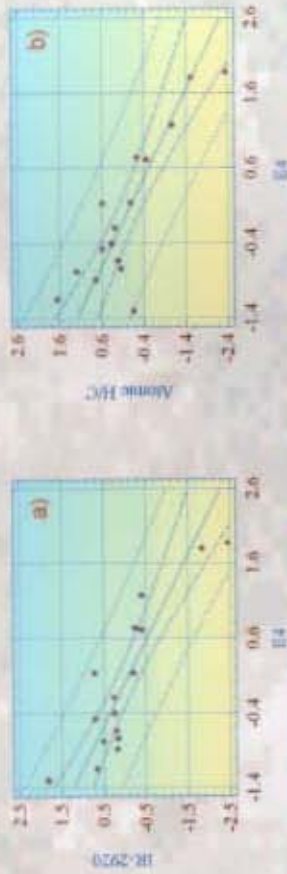


Fig. 2. Linear correlation between the specific extinction (E<sub>4</sub>) of humic acid and: a) intensity of the infrared alkyl stretching band (2920 cm<sup>-1</sup>); b) atomic H/C ratio calculated from the elementary composition.

Regarding the intensity of the major bands found in the FT-IR spectra, the E<sub>4</sub> values paralleled the intensity of the bands typically ascribed to aromatic structures (1510, 1620 cm<sup>-1</sup>), and decreases with the intensity of those assigned to alkyl (2920 cm<sup>-1</sup>) and oxygen-containing groups (3400, 1030 cm<sup>-1</sup>).

When using multiple regression models, most of the variability of the E<sub>4</sub> values was explained by the shared contribution of characteristics such as the low intensity of the alkyl stretching band in the FT-IR spectra (2920 cm<sup>-1</sup>), the yield of benzenecarboxylic acids, and the intensity of the valley at 620 nm in the 2<sup>nd</sup> derivative spectra (typically associated to polycyclic quinoid chromophors).

## Conclusion

Certain parameters (mainly E<sub>4</sub> and the H/C atomic ratio) seems to be appropriate descriptors to be used as surrogate indicators of the aromaticity degree of humic acids and to accurately distinguish systematic differences between environmental situations.

Table 1. Significant ( $P < 0.05$ ) linear correlation coefficients between specific extinction (E<sub>4</sub>) and a series of molecular analytical descriptors of soil HAs (supervised selection discarding the effect of outliers. Asterisks indicate  $P < 0.01$ ).

Descriptor	E <sub>4</sub>
NMR signal intensity 3-68 ppm	-0.6882*
NMR signal intensity 86-110 ppm	-0.7064*
NMR signal intensity 110-160 ppm	0.6553*
NMR signal intensity 0-25 ppm	-0.8180*
NMR signal intensity 25-40 ppm	-0.8211*
NMR signal intensity 40-60 ppm	-0.7502*
NMR signal intensity 60-90 ppm	-0.7729*
NMR signal intensity 90-110 ppm	-0.4832
NMR signal intensity 110-140 ppm	0.8020*
NMR signal intensity 140-160 ppm	0.5943*
NMR signal intensity 160-190 ppm	-0.4886
H/C atomic ratio	-0.6961*
Percentage of H	-0.8131*
Percentage of N	0.5743*
Benzenecarboxylic acids (perborate degradation)	0.8633*
Total aromatics (perborate degradation)	0.7028*
Total aliphatics (perborate degradation)	-0.4547
Benzenecarboxylic acid/phenolic ratio	-0.7529*
Aliphatic/aromatic ratio	-0.4919
Aliphatic/benzenecarboxylic ratio	-0.7743*
Syringyl-type compounds	-0.4001
Quercetyl-type compounds	-0.7182
Aromaticity	0.8777*
Total phenols (Calkal-oxid pyrolysis)	-0.6682*
Total 12 phenols (Calkal-oxid pyrolysis)	-0.6682*
OC200 intensity of the FT-IR band	-0.9331*
OC300 intensity of the FT-IR band	-0.8777*
OC450 intensity of the FT-IR band	0.6009*
OC510 intensity of the FT-IR band	0.6009*
OC560 intensity of the FT-IR band	0.9611*
OC1270 intensity of the FT-IR band	0.8191*
OC1370 intensity of the FT-IR band	0.5230
OC1500 intensity of the FT-IR band	-0.7544*