

## COMPARISON OF CuO OXIDATION, ANALYTICAL PYROLYSIS AND IR SPECTROSCOPY TO STUDY THE LIGNIN SIGNATURE IN SOIL HUMIC ACIDS

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One of the most common methodologies in the study of lignins in soils is alkaline CuO oxidation [1]. This method allows quantification of the released lignin-derived phenols, however, it is time-consuming and requires previous derivatization before GC. In order to compare the information on lignin composition provided by current routine techniques used on soil humic acids (HA), this research compares molecular assemblages obtained from 16 HA samples by using either CuO degradation, or analytical pyrolysis (Py), as well as a non-destructive technique such as infrared (IR) spectroscopy.

The HAs were extracted from 16 Mediterranean soils of central Spain. The alkaline CuO degradation [2] was applied to 30–50 mg of purified HAs. The digests were extracted with ethyl acetate, silylated with BSTFA and quantitatively analyzed by GC (FID detector). Cumulative concentrations of guaiacyl (G), syringyl (S) and cinnamyl (C) lignin units were calculated. On the other hand, Py-GC/MS applied to HA samples (0.5–1 mg) also released characteristic lignin-derived methoxyphenols of the G- and S-type [3], and the corresponding CG peaks were referred to the total ion chromatographic area. Finally, the second derivative IR spectra (4000 to 400  $\text{cm}^{-1}$ ) showed the variable intensity of the lignin band pattern in the HAs. The normalised intensities of IR bands *ca.* 1460  $\text{cm}^{-1}$  (asymmetric C–H bending in G and S  $-\text{OCH}_3$  groups), 1420  $\text{cm}^{-1}$  (G and S aromatic  $-\text{OCH}_3$  bending), were employed as surrogates of the total lignin amounts, and the intensities of the bands at 1330  $\text{cm}^{-1}$  and 1270  $\text{cm}^{-1}$  were used for the identification of S and G groups respectively [4].

The results of the three analytical procedures were compared by linear regression models. The concentration of lignin-derived compounds obtained by the CuO degradation, expressed as the G+S+C sum ( $\text{mg}\cdot\text{g}^{-1}$  HA), showed significant ( $P < 0.05$ ) correlation with the

pyrolysis results (integrated area of G+S phenols) as well as a very significant correlation ( $P < 0.01$ ) with the intensity of IR bands both at 1460 and 1420  $\text{cm}^{-1}$  (Figure 1a and 1b).

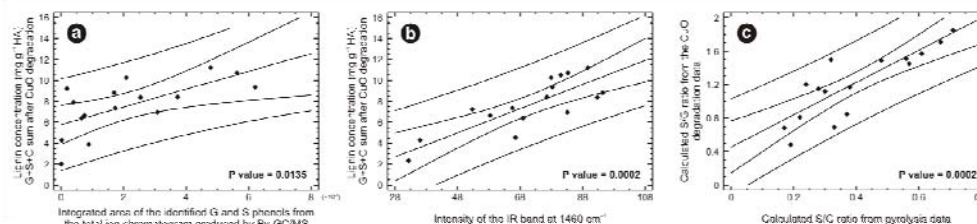


Figure 1. Linear regression between data from CuO degradation, pyrolysis and IR spectroscopy. Inner and outer curved lines show 95% confidence and prediction limits, respectively.

The S/G ratio of the studied HAs ranged from 0.71 to 1.85 with an average of 1.18 in the case of CuO oxidation. This ratio was also determined from Py and IR data, showing values from 0.17 to 0.71 for pyrolysis data, and from 0.15 to 0.96 from IR band intensities. In the pyrolysis case, this deviation was explained as the higher resistance to degradation of G-lignin domains as regards S-structures, the latter released in higher proportions by pyrolysis than by CuO. Concerning S/G ratios from IR data, the deviation was explained as a weak specificity of the bands at 1330  $\text{cm}^{-1}$  and 1270  $\text{cm}^{-1}$  for S and G units respectively. However, outstanding correlation ( $P < 0.01$ ) was found between S/G ratios from CuO and pyrolysis (Figure 1c).

Our results suggest that although alkaline CuO degradation method is probably the most accurate technique to study the lignin signature in HAs, analytical pyrolysis provides similar qualitative information, requires smaller sample amounts and avoids time-consuming pre-treatments or derivatisations. The IR spectroscopy has different limitations, but it also allows comparative analyses between samples as a first classification criterion in terms of the lignin pattern in the SOM.

## REFERENCES:

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