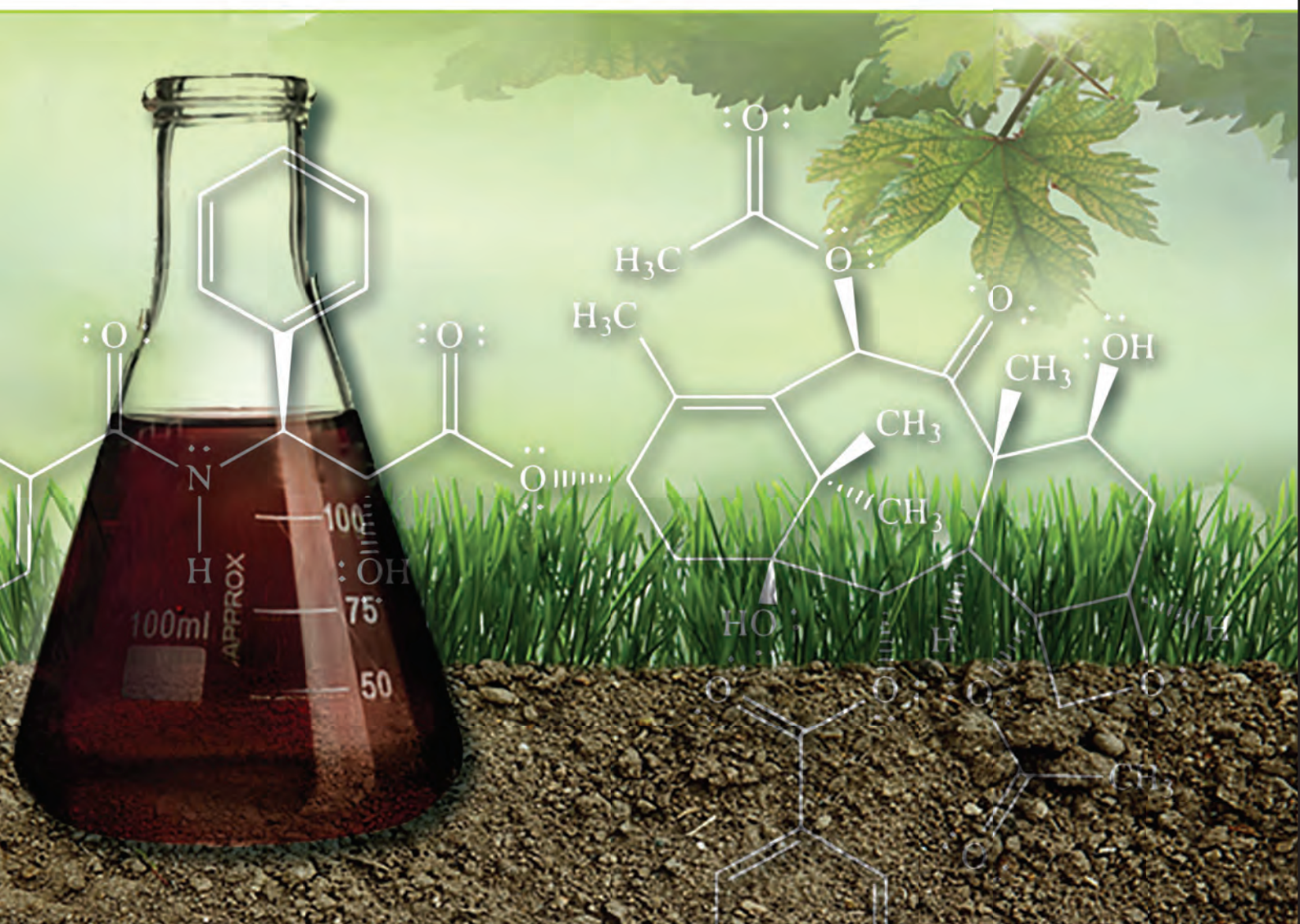


19th INTERNATIONAL CONFERENCE OF INTERNATIONAL HUMIC SUBSTANCES SOCIETY

Humic Substances and Their Contribution to the Climate Change Mitigation



16-21
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Albena Resort
Bulgaria



BOOK OF ABSTRACTS

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Evolution of Maize Residues into SOM in a study of two years incubation

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Abstract. Maize (*Zea mays*, a C4 plant) stover was incubated in a long-term cultivated soil for 24 months in order to better understand the transformation of crop residues into soil organic matter. Humic fractions, including humin, were isolated using an exhaustive sequential extraction procedure based on the charge density and polarity differences of the humic substances: Repetitive aqueous NaOH extraction was carried out at pH 7, 10.6, 12.6, and with 0.5 M NaOH. Then, an exhaustive 0.1 M NaOH + 6 M urea solution was applied to the soil residue to isolate the soluble 'urea humin' fraction. All isolates were further fractionated by the XAD-8 and XAD-4 resin-in-tandem procedure. Finally a concentrated sulphuric acid plus DMSO solvent was employed to the fine clay to isolate the most recalcitrant humin material. The samples were characterized by elemental and $\delta^{13}\text{C}$ analyses, FTIR and solid-state ^{13}C NMR spectroscopy with CSA-filter and DD spectral editing. The data indicated that ca 47%~60% and 28%~40% of transformed organic carbon was in the HAs and FAs fractions. The urea FA fractions and DMSO/acid FA fractions showed less ^{13}C enrichment compared to the fulvic fractions isolated in alkaline only solutions. The refractory humin was predominantly aliphatic and with low aromaticity.

Introduction

The return to soil of crop residues to maintain soil structure and increase soil fertility is common practise. There is now increased focus on its role in increasing soil carbon sequestration and in mitigating greenhouse gas emissions. The sequential extraction procedure can be used to study the compositions of components of soil humic substances (HSs).

In order to better understand the transformation process of the organic matter, maize stover was incubated for 24 months in a soil that had been under long-term cultivation to wheat for over 30 years. An exhaustive sequential extraction procedure was used to isolate a series of humic, and also humin fractions from the incubated soil [1, 2, 3, 4]. The humic fractions and humin were characterized using elemental and $\delta^{13}\text{C}$ analyses, FTIR and solid state ^{13}C NMR spectrometry. In some cases, the data are also compared with those for humic samples isolated from a 12 month incubation study under the same condition and with the control (unadvised) soil.

Materials and methods

Soil and experiment design. A cultivated soil [3] sample (32.5 kg) from the Teagasc, Carlow, Ireland, Oak Park site, was mixed thoroughly with chopped and ground maize powder (24.5 kg), and some NPK fertilizer (400 g) was added. The soil mixture was incubated in a plastic barrel in the laboratory, and stirred and watered regularly. The soil sample studied was taken after incubation for 24 months.

Extraction of HSs After H^+ exchanging and washing with water, the soil was exhaustively extracted in aqueous solutions (with pH adjustments using aqueous NaOH) at pH values of 7, 10.6, and 12.6, then with 0.5 M NaOH, and finally with a urea enhanced alkaline solution (0.1 M NaOH + 6 M urea) [1, 3]. Finally the organic matter-enriched fine clay was extracted with DMSO+6% conc. H_2SO_4 (v/v). For more details please refer to [1, 2, 3].

Results and Discussion

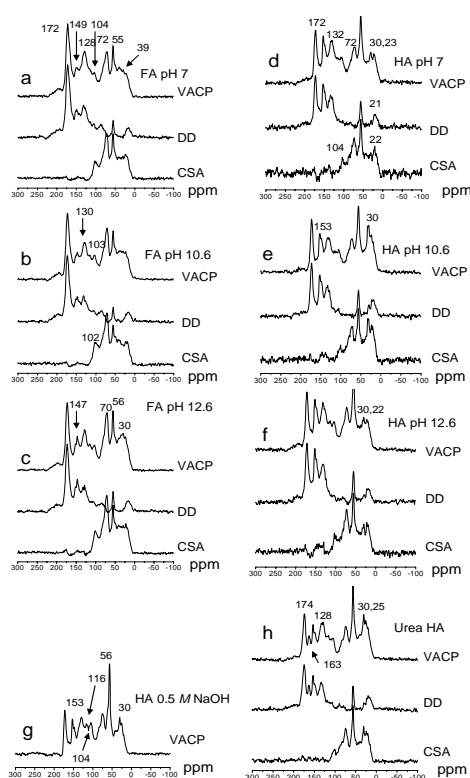
Yields, elemental and $\delta^{13}\text{C}$ analyses

The yields of humic fractions isolated were significantly different from those of the 1st year (Table 1; [3]). In the 1st year, the fractions isolated at pH 7 could be attributed mainly to the native soil. In the 2nd year, yields significantly increased due to transformations of maize to SOM. Yields of highly oxidized, well humified material at pH 7 were low compared to those for the less humified fractions isolated at pH 10.6 and pH 12.6 (which accounted for 47.2 and 38.7% of the yields, respectively). Compared to the significant increases for fractions isolated at pH 10.6 and 12.6, the urea isolates dropped to 1.5%. The maize material contributed to the major proportion of urea HAs in the 1st year, as revealed by high isotopic ^{13}C values. The yield of DMSO humin was about 64%.

A $\delta^{13}\text{C}$ enrichment in the urea isolates compared to the control soil was observed in the 1st year [3]. However, the urea FAs and DMSO/acid FAs fractions showed less ^{13}C enrichment compared to the fulvic fractions isolated in alkaline only solutions in the 2nd year. That was to be expected because the smaller molecule FA fractions isolated from "humin" were considered to be trapped in the humin matrix. From the results of $\delta^{13}\text{C}$ enrichment in the fine clay, and in humin (urea HAs, DMSO humin) (supported by another study), it may be considered that the accumulation of ^{13}C -enriched compounds resulted from the selective preservation of transformed plant residues following incorporation into the SOM, from the selective utilization of plant residues by microbes, as well as from some material directly derived from plant (maize) tissues. These results favour the in situ formation of SOM either by re-condensation of small molecules, or by selective preservation or utilization of biopolymers from soil microorganisms.

Table 1. Yields, C and N contents* and $\delta^{13}\text{C}$ values of the samples (*on a dry and free ash basis)

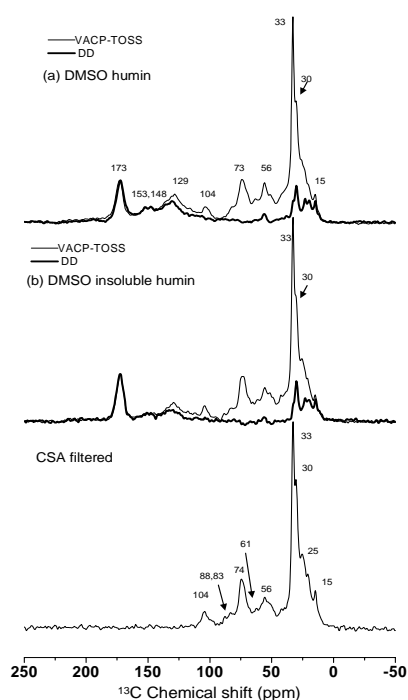
Fraction	Yield	C*	N*	$\delta^{13}\text{C}$
	g kg ⁻¹	%	%	‰
HA pH 7	1.16	50.2	4.74	-20.66
HA pH 10.6	13.44	63.86	5.83	-19.63
HA pH 12.6	10.86	58.42	5.59	-19.11
HA 0.5 M NaOH	0.52	57.63	5.97	-18.63
Urea HA	0.35	-	-	-
FA pH 7	1.04	55.1	4.94	-22.51
FA pH 10.6	0.91	50.09	4.42	-22.67
FA pH 12.6	0.9	49.11	3.59	-21.52
FA 0.5 M NaOH	0.12	52.96	4.45	-21.62
Urea FA	0.11	54.02	13.44	-23.4
XAD-4 acids pH 7	0.4	43.47	4.49	-21.99
XAD-4 acids pH	0.19	50.35	5.29	-21.69
XAD-4 acids pH	0.18	51.34	4.99	-21.51
DMSO FA*	-	42.12	5.45	-24.29

**Figure 1.** Full VACP-TOSS ^{13}C NMR spectra of FAs isolated from the incubated soil at pH7 (a), 10.6 (b), 12.6 (c), and of HAs isolated at pH7 (d), 10.6 (e), 12.6 (f), and 0.5M NaOH (g), and urea HAs (h) and corresponding DD and CSA spectra.

After two years of incubation, it is clear that lignin residues were still evident, especially in HAs, as revealed by sharp resonances for methoxyl at 55 ppm, and O-aryl C at 153 ppm (Fig. 1). The spectra of the HAs and FAs are relatively similar, though the FAs, are dominated by strong resonances for carboxyl, O-alkyl C, a sharp methoxyl, and relatively low aromatic C (110-140 ppm). The integrated areas data indicate that aliphatic C, methoxyl C from lignin units, and O-alkyl C (mainly from carbohydrate) gradually increased and the O-aryl C, the aryl and carboxyl groups progressively decreased as the pH values of the extracts

increased. The unprotonated aryl carbons in FAs varied between 73~79%, and these were greater than for the corresponding HAs fractions (DD spectra).

The spectral shapes for the HAs and urea HAs, are also similar. Significant sharp resonances are observed for: methoxyl, O-aryl C, and carboxyl groups, a strong aryl C resonance indicate significant contributions from lignin-type units, and the O-alkyl C resonance for carbohydrate is confirmed by the distinct anomeric C at 102 ppm in the CSA spectra. The percent of unprotonated C in the aryl C region decreased progressively as the extractant pH values increased, and varied from 64.6~53.8%. The urea HAs contained greater enrichment of aliphatic groups, and less methoxyl, O-alkyl C, and aryl C.

**Figure 2.** VACP-TOSS ^{13}C NMR spectra (thin line) of DMSO humin (a), DMSO insoluble humin (DIHU) (b) and the corresponding dipolar dephasing spectra (thick line), and chemical shift anisotropy (CSA) filtered- TOSS spectrum of DMSO insoluble humin (c).

The spectra (Fig. 2) for DMSO/acid humin are significantly similar, and show broad, but clearly resolved peaks. The VACP spectra have major peaks at 15, 30 (shoulder), 33, 56, 73, 104, 129 and 173 ppm. The largest peaks are in the aliphatic carbon region (0-46 ppm), indicating that both humin fractions are predominately aliphatic in nature) [1,4].

References

1. Song G., E. H. Novotny. A. J. Simpson et.al. Eur J Soil Sci. 59 (2008) 505-516.
2. Song G., M. H. B. Hayes, E. H. Novotny. A. J. Simpson. Naturwissenschaften 98 (2011) 7-13.
3. Song G., E. H. Novotny, J-D. Mao, M. H. B. Hayes. Sci Total Environ. 579 (2017)1843-1854.
4. Hayes M. H. B., R. Mylotte, R. S. Swift. Adv. Agron. 143 (2017) 27-138.