Table 1: Physical and chemical characteristic of Vertisols (Andalusian Blackearths)

Physikalische und chemische Charakterisierung von Vertisolen (Andalusische Schwarzerden)			
Profile	1	2	3
Colour	10 YR 3/1	10 YR 3/1	10 YR 2/2
Depth (cm)	0-15	0-15	0-15
Texture	sandy clay	clayey	sandy clay
Structure	polyhedral	crumby	crumby
pH (H ₂ O)	7.70	7.65	7.80
Org. matter (%)	1.13	1.28	2.0
C/N	9.6	14	11.3

The techniques used for the extraction and purification of the humic acids have been described elsewhere (Martin, 1970). Briefly, the soil samples were removed from roots and organic residues and were extracted with 0.1 N NaOH after decalcification with HCl. The humic acids solutions were precipitated with HCl and dissolved and precipitated several times. They were dissolved and centrifuged at 27 000 g, precipitated and treated with a dilute solution of HF-HCl (5 ml 48 % HF + 5 ml conc. HCl + 990 ml distilled water) for 24 hr, dialysed until they were free of Cl⁻. Finally they were dissolved in 0.1 N NaOH, passed over ion exchange resins (OH⁻ and H⁺ forms) and dried at 60 °C.

The humic acids were fractionated by gel filtration through Sephadex G-50. 0.5 g of each humic acid were dissolved in 50 ml of 0.1 N NaOH and passed through ion exchange resins, first Dowex 2 (OH⁻ form) and then Dowex 50 (H⁺ form) and made up to a volume of 250 ml with distilled water. The pH of the sol was 2.7.

A 10 ml aliquot of this sol was placed on the top of a 2.5 x 15 cm glass column, containing Sephadex G-50 and eluted with distilled water. Two fractions were obtained, that were collected at 1 ml portion in a fraction collector. This procedure was repeated until the 0.5 g were fractionated. The two fractions obtained, the first denominated F-I and the second F-II, were air dried at 60 °C.

The molecular weight (MW) was determined by Sephadex G-50 in a 2 x 40 cm glass column, according to the method of Swift and Posner (1971).

C and H were determined by dry combustion, N by the automated Dumas method. Oxygen was calculated by difference.

Total acidity was determined by the Ba(OH)₂ absorption method according to Brooks and Sternhell (1957), carboxyl by the calcium acetate method, according to Blom et al. (1957). In both determinations an automatic titrimeter (Radiometer) was employed.

Phenolic hydroxyls were assumed to be equal to the difference between total acidity and carboxyl groups.

Ultraviolet and visible spectra were measured in 0.1 N NaOH on a Beckman mod. DK 2A with automatic recording. IR spectra were taken on a Perkin Elmer mod. 621 in KBr discs. Electrophoresis was carried out according to Jacquín (1963).

Sonderdruck aus der
Zeitschrift für Pflanzenernährung und Bodenkunde
Journal of Plant Nutrition and Soil Science (Germany)
135. Band, Heft 1, 1973, Seite 1-96
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A water soluble fraction of humic acids from vertisols

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(Eingegangen: 21.11.1972)

Introduction

Soil humic matter is commonly divided into fractions with regard to the solubility in acid media. The term humic acids is applied to the insoluble fraction and the term fulvic acids to the soluble.

The comparative studies of both fractions show some differences in the elementary composition, optical density and behaviour towards electrolytes, but this classification is not concerned with the difference in well defined physical or chemical properties.

In recent years, by different methods, fractions with intermediate properties of humic and fulvic acids have been obtained, in such a manner that the actual classification, although convenient, is not the most suitable.

In the present paper, we report three cases of a fraction of humic acids extracted from vertisols and obtained by gel filtration through Sephadex. This fraction represents a considerable proportion of the total humic acids, is completely soluble in water and it has characteristics which allow it to be included in the group of the brown humic acids.

Material and methods

The humic acids were extracted from the upper horizon of three vertisols (Andalusian Blackearth). The physical and chemical characteristics of the soils are given in table 1.

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Results and Discussion

Figure 1 shows the distribution of humic acids from profile 1 (HA-1) by Sephadex G-50. Identical distributions were obtained with the three parent humic acids.

Figure 1

Distribution of humic acids from vertisol on Sephadex G-50

Verteilung von Huminsäure aus Vertisol on Sephadex G-50

In each case, the humic acids are separated into two peaks, the first eluted with the V_0 of the column, and the second just before the column volume V_t . Gjessing (1965) in the fractionation in acid media of humic substances from natural water, found also two peaks with distribution coefficients (K_d) of 0 and approximately 1, respectively.

As Swift and Posner (1971) do not consider the use of water as eluant, for a correct estimation of MW in humic acids, the F-I and F-II fractions were individually passed at neutral pH over G-50 and eluted with TRIS (2-amino-2-hydroxymethyl-propane-1,3-diol). The fractions remain unfractionated, having the same K_d as in acid media, proving their homogeneity. The same thing happened with the parent humic acids with respect to their K_d .

When the fraction F-I was filtered through Sephadex G-100, it was also completely eluted with the V_0 , therefore it probably has a MW > 100 000. When F-II was applied to a column of Sephadex G-25 one peak alone was obtained, and a MW ~ 5 000 was estimated for it.

Contrary to what other authors have stated (Gjessing, 1965; Posner, 1963 and Lindqvist, 1967) no irreversible adsorption was observed in the gel, when the humic acids were passed through it in acid media. However, humic acids extrac-

ted from the same soils by other extractants and also fractionated in acid media present reversible and irreversible adsorption (Vila et al. 1973, a).

In acid media it is obtained a clear separation between F-I and F-II fractions, with a distribution of 7:3 in weight respectively.

The fractions of smaller MW, F-II, are completely soluble in water in the three cases presented here and also are soluble those from humic acids extracted with 0.1 N NaOH from these type of soils.

Table 2 shows the elementary composition of the parent humic acids and their fractions.

Table 2: Elementary composition of parent humic acids and their fractions and O/C and H/C (atomic ratio)

Elementzusammensetzung von Ausgangs-Huminsäuren und ihrer Fraktionen sowie O/C- und H/C-Werte (Atomverhältnisse)

	per cent					
	C	H	N	O	ashes	H/C
HA-1	57.97	3.74	2.71	35.58	1.01	0.46
F-I	59.65	3.72	2.75	33.88	n.d.	0.42
F-II	54.06	3.78	2.61	39.55	n.d.	0.54
HA-2	57.33	4.11	4.20	34.16	1.11	0.44
F-I	58.43	4.63	4.65	32.39	n.d.	0.41
F-II	55.60	3.74	3.20	37.46	n.d.	0.50
HA-3	57.71	4.54	4.78	32.97	1.12	0.43
F-I	58.83	4.99	4.99	31.19	n.d.	0.40
F-II	56.14	3.83	3.24	36.79	n.d.	0.49

Oxygen was calculated by difference

The values of C, H and N of the parent humic acids are included among the normal values for humic acids that come from these soil types, with the exception of the N value of HA-1, smaller than the others.

In all cases, F-I has a higher value of C, H and N than F-II. Dell'Agnola and Ferreri (1971) in humic acids from a terra rossa found that the fractions of smaller MW have a higher value of C and N than those of higher MW. Roulet et al. (1963), Schnitzer and Skinner (1968) and Butler and Ladd (1969) found, on the contrary, an increase in the N-content with the MW. Rashid and King (1971) in humic acids from marine sediments also found an increase in C with the MW, but no order in the distribution of N between the different fractions was obtained.

Studying the diagram in which the atomic H/C ratio is plotted as a function of the atomic O/C ratio (Van Krevelen, 1952) the parent humic acids and the

fractions F-I and F-II are in the region which corresponds with non-condensed aromatic six-rings. In this diagram it can be seen that the fractions F-II may proceed from the parent humic acids or F-I when methane is split off. The main reaction can be a demethanation accompanied by a slight oxidation. The F-II from HA-1 can proceed by an hydration although a demethanation takes place also.

These processes may also be observed in the IR spectra of the parent humic acids and their F-I and F-II (figure 2). The band at 2930 cm^{-1} due to the aliphatic C-H stretching, is appreciably smaller in F-II than F-I and the band at 1720 cm^{-1} (C=O) higher.

Table 3 shows the values for total acidity, carboxyl groups and phenolic hydroxyls of the parent humic acids and their F-I and F-II.

Table 3: Functional groups of parent humic acids and their fractions
Funktionelle Gruppen von Ausgangs-Huminsäuren und ihrer Fraktionen

	me./g		
	Total acidity	Carboxyl	Phenolic OH
HA-1	6.90	4.40	2.50
F-I	5.77	4.00	1.77
F-II	5.09	3.48	1.61
HA-2	4.86	4.31	0.55
F-I	5.43	3.53	1.90
F-II	6.79	4.22	2.57
HA-3	3.95	3.91	0.04
F-I	5.54	4.04	1.50
F-II	6.00	4.12	1.88

It is specially interesting to point out the low content of phenolic hydroxyls in the parent humic acids, with the exception of HA-1, typical of humic acids from these soils (Martin, 1970). The fractions F-I and F-II have, with the exception of HA-1 too, more acidity than their parent humic acids and this increase in total acidity is due to a greater content of phenolic hydroxyls since the carboxyl groups are similar.

The total acidity tends to increase with decrease in MW. This increase may be associated with the solubility, in the same way, as the more soluble fraction of humic matter, the fulvic acids, have a greater total acidity.

Figure 2 shows the IR spectra of HA-2 and their F-I and F-II. The spectra are similar to those for humic acids and there are few differences between them, such as the greater intensity of the band at 2930 cm^{-1} in F-I, above mentioned, and the appearance of two bands at 1114 cm^{-1} and 1010 cm^{-1} in the F-II spectra. The bands near 1000 cm^{-1} have been attributed to Si-O, but in this case due to the low ash content may be also assigned to benzene ring substitution (Bellamy 1958).

IR spectra of a humic acid and its fractions
IR-Spektren von Huminsäure und ihrer Fraktionen

UV and visible spectra of HA-2 and their fractions. Note: The curves F-I and HA-2 have been displaced to avoid interesting

Spektren im UV und Sichtbaren von HA-2 und ihrer Fraktionen.

Anmerkung: Die Kurven F-I und HA-2 wurden verschoben, um Überschneidungen zu vermeiden.

The UV and visible spectra from HA-2 and their fractions are shown in figure 3. The other spectra are similar to these.

The visible spectrum failed to show characteristic peaks as normal in humic acids. In the UV region of HA-2 two inflections are shown at 295 m μ and 265–270 m μ . F-I has an inflection at 325 m μ , a saddle at 275 m μ and a well defined maximum at 255 m μ . F-II has only one inflection at 265 m μ .

It is very difficult to assign the bands in the UV spectra of humic acids because the polysubstitution on the aromatic nuclei causes displacement of the original bands. It is well-known that the bands in the region 265–275 m μ and in 295 m μ may be considered as a shift of L.E. band of benzene at 254 m μ (Scott, 1964). A shift of the bands is also possibly due to the effect of alkaline solutions on the spectra.

The peaks shown by F-I are also present in N-heterocyclic compounds (Morton, 1962). Martin et al. (1971) have found that part of the N content of humic acids from these soils is in heterocyclic form. Elsewhere fractions F-I are of greater N content (Table 2).

Table 4 shows the values of E_4/E_6 ratio and $\Delta \log K$.

Table 4: E_4/E_6 ratio and $\Delta \log K$ of parent humic acids and their fractions
 E_4/E_6 -Quotienten und $\Delta \log K$ -Werte von Ausgangs-Huminsäuren und ihren Fraktionen.

	E_4/E_6	$\Delta \log K$
HA-1	5.0	0.59
F-I	4.9	0.61
F-II	4.9	0.60
HA-2	6.4	0.69
F-I	6.3	0.75
F-II	4.5	0.59
HA-3	5.3	0.65
F-I	5.5	0.68
F-II	5.1	0.65

From the values of E_4/E_6 ratio it is deduced that all the parent humic acids and their fractions may be included in the so called brown humic acids. As in the other experimental data mentioned above, there is not a great difference in the values of E_4/E_6 ratio but those from F-II are smaller than those from F-I, this means according to Kononova (1966) that fractions F-II are more humified. The values for $\Delta \log K$ (Kumada, 1965) lead us to classify them as type B, and also this value shows that F-II are the most humified.

Studying the electrophoretograms, it is observed that the parent humic acids and their F-I and F-II give a well defined band with very little mobility towards the anode. F-II presents somewhat greater mobility. The band from the parent humic acids is broader than the fractions and its front and tail are comprised between the front of F-II and the tail of F-I. In all cases, moreover, there is a weak zone, visible under UV light, with very high mobility. No band remains at

the origin where the substances were initially deposited, which means that parent humic acids and their fractions are only constituted by brown humic acids.

Conclusions

The humic acids from vertisols (Andalusian Blackearth) extracted with 0.1 N NaOH and fractionated at acid pH by gel filtration on Sephadex present two fractions, one of high MW > 100 000 (70 % of the parent humic acids) and the other with a MW ~ 5 000 (30 % of the parent humic acid), the latter being completely soluble in water. As the 0.1 N NaOH extract 30 % of total C like HA (Vila et al. 1973, b), the latter fraction represents 9 % of total C in soil.

The chemical characterization of both fractions shows that there are not strong differences between them and both belong to the group of brown humic acids. Some of the experimental data show also that F-II, those from minor MW, are more humified.

The humification process may be a result of condensation of aromatic nuclei and an oxidation (or hydrolysis: HA-1) and further breakdown of humic molecules. From this breakdown, molecules of humic acids with smaller MW than fulvic acids can arise.

Perez and Martin (1970) have proved the penetration of humic acids between the clay layers, the increase of the d_{001} spacing being proportional to the MW of the humic matter. Non published data of these authors, show that fulvic acids from vertisols fractionated at acid pH through Sephadex G-50, are separated into two fractions, those from low MW represents 90 % of the total. This fraction induced a d_{001} spacing of a montmorillonite at 17.65 Å, while the F-II fraction produces a spacing at 14.70 Å. This shows that the latter has a smaller MW than the former.

Some authors (Schlichting, 1953; Kyuma, 1964; Kononova, 1966) have proved the existence of intermediate forms between humic and fulvic acids, called by Schlichting "humo-fulvic acids" with properties of both. The existence of a humic acid fraction, soluble in water may be an evidence of these intermediate forms, and of the artificiality of the division of the humic matter in humic and fulvic acids as pointed out by Kononova (1966) and Duchaufour, (1970).

Summary

Two fractions, one of them soluble in water, separated by gel filtration on Sephadex in acid media, from humic acids of Vertisols have been obtained. There are some differences between both fractions.

The molecular weight, determined by Sephadex, is of > 100 000 for the non-soluble fraction (70 % of the total) and ~ 5 000 for the soluble one (30 % of the total).

The elementary composition, functional groups and IR spectra are very similar, but the UV spectra of the non-soluble fractions have some inflection due probably to the greater N-content.

The analytical data, E_4/E_6 ratio and $\Delta \log K$ include both fractions in the so called brown humic acids, the water soluble fraction being more humified. This fraction may proceed from the parent humic acids or non-soluble fraction by demethanation and oxidation or hydrolysis as inferred from the *Van Krevelen* diagram and IR spectra.

The existence of a humic acid fraction soluble in water may be evidence of the intermediate forms between fulvic and humic acids and of the artificiality of the division of humic matter,

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- Eine wasserlösliche Fraktion von Huminsäuren aus Vertisolen
von F. Martin und C. Saiz-Jimenez
- Es wurden zwei Huminsäurefraktionen aus Vertisolen, eine davon wasserlöslich, durch Gelfiltration an Sephadex im sauren Medium hergestellt. Zwischen den beiden Fraktionen gibt es einige Unterschiede:
- Das Molekulargewicht, mit Sephadex bestimmt, ist > 100 000 für die unlösliche (70 % des Gesamtmaterials) und ≈ 5 000 für die lösliche Fraktion (30 %).
- Elementzusammensetzung, funktionelle Gruppen und IR-Spektren sind sehr ähnlich, aber die UV-Spektren der unlöslichen Fraktionen weisen einige Inflektionen auf, die möglicherweise von dem größeren N-Gehalt herrühren.
- Nach analytischen Daten, E_4/E_6 -Quotienten und $\Delta \log K$ -Werten gehören beide Fraktionen zu den sogenannten Braunkhuminsäuren, wobei die wasserlösliche Fraktion stärker humifiziert ist. Diese Fraktion kann aus den Ausgangs-Huminsäuren oder unlöslichen Fraktionen hervorgehen durch Entmethanisierung und Oxidation oder Hydrolyse, wie aus dem *Van Krevelen*-Diagramm und aus den IR-Spektren zu folgern ist.
- Die Existenz einer in Wasser löslichen Huminsäurefraktion kann ein Nachweis sein für Zwischenstufen zwischen Fulvo- und Huminsäuren wie andererseits auch für die Willkürlichkeit der Aufteilung von Huminstoffen.
- [3558]