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SOME PHYSICO-CHEMICAL ASPECTS OF THE INVESTIGATION OF HUMUS

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As a contribution to the discussion on the physico-chemical properties of humus and its constituents the need for adequate and uniform methods for extraction, fractionation and purification of the humus substances is strongly emphasized.

In accordance with literature, humic acids are assumed to be a globular, polydisperse, irregular polycondensate. The discrepancy of the data given in the literature regarding dimensions and structure of humic acid molecules is largely ascribed to the divergence in methods for isolating these substances.

Different methods used to determine the physico-chemical properties of these substances may give different results. In some cases it is questionable whether the method involved can give the pertinent information.

Critical remarks are made regarding the usual methods for differentiating between the types of acid groupings in humic acids, especially on those based on ion exchange.

The question of the nature of the bond between humus constituents and metal ions is briefly discussed. The occurrence of chelating compounds in the soil organic matter is an almost established fact but the detailed structure of these chelates is still mainly unknown.

The ion exchange behaviour of humus and humic acids was also discussed. These substances show a distinct selectivity for polyvalent cations compared with monovalent ones, which can be expressed in a selectivity coefficient. As yet, there has not been found an exchange law of general applicability, which is not surprising in view of the difficulties mentioned.

Au sujet des propriétés physico-chimiques de l'humus et des ses constituants, l'auteur insiste fortement sur le besoin de méthodes appropriées et uniformes d'extraction, de fractionnement et de purification des substances humiques.

D'après la documentation disponible, les acides humiques seraient des polycondensats globulaires, polydispersés et irréguliers. Si toutefois les données publiées diffèrent en ce qui concerne les dimensions et la structure de leurs molécules, c'est en grande partie à cause du manque d'uniformité des méthodes utilisées pour isoler ces acides.

Les résultats peuvent différer selon la méthode employée pour déterminer les propriétés physico-chimiques de ces substances. Il est parfois même douteux que la méthode utilisée puisse donner des résultats satisfaisants.

L'auteur procède à une évaluation critique des méthodes habituellement utilisées pour différencier les types de groupements acides des acides humiques, en particulier celles qui se fondent sur les échanges d'ions.

Il examine brièvement la question de la nature de la liaison entre les constituants de l'humus et les ions métalliques. Si l'apparition dans les matières organiques du sol de composés de chélation est un phénomène prouvé, la structure de ces complexes de chélation n'est pas encore établie en détail.

L'auteur étudie également le comportement de l'humus et des acides humiques sous l'angle des échanges d'ions. Ces substances manifestent une sélectivité bien plus marquée pour les cations polyvalents que pour les cations monovalents, propriété que l'on peut exprimer par un coefficient de sélectivité. Il n'a pas encore été possible d'établir une loi des échanges susceptible d'application générale, ce qui n'est pas surprenant vu les difficultés mentionnées.

Como contribución al estudio de las propiedades fisicoquímicas del humus y de sus constituyentes, se subraya insistentemente la necesidad de métodos apropiados y uniformes de extracción, fraccionamiento y purificación de las sustancias húmicas.

Según la literatura, se supone que los ácidos húmicos son policondensados globulares, polidispersos e irregulares. La discrepancia de los datos que aparecen en la literatura respecto a las dimensiones y la estructura de la molécula de los ácidos húmicos se explica mayormente por la divergencia de los métodos utilizados para aislar estas sustancias.

Los resultados pueden ser diferentes según los métodos que se utilicen para determinar las propiedades fisicoquímicas de estos ácidos. En algunos casos, es cuestionable que el método utilizado pueda proporcionar la información pertinente.

Se hacen observaciones críticas acerca de los métodos usuales para diferenciar los tipos de grupos ácidos en los ácidos húmicos, y especialmente acerca de los métodos que se basan en el intercambio iónico.

Se examina brevemente la cuestión de la naturaleza del enlace entre los constituyentes del humus y los iones metálicos. Se ha comprobado la presencia de compuestos quelantes en la materia orgánica del suelo, pero la estructura de los complejos quelantes no se ha establecido en detalle.

Se examina también el comportamiento del humus y de los ácidos húmicos desde el punto de vista del intercambio iónico. Estas sustancias presentan una clara selectividad para los cationes polivalentes en comparación con los monovalentes, propiedad que puede expresarse en un coeficiente de selectividad. Hasta la fecha, no ha sido posible establecer una ley de los intercambios que sea de aplicabilidad general, lo cual no es sorprendente si se tienen en cuenta las dificultades mencionadas.

1. INTRODUCTION

The physical and physico-chemical properties of soils, such as structure, water retention and ion exchange and consequently their fertility, are strongly affected by the soil organic matter. This has been recognized for a long time and therefore the relations between these soil properties and the amount and quality of organic matter has been the subject of many investigations.

Many conclusions have been drawn concern-

ing these relationships but they have not always been based upon data obtained from reliable experiments. Consequently much work remains to be done in this field.

Besides direct research into the effects of the organic matter as a whole on the physical and chemical properties of the soil, many investigators have examined the physico-chemical properties of certain fractions of humus, especially of humic acids, extracted from the soil. The latter investigations are certainly very useful for elucidating the mechanism of

the processes. It should be borne in mind, however, that the results are not always easily transferable to the soil as a whole, as there are often interrelationships between different properties, e.g. between the degree of swelling or dispersion (water content) and ion exchange behaviour.

An excellent comprehensive review of the literature up to 1960 is given by SCHEFFER and ULRICH⁽²⁰⁾ A valuable summary is also given by KONONOVA.⁽¹⁴⁾ Thus, I will confine myself to some general remarks and only enter into details on certain topics where recent investigations have been made or where important questions remain to be solved.

2. EXTRACTION OF ORGANIC CONSTITUENTS

Soil organic matter is a very complicated, polydisperse, multi-component system. The study of it is complicated by the fact that it never occurs in pure form but is mixed with and even partly linked to the mineral soil constituents. The presence of inorganic components involves, especially in the examination of different physico-chemical properties, an unknown factor which may even exert a dominating influence on these properties. For example, if one wants to examine the size of humic acid particles in solution, it is necessary to be sure that there are no inorganic substances present, acting as "bridges" between these particles.

Another problem is the lability of the soil organic material. Some components are easily decarboxylated,⁽⁴⁾ others sensitive to oxidation and subsequent condensation or breakdown reactions. It is evident that this may also have a distinct effect on the properties to be investigated, such as ion exchange, particle size, etc. This means that drastic chemical treatments should be avoided, however efficient they might be as to the amount of organic matter extract-

ed or as to the ash content of the extracted substances.

Therefore, the first difficulty encountered is how to extract, to fractionate, and to purify the organic constituents. It is often a matter of finding a compromise. In this respect there is a definite need for a technique by which the acceptability of a given treatment can be checked accurately. Spectroscopic measurements in the visible and ultraviolet region are of no help here as the absorption spectra obtained are highly uncharacteristic (an approximately linear curve of log extinction vs. wavelength). Recent investigations^(7, 9, 26) give hope that in this respect infrared spectroscopy might become a useful technique.

An indication of the difficulties inherent in the extraction of organic material from soil is the large number of extractants used (see the review given by SCHEFFER and ULRICH (Ref. 20, p. 56). Here, attention may be drawn to a new, in many respects promising, extraction method designed by BREMNER and HO⁽¹¹⁾, in which the chelation resin Dowex A-1 is used.

It would be a grave error to think that the extractants only differ as to the yield of extracted matter. There are also specific effects as can be concluded from the different C/N-ratios and ash contents of the humic acids in Table 1. This table (from unpublished data obtained by the author) also shows that the order of efficiency is not the same for different soils and that the effect of a pretreatment with a dilute solution of hydrochloric acid is different for different soils.

Despite the fact that a great deal of work has been done in this field, no definite extraction method can be recommended for all soils and for all purposes of investigation. It may prove impossible to develop such a uniform method. It would be, however, a very important step forward if we could at least agree to restrict

Table 1. A comparison of four extractants for humic acids from four different soil types, with and without pretreatment of the soil with 0.1 N HCl

Soil type	Extractant	Without pretreatment				After pretreatment with 0.1 N HCl			
		% C _{h.a.} in the soil	% N _{h.a.} in the soil	C/N h.a.	% "ash" in h.a.	% C _{h.a.} in the soil	% N _{h.a.} in the soil	C/N h.a.	"ash" in h.a.
Diluvial sandy soil	0.2 M NaF	0.20	0.070	3	4	0.24	—	—	9
	0.1 M Na ₄ P ₂ O ₇	0.76	0.058	13	12	0.69	0.052	13	18
	0.1 M NaOH	0.84	0.053	16	28	0.90	0.061	15	25
	* NaOH+NaF	0.65	0.048	14	23	0.68	0.068	10	26
Wood peat	0.2 M NaF	10.5	0.55	19	14	11.2	0.62	18	20
	0.1 M Na ₄ P ₂ O ₇	16.2	1.00	16	28	13.4	0.70	19	31
	0.1 M NaOH	20.4	1.28	16	31	21.4	1.34	16	31
	* NaOH+NaF	17.6	1.26	14	21	20.3	1.26	16	20
Chernozem	0.2 M NaF	0.20	0.016	12	56	0.41	0.029	14	71
	0.1 M Na ₄ P ₂ O ₇	0.70	0.045	16	87	1.10	0.082	13	79
	0.1 M NaOH	0.00	—	—	—	1.19	0.091	13	75
	* NaOH+NaF	0.16	0.017	9	59	0.47	0.032	15	20
Marine clay soil	0.2 M NaF	0.08	—	—	63	0.07	—	—	86
	0.1 M Na ₄ P ₂ O ₇	0.29	0.027	11	76	0.42	0.059	7	76
	0.1 M NaOH	0.05	—	—	64	0.41	0.027	15	62
	* NaOH+NaF	0.16	—	—	32	0.16	—	—	40

* A solution containing 0.1 M NaOH + 0.2 M NaF per litre.

ourselves to a limited number of well-described methods. In the present state of affairs the comparison of results of different investigators is in many cases absolutely impossible, the more so as some authors even omit an accurate description of their extraction procedure.

The same is also true for the purification of the extracted substances, though there is less diversity in methods.

3. SOME DISPUTED CHARACTERISTICS OF HUMIC ACIDS

3.1. Heterogeneity and Polydispersity

According to the prevailing conceptions, humic acids are formed by a complicated process of oxidation, condensation and addition reactions from compounds of poly-phenolic

and/or quinonoid character. These compounds are found in the soil in great variety as lignin and its degradation products and many metabolic products of micro-organisms. In this process nitrogen compounds, such as ammonia, amino acids and peptides may also be involved. Presumably humic acids can be best described as irregular "hetero-polycondensates". This explains their strictly amorphous character in dry condition and perhaps also their resistance to decomposition by micro-organisms.

As several of the humic acid precursors have more than two active sites, it is probable that a globular type of polycondensate is formed. The composition is presumably dependent on several "incidental" factors, such as type of plant residues, micro-organisms present, temp-

erature, moisture content, aeration, pH, composition of inorganic soil constituents, etc.

All these circumstances make it very probable that there will be not only a variation in molecular construction but also a rather wide variation in molecular or particle weight. At least part of the differences in composition of humic substances extracted with different solvents must be explained on this basis.

The fractionation of humatomelanic from humic acids with the aid of alcohol is nowadays seldom applied. The same is true for the fractionation by partial flocculation of "Braun-" and "Grau-" humic acids. Originally it was thought (e.g. SCHEFFER and WELTE⁽²¹⁾) that this partial "salting out" accomplished a separation of two essentially different types of humic acids, but recent publications of SCHEFFER and SCHLÜTER⁽²²⁾ and SCHARPENSEEL⁽¹⁹⁾ suggest that this partition is a rather conventional one.

In the last ten years much work has been done on fractionation of humic acids by means of partition chromatography and electrophoresis. In some cases the separation obtained was rather diffuse, in others, however, distinct zones were observed. Similar results were obtained by ultracentrifugation. It is not yet quite clear whether we are dealing here with a partition of substances with essentially different composition or substances differing in particle weight (or incidentally in size of micelles). Nevertheless it is now a definitely established fact that we are dealing with a poly-disperse system.

3.2. Size and Structure of the Particles

The values given by different investigators for the average molecular or particle weight vary as much as from about 500 to about 50,000. It is questionable whether this divergence can be ascribed to differences in origin of humic

acids (mineral soils, peats, composts, coals) and to the choice of extractant alone. It is very likely that some of the reported values are due to artefacts, caused by incomplete dispersion or purification or otherwise by a partial degradation of the particles before or during the determination. However, differences due to the method employed will undoubtedly explain some of the anomalies. Thus the number average molecular weight, derived from osmotic measurements, is always smaller than the weight average molecular weight, calculated from viscosity measurements. As to the latter calculation, it is questionable whether the application of Staudinger's rule, which is a limiting rule for loose structures, is valid for humic acids.

When computing the molecular weight from sedimentation velocity and diffusion in the ultracentrifuge, according to the formula used by Svedberg, the value obtained will usually lie between the number and weight average. Here it may be remembered that the use of this formula is only safe when the particles are compact and sediment separately without mutual interaction. There are indications that, notably in an acid medium, this condition is not met in the case of humic acids.

Some investigators calculated particle dimensions from diffusion measurements, applying the relation:

$$D = \frac{RT}{6\pi N\eta r}$$

where D is the diffusion coefficient and r is the particle radius. This relation, however, is only valid in case of spherical, uncharged particles. As to the shape of the particles the question still remains unanswered, but it is a well established fact that humic acids in solution are partly dissociated, and since the hydrated protons have a much greater diffusion constant than the large organic anions they drag the latter along, which

causes an increase in D (and thus an apparent decrease in r). This phenomenon will be stimulated by an increase of the dissociation of the acids as in alkaline medium. This has to be taken into account in a discussion of the dependence of D on the amount of alkali added to a humic acid solution as found by Scheele and discussed by FLAIG and BEUTELSPACHER.⁽⁸⁾

Concerning the shape of the particles or "molecules" similar comments can be made. Recently PIRET, WHITE, WALTHER and MADDEN⁽¹⁷⁾ carried out viscosity measurements on peat humic acids and they concluded that the "molecules" are non-spherical and probably hydrated. Assuming prolate ellipsoidal or rod-shaped molecules, an axial ratio of 1/11.2 was calculated. For disk-shaped molecules this ratio would even be 1/23. For both shapes the frictional ratio was estimated to lie between 1.60 and 1.86, which is of the same order of magnitude as that found by STEVENSON, VAN WINKLE and MARTIN⁽²³⁾ by ultracentrifugation.

It has to be regretted that PIRET *et al.*⁽¹⁷⁾ carried out their otherwise well designed measurements on a rather crude sample of humic acid; they mention a humic acid content of only 84.3 per cent, 8.3 per cent being "ash" and 7.4 per cent being "organic impurities". Furthermore they dried their humic acid for 2-4 days at 60° C which may affect its properties.

On the other hand, FLAIG and BEUTELSPACHER⁽⁹⁾ concluded from viscosity measurements and electronmicroscopic investigations that humic acid particles are of nearly spherical shape. They did not, however, allow for electroviscous effects as they carried out the viscosity measurements in salt-free solutions, which may affect the shape and size considerably. Theoretically, it may be that the dry particles are more or less spherical, which is the impression gained from electronmicrographs, but that the swelling is anisotropic, giving prolate ellipsoidal particles, whereas the mutual repulsion of the ionized

groups in its turn tends to cause a more spherical shape again.

All these considerations strongly emphasize the need for suitable methods for extraction, fractionation and purification.

Very interesting questions which need more detailed investigation are: the influence of the nitrogen content of the humic acids on their viscosity as reported by FLAIG and BEUTELSPACHER⁽⁸⁾ and the behaviour of humic acids in the presence of mineral acids and salts (aggregation and micelle formation). These questions cannot be discussed here in detail but I would like to say something about the particle structure.

According to electronmicroscopic studies by FLAIG and BEUTELSPACHER⁽⁸⁾ the humic acid particles show a loose, spongy structure, which is dependent on the nitrogen content, a higher nitrogen content giving denser (more cross-linked) particles.

HEIMANN⁽¹¹⁾ mentions that the exchange capacity of humic acids when determined with quinine and quinidine, appeared to be 30, and 70 per cent resp. lower than when determined with sodium hydroxide, which is ascribed to steric hindrance. We showed, however,⁽⁶⁾ that in well-dissolved humic acids, all acid groups are in a position such that they can bind very large organic cations (e.g. quaternary ammonium bases as methylquininium hydroxide and tetrabutylammonium hydroxide) in the same amount as small cations such as Na^+ . This indicates too a rather loose and perhaps flexible structure.

The importance of investigations into the shape, size, structure, aggregation tendency, etc., of humic acid particles under different conditions may be stressed by pointing out that the dependent properties are nature and selectivity of ion binding, effect on soil consistency, swelling and shrinkage.

3.3 Differential Analysis of Acid Groups

Humic acids are polybasic acids with at least two kinds of acid groups, viz. carboxyl and phenolic hydroxyl groups. For a detailed study of the ion exchange behaviour of humus constituents we need to determine not only the total exchange capacity as the sum of all the acid groups but we also need to obtain an exact differential analysis of these acid groups. The organic chemical methods proposed in the literature are tedious and inaccurate. Inorganic methods are most widely used and these are entirely empirical and partly even fundamentally wrong.

For example, the amount of acetic acid set free on adding an excess of calcium acetate to a suspension of humus or humic acids is often taken to be a measure of the carboxyl group content (see e.g. KONONOVA⁽¹⁴⁾ p. 273). These groups are then supposed to be "more strongly acid" than acetic acid which is still disputable when considering the last dissociating carboxyl groups. When sodium acetate is used the result may be correct. To answer that question the spectrum of dissociation constants for COOH- and OH-groups, the ratio of the numbers of these groups and the acetate concentration must be known. The result is arbitrary unless all this data is known.

A prerequisite, however, which is certainly not met when calcium acetate is applied, is that the affinity of the polyanion and the acetate ion for the Ca-ion are the same. Otherwise, it is not only the strength of the acids present but also this unequal affinity which determines the amount of acetic acid set free. With humic acids there are strong indications that the calcium ions or at least part of them, are attached with one valency to $-\text{COO}^-$ and with the other to $-\text{O}^-$. It is clear that in that case the results of this "calcium acetate method" are, to say the least of it, quite inaccurate. Similar

comments can be made on other methods based on ion exchange.

We tried to differentiate between the acid groups of different "strength" in humic acids by electrometric titration.⁽⁶⁾ For a discussion the reader is referred to the mentioned publication. We concluded that the sum of all groups, showing acid properties in aqueous medium, can be determined best by a conductometric titration in aqueous solution with barium hydroxide. Although definite proof is still lacking, we assumed from the obtained results that the ratio of stronger to weaker acid groups (presumably carboxyl and phenolic hydroxyl groups) can be determined by a high-frequency titration of humic acids dissolved in dimethyl-formamide with an anhydrous solution of sodium isopropylate as a titrant. The ratio of COOH and OH groups found in this way appeared to be about 1 : 4 but there were deviations. On average, the COOH-group appeared to be neutralized in aqueous solution at a pH somewhat above 5, and at pH 7 the COOH-groups and about two OH-groups were neutralized. This differs from what is often taken for granted in the literature.

4. THE PARTICIPATION OF HUMUS AND ITS CONSTITUENTS IN ION-EXCHANGE PROCESSES

The degradation of fresh organic matter, resulting in the formation of humus, is accompanied by a rapid increase of the cation exchange capacity to values varying from about 150 to 300 me/100 g dry humus. Because of its high exchange capacity compared with that of the mineral soil constituents the share of humus in the total cation exchange capacity of the soil is relatively high. It ranges according to SCHACHTSCHABEL⁽¹⁸⁾ from about 25–35 per cent in heavy clay soils to about 90 per cent in light sandy soils.

A problem, never quite solved, is the exact determination of the fraction of the total ex-

change capacity due to soil organic matter and that due to mineral constituents. It seems, however, that summation may be assumed as a good first approximation. This knowledge is necessary to understand the selectivity of the soil for binding cations since the selectivity depends on the nature and on the capacity of the ion exchange material, both very different for the organic matter and the mineral fraction.

The importance of these questions and that of the mobility of the organic salts is evident in both plant nutrition, especially the supply of micro elements, and the participation of humus constituents in soil genetic processes. For example, the higher the exchange capacity, the higher the selectivity for divalent cations compared with monovalent ions, which means a better protection against podzolization processes.

A recent review on "metallo-organic interactions associated with podzolization" is given by WRIGHT and SCHNITZER.⁽²⁴⁾ In this discussion only two questions may briefly be mentioned, viz. the nature of the bond between the organic anions and the cations and the calculation of the ion exchange equilibria.

4.1. Nature of the Bond between Humus Substances and Cations

In many publications the term "chelate" is used for the bond between the humic anions and polyvalent cations, especially from the transition elements as manganese, copper, zinc, iron. A review of the literature on this subject (126 references) was recently published by MORTENSEN.⁽¹⁶⁾

However, it appears that different authors who are describing this bond as a "chelate", nevertheless do not mean the same. There is some confusion as to the definition of a chelate. To eliminate nomenclatural misunderstandings, it is recommendable to keep to the rules drawn up by the International Union of Pure and Applied Chemistry in 1957. When applying these rules consequently, the assembly of humic

anions and di- or polyvalent cations most likely has to be called a "polynuclear complex", consisting of an unknown number of "chelate ligands", "bridging groups" and "nuclear atoms".

This description probably holds for all organic polyelectrolytes and ion exchangers, charged with polyvalent cations. It says no more than that a humic anion binds several polyvalent cations in such a (coordinating) way that these stick to the anion with at least two bonds per cation. So, this nomenclature in no way implies an opinion upon the nature of the bond (electrostatic or covalent) or upon the exact configuration of the chelate-cation-rings.

The stability of a complex consisting of a metal ion and a chelating compound is as a rule very high, especially with elements of the transition series at higher pH-values. As to the chelating compound, the stability of the complex depends on the number of atoms which form a ring with the cation, the number of rings of which the cation is a member, the nature of the ligands and the presence of certain substituents in the chelate.

Now, humic acids are indeed capable of binding di- and polyvalent cations very strongly, depending on the pH. But here is still an other factor which may be responsible for this stability. As shown by GREGOR *et al.*⁽¹⁰⁾ the strength of the bond may be much greater in case of a polymeric acid, as polyacrylic acid, than with the analogous monomers acetic acid and glutaric acid as a result of the strong electrostatic field inside of the polymer clew.

Furthermore, it is true that several humus constituents possess atoms and groups of atoms which are known to be electron donors, viz. N and O, e.g. in $R-NH_2$, heterocyclic N, $R-O^-$ and $R-COO^-$. However, as is observed by Mortensen (l. c.), electron donors in polymers "are not free to move and cluster around a metal ion in the way small ligands behave".

Undoubtedly part of them can indeed chelate metal ions, i. e. that more than one ligand is attached to the same cation, but besides there may be some "lonely" ligands, which only "function as centers for ion exchange". (From this point of view it is also important to get an insight into the structure and flexibility of the humic acid molecules.)

The study of ion binding to precursors and degradation products of humic acids will be very useful for elucidation of the nature of the bond between humic acids and metal ions. Hereby the possibility of the above mentioned electrostatic field effect is eliminated. It should be kept in mind, however, that these low molecular substances might be capable of a strong chelation but that sterical hindrance may weaken this bond in case of humic acids or even prevent it at certain places in the molecule. Therefore, the statement of LEWIS and BROADBENT,⁽¹⁵⁾ that "the chemistry of functional groupings attached to large molecules such as those that comprise the bulk of soil organic matter is usually, though not always, similar to that of much simpler systems" has to be applied here with great caution.

The order of stability of the bond between humic acids and metal ions can be recognized from titration curves, obtained potentiometrically with alkali hydroxide in presence of salts (see e.g. 5, 13). This order corresponds with that, found with several low-molecular chelates, which is in favour of the concept, that there is a similarity in structure.

Concluding, it might be stated that according to the present rules for the nomenclature soil organic matter contains many chelating compounds but that the detailed structure of these chelates is still mainly unknown.

4.2. Ion Exchange Equilibria

SCHEFFER and ULRICH (Ref. 20, p. 72) give some ion distribution curves for alkali and alka-

line earth ions, obtained with peat humus (not pure humic acids) calculated from experimental data given by ZADMARD.⁽²⁵⁾ We obtained similar curves in measurements with humic acids (to be published elsewhere). It must be pointed out that in the figures given by SCHEFFER and ULRICH the equivalent-fraction of the "in-changing" ion adsorbed by the peat humus, is given as a function of the equivalent-fraction of this ion in solution, e.g. in case of exchanging K in K-humus against Ca by suspending the K-humus in water and adding a solution of CaCl₂, the equivalent-fraction of Ca adsorbed by the humus and that remaining in solution are resp. given by

$$\bar{X}_{Ca} = \left(\frac{me \text{ Ca}^{++}}{me \text{ Ca}^{++} + me \text{ K}^+} \right)_{\text{humus}}$$

$$\text{and } X_{Ca} = \left(\frac{me \text{ Ca}^{++}}{me \text{ Ca}^{++} + me \text{ K}^+} \right)_{\text{soln.}}$$

This method was also applied by DEUEL and HUTSCHNEKER⁽³⁾.

For a given composition of the equilibrium solution the selectivity can be expressed by the selectivity coefficient $k_{\text{humus}_{\text{K}}^{\text{Ca}}}$ calculated as:

$$k_{\text{humus}_{\text{K}}^{\text{Ca}}} = \frac{\left(\frac{\frac{1}{2} [\text{Ca}^{++}]}{[\text{K}^+]} \right)_{\text{humus}}}{\left(\frac{\frac{1}{2} [\text{Ca}^{++}]}{[\text{K}^+]} \right)_{\text{soln.}}} = \frac{\frac{\bar{X}_{Ca}}{1 - \bar{X}_{Ca}}}{\frac{X_{Ca}}{1 - X_{Ca}}}$$

For humus and humic acids the selectivity-coefficient appears to be greater than 1, which means a preferential adsorption of bivalent ions compared with monovalent ions.

When only monovalent ions are involved in the exchange reaction the distribution of ions is about the same as predicted by the mass action law or by the Donnan concept (that means, determined by the activity coefficients of the participating ions).

However, this does not apply when monovalent ions are exchanged with divalent ions or the reverse. Distribution curves are obtained which

cannot be interpreted when the square root of the calcium ion concentration is used in the calculation (as is required when applying the Donnan principle or the mass action law) instead of equivalents of calcium.

In other exchange equilibria an exact application of these principles has also appeared to be only of restricted use, as is pointed out by BUSER *et al.*⁽²⁾

In some experiments the ion distribution curves appeared to be irregular, the sign of the selectivity coefficient being dependent on the value of the equivalent fraction in solution, e.g. a coefficient greater than one at a low equivalent fraction and one smaller than one at a high equivalent fraction of the inchanging ion (S-shaped curve).

Studying the ion exchange behaviour of humus or humic acids we come across some serious difficulties which may explain some of the anomalies found. When, for example, starting from a K-humate solution of pH 7 (at a higher pH hydrolysis phenomena are increasingly disturbing), the addition of a calcium chloride containing solution causes a lowering of the pH, partly because calcium ions are capable of displacing more protons than were neutralized with KOH at pH 7. This has, presumably, to be ascribed to a different nature of the bond between humate and K^+ -ions, and humate and Ca^{++} -ions respectively. In this way, however, we get a competition between three different ions instead of two.

Secondly, the conversion of dissolved K-humate into Ca-humate causes flocculation of the humate, which may affect the selectivity. It should be mentioned here that starting from a suspension of dry humus, as done by ZADMARD,⁽²⁵⁾ or of dry humates, may cause serious errors as the exchange capacity is strongly dependent on the degree of swelling and dissolution. This might very well explain the hysteresis mentioned by SCHEFFER and ULRICH⁽²⁰⁾ in the exchange reaction of Ca-humus with KCl compared with that of K-humus with $CaCl_2$. Therefore the experiments should preferably be performed with dissolved or freshly precipitated humates and maximally swollen humus as only then is the exchange capacity constant. The effect of swelling should be investigated separately.

DEUEL and HUTSCHNEKER⁽³⁾ give a summary of other factors affecting selectivity and they mention total concentration of ions in solution and kind of acid anion added as a partner of the "unchanging" cation as being important.

It is clear from what is stated above that in the case of humic acids it will be very difficult to find an exchange law of general applicability. A more detailed knowledge of empirically derived selectivity coefficients, however, would be of great value.

To use, at least at the end of this paper, the word "isotope", it may be mentioned that, as shown by HIMES and BARBER⁽¹²⁾ in the examination of the nature and the selectivity of the ion binding by humic acids the use of isotopes may be of value in these measurements.

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DISCUSSION

F. JAQUIN (*France*): Certain research workers (Kononova, Duchaufour) consider that elements such as iron and aluminium are an integral part of humic compounds. What is your opinion on this point?

H. VAN DIJK (*Netherlands*): Humic acids cannot be isolated ash-free from certain soils (especially clay soils). This is, however, an insufficient reason for assuming that mineral compounds are an integral part of humic substances as, for example, is nitrogen. From other soils, for example diluvial sands and peats, humic acids can be isolated in almost ash-free (or at least Fe- and Al-free) condition.

R. J. SWABY (*Australia*): (1) Dr. Posner at the Waite Agricultural Research has been using Sephadex gel to fractionate humic acid. He found two well-defined fractions, one high in molecular weight and the other low in molecular weight. Most of the polysaccharide is present in one of these fractions; but both are dark coloured. Have you any experience with Sephadex? (2) Our experience

in separating humic acids into fractions differing in zeta potential has been rather disappointing. We tried electrophoresis in gels of starch or Cyanogum 41, hoping to obtain discrete fractions. No discrete fractions were found, suggesting that the materials varied widely in electric charge (and also possibly in molecular weight, as these gels have a slight molecular sieving effect).

H. VAN DIJK: (1) I have no experience with Sephadex. (2) Similar results have been obtained by other investigators but there are also some claims that it is possible to separate discrete fractions by electrophoresis.

P. DUBACH (*Switzerland*): (1) I was very happy to hear you stress the fundamental importance of extraction, fractionation and purification. Contrary to what you said, we did not consider the decarboxylation of humic substances to be conclusive proof of the presence of COOH-groups, since CO₂ can also be split off from polycarbonyl compounds under the experimental conditions used. For this reason we conducted further studies on this problem. We consider, as do others, that the most conclusive proof of the presence of COOH-groups in humic substances is the displacement of the carbonyl absorption band on salt formation. We also attempted to determine -COOH-groups by reducing with diborane and measuring the increase in acetylatable -OH-groups.

(2) A publication on the application of the Sephadex gel filtration technique is about to appear. This technique is unsuitable for studying humic substances with molecular weights above about 70,000, since absorption phenomena become dominant above this limit. For lower molecular weight fractions this technique is very valuable; it is possible to determine the median molecular weight and the molecular weight range of humic fractions. The technique also proved useful for detecting changes in median molecular weight and molecular weight range after the humic substances had been given various treatments or subjected to purification.

H. E. OBERLÄNDER (*Austria*): I would like to have your opinion on the usefulness of organic solvents as extractants for humic substances.

H. VAN DIJK: Less organic matter is extracted by organic reagents than by, say, NaOH. One of the main disadvantages of using organic solvents is that you are never sure that the extractant is completely removed (with alkanolamines, for example, the extractant might have reacted with organic substances in the soil).

W. FLAIG (*Germany*): When Dr. White was working with us we examined his humic acids under electron microscope. The humic acid particles were found to be ellipsoidal.

E. POULSEN (*Denmark*): If you increase the organic matter content of a certain soil, would you then expect an increased absorption of potassium relative to calcium and magnesium by a crop?

H. VAN DIJK: It might be counterbalanced by an increased adsorption capacity of the plant root.

A. EVEN-HAIM (*Israel*): Do you think that competition between plants and humic acid for cations is important in view of the high cation exchange capacity of humic acid?

H. VAN DIJK: Dr. Wiersum, working in our Institute, found, as have other investigators, that the uptake of cations was *enhanced* by the presence of humates. The stability of the bond between humates and cations is strongly dependent on the pH (which might be low at the root surface) and this must also be taken into account.

H. HEIMAN (*Israel*): (1) How are the ion exchange properties of humic acid changed when it is linked to clay minerals? (2) what is your opinion on the exchange taking place in aqueous suspen-

sions between humic cation exchangers and "insoluble" compounds like BaSO_4 , apatite, and feldspar? (3) We measure the equivalent weight of humic acids by exposing the *solid* humic acid to ammonia vapor, removing the physically adsorbed ammonia, and then measuring the amount of NH_3 chemisorbed. I find that the results obtained are in good agreement with those obtained by conventional methods. What do you think of this method?

H. VAN DIJK: (1) This question is not yet completely settled; it seems, however, that the exchange capacity of both components is approximately additive. (2) Solid-solid contact exchange is often postulated but as far as I know there is no conclusive proof of this. Organic acids of low molecular weight could equally well "corrode" insoluble materials. (3) I have no experience with this method. The results will depend on the accessibility of the acid groupings in dry humic acids to gaseous NH_3 , i.e. on the pore size distribution of the dry aggregates.

W. FLAIG: At the Congress of the International Soil Science Society at Bucharest in 1964 one of the main tasks of Commission II will be to come to an agreement on methods for isolation and purification of humic substances. I would ask you all to send me any proposals you may have on this question.