# CATION BINDING OF HUMIC ACIDS

H. VAN DIJK

Institute for Soil Fertility, Haren-Groningen (The Netherlands)

## SUMMARY

The capacity of humic acids to bind metal ions is, at most, equal to the number of titratable $H^+$-ions divided by the valency of the concerning metal ion.

It is concluded that metal humates belong to the polynuclear chelates with cations binding predominantly to negatively charged ligand groupings of the humic acids.

Judging from the magnitude of the pH-drop on addition of inorganic salts, there is at pH 5 no large difference in bond strength for the divalent ions Ba, Ca, Mg, Mn, Co, Ni, Fe and Zn (in this order only slightly increasing). Pb (II), Cu (II) and Fe (III) ions, however, are (in this order) more firmly bound. At pH 5, Al ions seem to be quantitatively subject to the forming of hydroxide.

On several grounds it is supposed that the humates of metals of the transition series are forming hydroxocomplexes at high pH, H - ions dissociating from water molecules covalently bound by the metal ion. In the mentioned order, these metal ions are more difficultly displaced by alkali or alkaline earth ions.

As to the mechanism and the strength of the cation binding: there were only insignificant mutual differences between soil humic acids, notwithstanding differences in nitrogen content and in the ratio stronger and weaker acidic groups. The same holds for synthetic humic acids derived from polyhydroxibenzenes. Between these two groups, however, there are differences, presumably connected with a difference in the dissociation constants of the stronger acidic groups.

The bond between humic acids and ions of the transition elements undoubtedly has a partly covalent character. There are indications that this is also the case with alkaline earth ions.

"Competition" experiments between humic acids and some known chelators, and copper, gave some indications of the conditional stability constants of copper humates.

## INTRODUCTION

The exchange capacity of the soil organic matter, as usually determined, varies from about 150 to 400 mequiv./100 g dry organic matter,

which exceeds by far that of the mineral soil components. Of the total exchange capacity of the top layer of mineral soils, 25–90% is a result of the presence of the organic matter (Schachtschabel, 1940) and mainly due to its humic acid content. No wonder therefore, that the exchange behaviour of humic acids is studied by many investigators (see, e.g., the review given by Mortensen, 1963). We will refer to the relevant literature in the discussion of our results.

MATERIALS AND METHODS

The extraction and purification of humic acids from different soil types has been described in a former publication (Van Dijk, 1959): Extraction with a solution containing 0.1 mole NaOH and 0.2 mole NaF per litre, high-speed centrifugation, precipitation at pH 1.0, centrifugation, resolution in a 0.1 $M$ $Na_4P_2O_7$ solution, high-speed centrifugation, reprecipitation by acidifying and percolation of a suspension in water over Amberlite I.R.A. 400 (OH) and I.R. 120 (H), respectively. This method does not give the highest yields, but humic acids are obtained with relatively low ash contents, which was preferred.

Synthetic humic acids were prepared by oxidizing hydroquinone, pyrocatechol and pyrogallol in alkaline and in ammoniacal solution with gaseous oxygen.

As a method for studying the cation binding we used mainly the potentiometric titration in aqueous solution, as formerly described (Van Dijk, 1960). The concentration of humic acids was always 0.50 mequiv./60 ml, calculated from equivalent weights, derived by conductometric titrations with bariumhydroxide (Van Dijk, 1960). The cations of which the binding by humic acids were investigated, were usually added to the titration solution as nitrates (in some cases as sulphates), chloride ions as potential complex-forming ions being excluded.

The "competition" experiments, mentioned at the end of section "Some notes on the nature and the stability of the cation binding", were carried out as follows: To 0.50 mequiv. humic acids, dissolved in a 0.1 $M$ $KNO_3$ solution, 0.50 mequiv. Cu $(NO_3)_2$ was added and sufficient 0.2 $N$ KOH to bring the pH to exactly 6.0 (solution A). The solutions were made and kept in a nitrogen atmosphere.

Besides, 0.1 $M$ $KNO_3$ solutions were made containing 0.25 mmole aminotriacetic acid (ATA; nitrilotriacetic acid), 0.25 mmole aminodiacetic acid (ADA; iminodiacetic acid), 0.25 mmole salicylic acid, 0.50 mmole glycine or 0.50 mmole serine, respectively. Each of these solutions also contained 0.50 mequiv. $Ba(NO_3)_2$ and the pH was adjusted at 6.0 (solution B).

Then, a solution B was added to and thoroughly mixed with a solution A, and left standing overnight. (Up till then the experiment is carried out in a nitrogen atmosphere.)

Thereafter the mixture is centrifuged and copper is determined in the centrifugate with Na-diethyldithiocarbamate as described in Fiskell (1965, pp.1082–1083, method of Cheng and Bray). The copper and barium chelates of ATA etc. are soluble, whereas those of the humic acids are insoluble.

54

As a check, the humate precipitate is washed with distilled water, extracted with a 1 $N$ HCl solution and the copper extracted is again determined as mentioned. The agreement was satisfactory.

All experiments were made in duplicate.

RESULTS AND DISCUSSION

*The interpretation of the course of the titration curve of humic acids, in presence of inorganic salts*

Fig.1 shows the titration curves of humic acids I (without inorganic salt) and II–IV (in the presence of copper (II)-nitrate). The titration curve V of a pure copper nitrate solution is also given, with the ordinate 0.5 mequiv. shifted to the right. The latter curve shows a course which is normal when titrating inorganic copper salts: as soon as KOH is added (and the pH increases to 5.5), copper hydroxide, or more exactly, copper oxide hydrate is formed and the curve turns to horizontal. When 0.5 mequiv. KOH is added (and all copper is converted into oxide hydrate) a sharp increase of pH occurs.

The curves II and III, on the contrary, do not turn to horizontal at pH 5.5. This means that there are no free copper ions present. With curve IV 0.25 mequiv. Cu shows the behaviour of free copper ions; 0.50 mequiv. (i.e., 0.25 mg-atom) appears to be bound by the humic acids.

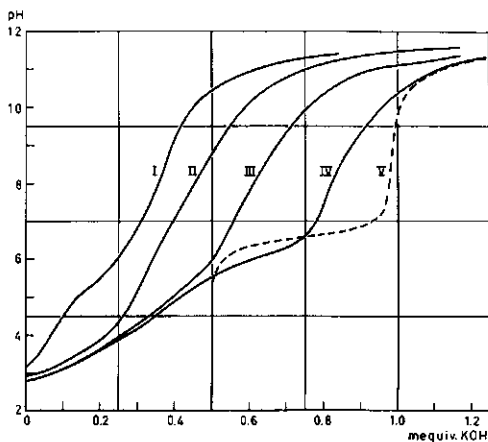The first important conclusion, therefore, is that the humic acids



Fig.1. Potentiometric titration of 0.50 mequiv. humic acids no. 082 I in 60 ml of water with a 0.3346 $N$ KOH-solution: I: without salts; II: in presence of 0.25 mequiv. $Cu(NO_3)_2$; III: in presence of 0.50 mequiv. $Cu(NO_3)_2$; and IV: in presence of 0.75 mequiv. $Cu(NO_3)_2$. [V: potentiometric titration of 0.50 mequiv. $Cu(NO_3)_2$ in 60 ml of water with KOH; ordinate 0.5 mequiv. shifted to the right.]

are able to bind so many copper ions as correspond with half of the acid groupings.

Fig.2 gives a more detailed insight into what occurs at a different pH: the addition of 0.10 mequiv. KOH brings the pH from 3.20 to 4.65. It is assumed that there occur at least 0.10 mequiv. anionic (probably carboxylate) groupings of the humic acids as charged groups. Nevertheless, on addition of 0.10 mequiv. copper nitrate the pH decreases again to 3.40 and 0.08 mequiv. KOH is needed to neutralize this decrease. Thus, the copper ions have displaced protons from the humic acids. (The pH is still too low for the forming of oxide hydrate.)
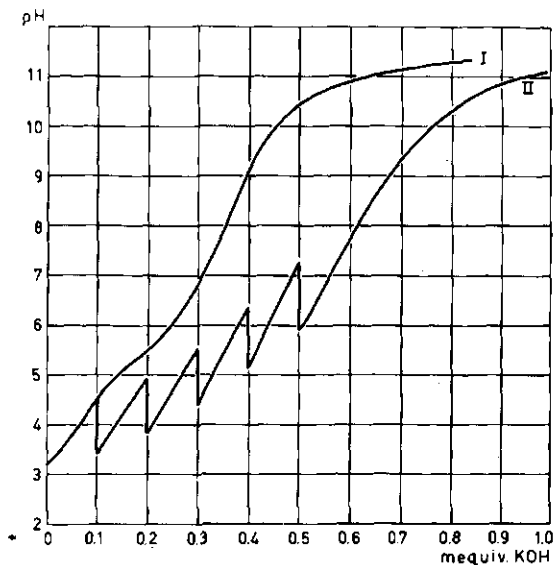


Fig.2. Potentiometric titration of 0.50 mequiv. humic acids no. 082 I in 60 ml of water: I: with KOH; and II: alternately titrated with KOH (ascending parts of the curve) and $Cu(NO_3)_2$ (vertical parts of the curve).

Most likely is a direct displacement, whereby one valence of the Cu ion is bound to a "free" anion group, and the other to a formerly protonated, weakly acidic group, e.g., a phenolic hydroxyl group. These groups are not free to move independently. To bind a copper ion, both ligand "dents" must have been already close together before. In that case, however, it is to be expected that one proton dissociates easily, whereas the second is much more firmly held, just as, for example, with salicylic acid of which the $pK_1$ is lower than the pK of benzoic acid, but $pK_2$ is higher than the pK of phenol.

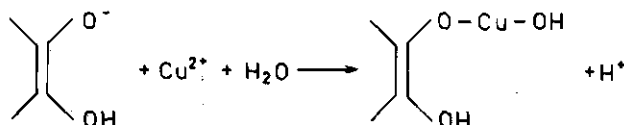As an example of what may have happened in this pH region, the following equation may be given:
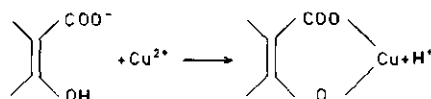
In principle, it may also have been two carboxylgroups (Schnitzer and Skinner, 1963) or two phenolic OH-groups, and both ligands need not implicitly be neighbouring groups of one ring or chain. In favour of the equation given is that, according to Himes and Barber (1957) and Schnitzer and Skinner (1965), the carboxyl groups and the phenolic hydroxyl groups are not independently participating in the binding of metal ions.

Upon further alternate addition of 0.1 mequiv. KOH and 0.1 mequiv. copper nitrate, a similar course of pH occurs (Fig.2, curve II). The displacement theory, holding at low pH, could only then hold well here if copper humate, contrary to potassium humate, is not subject to hydrolysis. A very strong argument against this theory, however, is that barium humate is also not subject to hydrolysis, but nevertheless there is no pH-drop when barium nitrate is added to the humic-acid solution after previous addition of 0.50 mequiv. KOH.

There can also be no question of a displacement of protons from "non-acidic" groups, as in that case an only slightly lower pH-drop should occur when besides copper nitrate, an excess of potassium nitrate is present.(Slightly lower, because of the somewhat decreased activity coefficient of $H^+$ at ionic strength 0.1.)In the latter case, however, a pH-drop was hardly observable.

Still another explanation for the pH-drop at the fifth titration step in curve II would be the occurrence of a "basic" copper humate, i.e. copper-hydroxide-humate, e.g.:

$$\text{>}\!\!<\!\!\begin{matrix} O^- \\ OH \end{matrix} + Cu^{2+} + H_2O \longrightarrow \text{>}\!\!<\!\!\begin{matrix} O-Cu-OH \\ OH \end{matrix} + H^+$$

However, we think this also improbable on the following grounds:

(1) The copper-humate bond appears to be very stable at this pH. Even at still higher pH there is no decomposition in copper-oxide hydrate and potassium humate. By a twenty-fold excess of Ba-ions, less than one third of the copper ions is exchanged at a pH of about 6, and if only 0.25 mequiv. (i.e., half equivalent amount) of Cu-ions were present, no copper ions were exchanged at all, although barium salts show less tendency for forming hydroxide salts (barium hydroxide is a strong base).

(2) In the order Mn, Co, Ni, Pb, Cu, Fe(III), an ever smaller amount of metal ions could be exchanged against barium ions. With inorganic salts, however, in this order there is an increasing tendency of forming of oxide hydrates!

(3) All humic acids are flocculated in presence of equivalent amounts of di-or trivalent metal ions, this being still the case when an equivalent amount of KOH already has been added. On further addition of KOH, however, the precipitate gradually redissolves completely, which is not to be expected when hydroxide humates would have been formed.

In our opinion, the most plausible reaction mechanism at low pH is the displacement of protons from acidic groups of the humic acids, whereas at a higher pH protons are dissociated from water molecules which were covalently bound to the copper ions, giving a hydroxo complex, e.g.:

$$\text{COO} \diagup \searrow \text{Cu}(H_2O)_x \quad (x=2?) \longrightarrow \quad \text{COO} \diagup \searrow \text{Cu} \diagup OH^- (H_2O)_{x-1} + H^+$$
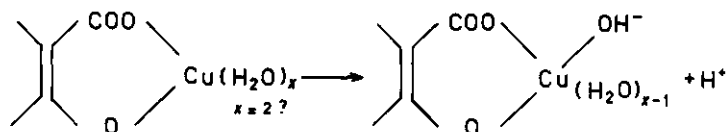
It is the latter complex which we suppose to be formed on adding the last 0.1 mequiv. of copper salt in the fifth titration step, the protons dissociating from the water molecules covalently bound by the copper ions being responsible for the drop in pH. (To a certain extent these protons were already dissociated from the hydrated copper ions, the salt solution being slightly acid, before the complex was formed.) (According to the I.U.P.A.C. nomenclature, 1959, this is a polynuclear chelate since one humic acid molecule is able to bind several copper ions.)

This supposition explains the high stability of the copper humate at higher pH (strictly speaking, this should be denominated a hydroxo-humato-cuprate).

Whether the copper ions, not exchangeable against barium at pH 6, are those which with one valency are bound to stronger acidic groups, cannot be said with certainty, but it seems not improbable since at a certain pH a complex is often more stable the weaker the acid (Martell and Calvin, 1953).

This explains also why the metal ions are more difficultly exchanged against barium in the order Mn, Co, Ni, Pb, Cu. This is about the order of increasing tendency of formation of hydroxo complexes.

Finally, this theory explains the resolution of the flocculated metal humates on addition of more KOH. The particles were flocculated while they had lost their negative charge by binding metal ions. On forming hydroxo complexes they got negatively charged again, resulting in a repeptization.


*Comparison of different cations*

In Fig.3, curve I shows the course of the pH on a similarly intermittent addition of 0.1 mequiv. calcium nitrate. At the start, here also there is a displacement of protons from the humic acids, although to a smaller extent than with copper nitrate, while calcium is bound less strongly. When 0.5 mequiv. KOH is added, the fifth addition of 0.1 mequiv. calcium nitrate only causes a very small pH-drop, as could be expected in view of the small tendency of calcium to form hydroxo complexes.

Curve II is the analogue obtained with Fe $(NO_3)_3$. However, here the first addition of 0.1 mequiv. iron nitrate may result in the formation of a "basic" salt, as is already the case with iron(III) nitrate at a pH lower

Fig.3. Potentiometric titration of 0.50 mequiv. humic acids no. 082 I in 60 ml of water: I: alternately titrated with KOH (ascending parts of the curve) and $Ca(NO_3)_2$ (vertical parts of the curve); and II: as I, but with $Fe(NO_3)_3$ instead of $Ca(NO_3)_2$. [III: potentiometric titration of 0.50 mequiv. $Fe(NO_3)_3$ in 60 ml of water with KOH.]

than 3 (see curve III). At higher pH, a further forming of a hydroxo complex is very probable again, e.g., as in the following hypothetical equation:



This interpretation can be considered as a refinement of the reaction course, suggested by Schnitzer and Skinner (1963). In one respect, however, our results strongly differ from theirs, viz. also in case of iron(III) humates we got no decomposition in K-humate and iron-oxide hydrate at high pH.

Fig.4 shows the titration curves of one of the humic acids in presence of various salts, added in equivalent amounts. Aluminium shows a behaviour, deviating from that of all the other metal ions, viz. at pH 4.1 the aluminium ions are quantitatively converted into Al-oxide hydrate, just as is the case with inorganic Al-salts. Thus it is found that humic acids (it was checked with other humic acids) are not able to "protect" aluminium ions from being converted into oxide hydrate. This result is in contradiction with a statement of Wright and Schnitzer (1963), but confirms the opinion of other authors (e.g., Scheffer and Ulrich, 1960, p.78), that aluminium ions are much less strongly bound than iron(III) ions.
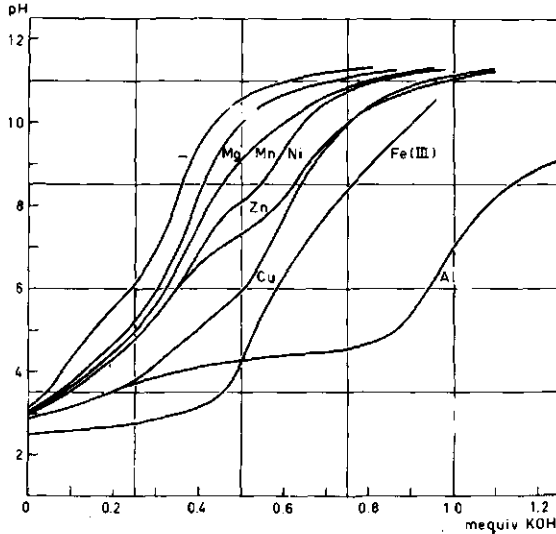


Fig.4. Potentiometric titration of 0.50 mequiv. humic acids no. 082 I in 60 ml of water with KOH (left curve), and in presence of 0.50 mequiv. inorganic Mg-, Mn-, Ni-, Zn-, Cu-, Fe(III)-, or Al-salt, respectively.

All cations mentioned in Fig.4 show a clearly visible effect on the course of the curves, the extent of the effect, however, is different and pH-dependent. At a pH of about 3 the differences between the cations are only small. At this pH the main part is still present as "free" ions. For example, in the presence of an equivalent amount of copper(II) ions at pH 3, about 70% of these ions are "free". (Practically all metal ions can be exchanged against $H^+$, as is also mentioned by Basu et al., 1964.)

In Table I we recorded the effect of the presence of metal ions as the differences in pH ("pH-drop") between the humic acid solution (0.50 mequiv./60 ml water) without any salt and this solution after adding equivalent amounts of salts, both after addition of 0.25 and 0.50 mequiv. KOH, respectively (marked as $s = \frac{1}{2}$ and $s = 1$, resp.).

Instead of this pH-drop, also the horizontal displacement of the curves can be taken as a measure of the effect of the presence of metal ions, i.e., the number of mequiv. of base to be added in excess in presence of these ions to achieve the same pH as in absence of these ions (in Table I recorded as $\Delta$ mequiv. base). Table I shows that, for humic acids, pH-drop and $\Delta$ mequiv. base qualitatively give the same information.

At $s = \frac{1}{2}$, the cations clearly diverge into two groups: Firstly, the

TABLE I

The effect of the addition of an equivalent amount of metal salt to the humic acids no. 082 I (0.50 mequiv. in 60 ml of water) measured after addition of 0.25 mequiv. KOH (s = $\frac{1}{2}$) and 0.50 mequiv. KOH (s = 1)

| Salt | s = | | | s = 1 | | | pH oxide hydrate formation[2] |
|---|---|---|---|---|---|---|---|
| | pH | pH- "drop" | Δ mequiv. base[1] | pH | pH- "drop" | Δ mequiv. base[1] | |
| None | 6.2 | – | – | 10.6 | – | – | – |
| $KNO_3$ | 6.2 | 0 | 0 | 10.6 | 0 | 0 | – |
| $Ba(NO_3)_2$ | 5.2 | 1.0 | 0.07 | 10.5 | 0.1 | 0.02 | – |
| $Mg(NO_3)_2$ | 5.2 | 1.0 | 0.07 | 10.0 | 0.6 | 0.08 | 10.5 |
| $Ca(NO_3)_2$ | 5.0 | 1.2 | 0.09 | 9.9 | 0.7 | 0.08 | – |
| $Mn(NO_3)_2$ | 5.0 | 1.2 | 0.09 | 9.2 | 1.4 | 0.12 | 8.5–8.8 |
| $Co(NO_3)_2$ | 4.9 | 1.3 | 0.10 | 8.4 | 2.2 | 0.15 | 6.8 |
| $Ni(NO_3)_2$ | 4.8 | 1.4 | 0.11 | 8.1 | 2.5 | 0.16 | 6.7 |
| $FeSO_4$ | 4.7 | 1.5 | 0.12 | 7.5 | 3.1 | 0.18 | 5.5 |
| $ZnSO_4$ | 4.8 | 1.4 | 0.11 | 7.3 | 3.3 | 0.19 | 6.8–7.1 |
| $Pb(NO_3)_2$ | 3.9 | 2.3 | 0.17 | 6.8 | 3.8 | 0.22 | 6.0 |
| $Cu(NO_3)_2$ | 4.0 | 2.2 | 0.18 | 6.1 | 4.5 | 0.26 | 5.3 |
| $Fe(NO_3)_3$ | 2.8 | 3.4 | >0.25 | 4.2 | 6.4 | 0.42 | 2 |

[1] Δ mequiv. base = amount of KOH to be added in excess to achieve the same pH as in absence of salts.
[2] I.e., the pH at which oxide hydrates of the corresponding metals begin the precipitate from 0.02 molar salt solutions. (After Kolthoff and Elving, 1959, p.806.)

group of Ba to Zn, which does not show much difference in affinity to humic acids at pH 5. The formation of hydroxo complexes does not yet play an important role. Secondly, the ions of Pb, Cu and Fe(III), for which the affinity is found to be considerably higher, especially for Fe(III).

At $s$ = 1 the mutual divergence is much larger. As stated before, we ascribe this to an increasing tendency for forming hydroxo complexes, bringing about a stabilization of the complex. The order of increase of the pH-drop is the same as found in the exchange experiments with barium ions.

The order of pH-drop is often said to correspond with the well-known series of Irving and Williams (1948) for the stability of metal complexes (e.g., Beckwith, 1955, and Khanna and Stevenson, 1962), from which is concluded that metal humates indeed are chelates. This correspondence, however, is not conclusive, as clearly appears from the fact that the metal ions mentioned in these publications, show the same order in respect to the pH at which oxide hydrates occur in solutions of inorganic salts of these metals.

Therefore, we choose a series of cations of which the order in the Irving-Williams series is different from the pH-order in which oxide-hydrate formation occurs. The results are shown in Fig.5. The order of pH-drop neither corresponds exactly with that in the Irving-Williams series, nor with that in the pH-order of forming oxide hydrates. This is not necessarily an objection to our assumption that, particularly at higher
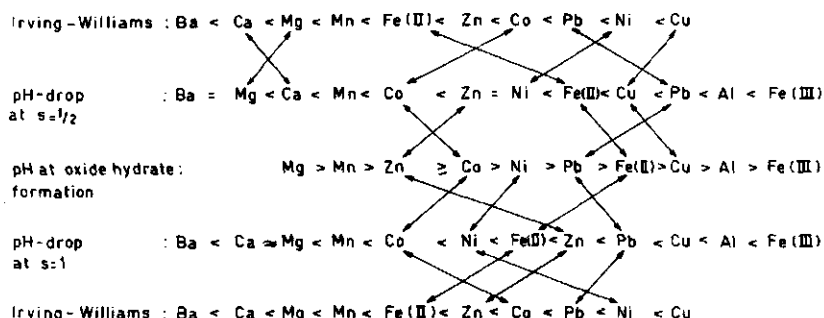
Irving–Williams : Ba < Ca < Mg < Mn < Fe(II) < Zn < Co < Pb < Ni < Cu

pH-drop at $s=\frac{1}{2}$ : Ba = Mg < Ca < Mn < Co < Zn = Ni < Fe(II) < Cu < Pb < Al < Fe(III)

pH at oxide hydrate formation : Mg > Mn > Zn ≥ Co > Ni > Pb > Fe(II) > Cu > Al > Fe(III)

pH-drop at $s=1$ : Ba < Ca ≈ Mg < Mn < Co < Ni < Fe(II) < Zn < Pb < Cu < Al < Fe(III)

Irving–Williams : Ba < Ca < Mg < Mn < Fe(II) < Zn < Co < Pb < Ni < Cu

Fig.5. Order of magnitude of the pH-"drop" of the humic-acids solution (0.50 mequiv.) upon adding the mentioned metal ions, after previous addition of 0.25 mequiv. KOH ( $s=\frac{1}{2}$ ) and 0.50 mequiv. KOH ( $s=1$ ), respectively, compared with the sequence in the Irving-Williams series and with the order of pH at which oxide hydrates are formed in solutions of inorganic salts of these metals.

pH, hydroxo complexes are formed, as it is well known that the concerning equilibria often are very complicated. In this direction points also the somewhat deviating course of the curves for nickel and zinc (Fig.4).

*Comparison of different humic acids*

Similar titrations were carried out with different humic acids. In Table II only those data are mentioned which refer to calcium and copper(II).

Firstly, the different behaviour of the natural and the synthetic acids may be mentioned. The average values and standard deviations are calculated for both groups. At $s = \frac{1}{2}$ the difference in pH-drop in presence of copper nitrate is highly significant ( $P = 0.01$ ). This difference is caused by the fact that the stronger acid groups (presumably carboxyl groups) of natural humic acids are considerably stronger acidic than those of the synthetic humic acids, as the curves in Fig.6 for humic acids, without salts, clearly show. This implies that in this respect the synthetic humic acids are not the closely resembling "model substances" of the natural product as they are often supposed to be.

A more interesting conclusion from Table II is, that the relatively small mutual differences between natural humic acids and also those between synthetic humic acids show no correlation with the amount of nitrogen or of "rest" (which is an approximation for the amount of oxygen) per 0.50 mequiv. humic acids. In the same respect, the different ratio of stronger (COOH ?) and weaker acid groups (phenolic OH?), if indeed real, seems not to play a role. For example, the pH-values in Table II of Ha.I b (without salt, with $Ca^{2+}$ and with $Cu^{2+}$, respectively) almost coincide with the corresponding values of Ha.II b. Also, only at a pH lower than 6, the pH-drop with copper of Ha.V deviates significantly from those of Ha.I b and Ha.II b. However, at 0.50 mequiv. of these humic acids, the amounts of nitrogen are 11.9, 7.3 and 0 mg, and the ratio "COOH/OH" was 1/5, 1/4 and 1/3.

TABLE II

Comparison of some data obtained from titration curves of equinormal solutions of thirteen humic acids in pure water (0.50 mequiv. in 60 ml) and in presence of an equivalent amount of calcium and copper(II) nitrate

| No. of humic acid | Origin | After addition of | | | | | | Per 0.50 mequiv. humic acids | | $T^2$ | "COOH/OH"[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.25 mequiv. KOH | | | 0.50 mequiv. KOH | | | mg N | mg O[1] | | |
| | | pH without salts | pH-drop with | | pH without salts | pH-drop with | | | | | |
| | | | Ca | Cu | | Ca | Cu | | | | |
| 082 I | peaty sand soils | 6.2 | 1.2 | 2.2 | 10.6 | 0.7 | 4.5 | 3.0 | 35.5 | 0.49 | 1/4 |
| 082II | peaty sand soils | 6.0 | 1.1 | 2.3 | 10.5 | 0.7 | 4.7 | 2.2 | 34.0 | 0.55 | 1/5 |
| 114 | sandy soils (arable land) | 5.8 | 1.0 | 2.1 | 10.5 | 0.5 | 4.8 | 3.8 | 36.0 | 0.46 | 1/2 |
| 094 B | heavy clay soil | 6.0 | 1.0 | 2.1 | 10.6 | 0.3 | 4.7 | 4.4 | 37.5 | 0.46 | 1/2 |
| 123b | wood peat,2. extract | 5.6 | 1.2 | 2.1 | 10.4 | 0.8 | 4.7 | 2.6 | 40.8 | 0.49 | 1/2 |
| 123c | wood peat,3. extract | 6.0 | 1.2 | 2.2 | 10.7 | 0.3 | 4.7 | 2.6 | 35.7 | 0.56 | 1/3 |
| 116 | black raised bog peat | 6.4 | 0.5 | 2.3 | 10.2 | 0.2 | 4.4 | 2.1 | 40.9 | 0.41 | 1/2 |
| 118 | ammoniated black peat | 6.2 | 1.2 | 2.8 | 10.6 | 0.7 | 5.4 | 11.5 | 31.0 | 0.49 | 1/3 |
| | Average | 6.02 | 1.05 | 2.25 | 10.51 | 0.5 | 4.7 | | | | |
| | Standard deviation (s) | 0.25 | 0.24 | 0.24 | 0.16 | 0.23 | 0.30 | | | | |
| I b | hydroquinone + NH$_4$OH + O$_2$ | 7.7 | 0.7 | 3.6 | 10.7 | 0.4 | 4.5 | 11.9 | 30.9 | 0.48 | 1/5 |
| II b | hydroquinone + NH$_4$OH + O$_2$ | 7.5 | 1.0 | 3.5 | 10.7 | 0.4 | 4.5 | 7.3 | 30.4 | 0.54 | 1/4 |
| V | hydroquinone + NaOH + O$_2$ | 7.8 | 0.8 | 3.0 | 10.6 | 0.3 | 4.3 | 0 | 29.3 | 0.62 | 1/3 |
| III | pyrogallol + NH$_4$OH + O$_2$ | 7.2 | 0.4 | 3.3 | 10.3 | 0.3 | 5.2 | 4.3 | 26.0 | 0.68 | 1/6 |
| VI | pyrocatechole + NaOH + O$_2$ | 8.4 | 1.1 | 4.1 | 10.7 | 0.6 | 5.0 | 0 | 30.0 | 0.57 | 1/2 |
| | Average | 7.72 | 0.8 | 3.5 | 10.60 | 0.4 | 4.7 | | | | |
| | Standard deviation (s) | 0.45 | 0.27 | 0.41 | 0.17 | 0.12 | 0.41 | | | | |

1 mg O = mg (loss on ignition – C – N – H).
2 T = mequiv. Ba(OH)$_2$ added at the endpoint of a conductometric titration, calculated per 100 mg ash-free humic acids.
3 "COOH/OH" = ratio of stronger and weaker acid groups according to high-frequency titration. (After Van Dijk, 1960.)
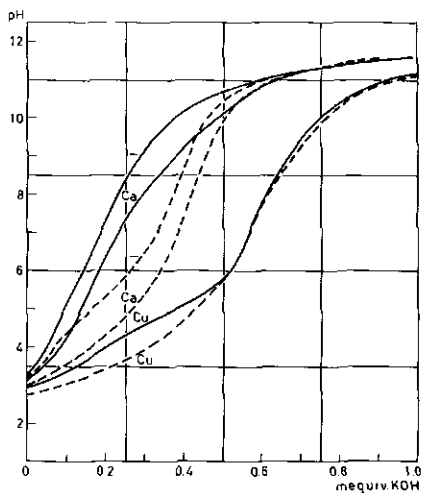
Fig.6. Potentiometric titration of 0.50 mequiv. synthetic humic acids no. I b (fully drawn curves) and natural humic acids no. 114 (dashed curves) in 60 ml of water, without salts (—), and in presence of 0.50 mequiv. Ca- or Cu-nitrate, respectively.

Although this is no conclusive proof, these facts suggest that the nitrogen in the humic acids does not play an important role in the cation binding. This binding seems to occur predominantly between the cation and 0-anion groupings, which are protonated in undissociated humic acids.

A possible explanation for the small mutual differences in cation binding between the humic acids is given by Kleist (1965), who states that there is an interaction between metal ions and stable free radicals, and that the content of the latter is not the same for all humic acids.

*Some notes on the nature and the stability of the cation binding*

According to the "Nomenclature of Inorganic Chemistry" of the I.U.P.A.C. (1959), the name "chelate" says nothing of the nature of the bond, but only that a bidentate ligand is attached to one central atom. The binding of cations to negatively charged groups of an ion exchanger is always, at least partly, caused by electrostatic forces (Coulomb forces). As a result of a "deformation" of the cation and eventually of the ligand, however, the bond may have a more or less strongly covalent character. Also on account of the titration results discussed above, we think there is no room for doubt, that the bond between metal ions of the first transition series and chelate ligands of the humic acids is partially covalent. (In this respect, therefore, we put it in other words than Martin and Reeve, 1958, and Khanna and Stevenson, 1962.)

As to be expected, the titration curves gave no indication of such a specific interaction with alkali ions. With alkaline earth ions, there was

64

a strong interaction, but this does not necessarily mean that it is partially non-electrostatic. Exchange experiments, analogous to those which Hutschneker (1955) carried out with ion exchangers, however, indicated that this interaction is indeed not purely electrostatic.

To have an idea of the magnitude of the stability of the metal-humate binding under certain conditions, we designed "competition" experiments (see end of section "Materials and methods") for copper between humic acids and a number of chelating substances of which the stability constants with copper were known. (The same kind of experiments, however, with soil and zinc, were made by Himes and Barber, 1957).

Part of the results are given in Table III. It is found that with ATA the greater part of the copper is bound by this compound. ADA, however, can only remove about 55% of the copper from the natural humic acids, and salicylic acid about 30%. This suggests that the logarithm of the conditional stability constant for copper humates (at pH 6) is about 7. This value agrees quite well with the logarithm of the mean conditional formation constant which Coleman et al. (1956) calculated for a copper-peat complex, viz. 6.5. A value of exactly 7.0 for the conditional stability constant of a humic acid-copper complex at pH 5.0 was found by Courpron (1967), using the method of Schubert (1948). Although then a value somewhat upwards of 7.0 has to be expected at pH 6, there is an excellent agreement with our estimate. However, since the conditional stability constants Courpron found for copper and zinc complexes of humic and fulvic acids are considerably lower than those found by Schnitzer and Skinner (1966) and Randhawa and Broadbent (1965), the question still cannot be considered as definitely settled.

With one of the humic acids (no.082 I) we performed the same experiment with glycine and serine as "competitors". (Amino acids, exudated

TABLE III

Partition of copper between humic acids and aminotriacetic acid, aminodiacetic acid and salicylic acid, when adding equivalent amounts of the latter acids to copper humates suspended in 0.1 N $KNO_3$, at pH 6.0

| Humic acid no.[1] | pH-"drop" with Cu | mg per 0.50 mequiv. | | "COOH/OH" | Percentage of copper retained by humic acids in presence of | | |
|---|---|---|---|---|---|---|---|
| | | N | "O" | | ATA(8.9)[2] | ADA(7.0)[2] | S.A.(3.5)[2] |
| 082 I | 4.5 | 3.0 | 35.5 | 1/4 | 9 | 45 | 73 |
| 116 | 4.4 | 2.2 | 40.9 | 1/2 | 8 | 40 | — |
| 118 | 5.4 | 11.5 | 31.0 | 1/3 | 17 | 47 | — |
| 117[3] | — | 2.7 | 33.2 | 1/6 | 8 | 44 | 64 |
| II b | 4.5 | 7.3 | 30.4 | 1/4 | 2 | 20 | 68 |
| V | 4.3 | 0 | 29.3 | 1/3 | 2 | 30 | 73 |

[1] For origin, see Table II.
[2] Values in parentheses: log conditional stability constants of the corresponding copper chelates, derived from data of Ringbom (1959) and Rosotti and Rosotti (1961).
[3] Extracted from a podzol B horizon.

from plant roots, might be potential carriers for microelements from humates to plant roots.) Although the logarithmic value of their conditional stability constants at pH 6.0 is 11.4, still 45 and 41 %, respectively, of the coppe: was found to be retained by the humic acids, which disagrees with the resul obtained with ATA and ADA. A possible explanation might be the forming of mixed complexes. The latter results indicate that the partition of copper between humic acids and a given chelating compound cannot be predicted from the conditional stability constants only.

Table III further suggests that there are no large differences in the stability constants of natural humic acids, notwithstanding different "COOH/OH"-ratios and "O"-contents. Humic acid no.118, extracted from ammoniated peat, seems to bind copper more strongly, which also becomes obvious from the pH-"drop" with copper. A possible effect of the high nitrogen content is not supported by the results with synthetic humic acids which, for that matter, again show a behaviour deviating from that of the natural products.

REFERENCES

Basu, A.N., Mukherjee, D.C. and Mukherjee, S.K., 1964. Interaction between humic acid fraction of soil and trace element cations. J. Indian Soc. Soil Sci., 12: 311–318.
Beckwith, R.S., 1955. Metal complexes in soils. Australian J. Agr. Res., 6: 685–698.
Beckwith, R.S., 1959. Titration curves of soil organic matter. Nature, 184: 745–746.
Coleman, N.T., McClung, A.C. and Moore, D.P., 1956. Formation constants for Cu(II)–peat complexes. Science, 123: 330–331.
Courpron, C., 1967. Détermination des constantes de stabilité des complexes organo-métalliques des sols. Ann. Agron., 18: 623–638.
Fiskell, J.G.A., 1965. Copper. In: C.A. Black (Editor-in-Chief), Methods of Soil Analysis, 2. Chemical and Microbiological Properties. Am. Soc. Agron., Madison, Wisc., pp.1078–1089.
Himes, F.L. and Barber, S.A., 1957. Chelating ability of soil organic matter. Soil Sci. Soc. Am. Proc., 21: 368–373.
Hutschneker, K., 1955. Ionengleichgewichte an Kationenaustauschern verschiedener Austauschkapazität. Thesis, Eidgenöss. Tech. Hochschule, Zürich, 78 pp.
Irving, H. and Williams, R.J.P., 1948. Order of stability of metal complexes. Nature, 162: 746–747.
I.U.P.A.C. (International Union of Pure and Applied Chemistry), 1959. Nomenclature of Inorganic Chemistry. Butterworths, London, 94 pp.
Khanna, S.S. and Stevenson, F.J., 1962. Metallo-organic complexes in soil, I. Soil Sci., 93: 298–305.
Kleist, H., 1965. Untersuchungen über Metall-Huminsäure-Verbindungen. Wiss. Z. Univ. Rostock, Math.-Naturwiss. Reihe, 14: 415.
Kolthoff, I.M. and Elving, P.I. (Editors), 1959. Treatise on Analytical Chemistry, I. Theory and Practice. Interscience, New York, N.Y., 1: 809 pp.
Martell, A.E. and Calvin, M., 1953. Chemistry of the Metal Chelate Compounds. Prentice Hall, New York, N.Y., 613 pp.
Martin, A.E. and Reeve, R., 1958. Chemical studies of podzolic illuvial horizons. J. Soil Sci., 9: 81–88.
Mortensen, J.L., 1963. Complexing of metals by soil organic matter. Soil Sci. Soc. Am. Proc., 27: 179–186.
Randhawa, N.S. and Broadbent, F.E., 1965. Soil organic matter-metal complexes, 6. Soil Sci., 99: 362–366.

Ringbom, A., 1959. Complexation reactions. In: I.M. Kolthoff and P.J. Elving (Editors), Treatise on Analytical Chemistry, I. Theory and Practice. Interscience, New York, N.Y., pp. 543–628.

Rosotti, F.J.C. and Rosotti, H., 1961. The Determination of Stability Constants. McGraw-Hill, New York, N.Y., 425 pp.

Schachtschabel, P., 1940. Untersuchungen über die Sorption der Tonmineralien und organischen Bodenkolloide und die Bestimmung des Anteils dieser Kolloiden an der Sorption im Boden. Kolloid–Beih., 51: 199–276.

Scheffer, F. und Ulrich, B., 1960. Lehrbuch der Agrikulturchemie und Bodenkunde. Teil III. Humus und Humusdüngung. Band I. Morphologie, Biologie, Chemie und Dynamik des Humus. Ferdinand Enke Verlag, Stuttgart, 226 pp.

Schnitzer, M. and Skinner, S.I.M., 1963. Organo-metallic interactions in soils, 1. Reactions between a number of metal ions and the organic matter of a podzol $B_h$ horizon. Soil Sci., 96: 86–93.

Schnitzer, M. and Skinner, S.I.M., 1965. Organo-metallic interactions in soils, 4. Carboxyl and hydroxyl groups in organic matter and metal retention. Soil Sci., 99: 278–284.

Schnitzer, M. and Skinner, S.I.M., 1966. Organo-metallic interactions in soils, 5. Stability constants of $Cu^{2+}$-, $Fe^{2+}$-, and $Zn^{2+}$-fulvic acid complexes. Soil Sci., 102: 361–365.

Schubert, J., 1948. Use of ion exchangers for the determination of physical-chemical properties of substances, particularly radiotracers in solution, 1. Theoretical. J. Phys. Colloid Chem., 52: 340–350.

Van Dijk, H., 1959. Zur Kenntnis der Basenbindung von Huminsäuren. Z. Pflanzenernähr., Düng. Bodenk., 84: 150–155.

Van Dijk, H., 1960. Electrometric titrations of humic acids. Sci. Proc. Roy. Dublin Soc., Ser. A., 1: 163–176.

Wagner, G.H. and Stevenson, F.J., 1965. Structural arrangement of functional groups in soil humic acid as revealed by infrared analysis. Soil Sci. Soc. Am. Proc., 29: 43–48.

Wright, J.R. and Schnitzer, M., 1963. Metallo-organic interactions associated with podzolization. Soil Sci. Soc. Am. Proc., 27: 171–176.