

# MEASUREMENT OF METAL-COMPLEXING ABILITY OF POLYFUNCTIONAL MACROMOLECULES: A DISCUSSION OF THE RELATIONSHIP BETWEEN THE METAL-COMPLEXING PROPERTIES OF EXTRACTED SOIL ORGANIC MATTER AND SOIL GENESIS AND PLANT NUTRITION<sup>1</sup>

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## ABSTRACT

Characterization of the metal-complexing ability of polyfunctional macromolecules through the determination of stability constants of their metallic complexes is discussed along with the validity of ascribing a relationship between the metal-ion complexing properties of organic matter extracted from the soil and the role of naturally occurring organic matter in soil in the processes of plant nutrition and soil genesis.

The type of complex obtained when mixing metal-ion and complexant solutions will be a function of the molar ratio metal-ion/complexant. If Schubert's original experimental conditions (e.g., complexant concentration  $\gg$  metal-ion one) are used, the MCh- or MCh<sub>2</sub>-type complexes will always be formed regardless of the polyfunctional character of the organic macromolecule (Ch). It is suggested that the overall stability constant of the saturated complex M<sub>n</sub>Ch would describe in a comprehensive way the metal-complexing properties of such complexants.

No reliable information about the role of soil organic matter in plant nutrition or soil genesis processes can be obtained by studying the metal-complexing ability of extracted soil organic matter. This is due to the fact that the metal-complexing properties of these materials—or the number of active anionic group on the extracted molecules—will depend on the extent of depolymerization suffered by the naturally occurring soil organic matter during extraction.

The methodology used up to the present to study the involvement of soil organic matter in plant nutrition or soil genesis processes through its metal-complexing ability urgently needs reevaluation.

Metal-complexing properties of extracted soil organic matter have been studied extensively in relation with soil genesis and plant nutrition. Through its ability to complex insoluble inorganic forms of metal ions, soil organic matter would be involved in soil genesis and also contribute to plant nutrition by increasing the availability of mineral nutrients (De Koek 1955; Levesque 1970; Schnitzer and Poapst 1967; Schnitzer and Skinner 1963, 1965, 1967). Implicit in the literature is the consideration that a higher stability of these complexes would increase the probability of involve-

ment of soil organic matter in both processes. In connection with this, the determination of the stability constant of metallic complexes formed with extracted soil organic matter seems to be at present an important topic in soil chemistry (Ardakani and Stevenson 1972; Bondarenko 1972; Schnitzer and Khan 1972; Stevenson and Ardakani 1972; Stevenson et al. 1973).

Numerous studies indicate that organic matter in soils may be bound to clays and to Al or Fe sesquioxides. Several mechanisms could be involved in these associations which have been recently reviewed (Greenland 1971). Studies of soil organic matter properties usually begin with the separation of the organic materials from the inorganic constituents of the soil. This is carried out using chemical extractants which are able to break the organic matter/inorganic colloids link-

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ages, thus extracting the organic matter (Greenlad 1971; Kosaka and Iseki 1956). Alkaline solutions (e.g., sodium hydroxide or sodium pyrophosphate) are the most commonly used extractants for this purpose (McLaren and Skujins 1971).

Soil organic matter is obtained in the form of negatively charged polymeric materials whose principal functional groups are COOH, phenolic and alcoholic OH, and CO (Felbeck 1971; Leenheer and Moc 1969; Schnitzer 1970). Due to this chemical configuration, Clark and Turner (1969) stated that the extracted soil organic matter will form metallic complexes when mixed with metal-ion solutions where the central group would be the organic molecule coordinated with more than one metal ion. Therefore, they criticized the use of the simplified approach of the ion-exchange equilibrium method to determine the stability of these complexes where polyfunctional macromolecular complexants are involved. These complexes were later described as  $M_aCh$ -type complexes (Zunino et al. 1972a).

Recently it was demonstrated (Zunino et al. 1972a) that some erroneous assumptions were made when the ion-exchange equilibrium method for the determination of stability constants of metallic complexes was developed (Schubert 1948). These assumptions compensate each other but only when the complex involved is of the  $MCh_b$  type, where the metal-ion acts as a central group coordinated to  $b$  molecules of complexant. From this, new equations were developed to interpret the ion-exchange equilibrium that exists when a complexant is competing with a cation exchanger for the free metal ions. The stability of the complex formed could be expressed in terms of the maximum complexing ability of the complexant (MCA).

A general method for determining the MCA of complexants in solution has been more recently developed (Zunino et al. 1972b). With this method, the equations cited above could be applied in determining stabilities of metallic complexes of polyfunctional macromolecular complexants like the soil organic matter.

At the same time, another method modifying the original ion-exchange equilibrium method was proposed (Ardakani and Stevenson 1972). Their approach is derived from a general equation proposed by Schubert (1948) where both coefficients  $a$  and  $b$  (or  $i$  and  $j$  in Ardakani and Stevenson's

work) appear. This equation defines the stability of a  $M_aCh_b$ -type complex.

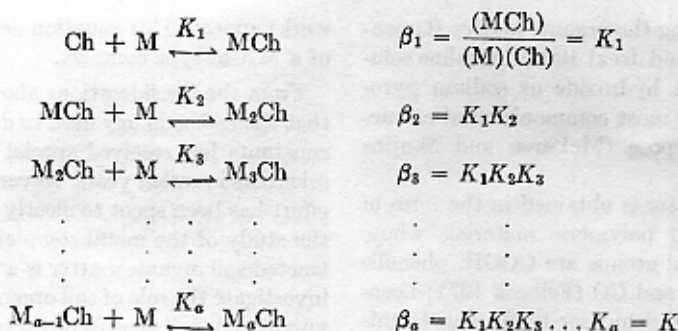
From the considerations above, it can be seen that the methodology used to determine stability constants has received special attention by soil scientists in recent years. Nevertheless, very little effort has been spent to clearly demonstrate that the study of the metal-complexing ability of extracted soil organic matter is a valid approach to investigate the role of soil organic matter in plant nutrition or soil genesis processes. The purpose of this paper is to thoroughly discuss:

1. The experimental conditions which have to be met in order to characterize the metal-complexing ability of polyfunctional macromolecules in terms of stability constant data.
2. The validity of the relationship claimed to exist between metal-complexing properties of extracted soil organic matter and the involvement of naturally occurring soil organic matter in plant nutrition or soil genesis processes.

#### *Formation of $M_aCh$ -type complexes and the determination of their stability constants*

Schubert's original experimental procedure (1948) can be used to determine the stability of  $MCh_b$ -type complexes where one metal ion acts as a central group coordinating  $b$  anionic molecules (where  $b \geq 1$ ). This method uses high complexant concentrations and very low and constant metal ion ones. Therefore, a sufficient amount of complexant exists for each ion to produce the complexation of nearly all of them. This will be regulated by the stability constant of the  $MCh_b$  complex formed. The measurement of  $\log K$  is performed carrying out a series of experiments in which increasing complexant concentrations are used, and the distribution of metal ion between the solutions and a cation exchange resin is measured.

It has recently been shown that where a polyfunctional complexant is involved in complex formation, the stepwise nature of this process must be taken into account (Stevenson and Ardakani 1972). Together with this, the effect of the metal ion/complexant concentration ratio on the type of complex formed is important and merits discussion. This can be done by using an approach similar to the well-known one applied to  $MCh_b$ -type complexes (Butler 1964), i.e.:



The curve brackets denote molar concentration. The valences of Ch and M have been left out for simplicity.

The overall formation constant  $K$  is represented by the oversimplified equation:  $a\text{M} + \text{Ch} \rightleftharpoons \text{M}_n\text{Ch}$ .

It can be seen from the equations above that the relative percentage of each complex species in any particular solution will be a function of the molar ratio of metal-ion/organic complexant. Clark and Turner (1969) focused on a similar point by examining mathematically the simplified Schubert's equation. They concluded that the observed slope of this equation in its linear form (representing  $b$  of the complex  $\text{MCh}_n$ ) "was a reflection primarily of the particular range of experimental conditions used".

All the available methods which can be applied to determine  $\log K$  of metal complexes where polyfunctional macromolecules are involved, i.e., ion-exchange, solvent extraction, and continuous variations, are based on obtaining the complex by mixing the metal-ion and the complexant solutions at a given ratio range. Therefore there is no certainty that the complex, whose stability is being measured, will be the same in each case unless certain well defined metal-ion/complexant ratio were used with any method.

The preceding statements may be clarified if some experimental data reported elsewhere are examined. Schnitzer (1965), using infrared spectrophotometric techniques, pointed out that one molecule of fulvic acid (FA) from a podzol could accept up to six  $\text{Fe}^{+3}$ . These were linked to the organic molecules through six COOH groups present in the molecule forming the  $\text{Fe}_6\text{FA}$ . However, in a later paper the same author claimed that the iron complex of the same FA has a 1/1  $\text{Fe}^{+3}/\text{FA}$  molar ratio (Schnitzer and Hansen 1970).

Schnitzer's data can be examined using the considerations already presented. In the first work he used several metal-ion solutions whose increasing concentrations were higher than the FA one, which was constant in all the experiments; therefore an  $\text{Fe}_6\text{FA}$  could be formed since the six COOH groups of the FA molecule are able to bind one  $\text{Fe}^{+3}$  each. In the second work he used different conditions, e.g., a FA concentration higher than the iron one, which was the same in all experiences. Actually, in the latter experiment there were approximately 1000 FA molecules for each  $\text{Fe}^{+3}$ . Due to this high number of available anionic sites, six per organic molecule without taking into account the other functional groups, each metal ion present was likely linked to a COOH group of a different FA molecule. This was because the probability that a second metal ion was bound to the same molecule is very low. Furthermore, this second linkage would require a higher energy level than the first one did. In this way, only the  $\text{FeFA}$  complex could have been formed in this experiment.

After the proposal of his general equation interpreting the ion-exchange equilibrium method, Schubert developed a simplified approach to apply the method to  $\text{MCh}_n$  complexes (1950). This simplified approach was first used by Miller and Ohlrogge (1958) in soil chemistry. Surprisingly, soil scientists still attribute this simplified approach to Martell and Calvin (1952). Clark and Turner (1969) criticized the way this approach had been applied to determine the stability constant of the metallic complexes of soil organic matter.

The new interpretation of the ion-exchange equilibrium method made by Ardakani and Stevenson (1972) was developed from the general equation presented by Schubert (1948) and basically maintains the original experimental pro-



cedure outlined by this investigator. Consequently it is based on the same erroneous assumptions Schubert made for interpreting the equilibrium existing between a solution containing metal-ions and complexants, and a cation exchanger (Zunino et al. 1972a). Therefore, the Ardakani and Stevenson procedure will be applicable only to MCh- or MCh<sub>6</sub>-type complexes.

Using the experimental conditions of Schubert's method (1948) the MCh complex will be easily formed and the original Schubert's equations, simplified or not, as well as those proposed by Ardakani and Stevenson (1972) are valid. In other words, always the MCh- or MCh<sub>6</sub>-type complexes will be formed regardless of the polyfunctional character of the complexant Ch. This is due to the large deficit of metal ions compared to the amount of available complexant molecules present. This point was not adequately considered before (Zunino et al. 1972a).

In our opinion the results of Ardakani and Stevenson (1972) confirm the statements above. They found that the metallic complexes of the humic acids they used were made up of one metal ion and a single complexing site (*i* and *j* coefficients nearly equal to 1). This means that despite the polyfunctional character of the humic acid used, the complexes formed were of the MCh type. The same findings have been reported by other investigators who used the simplified Schubert's approach to calculate the stability of metallic complexes of other humic acids (Randhawa and Broadbent 1965; Matsuda and Ito 1970; Schnitzer and Hansen 1970).

In view of all the preceding considerations it seems to the authors that the best way to express, in terms of stability data, the ability of a polyfunctional macromolecule to complex metal ions would be to determine the overall constant *K* for the saturated complex M<sub>6</sub>Ch. In so doing, the experimental data obtained for different complexants could be standardized and compared.

Overall stability constants have to be calculated experimentally by using increasing concentrations of metal ion and a constant concentration of the complexant. In this form the saturated complex M<sub>6</sub>Ch could be formed and the *K* values obtained will have thermodynamic meaning. It is evident that Schubert's experimental procedure cannot be applied to this purpose. Therefore, the development of new methods which meet the requirements already cited is needed.

It is well known that soil organic matter is a mixture of molecular species differing in molecular weight as well as number and distribution of functional groups. Any method applied to such a mixture of complexants in order to determine the stabilities of their metallic complexes will give data which will only estimate some average value. However, the estimation of some average value could be appropriate to study the involvement of soil organic matter in plant nutrition or soil genesis processes, providing that the metal-complexing properties of the organic materials tested really represent the actual complexing ability of the organic matter in the soil. If, however, more accurate data were needed, the vast mixture of macromolecular species constituting the soil organic matter could be easily fractionated on the basis of molecular weights. There are several physical, nondestructive processes available for this purpose, the principal one being the use of gel filtration (Carrol 1972; Khan and Schnitzer 1971; Holty and Heilman 1971; Swift and Posner 1971; Pavlotskaya et al. 1972).

*Metal-complexing properties of extracted soil-organic matter and their relationship with plant nutrition and soil genesis*

As has been pointed out in several publications, the composition of organic matter extracted from the soil is affected by the chemical extractant used (Haworth 1971; Gascho and Stevenson 1968; Jenkinson 1971; McLaren and Skujins 1971; Ortiz de Serra and Schnitzer 1972). Autooxidation or polymerization of organic materials, produced mainly with alkaline extractant solutions, may be avoided if the extraction is carried out under nitrogen, which displaces the atmospheric molecular oxygen from the alkali-soil suspension. However, hydrolysis or depolymerization processes are always involved in the organic matter alteration regardless of the extractant used. As a result of these processes the linkages between molecules forming part of the macromolecules occurring in soil will be broken, thus liberating several functional groups.

The large amount of information accumulated using several extraction procedures constitutes a valuable contribution to the knowledge of the soil organic matter structure. However, from the point of view of metal-complexing properties of soil organic matter and its relation to plant nutrition and soil genesis, some questions remain.

Several authors have identified and determined the concentration of functional groups present in soil organic matter extracts. It has been suggested (Felbeck 1971) that approximately 26 percent of the total humic acids are constituted by the five following groups: COOH, phenolic OH, alcoholic OH, CO, and methoxy, the principal one being the carboxylic group with 11.2 percent. This means that the number of free functional groups for each molecule of humic acid extracted from the soil is enormous considering the high molecular weight of the humic acids. Therefore, when humic extracts and metal-ion solutions are mixed, the number of metal ions which can be bound to one organic molecule may vary widely (from 1 to  $a$ , where  $a$  equals the total number of available anionic sites). A similar situation may be supposed to occur with other soil organic matter fractions.

As previously discussed, the number of available anionic sites on the extracted organic molecules will be closely related to the extent of hydrolysis or depolymerization undergone by the organic macromolecules during the course of the extraction process. The type and number of functional groups, and the molecular weight of the organic molecules obtained, will depend principally on the nature of the extractant used, its concentration, temperature, and time of contact, just as they will when any other organic macromolecule is hydrolyzed. The metal-complexing ability of a polyfunctional macromolecule is closely related to the nature and number of its functional groups. Therefore, the original ability of soil organic matter to complex metal ions could be completely changed during the extraction, the extent of the changes depending on the conditions used.

Which of the free functional groups found in the extracted soil organic matter were liberated during the extraction? Which available anionic sites on the organic molecules could have participated in plant nutrition or soil genesis processes in the soil? There are no answers to these questions because these conditions are impossible to measure at present. This means that the anionic site concentrations considered in laboratory work to obtain the stability data may be completely different from the ones participating in plant nutrition or soil genesis processes in the soil from which the organic matter was extracted.

It seems rather superfluous to accumulate

stability data of metallic complexes formed with organic complexants which have been extracted from the soil without taking into account the effect of the extraction procedure on the concentration of free functional groups. Therefore, the relationship between the metal-complexing ability of organic materials extracted from the soil, and the role of naturally occurring organic matter in soil, is doubtful. This relationship has been unquestionably accepted as valid by many investigators. Even if we use an ideal method to overcome the experimental limitation in the measurement of the metal-complexing properties of polyfunctional macromolecules, discussed in the preceding chapter, the data obtained with extracted soil organic matter would not have any meaning with respect to the actual behavior of soil organic matter on soil genesis or plant nutrition processes.

It seems rather difficult to isolate the soil organic fraction without disturbing its composition. Any chemical procedure could alter the organic matter which is being extracted and besides there is no way to measure this alteration on an absolute basis. This suggests that it is necessary to develop new approaches to study the metal-complexing properties of soil organic matter. One of them might be to study the behavior of soil organic matter *in situ* without previous separation from the inorganic fraction; in relation to this, some recent soils containing high amounts of organic matter loaded with free functional groups would be interesting to work with. Another approach could be to study the metal-complexing properties of some microbial-humic materials. These materials can be easily isolated from culture solutions employing nondestructive physical methods (i.e., dialysis, solvent extraction). The naturally occurring soil organic matter has been claimed to be at least partially of microbial origin (Haider and Martin 1968; Martin and Haider 1971; Vol'nova and Mirchnik 1972).

All of the considerations above indicate that the methodology which has been used up to the present to study the involvement of soil organic matter in soil genesis and plant nutrition processes has to be reevaluated. In particular, modification of the soil organic matter has to be overcome. At the same time, it seems advisable to standardize the experimental procedures used to determine stability constants of the complexes where polyfunctional macromolecular complexants are involved. If these conditions are

met, reliable relationships valid for conditions existing in the soil can be drawn and the role of soil complexants in plant nutrition and soil genesis be ascertained.

The role of soil organic matter through its metal-complexing properties in soil genesis and plant nutrition has been widely described and can easily be visualized, but up to the present has not been adequately demonstrated. The suggestions given in this paper may contribute toward better approaches for future investigations.

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