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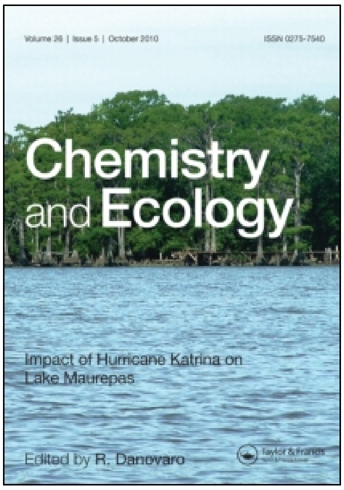

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INFLUENCE OF DISSOLVED ORGANIC MATTER ON THE BIO-AVAILABILITY AND TOXICITY OF METALS IN SOILS AND AQUATIC SYSTEMS

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Cations in soil are essential for the growth of plants and micro-organisms. Their availability is dependent on soil organic matter. Soil organic matter (SOM) is heterogeneous comprising amino, aliphatic and phenolic acids, but particularly humic substances. All these substances can complex cations selectively. Mechanisms of complexation with dissolved organic matter are discussed. Such complexation can lead to the apparently contradictory observations that dissolved organic matter (DOM) can either increase the concentration of some less soluble nutrients, making them more available for plant uptake, or make them less available and hence less toxic. The importance of DOM is discussed in relation to soil solution, particularly the rhizosphere, and also in relation to aquatic systems. The latter systems contain mainly dissolved humic substances whereas in the soil, non-humic substances assume a greater importance.

SOM in the rhizosphere is derived from plant, microbial and animal remains but much, especially the water-soluble compounds, are acquired through root exudation. Exudation has important consequences for enhanced nutrient availability as a result of the production of non-humic substances such as amino, aliphatic and phenolic acids. In future, the role of root exudation in relation to DOM and nutrient availability should be investigated more fully, particularly as predicted elevated CO_2 levels are likely to have a major impact on root exudation, nutrient availability, and possibly ecosystem community structure and functioning. It is likely that more information will become available on aquatic systems as more highly sensitive techniques and equipment capable of dealing with low concentrations of DOM in these systems become available.

KEY WORDS: aquatic systems, cations, complexation, DOM, humic substances, nutrients, rhizosphere, root exudation

INTRODUCTION

Soil organic matter (SOM) is one of our most important natural resources, playing a vital role in maintaining or improving soil fertility (Campbell, 1978; Vaughan and Ord, 1985). It is likely that the main contribution towards fertility results from an influence on the physical, chemical and biological properties of the soil (Vaughan and Ord, 1985). One of these properties is the effect of SOM on nutrient availability for higher plants. In this context, insoluble humic substances, which form the bulk of the organic components, can act as a reservoir for plant nutrients and prevent leaching of elements which are vital for plant growth. Much of this is due to the considerable cation exchange capacity of humic materials which can be in excess of 3.0 mol kg^{-1} (Sposito, 1989).

SOM is heterogeneous, covering a range from low molecular size substances such as aliphatic, amino and phenolic acids to high molecular size humic substances (Vaughan and Ord, 1985). Part of the SOM is soluble in water as dissolved organic matter (DOM) which plays a key role in soil solution chemistry (Stevenson, 1982;

Linehan, 1985). Thus, in addition to acting as a nutrient reservoir, SOM can form stable complexes with some metals and thus influence their availability to plants and microorganisms (van der Werff, 1984; Linehan, 1985; Uren and Reisenauer, 1988; Marschner, 1991).

Individual DOM components bind metals to different extents. Binding is influenced by the affinity of the metal for organic functional groups, competition between cations and the pH and ionic strength of the solution (Livens, 1991). In this review, we concentrate on the nature of the binding and its application to DOM in two distinct environments. In one of the environments, the aquatic system, humic substances predominate, while in the other, the soil rhizosphere, non-humic substances become of increasing importance. For clarity and completeness the plant's need for cations is considered, together with the composition of DOM in the context of cation availability and toxicity.

Plants contain mainly C, H and O which together with N, P, S, K, Ca and Mg are termed as the macronutrients. In addition, there are micronutrients comprising Cu, B, Fe, Mn, Mo, Co and Zn. With the exclusion of C, H and O, all the other nutrients combined contribute 5–6% of the dry weight of the plant (Sutcliffe, 1962). All the elements listed above are essential for growth and development (Marschner, 1986; Lawlor, 1991) and some of their functions are listed in Table I. All these elements form either a part of the cell components, or are essential for providing conditions needed for metabolism, or both (Lawlor, 1991). Thus carbon is a constituent of all proteins, potassium is not covalently linked to any organic molecule (Rains, 1976), while magnesium is present as a constituent of chlorophyll and is also involved in the regulation of enzyme activities.

Table I Some functions of nutrients required for plant growth.

Element	*Role in cells*
Macroelements	
Carbon	A building block of cells; required for oxidation/reduction processes
Hydrogen	As for carbon
Oxygen	As for carbon
Nitrogen	Constituent of proteins, nucleic acids; involved in nitrogen fixation
Potassium	Ionic and osmotic regulation, required for protein synthesis
Calcium	Present in cell wall pectinates, regulation of cell metabolism, membrane stability
Magnesium	Constituent of chlorophyll, co-enzyme factor
Phosphorus	Constituent of nucleic acids and lipids; required for cell energy processes and co-enzyme regulation
Sulphur	Constituent of proteins and sulpholipids; required for energy transfer reactions; thiol groups
Microelements	
Boron	Probably enzyme regulation (Dugger, 1983)
Chloride	Chloroplast photosystem 11; metabolism
Iron	Energy transfers, cytochromes
Manganese	Co-factor in water splitting enzyme, aminopeptidase
Zinc	Enzyme co-factor, carbonic anhydrase, superoxide dismutase, alkaline phosphatase
Copper	Constituent of plastocyanin, ascorbic acid oxidase, superoxide dismutase
Molybdenum	Constituent of nitrate reductase
Cobalt	Vitamin B_{12}

It is, therefore, essential that there is an adequate supply of all the nutrients listed in Table I for the continuous growth of plants. Although plants obtain all their carbon, and in some cases their nitrogen, directly from the atmosphere by fixation, most of their nutrients come from the soil. This review concentrates on the essential cations (all microelements) which can form complexes with DOM components, particularly iron, copper, zinc, manganese and cobalt. The importance of complexation reactants between aluminium and DOM is also considered.

DISSOLVED ORGANIC MATTER (DOM)

An important consideration when discussing DOM is the distinction between fine particulate or colloidal material and that which is actually in solution. The distinction is arbitrary but generally accepted on the basis that dissolved material passes through a 0.45 μm filter (Aiken, 1985; Buffle *et al.*, 1992). It must be acknowledged, however, that many colloidal species will also pass through the filter (Aiken and Leenheer, 1993).

Conveniently, DOM can be divided into two broad categories of humic and non-humic substances. The non-humic substances are well defined, comprising proteins, carbohydrates, hydroxamate siderophores, polyphenols, and amino, aliphatic and phenolic acids (Stevenson, 1982; Kuiters, 1993). The humic substances are acidic brown-coloured polyelectrolytes distinguished, on the grounds of solubility, as humic and fulvic acids. Humic acids (HA) are soluble in alkaline and insoluble in acid solutions at pH <2.0 whereas fulvic acids (FA) are soluble in both acid and alkaline solutions (Kononova, 1961). Humic substances are frequently associated with non-humic material, particularly those extracted from agricultural soils (Vaughan and Ord, 1985).

Typically DOM in surface waters comprise 50% FA, 5% HA, 25% low molecular weight acids, 7% hydrophobic and 8% hydrophylic neutral substances and <4% basic compounds (Malcolm, 1985). It is the humic substances which colour peaty water and some lakes, and the degree of coloration is often used as an index of DOM concentration in aquatic systems (Lundin, 1991). In these coloured waters, humic acids may represent >10% of the humic substances.

DOM plays a substantial role in the carbon cycle (Aiken, 1985), but despite a large literature (see Thurman, 1985), knowledge of its composition, structure and ecological behaviour in aquatic systems is limited due to low concentrations and limitations of analytical techniques. Most of the information available comes from soil solutions, or chemical extractions of soils, where individual components are at a higher concentration than in aquatic systems. For the majority of soil solutions, current analytical techniques are not limiting.

From a metal complexation perspective, humic and non-humic substances are very distinct and will be considered separately in the section dealing with complexation. Fully characterised organic substances such as amino and phenolic acids, and other biochemical products in the soil, are important in the chelation of trace elements (Linehan, 1985). These organic substances, which have short residence times, occur in greater concentration in the soil solution of the rhizosphere, often resulting from root exudation (Marschner, 1991). Humic substances, which contribute over 70% of the total SOM (Stevenson and Ardakani, 1972; Schnitzer, 1986) are mainly products of biochemical transformations (Sparling, 1985; Vaughan and Ord, 1985; Zsolnay and Steindl, 1991) and are especially present in the top soil. However, with the exception of some soils which are peaty or acid and close to peat deposits, such as the dystric histosols (Vaughan and Ord, 1993), humic substances are only sparingly soluble in soil solutions. Although under laboratory conditions we have found that a chemically isolated FA is very soluble in water (0.82 g 100 cm^{-3}), this humic substance in the form occurring in soil is relatively insoluble (Linehan, 1978). HA is rarely found in soil solutions, and when present is in a colloidal form. Although they are usually absent in DOM in agricultural soils, humic substances possess functional groups (Figure 1) which are active in complexing many cations. The amounts of these groups vary between HA and FA, but their total acidity, indicated by carboxylic acids, is higher in fulvic acids from temperate soils than in the corresponding humic acids (Table II). These functional groups are also important in well defined substances such as amino acids. It should be borne in mind that only a meagre amount of information is available on the influence of naturally occurring DOM *per se* on nutrient availability, a point also emphasised by Kuiters (1993).

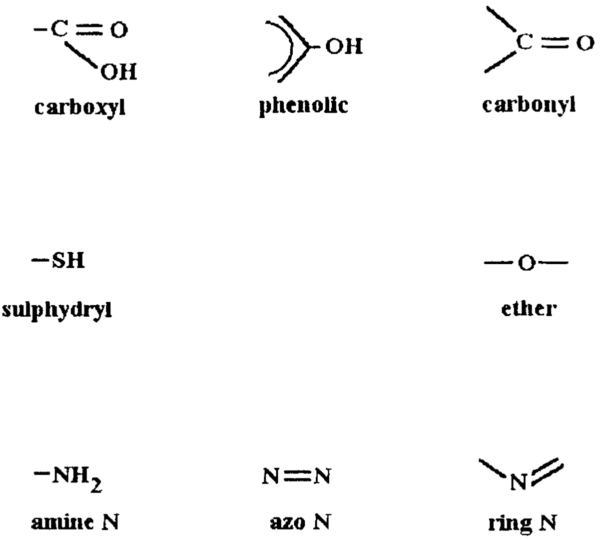

Figure 1 Functional groups of organic matter having potential to bind cations.

Table II Functional groups of humic and fulvic acids extracted from cool, temperate acid soils (after Schnitzer, 1978).

Functional group	*Humic acid*	*Fulvic acid*
	mol kg^{-1}	
CO_2H	1.5–5.7	6.1–8.5
Phenolic OH	3.2–5.7	2.8–5.7
Alcoholic OH	2.7–3.5	3.4–4.6
Quinoid C=O	0.1–1.8	1.7–3.1
Ketonic C=O		
OCH_3	0.4	0.3–0.4

The complexation of metals by DOM has important implications for plant nutrition because it controls several interrelated processes that influence the bioavailability of metals in soil solutions and waters (Morrison, 1989). These processes include the speciation of metals in solution and the dissolution of metals from relatively insoluble mineral phases brought about through complexation reactions with organic ligands. Speciation describes the distribution of chemical forms in which an element occurs. The concentration of free (i.e. uncomplexed) metals in solution is directly related to their potential toxicity (Florence and Batley, 1988; Parker *et al.*, 1988). Thus DOM can ameliorate the toxic effects of aluminium in acidic soils (Hargrove and Thomas, 1981; Asp and Berggren, 1990). Similarly, the toxicity of cadmium to barley plants was reduced by the formation of a complex with HA (Cabrera *et al.*, 1988).

Furthermore, the complexation of metals by DOM can maintain concentrations of total metal in solution greater than would be predicted from the solubility of metal-containing soil minerals. A good example of this is the favourable concentrations of ferric ions maintained in solution which are far higher than would be predicted from the solubility of ferric oxyhydroxide minerals such as goethite and lepidocrocite. In contrast, despite the greater amount of the metal in solution, only the uncomplexed form of aluminium is toxic, so that the total amount in solution is no guide to its harmful effects. Hence the toxicity of aluminium can be greatly decreased by complexing with organic acids (Gjessing *et al.*, 1989; Bessho and Bell, 1992). But the molecular weight of the organic ligand is also important in determining the uptake of the complexed metal into the plant. Hence the uptake of zinc from HA–Zn complexes was greater from medium molecular size compared with high molecular size complexes (Mirave and Orioli, 1989). It is now pertinent to consider how complexes are formed between metals and DOM.

INTERACTIONS BETWEEN DOM AND CATIONS — SOME BASIC PRINCIPLES

Metals in natural waters such as soil solution or aquatic environments may exist either as free (uncomplexed) ions or as various complexes with both inorganic and organic ligands. Metal organic complexes are formed by the interaction of a metal ion and a coordinating species known as a ligand (Bell, 1977). In natural systems, the organic ligands can either be well-defined simple organic molecules, or complex heterogeneous molecules such as those found in humic substances.

Before considering the interaction of metals with organic ligands, the behaviour of the metal with the most abundant ligand in natural systems, i.e. water, must be considered. Metals in aqueous media behave as weak acids and cause the hydrolysis of water resulting in the formation of several soluble hydroxy-inorganic species. The reaction of a divalent metal ion (Me^{2+}) with water is usually written as:

$$Me^{2+} + H_2O \rightleftharpoons MeOH^+ + H^+$$

and a number of hydrolysis reactions are possible, e.g.

$$Me^{2+} + 2H_2O \rightleftharpoons Me(OH)_2^0 + 2H^+$$
$$Me^{2+} + 3H_2O \rightleftharpoons Me(OH)_3^- + 3H^+$$
$$Me^{2+} + 4H_2O \rightleftharpoons Me(OH)_4^{2-} + 4H^+$$

The complexes such as $MeOH^+$, $Me(OH)_2^0$ etc. are referred to as species and the total concentration of metal in solution $[Me]_t$ is the sum of the various species. The tendency of a metal to undergo hydrolysis is quantified by the use of formation hydrolysis constants (thermodynamic equilibrium constants), e.g. the first hydrolysis constant β_1 of a divalent metal is defined as:

$$\beta_1 = \frac{\{MeOH^+\}\,\{H^+\}}{\{Me^{2+}\}}$$

therefore

$$\{MeOH^+\} = \frac{\beta_1\{Me^2\}}{\{H^+\}}$$

similar expressions can be written for the other hydrolysed species.

Thus for a metal in pure water, the total concentration of metal $[Me]_t$, is given by:

$$[Me]_t = Me^{2+} + MeOH^+ + Me(OH)_2^0 + Me(OH)_3^- + Me(OH)_4^{2-}$$

The distribution and concentration of these species will vary with pH and can be calculated if the relevant formation constants and total metal concentration are known. The hydrolysis products of Fe (III) in water are shown in Figure 2. As can be seen, the free metal ion exists at low pH but decreases as the hydrolysed species become more dominant as pH rises. Not all metals hydrolyse to the same extent; the values of log β_1 for a number of metals are given in Table III. Metals such as iron and aluminium are considered highly hydrolysable whereas hydrolysed species of metals such as nickel, cadmium and manganese will be present in significant amounts only at pH's greater than 9. The tendency to hydrolyse is an indicator of the metals' tendency to form bonds with oxygen and therefore, as a general rule, the highly hydrolysable metals will have a stronger association with the oxygen containing functional groups on organic molecules.

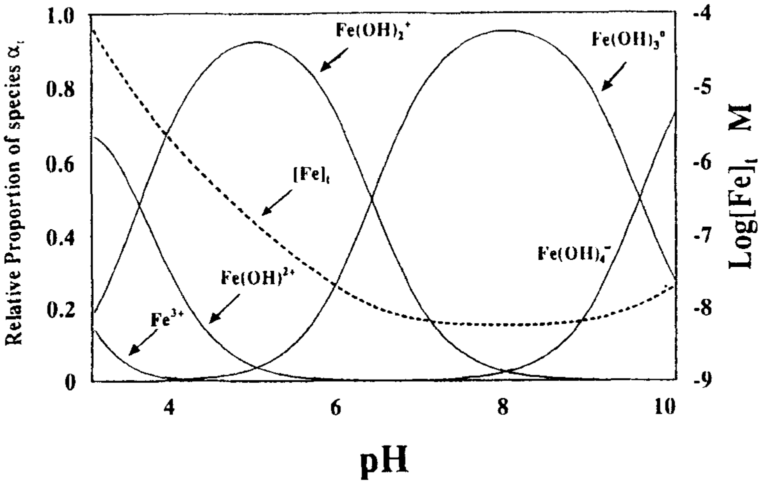

Figure 2 The proportions α, of hydrolysed species of Fe(III), shown on the left Y axis, and log $[Fe]_t$, shown on the right Y axis, in equilibrium with an amorphous ferric hydroxide mineral at an ionic strength of 5 mM. Fe(III) hydrolysis constants from Turner *et al.* (1981) and log $^*K_{so}$ amorphous $Fe(OH)_3$ = 3.54 from Lindsay (1979).

Table III The first hydrolysis constants of a number of metals.*

Metal	*Hydrolysis constants* log β_i
Fe^{3+}	-2.19
Al^{3+}	-4.97
Pb^{2+}	-7.71
Cu^{2+}	-8.00
Zn^{2+}	-8.96
Co^{2+}	-9.65
Ni^{2+}	-9.86
Cd^{2+}	-10.08
Mn^{2+}	-10.59
Mg^{2+}	-11.44
Ca^{2+}	-12.85

Hydrolysis constants for the reaction $Me^{z+} + H_2O == MeOH^{(z-1)+} + H^+$
* Values from Turner *et al.*, 1981 and Stumm and Morgan, 1981.

Metals, in the presence of organic ligands, may also exist as organic complexes. For example, the reaction of the metal with a ligand (L) such as a diprotic organic acid can be represented by the following reaction:

$$Me^{2+} + H_2L == MeL^0 + 2H^+ : \beta = \frac{\{MeL^0\}\{H^+\}^2}{\{Me^{2+}\}\,\}H_2L\}}$$

where β is the formation constant of the metal organic complex. In this case $[Me]_t$ will be given by:

$$[Me]_t = Me^{2+} + MeOH^+ + Me(OH)_2^0 + Me(OH)_3^- + Me(OH)_4^{2-} + MeL^0.$$

Because the above reactions involve protons, they can be viewed as competition reactions between the proton and the metal ion for the ligand or, at higher pHs, between OH and the organic ligand for the metal. This the extent of metal organic complexation in part will depend upon the relative concentrations of metal to ligand, the pH, and the affinity of the metal to associate with the available organic ligands, as may be quantified by the formation constant.

The formation of complexes with the constituent cations of a mineral phase is able to increase the solubility of the mineral. Thus process is important for maintaining levels of $[Me]_t$ in solution that would otherwise be vanishingly small. For example, consider the total amount of ferric ion, $[Fe]_t$, in equilibrium with a soil mineral phase such as an amorphous $Fe(OH)_3$. In the absence of complexing organic ligands, the activity of Fe^{3+} ion in solution will be controlled by the dissolution reaction of the mineral phase, e.g. the solubility of amorphous ferric oxyhydroxide is given by Lindsay (1979) as:

$$\text{(amor) } Fe(OH)_{3(s)} + 3H^+ == Fe^{3+} + 3H_2O : \log {}^*Kso = 3.54,$$

where *Kso is the solubility product of (amor) $Fe(OH)_3$ (s),

$$Kso = \frac{\{Fe^{3+}\}}{\{H^+\}^3} \text{ and } Fe^{3+} = Kso\ \{H^+\}^3.$$

The total concentration of metal $[Fe]_t$ in solution will be the sum of the free metal plus the hydrolysed species; for ferric ions:

$$[Fe]_t = Fe^{3+} + Fe(OH)^{2+} + Fe(OH)_2^+ + Fe(OH)_3^0 + Fe(OH)_4^-.$$

By substituting values for the hydrolysis constants, corrected for the ionic strength of the solution, this can be expressed as:

$$[\mathrm{Fe}]_t = \mathrm{Kso}\ \{\mathrm{H}^+\}^3 \left[1 + \frac{\beta_1}{\{\mathrm{H}^+\}} + \frac{\beta_2}{\{\mathrm{H}^+\}^2} + \frac{\beta_3}{\{\mathrm{H}^+\}^3} + \frac{\beta_4}{\{\mathrm{H}^+\}^4}\right].$$

For any pH, the expression:

$$\left[1 + \frac{\beta_1}{\{\mathrm{H}^+\}} + \frac{\beta_2}{\{\mathrm{H}^+\}^2} + \frac{\beta_3}{\{\mathrm{H}^+\}^3} + \frac{\beta_4}{\{\mathrm{H}^+\}^4}\right]$$

is a constant, therefore the total metal solution is dependent upon the value of Kso and the extent of hydrolysis. The distribution of these ferric species and the total ferric iron in solution in equilibrium with an amorphous ferric oxyhydroxide mineral is shown in Figure 2. As the pH rises, the amount of total iron in solution falls, reaching a minimum at pH 8, but increases beyond pH 8 due to the formation of $\mathrm{Fe(OH)_4^-}$. At a pH of 6, the total iron in solution is log $\mathrm{Fe_t}$ −7.74 (0.0182 μM). In the presence of a complexant organic ligand (e.g. a triprotic organic acid such as citric acid) a number of ferric citrate complexes can form:

$$\mathrm{FeH_2L^{2+}} = \beta_5\ \{\mathrm{Fe^{3+}}\}\frac{\{\mathrm{H_3L}\}}{\{\mathrm{H^+}\}}$$

$$\mathrm{FeHL^{+}} = \beta_6\ \{\mathrm{Fe^{3+}}\}\frac{\{\mathrm{H_3L}\}}{\{\mathrm{H^+}\}^2}$$

$$\mathrm{FeL^{0}} = \beta_7\ \{\mathrm{Fe^{3+}}\}\frac{\{\mathrm{H_3L}\}}{\{\mathrm{H^+}\}^3}.$$

If these organically complexed species are present then the total iron in solution will be given by the expression:

$$\mathrm{Fe_t} = \mathrm{Kso}\ \{\mathrm{H}^+\}^3 \left[1 + \frac{\beta_1}{\{\mathrm{H}^+\}} + \frac{\beta_2}{\{\mathrm{H}^+\}^2} + \frac{\beta_3}{\{\mathrm{H}^+\}^3} + \frac{\beta_4}{\{\mathrm{H}^+\}^4} + \frac{\beta_5\{\mathrm{H_3L}\}}{\{\mathrm{H}^+\}} + \frac{\beta_6\{\mathrm{H_3L}\}}{\{\mathrm{H}^+\}^2} + \frac{\beta_7\{\mathrm{H_3L}\}}{\{\mathrm{H}^+\}^3}\right].$$

In this case the term inside the brackets has increased due to the formation of the organic complexes, therefore the amount of total iron in solution will now also depend upon the values of β_5 β_6 β_7 and $\mathrm{H_3L}$. The total iron in equilibrium with a ferric oxyhydroxide mineral in the presence of three different concentrations of citrate is shown in Figure 3. Compared to the example with no organic complexing agent, it can be seen that the presence of the citrate at 0.1 mM has increased the total iron in solution by a factor of about ten at pH 6.0. In addition to increasing the total metal in solution, the presence of mono or zero valent species (e.g. $\mathrm{FeL^0}$) can increase the diffusive flux through soil solution, increasing the uptake by plants (Lindsay, 1979).

The above examples illustrate how the presence of an organic complexing ligand can maintain levels of metal in solution above that predicted from the solubility of a mineral phase alone.

Two types of complex are recognised between metals and organic complexant ligands. Outer sphere complexes are relatively weak electrostatic associations in which each of the species retains a hydration shell and is charged. Often in the

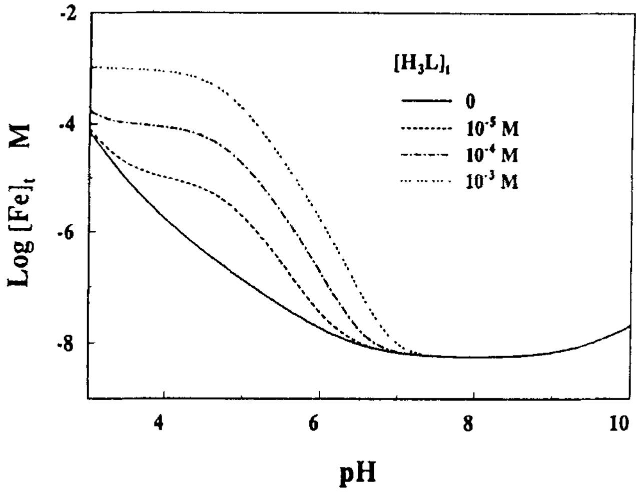

Figure 3 The solubility of amorphous $Fe(OH)_3$ expressed as log $[Fe]_t$ (molar) with or without different molar concentrations of citric acid $[H_3L]_t$.

literature, metals in outer-sphere complexes are referred to as exchangeable cations (Sposito, 1989). In contrast, inner-sphere complexes are stronger interactions in which a covalent bond is formed between a metal ion and a ligand. Examples of metal containing inner-sphere complexes would include the complexes of Fe(III) and Cu(II) with organic ligands (Senesi *et al.*, 1977; McBride, 1978). Metals forming weaker outer-sphere complexes would include Na^+, K^+. Electron Spin Resonance work suggests that the Mn–FA complexes are outer-sphere complexes (Cheshire *et al.*, 1977; Senesi, 1990). The evidence for the existence of such complexes involves detailed spectroscopic studies, but there is still debate over the exact nature of many of the associations between DOM and metals in soil solutions (Stevenson and Fitch, 1986; Livens, 1991).

Complexes that form with multidentate ligands are often referred to as chelates, from the Greek word χελε (chele) meaning claw. These types of complexes have often been used to increase the availability of plant nutrients, e.g. a well known chemically synthesised chelating agent is ethylenediaminetetraacetic acid (EDTA) used in nutrient solutions. The most stable metal organic chelates are those which form five membered rings. Thus, the dissolution of aluminium from an aluminium oxide mineral brought about by complexation with organic acids was found to increase for acids in the order oxalic>malonic>succinic as the ring size of the chelate increased from 5 to 6 to 7 (Stumm and Furrer, 1987).

To understand the behaviour and the factors influencing the formation of metal DOM complexes it is necessary to consider the complexant organic ligands and the metals with which they associate.

DOM is a complex mixture of non-humic and humic materials which are distinct in their metal complexation behaviour. Buffle (1988) has summarised the important distinctions between humic and non-humic materials. The following properties characterise non-humic substances:

(i) the molecular structure is well defined for all solution conditions;

(ii) the amounts of compound and its complexes can be expressed in molar concentrations;
(iii) the stoichiometry of each complex is considered known;
(iv) the free energy of formation of each metal–ligand complex is known, and has a unique value over a range of solution conditions.

In contrast, humic substances are less clearly chemically defined with regard to the following properties:

(i) the molecular structure is poorly defined;
(ii) the concentration cannot readily be represented in molar concentrations;
(iii) the stoichiometry of the complexes is unknown;
(iv) free energy of formation of metal–ligand complexes is poorly defined.

Humic substances differ from the simple complexant ligands in that they are polyelectrolyte macromolecules which develop a pH dependent surface charge and exhibit polyfunctionality (i.e. having many coordinating sites of differing nature present on the same molecule). These materials have high molecular weights, which range from 500–1500 daltons for FA to 1500–300,000 daltons for the HA fraction. Importantly, they contain a number of functional groups (Figure 1) that can form inner-sphere complexes with metals, the predominant ones being the oxygen containing carboxylic and phenolic groups (Linehan, 1985). The properties of the fulvic and humic acid fractions are summarised in Table II. Because of the high proportion of oxygen-containing functional groups (carboxylic and phenolic), especially in the fulvic acid fraction, they are likely to dominate the metal complexation chemistry even though nitrogen and sulphur containing groups have a high affinity for metals such as cadmium and copper. Most of the functional groups of these humic materials are acidic in nature (Perdue, 1985) and, therefore, tend to dissociate protons with increasing pH, thus developing negative charge on the surface of these molecules. The development of surface negative charge allows, in addition to the covalently bonded inner-sphere metal complexes, the retention of metals as "exchangeable" outer-sphere complexes on the same humic molecule.

As the concentration of humic complexant ligands cannot be expressed readily in molar concentrations, the value of the titratable acidity is often used to estimate the complexation capacity of DOM. The pKa values of the acidic functional groups on humic substances have been discussed by Perdue (1985).

Much of the work on complexation of cations by SOM in aqueous solution has been focused on humic substances. In recent years, however, more work has been done on the rhizosphere because processes in this environment are directly involved in plant nutrient availability and uptake. Within the rhizosphere much of the soluble organic material arises from plant root exudation.

DISSOLVED ORGANIC MATTER IN THE RHIZOSPHERE

Most of the information on metal complexation by soil organic matter applies to the bulk soil and there has been, with few notable exceptions (e.g. Marschner, 1991), little attempt to consider metal binding in the immediate vicinity of the root, termed the rhizosphere.

As much as 30% of assimilated carbon is lost from plant roots to the rhizosphere (Whipps and Lynch, 1986). Much of this organic carbon is lost passively as water-soluble exudates such as polysaccharides, proteins and low molecular weight components. Among the low molecular weight aliphatic acids identified are oxalic, citric, fumaric, tartaric, malic and succinic acids. Aromatic acids which have been reported include p-hydroxybenzoic, ferulic, vanillic, p-coumaric, protocatechuic, gentistic, gallic, sinapic, salicylic, caffeic, syringic acids (Stevenson and Fitch, 1985; Sposito, 1989; McKeague *et al.*, 1986).

Hydroxamate siderophores are produced by soil microbes and are chelators of cations in soil (Akers, 1983; Buyer and Sikora, 1991). The siderophores are low molecular weight peptide derivatives that chelate Fe^{3+} by hydroxamate or di-ortho-phenol groups (McKeague *et al.*, 1986), they are also able to chelate other metal cations such as zinc, manganese and copper (Sugiura and Nomoto, 1984; Crowley *et al.*, 1987).

Most of these non-humic substances are soluble in the soil solution (Vaughan and Ord, 1991) and have functional groups which can chelate cations. Typical concentrations of these substances have been summarised by Stevenson and Fitch (1986) as:

(i) simple organic acids (1 mM to 4 mM);
(ii) amino acids (0.08 mM to 6 mM);
(iii) phenolic acids (0.05 mM to 0.3 mM);
(iv) hydroxamate siderophores (0.08 μM to 1 mM).

In the rhizosphere, it is the dissolved non-humic substances which are of increasing importance in comparison with the dissolved humic substances. For example, organic acids of the citric acid cycle are effective trace metal chelating agents and have been isolated from root exudates of barley, bean and wheat (Vancura and Hovadik, 1965). Root exudates of several species also contain phenolic acids (Vaughan and Ord, 1991; Marschner, 1991) and such phenolics are found widely in the soil solution (Hartley and Whitehead, 1985; Kuiters and Denneman, 1987). Furthermore, the amounts of soluble simple substances such as phenolic acids in the soil solution vary according to the predominant plant species (Table IV). Water-soluble root exudates play a significant role in nutrient uptake (Marschner, 1991); thus the crop species, which determines the amounts of phenolic acids in the root exudates (Table IV) can have a profound effect on metal complexation in the rhizosphere.

Table IV Amounts of three phenolic acids extracted by water from soil, under different plant species (after Whitehead *et al.*, 1982).

Plant species	*Organic C (% of dry soil)*	*HBA**	*Vanillic acid*	*p-Coumaric acid*
			$\mu g\ g^{-1}$ soil organic C	
Elymus repens	3.06	1.05	0.26	0.21
Agrostis stolonifera L.	2.19	0.55	0.05	0.05
Holcus mollis L.	2.42	0.38	0.09	0.07
Cicerbita macrophylia	1.80	0.60	0.19	<0.01
Petasites fragrans	2.06	0.50	0.10	0.04
Urtica dioica L.	3.23	0.33	0.04	0.02
Pteridium aquilinum (L)	2.56	0.15	0.01	0.03

* HBA = phydroxybenzoic acid

Uren and Reisenauer (1988) consider that root exudates influence nutrient stability and uptake directly by acidification, chelation, precipitation and oxidation-reduction. Root exudates are effective in the dissolution of hydrates, MnO_2 (Godo and Reisenauer, 1980), and $Fe(OH)_3$ (Olsen and Brown, 1980) through chemical reduction and chelation. Such mechanisms could facilitate the transport of those nutrients in the rhizosphere (Nye and Tinker, 1977). In terms of specific exudate components, malic acid forms chelates with hydrated oxides of manganese (Stevenson, 1967; Jauregui and Reisenauer, 1982). Enhanced release of soluble phenolic compounds such as caffeic acid into the soil solution may occur in response to iron deficiency in many plant species (Marschner, 1991). These phenolic acids mobilise sparingly soluble inorganic Fe(III) compounds (Marschner *et al.*, 1986b) in addition to manganese from MnO_2, most probably by reduction in the rhizosphere. The mobilisation of iron in the rhizosphere has been reviewed by Linehan (1985), Romheld and Marschner (1986) and Uren and Reisenauer (1988). The nutritional status of the environment can influence root exudation qualitatively to produce exudate components which enhance cation availability (Marschner *et al.*, 1986a). Under some conditions such as iron deficiency in grasses, non-proteinogenic amino acids, phytosiderophores, are released (Takagi *et al.*, 1984; Marschner, 1991) which are very efficient in facilitating the uptake of iron by plant roots. In roots of grasses, Fe(III)–phytosiderophore complexes are taken up by a specific transport system 100–1000 times faster than those for Fe-synthetic complexes such as Fe-EDTA. Phytosiderophores also form chelates with other heavy metals such as zinc, manganese and copper (Sugiura and Nomoto, 1984; Crowley *et al.*, 1987) thus mobilising these cations in the soil solution (Takagi *et al.*, 1988; Treeby *et al.*, 1989). The role of root exudates in the acquisition of nutrients other than iron and manganese is unknown. However, roots excrete protons (Marschner, 1991) with consequent effects on the extent of complexation between the organic matter and cations as described above.

DISSOLVED ORGANIC MATTER IN AQUATIC SYSTEMS

Unlike the rhizosphere, in aquatic systems dissolved humic substances predominate over non-humic substances. Substantial amounts of DOM are carried from the land in rivers and streams towards lakes or the sea. Thus in the Gulf of Bothnia (between Sweden and Finland), it has been calculated that about 1.8 million tonnes of humic substances are deposited annually (Allard *et al.*, 1991). Humic substances are a major component (55–80%) of DOM in aquatic systems of which at least 85% is FA (Thurman, 1985; Pouravuori and Pihlaji, 1991). Malcolm (1985) has emphasised that the DOC of organically coloured stream water is very variable, ranging from 5 mg C to >50 mg C dm^{-3} but always comprises about 90% FA and <10% HA. Generally stream and river FAs are thought to originate solely from SOM, but this is not the only source of humic substances (Malcolm, 1985).

Stream humic substances are very resistant to both biological and chemical breakdown (Midwood and Felbeck, 1968) so are unlikely to be an important food source for aquatic organisms. Because of its solubility in water, FA is the most important metal binding organic ligand in surface waters and plays a key role in controlling metal speciation, transport, and the bioavailability of metal cations in aquatic systems (Thurman and Malcolm, 1981; Buffle, 1988). This has been emphasised by David *et al.*, (1991) who stated that DOM in lakes and streams in temperate ecosystems has a high FA content rich in carboxylic functional groups which play a key role in metal binding.

Metals in the aquatic environment can be brought into solution by several different processes as described under the metal complexation section, above. Strong complexation or chelation of DOM is one process for lead, iron and aluminium, but loss of metals from solution can be explained by precipitation of DOM–metal complexes (La Zerte, 1991) as in soil solutions. Metals such as copper, iron and aluminium that bind tightly to fulvic acids as covalent complexes or chelates (Gamble *et al.*, 1970) tend to follow the same pattern of transportation as DOC. They tend to accumulate where the humic substances accumulate and migrate with these substances. Some metals following this pattern may also be influenced by redox processes (e.g. iron) and some by induced dissolution of hydroxides (e.g. aluminium). This can be illustrated by the model proposed by Theis and Singer (1975), relating to the precipitation of Fe in aquatic systems (Figure 4) where there is DOC and dissolved oxygen. At pHs near neutrality, Fe(II) is oxidised to Fe(III) which precipitates as $Fe(OH)_3$, shown in reactions 1 and 2. If sufficient concentrations of DOM are present, the complexing reaction 3 will compete effectively with the oxidation reaction. Fe(III)–OM complex is formed from the Fe(II)–OM complex very slowly (reaction 4). The Fe(III)–OM complex is, however, very unstable and becomes reduced by the DOM (reaction 5), producing Fe(II) which may then undergo oxidation as in reactions 1 and 4. The reactions between iron and humic substances are important in relation to the precipitation of iron, as ochre, in streams near peaty soils. We have found that in a stream 10 km south of Aberdeen, Scotland, the amount of dissolved humic substances can vary considerably throughout the year with consequences for iron precipitation. In a laboratory experiment, water samples from the stream, pH 4.9, were filtered using a 0.45 μm filter and incubated in stoppered flasks at pH 6.0 for 5 days, after which time the amount of ferrous iron remaining in solution was determined (Vaughan and Ord, 1993). In January 1987, no humic substances were found in the water, but in subsequent months these substances were detected, reaching a very high value in September, thereafter declining (Table V). The amount of complexed iron increased with increasing concentrations of humic substances and the

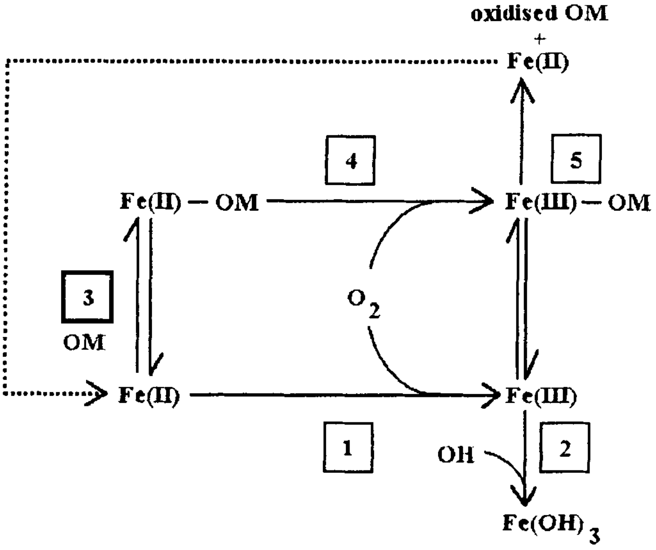

Figure 4 Scheme showing the oxidation of iron in aquatic systems in the presence of dissolved organic matter and oxygen (after Theis and Singer, 1975).

amount of iron precipitated was related to the amount of the complexed metal, being least in September.

Table V Relationship between iron bound to humic substances in the drainage waters, Cairn Robin farm, Aberdeen, Scotland, at different seasons, and its precipitation on incubation for 5 days at pH 6.0.

Sampling date (1987)	*Humic substances ($\mu g\ cm^{-3}$)*	*Free Fe(II)*	*Bound Fe*	*Amount of total Fe precipitated (%)*
January	0	14.1	0	99.6
May	59	12.0	2.2	78.9
September	112	10.1	4.8	62.2
November	21	12.5	1.8	90.2

Other metals appear to behave more independently of DOM (e.g. cadmium, zinc and manganese) probably because of their weak electrostatic bonding to fulvic acid (Gamble *et al.*, 1976). These observations between weak and strong binding have been confirmed by La Zerte (1991) who showed that lead, copper and iron mobilisation in dilute waters of the Precambrian Shield in Canada closely followed the patterns of DOC mobilisation, whereas cadmium, zinc and mangenese were not correlated with DOC concentrations or movement.

PERSPECTIVES FOR FUTURE RESEARCH

DOM is important for nutrient availability both *indirectly* as a result of biochemical mineralisation and *directly* through its impact on soil solution chemistry and ion uptake mechanisms in root cellular membranes. Much of the previous work was done using humic substances extracted from soil using harsh chemical extraction techniques and is open to the criticism that these substances do not occur naturally. Increasingly more work is being done on solution chemistry, using more natural conditions such as soil solutions and aquatic environments and this will continue. This is because both the aquatic environment and soil solution can contain extremely low concentrations of DOM and the more sensitive analytical techniques now becoming available should give a considerable boost to future research. Such sensitive techniques can also be applied to qualitative measurements in root exudation. There is considerable scope to expand work on nutrient availability in the rhizosphere, in relation to cations resulting from the projected enhancement of atmospheric CO_2 levels. In addition to increased CO_2 levels, changes in management practices are likely to have a major impact on root exudation, qualitatively and quantitatively, and hence ultimately soil fertility via soil processes, changing the availability to plants of carbon, nitrogen and phosphorus.

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