CHAPTER - I

Introduction and Review of previous works.

INTRODUCTION

It is well-known that the colloid-sized particles play an important role in determining the various physical and physio-chemical properties and reactions that occur in soils. The soil-colloids can roughly be divided into two groups viz. organic and inorganic. The inorganic part may consist either of residues of the original minerals present in parent material or of new formations produced during the weathering of these minerals. The organic part consists of a large group of compounds which are formed by the decomposition of the plant and animal residues added to the soils. These are characterised by their dark colour and referred to as humus substances.

According to Staudinger (1935)[1], the humus substances are essentially large polymers called bio-polymers, mostly made by the aggregation of a large number of atoms through chemical bond rather than colloidal assemblage of smaller molecules. The humus substances or more commonly known as humus, which, because of their peculiarities in nature, cannot be related directly to any of the major groups of compounds in organic chemistry (viz. Protein, Nucleic Acids, Fats, Carbohydrates, etc.), constitute an important part of the soil organic matter.

These soil organic matters are the chief sources of plant nutrients. There is in fact conclusive evidence that small amount of certain organic substances like highly dispersed humus have a definite and positive effect on the growth and development of the plant. Besides being an important factor in structure formation, organic matter has also a fundamental effect on the physical properties of soil viz. water holding capacity, heat regime, aeration etc. Various physical, chemical as well as the biological properties of soil depend largely on the interaction of clay minerals, metal ions and hydrous metal oxides with the soil organic matter.

The humification process of plant and animal residues in soil can as a whole be considered as the activity of various representatives of the microflora possessing differentiated fermentation mechanism and the work of microscopic and macroscopic animals in processing organic residues[2]. The polymeric materials e.g., polysachharides, lignins, proteins etc. present in the plants and animals are first degraded to simple compounds in soils. These smaller units, which are then utilised by various soil micro-organisms in their metabolic pathways, undergo enzymatic or chemical reactions to form new polymers. Some of these polymers are relatively resistant to further decomposition or form stable complexes with metal ions and clays and thus constitute an important part of the soil humus.

The early investigations of soil humus during the 18th century merely give~~s~~ a confused idea that humus is a soil constituent and it has some importance in soil fertility.

Sprengel (1826)[3] is the first to begin a systematic study of chemical nature of humus. Berzelius (1883)[4], Hoppe-Seylers (1889)[5] and later Oden (1912)[6] have extended the investigation on humus. According to these scientists humus substances are heterogeneous mixture of some organic acids viz., fulvic, humic and hymatomel~~o~~anic acids and humins. Fractionation of these substances is due to Oden[7] who has primarily depended on the solubility of different fractions of humus in acids, alkali or alcohol. Bemmelen (1888)[8] from his studies also concludes that these acids including humins are heterogeneous substances and molecular formulae to represent them have no real significance. He has further assumed that these substances are amorphous and colloidal in nature. Baumann et al.(1910)[9] have found that the acid properties of humus are not due to the presence of compounds with functional groups but mainly due to its colloidal properties. Thus the nature of humus and its actual role in soil has remained obscure for quite some time.

The aromatic character of humic acids has been first established by Shmuk[10] although many other investigators[5,11] put forward evidences in support of the fact that aromatic compound can be converted into humus like substances by means of their oxidation to quinones with the formation of dark-coloured complex compounds.

Only a small part of the humus exists freely in soils. The main fraction is in the form of metallo-organic compounds[12,13,14].

More recent studies[15,16,17,18,19,20] indicate that humic and fulvic acids exist in the soil as (i) humates and fulvates of alkali and alkaline earth metals, Iron, Aluminium and Ammonium etc., depending on the characteristic components of the soil; (ii) the group of complex compounds formed between soil organic matter and Iron, Aluminium, Manganese, Copper and other trace elements present in the soil. The complex may be one in which each radical is linked by one co-ordination bond to the central metal or may be 'Chelates' in which a number of radicals of a molecule are linked to the central ion by several co-ordination bonds.

According to Felbeck (1971)[21] a major part of the humic acids is made of amino-acids, hexosamines, polycyclic aromatics and oxygen containing functional groups. The remaining part is believed to contain easily oxidis~~e~~able heterocyclic compounds having higher degree of unsaturation which is responsible for the dark colour of all types of humic substances.

Several investigators[22,[illegible],24,26] have demonstrated the polyelectrolytic character of humic and fulvic acids. This was later supported by other workers[25]. The analogy of the humus like substances with the condensation products of different quinones and amino acids have been noted by some workers[28,29,30,31]. The formulae for the synthetic acids proposed by Eller (1923)[32] correspond to a polymeric hydroxy p-benzoquinones. From the investigations with electron peramagnetic resonance spectromet it has been found that the humic acids contain semiquinone rad together with some quinhydro~~s~~nes, which may possibly be derive from o-and-p-benzoquinones[33,34]. The redicals are stable against chemical attack and the stability increases in the order: lignin > degraded lignin > fulvic acids > humic acids. These results agree with the conceptions concerning the chemical processes occurring during the transformation of lignin or its degraded products to fulvic and humic acids.

Isolation from soil

The humus content of the soil varies greatly and is generally in the range of 0.2 to 20.0%. The organic components of soil usually decrease with depth, being maximum at the surface (0 to 20 cms). The major fraction of the organic matter cannot be extracted from the soil by water only. Suitable chemicals such as acids, alkali and some complexing agents are employed for the purpose. The humic substances are in intimate contact with the clay and other polyvalent cations in the soil.

Different investigators[35,36] have questioned the common method of employing dilute aqueous NaOH as the extracting reagent. These authors and others[37] have used various other inorganic and organic solvents for the purpose. Sodium pyrophosphate has been proved to be one of the successful reagents for isolating humus substances from soils. It has been found[38] that a mixture of sodium pyrophosphate and sodium fluoride successfully isolates a large portion of humus from the B_h-horizon of a podzolic soil but they have failed to isolate equal amount of humus from A_0 horizon of the same soil using the mixture. According to Drozdova[39] sodium pyrophosphate extracts metal complexes from peat soils in addition to free humus. Scheffer and Welte[40] and Welte[41] have found no essential difference in the visible absorption curves for solutions of humic acids isolated by means of 0.50% sodium hydroxide and those isolated with the help of 1% sodium fluoride. Elementary composition and content of carboxylic groups in the humic acid have been found to show a negligible change whether isolated with 1% NaF or with 0.1 (M) NaOH[42]. Posner (1966)[43] has compared 0.50 (M) NaOH, 0.10(M)$Na_2P_2O_7$ and 0.50(M) Na_2CO_3/ $NaHCO_3$ as extractants for humus. Schnitzer and Skinner (1968)[44] did not observe any appreciable change in the organic matter caused by 0.50 (M) NaOH in the podzol B_h horizon. Dowex-1 resin has also been employed to extract organic matter[45] from soils, the latter is comparable with that of the sodium hydroxide extraction method.

The general problems of extraction have been very successfully reviewed by Van Dijk (1966)[46]. In view of the above as also from experiences in our laboratory[47], we consider the alkali extraction method to be the best for isolating organic components of the soils.

Fractionation of the Humic Substances and their purification

Generally the humus after extraction from the soil is precipitated with the help of HCl or H_2SO_4, from which fulvic acid, soluble carbohydrates etc. separate out. From the precipitate humus the hymatomelanic acid is separated by dissolving it with boiling alcohol in a soxhlet. The fulvic acid is separated by precipitating with Barium, Copper or Iron salts[48,49,50].

For further fractionation of humic substances many attempts have been made which include fractional precipitation[51,52] from solutions at different pH's, Chromatographic technique[53,54], Electrophoresis[55], Gel-filtration[56,57], etc.

Kononova and Belchikova[53] have isolated humic and fulvic acids by fractionation with partition paper chromatography. Scheffer et al.[58] by means of their experiment on the influence of electric field on humic acids have established that using a higher potential and longer times, humus substances may be separated into a large number of fractions. Zeichmann (1967)[59] has separated three fractions by low voltage electrophoresis. Sephadex gel has been found to be suitable for separation of humic acid fractions. Schnitzer and Skinner[44] have prepared seven fractions of a podzol fulvic acid by using the sequence of sephadex gel. These fractions have been found to differ in the number average molecular weight, Nitrogen content, - COOH, and phenolic -OH content, I.R. and N.M.R. spectra. Recently Sephadex G - 25 gel has also been used[60] for fractionation of humic acids.

The humic substances after isolation from the soils are made free from contaminated clay particles, sesquioxides, mineral salts etc. The humic acids are usually purified by repeated precipitation and redissolving in dilute NaOH. Fulvic acids prepared by retaining them on activated charcoal and then elluting the same with 0.2 (N) NaOH have been shown[61] to contain iron chelates.

Ion-exchange resins have been used for purifying humic and fulvic acids[62,63]. The acids purified by resins have been found to be of a very low ash content. Commonly used resins are Amberlite. I.R.- 120 (H) for the cations and Amberlite I.R.A.- 400 (OH) for the anions.

Molecular Weight

It is beyond doubt that humus substances exist as a mixture of high molecular weight heterogeneous compounds. It is thus very difficult to obtain precisely the molecular weight, rather an average of the molecular weights are generally obtained. The values obtained so far vary widely depending on the source and methods adopted for the purpose.

Fuchs et al.[64] have reported a value for the molecular weight ranging around 1400 by measuring the elevation of boiling point of a few acetone soluble fractions of humic acids. Flaig and Beutelspacher[65] using ultracentrifuge have found the molecular weights in the range 30,000 to 50,000 for humic acids. Schnitzer and Skinner[44] obtained molecular weights 5813 by ultracentrifuge technique and 5819 by vapour pressure osmometry for the same fraction of fulvic acids isolated from the B_h horizon of a podzol. Posner[56] obtained two fractions of humic acids, the molecular weights of which have been found to be 70,000 and 200,000. Several other workers[66,67] have investigated the fractionation and molecular weight distribution in humic acid fractions. In general it has been found that the molecular weights determined by cryoscopic or ebuliscopic methods are very much lower than those determined by other methods like osmometry or ultracentrifuge.

Potentiometric Titrations

Polybasic character of different fractions of humus i.e., the humic, fulvic, hymatomelanic acids are due to various functional groups such as carboxyl, hydroxyl, enolic, amino and imino groups. Potentiometric titrations are often applied to such acids in order to ascertain the relative acidity of the different groups and the mechanism of interactions between metal salts and the humic acids.

Gillam (1940)[68] has reported two distinct inflexion points in the potentiometric titrations of soil organic matter with sodium hydroxide - the first due to carboxylic and the second due to phenolic hydroxyl groups. Chatterjee and Bose (1952)[69] have found that the base exchange capacity depends on the kind of base used for titrations, which they have accounted as 'Cation effect'. The reactivity of the bases are in order : Calcium hydroxide > Barium hydroxide > Sodium hydroxide. The titration curves in absence of natural salts have shown irregular cation effects. In the presence of neutral salts, however, the titration properties of different humic acids with bases are similar. From hysteresis of the potentiometric curves Halla and Ruston[70] have assumed an exchange reaction of the humic acid if the base-titrated solution is back-titrated with acid. Martin and Reeve (1958)[71] have demonstrated that the shape of the titration curves of an acetyl acetone extracts of organic matter with 0.05(M) NaOH in the presence of 0.10(M) KCl as well as the average pK values are influenced by the Aluminium ions present in the reaction mixture.

From the observation that there is always a drop of pH on addition of metal salts and the drop is greater for the metal with stronger complexing ability, Van Dijk (1971)[108] is of the opinion that the pH drop originates from the dissociation of the weakly acidic groups and possibly from the release of protons from the functional groups which are normally not ionised.

Schnitzer and Desjardins[74] have proposed a molecular formula $C_{20}H_{12}(COOH)_6(OH)_5(CO)_2$ for these organic substances. From their results of potentiometric titrations, they have proposed that at pH 3, one of the carboxylic acid groups, at pH 6 five groups and at pH 8 all six carboxylic acid groups are neutralised. The phenolic hydroxyl groups are titrated at about pH 10. Schnitzer and Skinner[75] have studied the complexing properties of organic substances, isolated from B_h horizon of a podzol soil with Iron, Calcium, Magnesium, Copper and Nickel cations by potentiometric titrations.

Posner (1964)[76] has titrated humic acids in presence of different concentrations of LiCl, NaCl and KCl and has observed similarities in titration properties. Similarities are also obtaine when the humic acids are titrated in presence of divalent neutral salts like $CaCl_2$ and $BaCl_2$. At very high concentration of divalent salt (≈IM) the titration curve resembles that of a strong acid, which indicates that almost all of the protons from humic acids are released by these high concentration of divalent cations.

Posner (1966)[77] and later Stevenson (1975)[78] have divided the potentiometric curves of humic acid into three parts. Region I from pH 3 to 7 has been assigned to carboxyl groups, region II from pH 7 to 8 to amino nitrogen and region III from pH 8 to 12 for the phenolic hydroxyls. Dutta and Mukherjee (1968,1970)[79,80] have carried out several potentiometric investigations on humic, fulvic and hymatomelanic acids and are of the opinion that these organic acids are weak polyelectrolytes having c.e.c. ranging from 200 - 800 meq./100 gm of the dry acid.

Conductometric Titrations

Several workers have used conductometric titration as one of the methods of characterisation of humic and fulvic acids. Chatterjee and Bose[69] obtained three breaks in the titration curves of Merck humic acid while only one break for soil humic acid. The neutralisation of humic acids by bases follows the order $Ca(OH)_2 > Ba(OH)_2 > NaOH$. Roy (1957)[81] obtained two breaks in the conductometric titration curves for 'Merck' humic acids while the soil humic acid reveals only one. The neutralisation reactions follow the order $LiOH > Ba(OH)_2 > KOH$, in agreement with the result from potentiometric titration. According to Van Dijk[72] synthetic and natural humic acids show similarities in their titration properties.

Schnitzer and Skinner[75] assumed the minimum of the sodium hydroxide titration to be the points of neutralisation of two strongly acidic carboxyl hydrogen atoms. The additional three breaks in the conduct~~i~~ometric titration curve for the $Ca(OH)_2$ titration ~~is~~ are explained by a stepwise change of conductance of carboxyl groups as the acidity changes. They, however, have failed to account for the participation of phenolic hydroxyl or other groups in the neutralisation process.

Mukherjee et al.(1968)[79,80] carried out several investigations on fulvic and hymatomelanic acid by conductometric titrations. These authors have compared the titration curves with those of salicyclic, phthalic and succinic acids. They have obtained a higher cation exchange capacity from conductometric titration than the corresponding value in potentiometric titration.

Viscosity Measurements

Many investigators[22,24] have shown polyelectrolytic nature of coal humic acids by means of viscosity measurements. Polyelectrolytic character, as evidenced by the viscosity and sedimentation behaviour of peat humic acids has also been demonstrated by Piret et al.[26]. These observations are not in conformity with those made by Flaig et al.[65,84,85] who have shown by Viscometric, ultracentrifugation and electron microscopic studies that the humic acids have a spherical shape. Mukherjee et al.[25,82], on the other hand, confirmed with the aid of viscosity measurements, the polyelectrolytic character of soil and synthetic humic acids. However, Wershaw et al. (1967)[66] have proposed that the particles not only have variable size but also they vary in shape. This is further supported by Khan[86] who has considered that humic acids contain a mixture of both linear and sphero-colloids. Chen and Schnitzer[27] have calculated the molecular weight of humic and fulvic acids from viscosity measurements. They have also shown that humic acids at pH 7 and fulvic acids at a very low pH behave like uncharged or non-electrolytic polymers.

Visible Spectroscopy

From the study on the optical properties of these dark coloured compounds, it has been claimed that light absorption of humic substances appears to increase with increase in (i) the degree of condensation of the aromatic rings in the humus[2], (ii) total carbon content[87], (iii) molecular weight[87] and (iv) the ratio of the carbon in aromatic nuclei to carbon in aliphatic or alicyclic side chains[88].

The ratio of the optical densities at 465 and 665 nm for the characterisation of humic acids is sometimes used as an index of humification. In general, the ratio E_4 (extinction at 465 nm)/ E_6 (extinction at 665 nm) decreases with increasing humification[2] and is independent on the concentration of the humic compound. Campbell et al. (1976)[89] have found that the humic material with the lowest mean residence time had highest E_4 / E_6 ratio.

When humic and fulvic acids are excited with visible lights of suitable wavelength fluorescence is exhibited. This fluorescence property has been utilised by several workers[145,146] to ascertain the progress of humification. Mukherjee et al.[90,91] have obtained fluorescence emission spectra of humic, fulvic and hymatomelanic acids with maxima in the region 500 - 540 nm. From the nature of the spectra which are almost similar in all cases, it is concluded that these acids are more or less similar, probably representing the intermediate stages of a polymer series.

Infra-red Spectroscopy

The infra-red spectroscopy has also been used by many workers for characterising these acids[92,93]. Schnitzer et al.[94,45] have investigated the nature of the absorption band after addition of polyvalent metal ions to humic and fulvic acids. In general, due to overlapping of various absorption bands, no specific band has been assigned to these acids. But the features of the overall curves are almost similar in all cases. There is some evidence that the carboxylic group is conjugated or directly bound to the aromatic nucleus. Some differences in the absorption bands sometimes arise which may be caused by different degrees of condensation during formation of humic substances.

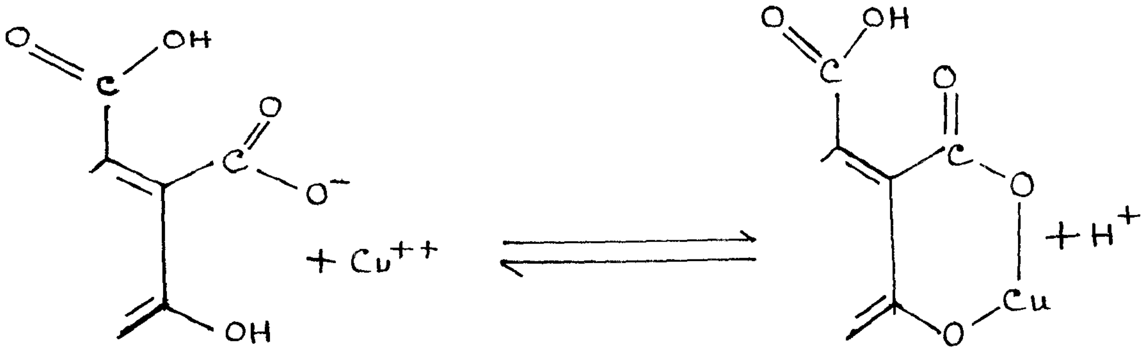
Other techniques used for the investigation of the nature and properties of humic and fulvic acids, are Polarography[96,97,98], X-ray[99,100], Differential Thermoanalytic methods[101,102], E.S.R. studies[103,147] and Electron microscopic studies[84].

Interaction of metal ions with Humic and Fulvic acids

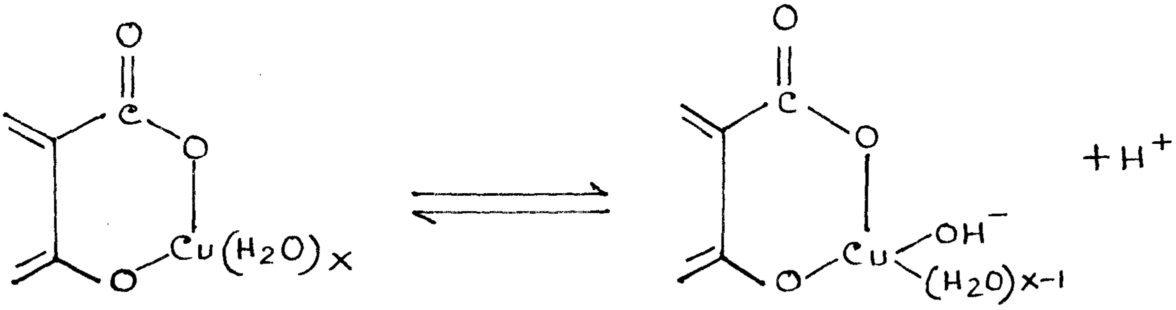
Nature of the complexing sites:

Humic acids may be defined as polydispersed mixtures of higher molecular polyanions, which are mainly but not exclusively formed by reaction of phenolic nitrogenous compounds in soils[2]. They form complex linkage of various kinds with metals by ion-exchange, adsorption on the surface and in many cases 'chelates'[104,105,106]. In the study of the interaction of metals with humic and fulvic acids many authors use the term 'complex' or 'chelate~~s~~' for the linkage involved in the reaction product[104,107,108]. This is because, in addition to the electrostatic forces operating in the linkage, there are evidences which show that the bond between the metals of the first transition series and humic acids have partial covalent character. Even with the alkaline earth ions this bond may have some covalent character[108]. Unlike clay minerals, the humic and fulvic acids have no well defined cation exchange capacity. The exchange or complexing capacity vary from source to source and is influenced by the charge of the metal[109]. The exchange capacity for a metal with these organic substances generally increases with increase in pH. This is because of the larger dissociation of the acidic sites[110]. It has been observed that below a certain pH adsorption ceases to occur regardless of the amount of the exchanger present[111]. The method of extraction has some influence on the exchange capacity of humic acid. Posner[77] has found that the exchange capacity ranges from 217 to 409 meq/100 gm. of dry organic matter for a red-brown soil humic acid.

There is a direct relationship between the contents of the lignin-like substances of the soil organic fraction and its cation exchange capacity. This has led to the assumption that phenolic hydroxyl groups are the most active sites in the humic and fulvic acids. Broadbent and Bradford[110] have reported that the carboxylic acid groups as well as the phenolic hydroxyl groups are responsible for the exchange with metals. In 1957 Himes and Barber[112] have found that the blocking of non-carboxylic acidic functional groups reduce Zn^{++} retention just as effectively as in the blocking of carboxyl groups. Thus they have emphasised the importance of carboxyl and other noncarboxylic groups in forming complexes with metals. These workers and others[107] are of the opinion that probably salicylate type of chelates are formed under natural conditions during the interaction, e.g.,



Schnitzer and Skinner (1965a)[95] from their experiments by blocking the groups selectively have shown that the largest reduction in metal ion retention by the fulvic acids occur when the carboxyl and phenolic hydroxyl groups are both blocked (ii) the second largest effect is obtained when either carboxyl or phenolic hydroxyl groups are blocked and (iii) no effect results from the blocking of alcoholic hydroxyl groups. Van Dijk (1971)[108] has proposed a salicylate type of chelate for copper humic acid interaction and is of the opinion that the most plausible reaction mechanism at low pH is the displacement of protons from the acidic groups of the humic acids, whereas at a higher pH protons are also liberated from water molecules which are covalently bound with the metal ions (e.g. Cu^{++}) giving a hydroxocomplex, e.g.,



Recent researches of Stevenson[78,113] have supported this view. Although there is no direct evidence that the carbonyl or other groups (e.g., $-NH_2$ or $=NH$ etc.) participate in the chelation process, their contribution towards a bond formation with a metal ion cannot be completely ruled out[108,78,95].

Stability of the metal Soil Organic matter complexes

The stability of the complex or chelates formed by the metal ions with soil organic matter has drawn considerable attention of the soil scientists and mineralogists. A good number of workers[114,116,121,123] have reported the stability constant values for the complex formed between polyvalent metals and humic or fulvic acid fractions of soil organic matter. From these values we may have some quantitative idea on the affinity of a metal for these types of complex molecules.

Ion exchange equilibrium method proposed by Schubert[120] (1948) and later simplified by Martell and Calvine (1952)[119] is the most extensively used method for the evaluation of stability constants for the soil organic matter-metal complexes. The method depends primarily on the fact that the quantity of a cation bound to a definite amount of cation exchange resin at equilibrium is proportional to the concentration of free ions in the solution even in presence of chelating species (e.g., humic acid) over a range of concentrations of the metal.

The equilibrium reaction for the chelate or complex formation can be represented as:

$$M + xCh \rightleftharpoons M(Ch)_x$$

where M is the metal and Ch the complexing agent. x is the number of moles of complexing agent bound per mole of metal ions.

The equilibrium constant has been formulated as

$$K = (\lambda o/\lambda - 1)/(Ch)^x$$

$$\text{or } \operatorname{Log}[(\lambda o/\lambda) - 1] = \operatorname{Log} K + x \operatorname{Log}(Ch)$$

λo = Distribution constant in absence of humic or fulvic acid.

λ = Distribution constant in presence of humic or fulvic acid.

K = The Equilibrium or the stability constant for the complex.

Thus plotting $\operatorname{Log}\left(\frac{\lambda o}{\lambda} - 1\right)$ against log (Ch), the values for log K and x can be easily ascertained from the intercept and slope respectively.

Himes and Barber (1957)[112] have obtained an estimate of stability of Zinc-Organic matter complex by addi~~ti~~ng radioactive Zinc (Zn^{65}) to a soil suspension. By treatment of the soils with peroxide, these workers have demonstrated that these complexing reactions are significantly associated with the chelating ability of the organic soil substances. Basu et al.[121] have indicated the formation of mixed type of bonds in the interactions of Cu^{++}, Ni^{++}, Zn^{++} and Mn^{++} with humic acids from the observation that 'adsorption isotherms' of humic acids for these metals are generally higher than the 'release isotherms'.

Lewis and Broadbent[121,111] have shown that all ~~the~~ ions of a certain metal adsorbed by humic acids are not bound with equal strength. This has also become evident from the investigations of Ennis and Brogan[124] which have shown that only the most strongly bound copper is virtually unavailable to oat plants.

Randhawa and Broadbent[125] have calculated the pH dependent stability constants for $Zn^{(II)}$- humic acid complexes. Log K values at pH 3.60 is 4.42, at pH 5.60 it is 6.18 and at pH 7.00, 6.80. These authors and others[128] consider the participation of both monovalent and divalent form of the ions in the complexing reaction. At lower pH values the monovalent form is predominant. It is thus concluded that more ligands are made available for complex formation as the pH of the solution is increased. Miller and Ohlrogge[128] have chosen the maximum complexing ability at a particular pH in the determination of ligand site concentration and in this way they have found the 'apparent' stability constant for the Zn-manure extract complex to be 7.80.

Schnitzer and Skinner[95,114,115] have investigated the complexing properties of different heavy metal ions with fulvic and humic acids. Adopting Schubert's ion exchange technique they have calculated the stability constants (Log K) for these metals with fulvic acid at pH's 3.50 and 5.00. The stabilities are in the order at pH 3.50 :

$$Cu^{++} > Fe^{++} > Ni^{++} > Pb^{++} > Co^{++} > Ca^{++} > Zn^{++} > Mn^{++} > Mg^{++}$$

and at pH 5.00 :

$$Cu^{++} > Pb^{++} > Ni^{++} > Mn^{++} > Co^{++} > Ca^{++} > Zn^{++} > Mg^{++}$$

The x values for these metals ranged between 0.50 to 1.50 at pH 3.50 and 0.79 to 2.00 at pH 5.00. Courpron[129] has obtained the values for log stability constants at pH 5 of zinc and copper-humic acid complexes to be 2.87 and 7.00 respectively. Matsuda and Ito[117] have attempted to find a correlation between degree of humification and stability constants. Log K of zinc-humic acid complexes are within the range 10.33 to 4.20 and that of zinc-fulvic acid complexes 9.30 to 3.88 at pH 7.00. These authors have found a positive correlation between the stability constants of zinc-humic acid complexes and RF values, but no such correlation has been observed for fulvic acids.

Schnitzer and Hansen[126] have reevaluated their earlier work by applying an improved analytical approach and are of the opinion that at an ionic strength of 0.10 and at pH 3.00 and 5.00 the values of 'x' are unity. At lower ionic strength the value of 'x' deviates from unity due to the formation of mixed polynuclear complexes.

Soil organic matter is obtained in the form of negatively charged polymeric materials whose principal functional groups are -COOH, phenolic-OH, = CO etc[21]. Considering this chemical configuration Clark and Turner[127] have suggested that the organic matters isolated from soils form complexes with metals in a way that the central group would be the organic molecule co-ordinated with more than one metal ion. They have, therefore, questioned the use of the ion exchange technique to such systems where polyfunctional macromolecular complexants are involved. Zunino et al.[130]

have described these complexes as M_aCh - type of complexes. In a later publication Zunino et al.[131] have suggested that the best way to express, interms of stability data, the ability of a polyfunctional macromolecule to complex metal ions would be to determine the overall constants K for the saturated complex M_aCh. Thus overall stability constants should be calculated experimentally by using increasing concentration of metal ions and a constant concentration of the complexant.

The modified ion exchange technique is described by Ardakani and Stevenson[132] and following this approach they have calculated the Log K values for zinc-humic acid system within the range of values 3.13 to 5.13. This method also suffers from some limitations and is only applicable to MCh- or MCh_b -type of complexes[130,131].

Job's method of continuous variation[133] has been applied by several investigators[75,19]. The method is based on variations of optical densities of solutions containing different ratios of metal ion and complexing agent, while simultaneously maintaining a constant total concentration of reactants.

Schnitzer and Skinner[75] have used Job's method in an attempt to determine the composition of complexes formed between fulvic acid and metal ions. Courpron[129] has also used this technique to determine the composition of fulvic acid and humic acid metal complexes. Schnitzer and Hansen (1970)[126] have employed Job's method to determine both composition and stability constants of fulvic acid metal complexes. Adhikari et al.(1972)[134] have tried to establish the composition of some fulvic acid-metal complexes using this technique.

MacCarthy and Mark[135] in a recent publication summarised the limitations for the applicability of the Job's method of continuous variation in the study of soil organic matter - metal complexes.

Cheam[136] has investigated the chelation reaction between Cu^{++} and fulvic acid employing a relatively direct approach, that of titration with a cupric ion - selective electrode. Cheam and Gamble[118] in a later publication have reported the conditional stability constant values of Mercury-, Cadmium-and Copper- ~~and~~ fulvate complexes using specific ion-selective electrodes.

Very recently Saha[47] has evaluated the stability constants for the interaction of metal ions with humic and hymatomelanic acid from polarographic measurements. In his studies he has utilised the idea of Tanford[138] which envisages that in a system where factors such as adsorption on mercury surface and viscosity do not affect the diffusion current, the ratio I_d/I_{d_o} (where I_d = diffusion current in presence of the complexant and I_{d_o} = diffusion current in absence of the complexant can be taken as a measure of the extent of binding of metal ions to the substrate. Total complexing sites in moles/litre for a particular metal ion and particular complexant are determined by measuring the maximum complexing ability for that metal with the particular substrate at a given pH.

Potentiometric titrations of humic and fulvic acids in presence of various metal ions have attracted a number of workers. In course of these investigations much insight has been gained into the nature and strength of bonds between metal ions and humic and fulvic acids obtained from different sources. Beckwith[139] has found that the order of decrease of pH upon addition of metal salts to soil organic matter suspension generally follows the Irving-Williams series (1948). He further states that the metal chelates are formed as co-ordination compounds with partly covalent character. Martin and Reeve[71] have suggested the possibility that protons titrable in presence of metal ions may also originate from the hydrated metal ions in addition to the organic acids.

Khanna and Stevenson[73] have observed that the magnitude of the pH drop upon addition of metals of the 1st transition series follows the order $Mn^{++} < Co^{++} < Ni^{++} \ll Cu^{++} > Zn^{++}$ which agrees with the Irving-Williams[2] stability series. Further, these authors[73] state that the slight lowering of the pH for the organic matter isolates examined in the study may result from the shift in the ionization equilibrium of weak acid groups, rather than through release of protons from groups which normally do not ionise, e.g., amino, alcoholic hydroxyl, etc.. Khan[141] has indicated the formation of metal-humic acid complexes from potentiometric titrations of these acids in presence of different heavy metal ions. In his experiment, the order of magnitude of pH drop on addition of metals is

$Mn^{2+} < Co^{2+} < Ni^{2+} < Zn^{2+} < Cu^{2+} < Al^{3+} < Fe^{3+}$.

According to Van Dijk[108] at pH 5 there is no large difference in bond strength for the divalent ions Ba^{++}, Ca^{++}, Mg^{++}, Mn^{++}, Co^{++}, Ni^{++}, Fe^{++} and Zn^{++} (slightly increasing in this order). Pb^{++}, Cu^{++} and Fe^{++} ions are, however, more firmly bound. To have an idea of the magnitude of the stability of the metal - humate binding under certain conditions, Van Dijk[108] has carried out 'competition' experiments for copper between humic acids and a number of chelating agents of which the stability constants with copper ions are known. The logarithm of the 'conditional' stability constant at pH 6 is about 7 for humic acids in his experiments.

Stevenson et al.(1973)[116] and Stevenson (1976)[78] have determined stability constants for Lead and Copper complexes with humic and fulvic acids from potentiometric measurements. In these papers the number of titrable functional groups have been used to represent the concentration of humic or fulvic acid as complexant. Bjerrum's (1941)[148] approach as modified by Gregor et al.(1955)[143] is adopted for the determination of formation constants for the metal organic matter complexes.

In a later publication Stevenson (1977)[113] has adopted a modified technique in which he has calculated pH dependant stability constant data (Log K), from the Bjerrum's formation constant B_2 , for some divalent heavy metal complexes with humic acids.

Recently Takamatsu and Yoshida (1978)[149] have proposed a relatively direct approach for the determination of apparent stability constants (successive stability constants and overall stability constants). The technique is based on the combined use of potentiometric titration and Ion-selective electrodes. In this study the order of the values of stability constants is, $Cu^{++} > Pb^{++} > Cd^{++}$. The values of the stability constants as obtained from potentiometric measurements are in general much greater than those estimated by other methods, viz., Job's method or Ion-exchange Equilibrium method.

Several methods are proposed for the determination of stability constant data for the metal organic matter complexes and many attempts are made to find some correlation between these stability data with those of other factors, such as degree of humification[117,149], participation of groups in forming chelates (-COOH, Phenolic -OH, alcoholic -OH, $-NH_2$, = NH or other groups)[107,113] in the complexes, etc., However, none of a single method seems to be unequivocal.