INTERACTIONS BETWEEN METAL IONS AND HUMIC FRACTIONS EXTRACTED FROM AN ALFISOL AND A VERTISOL IN RELATION TO THEIR PLANT AVAILABILITY

By

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This is to certify that the thesis entitled **INTERACTIONS BETWEEN METAL IONS AND HUMIC FRACTIONS EXTRACTED FROM AN ALFISOL AND A VERTISOL IN RELATION TO THEIR PLANT AVAILABILITY** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N.G.Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Ms. M. SAILAJA** under my guidance and supervision. The subject of the thesis has been approved by the student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, **Ms. M. SAILAJA** hereby declare that the thesis entitled **INTERACTIONS BETWEEN METAL IONS AND HUMIC FRACTIONS EXTRACTED FROM AN ALFISOL AND A VERTISOL IN RELATION TO THEIR PLANT AVAILABILITY** submitted to Acharya N.G.Ranga Agricultural University for the degree of **MASTER OF SCIENCE IN AGRICULTURE** is the result of the original work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

Studies on **"Interactions between metal ions and humic fractions from an Alfisol and a Vertisol in relation to their plant availability"** were conducted with a view to characterise the nature of organic matter, the complexes formed by interaction of humic fractions with Cu(II), Zn(II), Fe(II) and Mn(II) and to evaluate the relative effectiveness of zinc carriers in improving zinc nutrition of rice and maize crops. The organic carbon mapping of soils of fields belonging to research farms under Directorate of Rice Research and National Research Centre for Sorghum was also taken up and the soils were characterised into low, medium and high categories.

Vertisol had higher amount of organic matter, total nitrogen and available nitrogen than that of the Alfisol. In both soils these values decreased with depth. C/N ratio was high in Vertisol than in Alfisol and these values were found to increase with depth.

The contents of total acidity, carboxyl and phenolic-OH groups were higher in humic acid obtained from Vertisol as compared to humic acid obtained from Alfisol. The total acidity and carboxyl group contents increased with depth while the phenolic-OH groups decreased with depth.

Potentiometric titration curves of humic acids extracted from an Alfisol and a Vertisol were similar irrespective of the sources from which they were extracted and the curve obtained was sigmoidal in nature. The UV spectra of humic acids were featureless showing a decreasing optical density with an increasing wavelength.

Potentiometric titration curves of metal humates showed no inflection indicating that formation of metal hydroxides was suppressed when metal ions were added to humic acid to form the complex. Reduction in pH on titration of humic acids in presence of various metal ions followed the order: Cu(II) > Zn(II) > Fe(II) > Mn(II) at pH 7.5 and Zn(II) > Cu(II) at above pH 7.8. Job's plot indicated that formation of 1.5:1 HA-metal complexes for Cu(II), Fe(II), Zn(II) and Mn(II).

Stability constants of humic acids with Cu(II), Fe(II), Zn(II) and Mn(II) were determined at pH 7.0 and 30°C by Schubert's ion exchange method. The stabilities of metal complexes followed the order: Cu(II) > Fe(II) > Zn(II) > Mn(II). The negative ΔGr^0 values indicated that the reactions were spontaneous.

Among the zinc carriers, Zn chelate and zinc humate were evaluated to see the effect of this application on zinc nutrition of rice (Tellahamsa) and maize (DHM-105) and were compared with $ZnSO_4$ at 2.5 and 5.0 ppm levels of applied Zn. The dose of 2.5 ppm zinc was found to be optimum for rice and maize crops grown under pot culture conditions. The effectiveness of different zinc sources followed the order: $ZnSO_4$ > Zn-humate > Zn-chelate.

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SYMBOLS AND ABBREVIATIONS

ANGRAU	:	Acharya N.G. Ranga Agricultural University
ARI	:	Agricultural Research Institute
Available N	:	Available Nitrogen
$Ba(OH)_2$	:	Barium hydroxide
Be	:	Beryllium
Ca	:	Calcium
$Ca(OAC)_2$	:	Calcium acetate
Cd	:	Cadmium
CD	:	Critical Difference
CEC	:	Cation Exchange Capacity
CH_3COOH	:	Acetic acid
cm	:	centimeter
CO_2	:	carbondioxide
conc.	:	concentrated
COOH	:	Carboxyl
Cu	:	Copper
^0C	:	degree celsius
DRR	:	Directorate of Rice Research
dSm^{-1}	:	decisiemens per meter
DTPA	:	Diethylene triamine pentaacetic acid
FA	:	Fulvic acid
Fe	:	Iron
Fig.	:	Figure
g	:	gram
$gPot^{-1}$	:	gram per pot
h	:	hours

H_2O	:	Water
H_2SO_4	:	Sulphuric acid
ha	:	hectare
HA	:	humic acid
HCl	:	Hydrochloric acid
$HClO_4$	:	Perchloric acid
HF	:	Hydrofloric acid
HNO_3	:	Nitric acid
i.e.,	:	which is to say in other words
ICAR	:	Indian Council of Agricultural Research
IR	:	Infrared
K	:	Potassium
KCl	:	Potassium chloride
Kg	:	Kilo gram
m	:	meter
me g^{-1}	:	milli equivalents per gram
Mg	:	Magnesium
mL	:	milli litre
ML^{-1}	:	Moles per litre
mm	:	millimeter
Mn	:	Manganese
MOP	:	Mureate of potash
NaOH	:	Sodium hydroxide
Ni	:	Nickel
nm	:	nanometers
NRCS	:	National Research Centre for Sorghum
OH	:	hydroxyl
Pb	:	Lead

ppm	parts per million
%	per cent
rpm	revolutions per minute
Std.	standard
S.Ed.	Standard error deviation
SSP	Single super phosphate
Total N	Total Nitrogen
TV	Titre value
μgg^{-1}	microgram per gram
µmoles	micromoles
UV	Ultra Violet
VIS	visible
vs	versus
wt.	weight
Zn	Zinc
$ZnSO_4$	Zinc sulphate

INTRODUCTION

CHAPTER I

INTRODUCTION

Soil organic matter consists of a complex system of substances, ranging from components of organic residues undergoing decomposition, metabolic products of microbes, products of secondary synthesis and humic substances.Soil organic matter serves as soil conditioner, nutrient source, substrate for microbial activity, preserver of the environment and major determinant for sustaining and increasing agricultural productivity. The quantity and quality of soil organic matter vary with the conditions of soil formation. In general, the soils of tropical or subtropical regions are low in organic matter. Deforestation and cultivation also brought about a lot of changes in quality and quantity of soil organic matter.

The humic fractions of soil are highly reactive polymers, due to high content of oxygen containing functional groups like carboxylic, phenolic-OH, enolic-OH, aliphatic and carbonyl groups.

Soil organic matter fractions are capable of forming complexes with metal ions (Hodgson, 1963). These complexes are of prime importance in soil formation and plant nutrition (Stevenson, 1982). During complexation, numerous compounds including Humic Acid (HA) and Fulvic Acid (FA) are involved, which control the distribution and supply of micronutrients to plant roots and interact with metal ions through their functional groups to form metal complexes of varying stabilities. A conclusive evidence for the complexation of micronutrient metal ions with HA and FA has been demonstrated by several workers employing chemical methods and IR spectroscopy (Schnitzer and Hansen, 1970; Chakravarthy *et al.*, 1984; Kipton *et al.*, 1991; Stevenson *et al.*, 1993; Sujana Reddy *et al.*, 1998). The formation and stability of various complexes were extensively studied by Rajkumar *et al.* (1997).

The Farm of Directorate of Rice Research has 16 ha of land under cultivation. On this farm, rice is grown under submerged conditions. The National Research Centre for Sorghum has 41.9 ha of land under cultivation of sorghum crop. These two farms have witnessed lot of changes due to intensive cultivation practices adopted during the past 4-5 decades. Hence, it was thought desirable to prepare organic carbon maps of these two farms to indicate changes in contents of their organic matter. The available information on complexation behaviour of humic fraction extracted from an Alfisol and Vertisol of ANGRAU, Rajendranagar campus with metal ions and its availability to crop plants is scanty and meagre. Hence, the present investigation entitled **"Interactions between metal ions and humic fractions extracted from an Alfisol and a Vertisol in relation to their plant availability"** was carried out with the following objectives:

1. To prepare the maps of soil organic carbon status of the research farms of Directorate of Rice Research (DRR) and National Research Centre for Sorghum (NRCS) located at the main campus of Acharya N.G. Ranga Agricultural University, Rajendranagar,

2. To characterise the humic acid with respect to oxygen containing functional groups and spectral properties,

3. To characterise the metal complexes by potentio-metric, conductometric and spectrophotometric methods,

4. To study the behaviour of complexes formed with metals [Zn(II), Cu(II), Fe(II), Mn(II)] and to determine their stabilities, and

5. To evaluate the relative effectiveness of metal complexes in nutrition of rice and maize crops grown on an Alfisol under pot culture conditions.

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REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Soil organic matter influences plant growth through its effects on physical, chemical and biological properties of soils (Stevenson, 1982). It has a physical function in that it promotes good soil structure, thereby improving tilth, aeration and moisture movement and retention. Its chemical function is manifested by its ability to interact with metals, metal oxides and hydroxides and clay minerals to form metal-organic complexes and act as ion exchanger and store house of N, P and S (Prakash and MacGregor, 1983). Soil organic matter has been reported to stimulate various physiological and biochemical processes associated with the cellular metabolism and to attenuate the toxicity of heavy metals and toxic organics (Schnitzer, 1991).

The present review is aimed to include those aspects of studies on soil organic matter which are most relevant to the problem studied here under the following heads.

2.1 COMPOSITION OF SOIL HUMUS

Soil organic matter consists of a mixture of plant and animal residues in various stages of decomposition and is divided into groups viz., humic substances and non humic substances (Schnitzer and Khan, 1972). Non humic substances include carbohydrates, proteins, peptides, amino acids, nucleic acids, purines, pyrimidines, fatty acids, waxes, resins, pigments and other low molecular weight organic substances. The bulk of soil organic matter consists of humic substances. These are amorphous, dark-coloured, partly aromatic, mainly hydrophilic, chemically complex, polyelectrolyte-like materials, which range in molecular weight from a few hundred to several thousand (Schnitzer and Khan, 1972). Based on solubilities, the humic substances are broadly divided into three classes.

(a) humic acid (alkali soluble and acid insoluble)
(b) fulvic acid (acid and alkali soluble) and
(c) humin (insoluble in both acid and alkali)

Chaudhury and Saha (1973) studied the distribution and nature of organic matter among profiles of some soils of West Bengal and reported remarkable variations in the organic matter and total nitrogen contents of top (0-15 cm) layers; the organic matter and total N contents being more in top layer than the lower (15-30 cm) layer.

Banerjee and Chakraborthy (1977) studied the nature, distribution and composition of organic matter in surface soils of West Bengal that a moderate moisture regime, slightly acidic to neutral reaction and fairly intensive microbiological activity were the main factors responsible for formation of humic acid while high moisture regimes, alkaline reaction and microbiological activity resulted in the formation of fulvic acid.

Tomar *et al.* (1986) characterised the organic matter present in soils of widely different climatic zones and observed that humus content was highest in pine forest Ustifluvent of morrin hills and lowest in Tripura under maize-jute rotation.

Nagamadhuri *et al.* (1998) studied the status and changes in organic matter under natural vegetation and continuous cultivation in soils of Acharya N.G. Ranga Agricultural University, Rajendranagar campus, Hyderabad and observed that the soils under continuous cultivation contained less amount of organic carbon and organic matter. This trend was observed both in surface and sub-surface horizons. The sub surface layer had a lower contents of organic carbon and organic matter. Similar results were reported by Sujana Reddy (1997).

2.2 EXTRACTION, FRACTIONATION AND CHARACTERISATION OF HUMIC SUBSTANCES

The humic substances formed during humification consist of a mixture of various compounds. As these compounds have similar properties, the extraction of substances with uniform composition is difficult. It is also possible that organic matter is held to cation exchange sites on clay mineral and colloidal sesquioxides. A pretreatment with HCl and/or HCl+HF is applied for desorption of humic substances from sesquioxides, free silicic acid, clays and for removal of carbonates, exchangeable bases followed by leaching with water until complete decalcification leads to increased solubilities in alkali and increases extraction efficiency (Stevenson, 1982).

Based on the differences in solubilities in aqueous solution, humic substances are being fractionated. The isolation of specific organic fractions has been described in detail by Kononova (1966), Schnitzer and Khan (1972)and Stevenson (1982). The exhaustive extraction of organic soil constituent followed by acid treatment to separate the extract is acid soluble fraction (fulvic acid) and the acid insoluble fraction (humic acid). Humic acids are purified by dialysis.

2.2.1 Functional group content of humic fractions and spectral properties

The suitability of the $Ca(OAc)_2$ and $Ba(OH)_2$. methods for the determination of carboxyl and of total acidity was compared by Schnitzer and Gupta (1965). Their results showed that $Ca(OAc)_2$ method was more suitable for the analysis of COOH groups. The $Ba(OH)_2$ method was found to react with acidic hydrogens in both aliphatic and aromatic compounds and was not specific for phenolic OH groups.

Schnitzer and Skinner (1965) found that blocking of either carboxyls (or) phenolic hydroxyls caused significant reductions in metal retention.

Separation and functional group analysis of soil organic matter was carried out by Leenheer and Moe (1969) and they reported that Romney organic matter was characterised by its high carboxyl and carbonyl contents. Whereas Zanvesville organic matter exhibited a high aliphatic hydroxyl groups. This comparison of functional groups composition indicates a greater state of humification for the Romney organic matter.

Characterisation of humic and fulvic acids extracted from different Indian soils was attempted by Nand Ram and Raman (1981) and their results indicated that humic acids contained more carbon and less oxygen than fulvic acid. Fulvic acids showed higher O/H values but lower C/H values than humic acids. Fulvic acids were more acidic than humic acids. Carboxyl groups were more in fulvic acids as compared to humic acids. From the study of molecular formulae, it was evident that the cultivated soils show a much lower carbon and hydrogen contents than the corresponding forested soils.

The functional group composition of humic and fulvic acids isolated from forest and cultivated soils was studied by Dkhar *et al.* (1986). Their data showed that the contents of total acidity, carboxyl and phenolic-OH of fulvic acids were more than those of humic acids. There was higher content of total acidity and carboxyl groups in the forest surface soils than that of cultivated surface soils. The contents of total acidity and carboxyl groups were higher in humic acid (or) fulvic acids extracted from cultivated sub-surface samples than from those of forest sub-surface soils. Similarly humic and fulvic acids of well drained forest and cultivated soils possessed higher contents of total acidity and functional groups in comparison with their poorly drained counter parts.

Piccolo and Camici (1990) compared two methods of total acidity determination of humic substances viz., the $Ba(OH)_2$ method and a direct potentiometric titration after elution of humic substances through a cation exchange resin. Their results showed that $Ba(OH)_2$ method was more accurate than direct potentiometric titration method.

Sujana Reddy (1997) observed that the contents of oxygen containing functional groups i.e., carboxyl, phenolic-OH and total acidity were higher in soils under natural vegetation than in soils occurring under continuous cultivation at Acharya N.G. Ranga Agricultural University, Rajendranagar campus in the same type. Total acidity and carboxyl groups were higher in sub-surface layer than in surface layers, while phenolic-OH groups of subsurface layers were less in both the soils. Similar results were reported by Nagamadhuri *et al.* (1998).

The partition coefficients (Kd) and E_4/E_6 ratios of fulvic and humic acid fractions were studied by Prasad and Sinha (1982). Their results indicated a highly significant relationship between the values of Kd and E_4/E_6 ratio. The E_4/E_6 ratio showed inverse relationship with molecular weight of fulvic acid fraction.

According to Dkhar *et al.* (1986), the IR spectrum of fulvic acids differed from those of humic acid mainly in 1700 cm^{-1} and in the region of 1200 cm^{-1}. IR spectra and E_4/E_6 ratio indicated that humic and fulvic acids were more aromatic in nature in cultivated soils than those of forest soil.

Udupa *et al.* (1990) observed that the E_4/E_6 i.e., extinction ratio of humic acids was largely governed by total carbon, exchangeable acidity, phenolic-OH and total iron contents. The ratio increased markedly in the sub soil compared to top soil.

The humic and fulvic acids extracted from some soils belonging to Inceptisol and Alfisol of Assam were characterised by E_4/E_6 ratio, UV and IR spectroscopy (Sarmah and Bordoloi, 1993). The UV spectra of humic acid showed high adsorption than fulvic acids. The IR-spectra of humic acids differed from fulvic acids mainly in respect of a medium adsorption band at 1725 cm^{-1} and absence at 1540 cm^{-1} band and a band near 1025 cm^{-1}.

The humic acids extracted from Alfisol and Vertisol were characterised by spectral properties (Nagamadhuri, 1996; Sujana Reddy, 1997). The optical densities of humic acids obtained from sub-surface layer were higher than those obtained from surface layers. This could be due to the fact that humic acid of sub-surface layer possesses higher degree of aromaticity in the carbon atoms than those occurring in the top layers. The UV spectra of humic acid were featureless with a decrease in optical density with an increase in wave length.

2.3 ORGANIC MATTER REACTIONS WITH METAL IONS

The role of organic matter in relation with metal ions has been a subject of wide interest. Basically four ways have been used to assess the contribution of organic matter to the chemistry of metals in soils.

1. Association of organic matter content with the distribution and availability of metals in soils
2. The effect of organic matter removal on the reactivity of soils with metals
3. A direct assessment to assess the amount of element present in organic form, and
4. Characterization of organic matter and its reaction sites.

Organic matter contributes significantly towards CEC of many soils. Because the functional groups indicate the weak acids and their configuration offer an opportunity to chelate, these groups bind heavy metals very strongly (Hodgson, 1963).

Humic substances tend to form both the soluble and insoluble complexes with polyvalent cations depending upon the degree of saturation. Because of their high acidity and relatively low molecular weight, the metal complexes of fulvic acid are more soluble than those of humic acids (Stevenson, 1982). Natural complexing agents are of considerable importance in weathering process and movement of nutrients in the soil. It was also reported that the association of polyvalent cations with humic substances might completely modify the characteristic movement of HA (Juste and Delas, 1970).

Most of the metals have been shown related to organic matter distribution in many soils. Little is known about the nature of ligands in polymeric compounds of soil organic matter, which chelate with metals. However, carboxylic, phenolic and carbonyl groups are important in chelating abilities. Schnitzer and Skinner (1963) noticed that in the lower complexes, iron occurred as $Fe(OH)^{+2}$ and in the high complex as $Fe(OH)^{+}$. Aluminium occurs as $Al(OH)^{+}$ and Al(OH)(II).

Reactions of zinc with model compounds and humic acid were studied by Randhawa and Broadbent (1965). They reported that complexing of zinc with humic acid was very strong. Normal KCl and 0.01 N acetic acid were approximately equal in their ability to displace zinc adsorbed on humic acid. They also concluded that zinc was more strongly bound on humic acid than calcium but was less strongly held than copper (or) ferrous ion. The complexation of zinc with humic acid varied with pH. At pH 7.0; 70 per cent of the zinc was retained by humic acid as divalent species and at pH 3.6, 75 per cent of the zinc was monovalent.

Meisel *et al.* (1978) observed that the CEC of low land peat humic acid depended on the degree of polymerisation of humic acid. The interactions between humic acid and metal ion involved not only physical adsorption but also chemical (or) ionic (or) chelate bonds. The lower molecular weight fractions of humic acid show maximum metal binding capacity and organo metallic complexes have the residual acidity because all the functional groups are not involved in complexation (Banerjee and Mukherjee, 1972).

The interaction of metal ions with humic substances under sterile conditions and in the absence of non-humic substances was studied by Ghosh *et al.* (1983) and they concluded that apart from the simultaneous formation of well known chelates, humic substances are independently able to change the valence state of metal ions.

Keefer *et al.* (1984) observed that the amounts of metal associated with the hydrophobic and hydrophillic fractions depended on the metal examined, extractant used, the pretreatment and the kind of sludge added to the soil. Cadmium was not detected in any of the organic fractions. Cu(II), Zn(II) and Ni(II) were generally recovered least efficiently from the soil treated with a sludge, which is rich in the metals.

The experiment was prompted by Hargrove (1986) to show that A_l organic matter complexes may be solubilized in the pH range of 5 to 7 and thereby become available to plant roots and account for Al-toxicity effects at neutral pH values.

2.4 CHARACTERISATION OF METAL COMPLEXES

Potentiometric titrations, absorption spectroscopic methods and stability constants have been widely used for characterisation of metal complexes formed with humic substances.

2.4.1 Potentiometric titrations

The magnitude of pH drop on addition of metal ion to an aqueous solution of humic acid and fulvic acid may be taken as an indication of complex formation. The order of magnitude of pH drop on addition of metal by first transition series was reported by Khanna and Stevenson (1962) and followed the order Mn(II) < Co(II) < Ni(II) < Cu(II) < Zn(II) (pH less than 7.5). They assumed that the pH drop was due to ionisation of weakly acidic groups and also possibly due to the release of protons from functional groups, which are not ionised.

The titration curves of Schnitzer and Skinner (1963) and complexing properties of organic matter with Fe(III), Al(III), Co(III), Mg(II), Cu(II) and

Ni(II) showed the formation of metal hydroxides by distinct inflection point but the absence of such inflection in the presence of organic matter suggested the index of complex formation.

Trikha *et al.* (1966) observed the dissociation constant of phenanthrenequinone monoxime and formation constants of some of its metal complexes, which were determined potentiometrically in 75 per cent dioxene at constant ionic strength (0.1 M). The order of stability of the complexes was Cu(II) > Ni(II) > Be(II) > Pb(II) > Zn(II) > Cd(II) > Mn(II) > Mg(II).

The interactions between metal ions and fulvic acid of an alluvial soil from West Bengal were studied by absorption spectroscopy and electrometric titrations (Adhikari *et al.*, 1972). The conductometric studies showed one type of complex with trivalent metal ions viz., Fe(III) and Al(III) and two to three different types of complexes with bivalent metal ions viz., Cu(II), Zn(II) and Ni(II). Potentiometric titrations showed a formation of hydroxylated fulvic acid metal complex. The stabilities of these complexes followed the order Fe(III) < Al(III) for trivalent ions and Ni(II) < Zn(II) < Cu(II) at pH < 7.0 and Cu(II) < Ni(II) at pH 8 for bivalent ions.

Banerjee and Mukherjee (1972) reported that stability of the humic acid and fulvic acid metal complexes deduced from the potentiometric titraton curves of the acids in presence of metal salt in the order of Cu(II) > Fe(II) > Zn(II) > Co(II) > Mn(II). The decrease in intensity of fluorescence excitation spectra confirmed the formation of complexes, which were more or less nonionic.

The isolation and characterisation of stable metal-organic complexes was studied by Griffith and Schnitzer (1975) on samples collected from tropical volcanic soils. They found that 22.8 and 70.4 per cent of soil organic carbon occurred in purified complexes. The most prominent metal in all complexes was Al, which constituted between 3.45 and 7.17 per cent of the air-dry weights. The Al(III) and Fe(III) in the metal-organic complexes appeared to be present as $Al(OH)^{+2}$ and $Fe(OH)^{+2}$ bounded to negatively charged carboxyl and phenolic hydroxyl groups on the ligands.

Shah *et al.* (1976) carried out potentiometric titration of humic acid with Al(III), Fe(III), Cu(II), Zn(II), Ni(II), Co(II), Mn(II) and K(I) and noticed greatest drop in pH with Al(III) and Fe(III). In the modified potentiometric method, Stevenson (1977) observed that at pH values below the point of oxide hydrate formation and the drop in pH resulted in release of proton from participating reactive groups, while at high pH value and higher metal to humic acid ratio, additional proton was released from hydration water of metal held in 1:1 complex.

The nature of binding of divalent metal ions by modified potentiometric titration procedure involves sequential addition of metal ion to solution of humic acid at a constant pH. At pH values below the point of complex hydrate formation, the drop in pH resulted from participating reactive protons and at high pH values the negligible sites were involved in complex formation (Stevenson, 1977).

The characteristics of water-soluble fulvic acid- copper and fulvic acid - iron complexes were studied by Schnitzer and Ghosh (1981) by chemical, spectroscopic X-ray and thermal analysis methods. They found that substantial portions of the metals in the complexes formed inner sphere complexes with the fulvic acid. From X-ray diffraction pattern, it appeared that metals brought fulvic acid molecules closer together by bridging adjacent fulvic acid molecules. The major functional groups involved in complexing are -COOH and phenolic -OH groups.

Chakravarthi *et al.* (1984) studied the formation of complexes of Cu(II), Pb(II), Zn(II), Co(II), Ni(II) and Cd(II) humic acid at pH 4.0 by potentiometric investigations and observed that they followed the order. Cu(II) > Pb(II) > Ni(II) > Cd(II) > Zn(II). This is because at high pH, extra protons are released either through dissociation of hydration water or from very weak acidic functions not-titrable within the neutralisation of humic acid.

Kipton *et al.* (1991) studied the interaction of humic substances with stable hydrophobic copper (II), complexes $Cu(PAN)_2$ and $Cu(Oxine)_2$ at pH 8.2 by DC-anodic stripping voltametric measurements. They found that the apparent stability of the Cu(II) complexes decreased in the presence of both humic substances. It was greater for humic acid than fulvic acid i.e., 2.8×10^{-7} Mol L^{-1} $Cu(oxine)_2$ at 10 $\mu g/mL^{-1}$ HA or 50 $\mu g\ mL^{-1}$ FA.

The thermodynamic adsorption of Fe(III) - fulvic acid complexes was studied by Pandeya (1991) by fitting into Langmuir equation. These complexes indicated that the interaction or adsorption involves physical and metal ion co-ordinate bonding on clay surfaces. The activation energy was an inverse function of the amount of absorbate adsorbed by the soils.

Sujana Reddy *et al.* (1998) characterized the metal-humic complexes Cu(II), Zn(II), Mn(II) and Ca(II) by potentiometric titration. They observed that the titration curves of metal humates showed no inflection indicating the formation of metal hydroxides and it was suppressed when metal ions are added to humic acid indicating the formation of a complex. The reduction in pH on titration of humic acid in presence of various metal ions followed the order: Cu(II) > Zn(II) > Mn(II) > Ca(II) at a pH 7.5 and Zn(II) > Cu(II) above 7.8.

2.4.2 Spectroscopic methods

This method was developed by Job (1928) based on variations of optical densities of solutions containing different ratios of metal ions and complexing agents while simultaneously maintaining a constant total concentration of reactants and has been used by soil scientists for studying complex formation between humic substances and metal ions.

Schnitzer and Skinner (1963) used Job's plot for complexes between humic substances and Cu(II), Fe(III) and Al(III) and reported a molar ratio of 1:1 at pH 3.0 while at pH 5.0, Cu(II) and Fe(III) formed 2:1 complexes. Gamble *et al.* (1970) carried out a type of Job's experiment on Cu-fulvic acid system by plotting the concentration of Cu complex vs concentrations of fulvic acid obtained a "blunt maxima" indicating a molar ratio of 1:1 of Cu(II) to binding sites and reported that their results were consistant with the conclusions of Schnitzer and Skinner (1963) and Schnitzer and Hansen (1970). Adhikari *et al.* (1972) reported that fulvic acid formed complexes at pH 4.0 to follow a composition of 1:1 for Fe(III), 0.33:1 for Cu(II) and 0.72:1 for Ni(II).

Stevenson *et al.* (1993) used a continuous distribution model based on the Scatchard plot for complexes between humic acid and Cu(II) and indicated the formation of 1:1 and 2:1 complexes (i.e., CuL^{-1} and CuL^{-2}, where 'L' is the reactive site of the macro molecule) at pH 4.0.

Sujana Reddy (1997) applied Job's plot for complexes between humic substances and Cu(II), Zn(II), Mn(II) and Ca(II). She reported that maximum absorbance occurred at X=0.6 for Cu(II), Zn(II), Mn(II) and Ca(II). She also concluded that humic acid formed 3:2 molar complexes with Zn(II), Mn(II), Cu(II) and Ca(II).

2.5 STABILITY CONSTANTS OF METAL-HUMIC ACID INTERACTIONS

Stability constant provides a quantitative measure of the ability of metal ion for complexing with the functional groups of humic substances. The ion exchange equilibrium method originally developed by Schubert (1948) was first applied to water soluble soil organic matter complexes by Miller and Ohlrogge (1958), Randhawa and Broadbent (1965) and Schnitzer and Hansen (1970).

Clark and Turner (1969) examined the resin exchange method for determination of metal-HA complexes. These two special cases indicated that a plot of log (λ_o/λ-1) vs log K_{et} (where λ & λ_0 are distribution constants of metal ion in the presence and absence of HA, K_{ct} is the total concentration of complexing agent). The curve is essentially a straight line even though the complex formed was non monomolecular with respect to control metal ion. The slope of plot varies from one to a negative value. The ion exchange method has been studied by many workers (Clark and Turner, 1969; Ardakani and Stevenson, 1972; Stevenson, 1976; and Nand Ram and Raman, 1981).

Randhawa and Broadbent (1965a) studied the stability constants of zinc-humic acid complexes at different pH values and reported that the species of zinc complexed by the humic acid was found to vary with pH. Total zinc retained increased with increasing pH to a maximum at about 8.5 but declined at higher pH values. Stability constants for the zinc-humic acid complex were found to be 4.42 at pH 3.6, 6.18 at pH 5.6 and 6.8 at pH 7.0.

Stability constants of Pb(II), Ni(II), Mn(II), Co(II), Ca(II) and Mg(II)-Fulvic acid complexes were studied by Schnitzer and Skinner (1967) and according to them, stabilities of complexes formed between fulvic acid and metal ions following the order: Ni(II) > Pb(II) > Co(II) > Ca(II) > Mn(II) > Mg(II) at pH 3.5. At pH 5.0, the order changed to Cu(II) > Pb(II) > Ni(II) > Mn(II) > Co(II) > Ca(II) > Mg(II).

Ardakani and Stevenson (1972) used the modified ion-exchange technique for the determination of stability constants of metal-soil organic matter complexes and observed that apparent stability constants (log K) of Zn(II) - humic acid complexes ranged from 3.13 to 5.13 at pH 6.5.

Stability constants of Cu(II), Pb(II) and Cd(II) complexes with humic acids from three diverse sources were studied by Stevenson (1976) and reported that complexes of Cu(II) and Pb(II) were more stable than those of Cd(II). Log K_2 values for the three humic acids were 8.9 for Cu(II), 8.7 for Pb(II) and 6.9 for Cd(II). Differences between humic acids in their ability to bind metal ions were slight. At least two major sites were involved in the binding of metal ions.

A study was conducted by Bresnahan *et al.* (1978) to determine the stability constants for the complexation of copper(II) ions with water and soil fulvic acids. Stability constants were 1×10^6 and 8×10^3 for soil and water fulvic acids, respectively. In both cases, total number of binding sites increased from pH 4.0 to 6.0. Most of the increase occurred between pH 5.0 and 6.0.

Takamatsu and Yoshida (1978) determined the stability constants of metal-humic acid complexes by potentiometric titration and ion-selective electrode. Stability constants increased with an increase in pH and the order of values obtained was Cu(II) > Pb(II) > Cd(II). The overall stability constants increased with increasing humification.

Challa and Raman (1984) carried out stability studies under different pH and thermal conditions. The humic and fulvic acid metal complexes showed little variation in stability with pH or temperature. Humic acid complexes showed higher degree of stability than fulvic acid. The order of stability of the humic acid complexes was Cu(II) > Zn(II) > Ca(II) > Mg(II) and Ca(II) > Zn(II) > Mg(II) in case of fulvic acid metal complexes.

Relan *et al.* (1986) studied the stability constants of Cu(II), Pb(II), Zn(II), Mn(II), Fe(II) and Cd(II) complexes with humic acid from farm yard manure by potentiometric titration. The values of stability constants (log K_2) between bivalent metal cations and humic acid were Pb-HA: 7.19, Fe-HA: 7.39, Zn-HA: 7.44, Cu-HA: 7.99, Cd-HA: 7.18 and Mn-HA: 6.36. The order of stability was Cu > Zn > Fe > Pb ~ Cd > Mn.

Free energy change during complexation of metals with humic and fulvic acids was studied by Nand Ram and Raman (1988) and they reported that the negative values of standard free energy change (ΔGr^0) suggested that the complexation reaction between metal ions Ca(II), Mn(II), Zn(II) and Cu(II) and humic (HA) and fulvic acids (FA) is spontaneous. Relatively, higher negative values of ΔGr^0 in case of Cu(II) showed the higher thermodynamic stability of Cu-HA and Cu-FA complexes formed. Computation of standard entropy change (ΔSr^0) revealed higher stability of Cu-HA complexes owing to the formation of comparatively stronger bonds and more ordered atomic arrangement in the complex with least steric hindrance.

Formation constants of some micronutrients and heavy metal complexes with fulvic acid from Farm yard manure were studied by Relan *et al.* (1990). Formation constants showed the order of Cu-FA (9.58) > Fe-FA (9.30) > Zn-FA (9.08) > Mn-FA (9.05) > Pb-FA (9.03) > Cd-FA (8.72) and they did not follow the order of stability of the Irving-William stability series.

Stevenson and Chen (1991) applied modified potentiometric titration for stability constants of copper(II) - Humate complexes and reported that the binding affinity of some humates for Cu(II) followed the order: Soil humic acid (SHA) > Peat humic acid (PHA) > lignite humic acid (LHA) > soil fulvic acid (SFA) ~ fungal melanin. Intrinsic stability constants (log K_{int}) at pH 4 and an ionic strength of 0.01 M for the five humate preparations were 8.3, 7.9, 7.4, 7.2 and 6.9, while at pH 5 the values were 8.5, 8.5, 7.9, 7.6 and 7.6, respectively.

Cheng-Fang Lin *et al.* (1993) used the Real-time full spectra fluorescence spectrophotometry for stability constant of dissolved organic matter and stated that use of real-time full spectra fluorescence spectrophotometer provides simultaneous observations on rayleigh scattering and full spectral intensity.

Manunza *et al.* (1995) applied bimodal Gaussian distribution to the study of the potentiometric titration data of metal-humate complexes. Its application to the potentiometric titration data showed that different classes of sites were involved in the binding of the metal ions. The affinity to the humic matrix followed the order: Cu(II) > Pb(II) > Mn(II) >> Cd(II). Phenolic groups played a relevant role in Cu binding while Cd appeared to bind exclusively to carboxylic groups.

Rajkumar *et al.* (1997) studied the complexation of trace metals with humic acids from soil, sediment and sewage by Scatchard plots at different pH values and ionic strengths. According to them, stabilities of metal humic acid complexes followed the order: Cu-HA > Pb-HA > Cd-HA for humic acids from any single source and increased with a rise in pH and fall in ionic strength of the medium. The humic acids extracted from the soil and the sediment were stronger complexing agents compared to that was extracted from sewage.

2.6 PLANT RESPONSE TO METAL-COMPLEXES AND HUMIC SUBSTANCES

Zinc nutrition is the key to rice production in many parts of the world. Zinc deficiency in rice is known as "Khaira" which was first reported in Mollisols of India (Nene, 1968). It shows the symptoms of (1) stunted plants (2) attenuated root system (3) reddish brown discolouration of leaves preceded by interveinal chlorosis at the base. Effected plants produce very little grain and it could be completely cured by foliar spray of zinc sulphate. Results obtained from foliar sprays in different parts of India show response of 0.1 to 5.6 t/ha to varying level of Zn (10 to 100 kg $ZnSO_4$/ha) (Chibba *et al.*, 1989; Muralidharudu and Singh, 1990).

The response of rice to Zn application was more under flooded conditions than under upland conditions (Gangwar and Mann, 1972). A fairly differential response of rice cultivars to zinc application was reported by Randhawa and Takkar (1975).

Subramanyam and Mehta (1974) obtained 11 per cent increased dry matter yield of rice over control when 5 ppm Zn was applied. Giordano and Mortwedt (1974) obtained high zinc concentration in most responsive zinc cultivars than the least zinc responsive cultivars. Zinc from water soluble source was more available than the non-soluble source (Kang and Okora, 1976). Crop response to zinc application is modified to a large extent by the environment. Zinc deficiency in rice is acute in cold weather and mild (or) absent in warm weather.

Several zinc sources such as $ZnSO_4$, ZnO, $ZnCO_3$, Zn fritts (silicates), $Zn_3(PO_4)_2$ and zinc chelates have been used for ammending zinc deficiencies. The water soluble zinc salts and natural (or) synthetic chelates of zinc generally are more efficient than inorganic zinc salts. Randhawa *et al.* (1977) reported the order of effectiveness of various zinc carriers in improving the tissue concentration of zinc in rice as Zn-DTPA > Zn-EDTA > Zn-fulvate > Zn-citrate > $ZnSO_4$ > ZnO > Zn-fritts.

Wallace (1963) proved chelating agents to be useful in supplying Fe and to a lesser extent Zn and Mn to plants under conditions in which plants were subjected to deficiency of these metals. The chelating agents keep the metals soluble in soil and are taken up by plants. Humic substances are capable of chelating metal ions, retain the soil nutrients in exchangeable forms to be made available for plant growth as required and the very high exchange capacity of humate may bring the buffer utilization of nutrients available in the root zone.

The nutrient cations in soil are influenced by the presence of synthetic chelates and soluble organic matter capable of forming organo metallic complexes. Hodgson (1969) demonstrated a practical model to show the contribution of metal complexes to nutrition of plants.

Vaughan (1974) reported that HA enhanced the cell elongation in pea by virtue of its ability to complex with Fe within the plant tissue. Prasad *et al.* (1976) reported that chelates, either natural (or) synthetic, play an important role in the diffusion of zinc and suggested that organic amendments (or) chelated zinc fertilizers are more effective than soluble zinc salts in developing zinc deficiencies. The chelates enhanced the uptake of both native and applied sources (Singh and Sinha, 1977). Humic acid significantly increased the dry matter yield of barley at all zinc concentrations used in sand culture and plants grew normally (Elgale *et al.*, 1978).

The behaviour of ferric EDTA and ferric citrate in nutrient solution and their interaction with humic acid was investigated by Linehan (1978). According to him, some iron present in solutions of both ferric EDTA and ferric citrate was bound by humic acid at all pH values from 5.0 to 7.0. Wheat roots absorbed more iron from ferric EDTA than from ferric citrate at all pH values. Increasing pH between 5.0 and 7.0 resulted in a progressive decrease in the uptake of iron in both cases. The presence of humic acid depressed iron absorption from both solutions at all pH values.

The effect of humic acids on nutrient uptake and growth of corn plants (*Zea mays* L.) was attempted by Tan and Nopamornbodi (1979) and their results indicated that dry matter yield in corn shoots was stimulated by humic acid. Moderate applications of humic acid resulted in a significant increase in N content while large amounts of HA had a tendency to reduce the N concentration in corn shoots. Phosphorus concentration was decreased with HA treatments. The contents of K and Mn were non significantly different among the treatments. Zinc content showed a tendency to increase with increasing applications of HA.

The application of fulvic acid to a saline-sodic soil augmented the solubility and diffusion of zinc in the soil. Application of gypsum, zinc sulphate and fulvic acid significantly increased dry matter yield and uptake of zinc by rice crop. Application of gypsum with pressmud or with fulvic acid and zinc sulphate resulted in significantly higher yield and zinc uptake than in other treatments (Milap Chand *et al.*, 1980).

Singh *et al.* (1980) conducted an experiment to study the effect of fulvic acid on the diffusion of zinc to a stimulated root system and observed that fulvic acid enhanced the diffusion of zinc to the stimulated root system. A constant flux of fulvic acid and water in the presence of applied zinc, significantly enhanced the dry matter production and uptake of zinc by corn (*Zea mays* L.).

Effects of a soil fulvic acid on the growth and nutrient content of cucumber plants was studied by Rauthan and Schnitzer (1981). Their results showed that the addition of 100 to 300 ppm of FA produced highly significant increase in the growth and development of above and below ground plant parts, in the uptake of nutrient elements (N, P, K, Ca, Mg, Cu, Fe and Zn) and in the formation of number of flowers per plant. Effects of adding 500 and more ppm of FA were less beneficial.

Application of fulvic acid increases the zinc movement in soil and its uptake by rice and wheat plants in an Alluvial soil (Gupta and Deb, 1985).

Lobartini and Orioli (1988) carried out studies on absorption of iron from Fe-humate in nutrient solution by plants and reported that the Fe-humate was a good source of iron which was readily absorbed and transported to the shoot.

Seedling root growth of lettuce, onion and cantaloupe was stimulated by coal-derived sodium humate (Van de Venter *et al.*, 1991).

David *et al.* (1994) found that humic acid improved growth of tomato seedlings in solution culture. The addition of 1280 mg HA/litre increased shoot accumulation of P, K, Ca, Mg, Fe, Mn and Zn as well increased accumulation of N, Ca, Fe, Zn and Cu in roots. Fresh and dry weights of roots were also increased.

Response of sunflower (*Helianthus annuus* L.) to phosphorus, sulphur, micronutrients and humic acid was studied by Vasudevan *et al.* (1997) and they observed that both nutrients and humic acid increased the seed yield, achene and kernel oil contents and seed-protein contents.

An experiment was conducted by Young and Chen (1997) to study the effect of polyamines in humic acid on radical growth of lettuce seedlings and their results showed that the purified humic acid significantly affected the radicle growth of lettuce seedlings, but not the shoot as noted for polyamines authentic.

Pandeya *et al.* (1998) observed the uptake of Fe by the rice crop and the percentage of tissue iron contents derived from fertilizer were higher in the case of Fe-FA in comparison with $FeCl_3$, indicating the superiority of organically complexed Fe fertilizers over inorganic salts.

Influence of humic acid on the yield and nutrient uptake by sesame was explained by Singaravel *et al.* (1998). Their results showed that application of HA @ 20 kg ha^{-1} increased the growth and yield components over other levels of HA. This might be due to the direct effect of HA on plant growth through its auxin activity which increased growth and yield components. Humic acid @ 20 kg ha^{-1} increased the N, P, K and micronutrients uptake.

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MATERIALS AND METHODS

CHAPTER III

MATERIAL AND METHODS

3.1 AREA OF STUDY

Acharya N.G. Ranga Agricultural University, Rajendranagar campus, Hyderabad is located on the Hyderabad-Bangalore National Highway in the Ranga Reddy district of Andhra Pradesh. The campus is located at an altitude of 542.6 m above mean sea level, 77°5'E longitude and 18°59'N latitude. The mean maximum temperatures vary from 28.4 to 41.9°C, while the mean minimum temperatures vary from 7.7 to 20.1°C. The normal annual rainfall of the location is 816.7 mm.

3.1.1 Directorate of Rice Research (DRR)

Directorate of Rice Research (ICAR), an institute connected with Rice Research is located at Acharya N.G. Ranga Agricultural University, Rajendranagar campus. The Institute has a farm with 16 ha of land under cultivation. The soils are clayey and clay loam in texture belonging to the soil order Vertisols. These soils have been subjected to continuous cultivation by growing rice under submerged conditions.

3.1.2 National Research Centre for Sorghum (NRCS)

National Research Centre for Sorghum (ICAR), which is carrying out research on sorghum crop is also located at Acharya N.G. Ranga Agricultural University, Rajendranagar campus. In this farm, an area of 41.9

ha of land is under cultivation. Soils of this farm vary from sandy loam to clay loam in texture and have been classified under the soil orders Alfisols and Vertisols respectively.

3.2 ORGANIC CARBON MAPPING

In order to prepare organic carbon map, soil samples were collected from the farms of Directorate of Rice Research (DRR) and National Research Centre for Sorghum (NRCS). Representative surface (0-15cm) soil samples collected from different plots were air dried, ground to pass through 0.5 mm sieve and were stored in polythene bags. The organic carbon content was determined by wet digestion method (Walkley and Black, 1934). Based on the organic carbon content, organic matter content was computed using the factor 1.724 (Jackson, 1967).

3.3 PHYSICO-CHEMICAL AND CHEMICAL PROPERTIES OF SOILS

The soil samples collected for isolation, fractionation and characterization of humic substances were analysed for different physico-chemical and chemical properties by adopting standard procedures as given in Table 1.

3.4 ISOLATION, FRACTIONATION AND CHARACTERISATION OF HUMIC SUBSTANCES OF SOILS

For the purpose of isolation, fractionation and characterisation of humic substances, an Alfisol from Student's Farm and a Vertisol from

Table 1: Procedures followed for Physico-chemical properties of soils

Character	Reference
pH	1:2 soil water suspension (Jackson, 1967) by using combined glass electrode pH meter (Digisun DI-707)
EC (dSm^{-1})	1:2 soil water extract by using Digisun DI-909 EC meter
Organic carbon (%)	Wet digestion method (Walkley and Black, 1934)
Total nitrogen (%)	Macrokjeldahl digestion and distillation method (Jackson, 1967)
Available nitrogen (%)	Alkaline permanganate method (Subbaiah and Asija, 1956)

Agricultural Research Institute (ARI), Acharya N.G. Ranga Agricultural University, Rajendranagar campus, which were under continuous cultivation, were selected . Soil samples were collected from two depths i.e. surface (0-15 cm) and sub-surface (15-30 cm) layers and were labelled as follows :

Soil Order	Location	Abbreviations used	
		0-15 cm	15-30 cm
Alfisol	Students Farm	A1	A2
Vertisol	Agricultural Research Institute	V1	V2

3.4.1 Extraction, purification and fractionation of humic substances

The humic substances were isolated and determined by Tyurin's method (schematic diagram) as described by Kononova (1966).

3.4.1.1 Decalcification of the sample:

The soil samples after weighing were treated with 0.5N HCl and were covered with 600-800 mL of 0.05N HCl. The contents were stirred occasionally and rootlets floating were removed. After stirring, the clear supernatent was decanted. This treatment was repeated for 5-6 times, till the supernatent was free from Ca(II). Following this, the soil was washed with 0.1N H_2SO_4.

3.4.1.2 Extraction of humic acid:

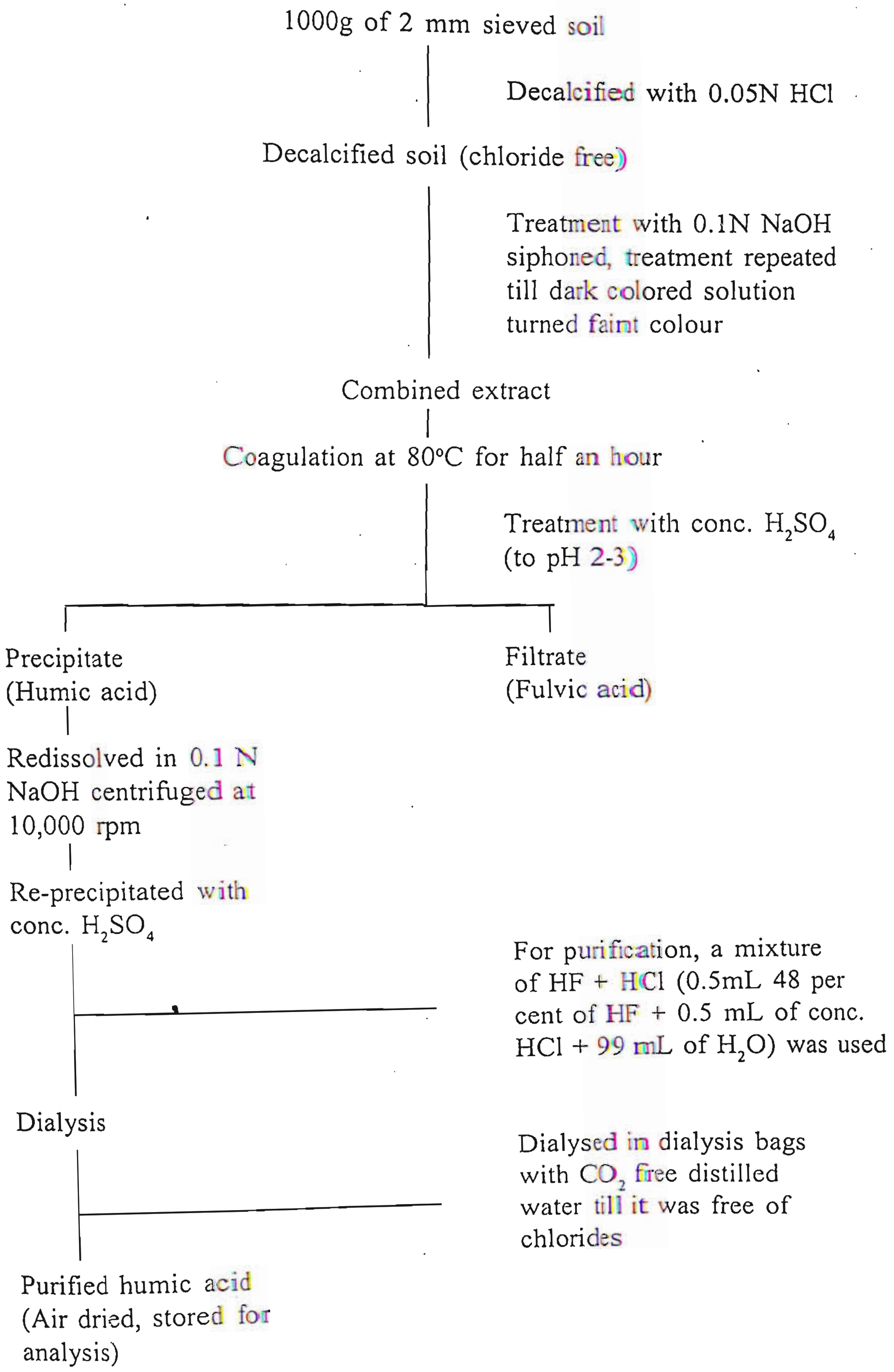
The decalcified sample was covered with 0.1N NaOH and was kept aside for 4-6 hours with occasional stirring. Sodium sulphate was added at the rate of 10g/500 mL and stirred vigourously till the salt was dissolved. The solution was allowed to settle for 18-20 hours. The dark coloured supernatent was siphoned out. The clay coming along with humic acid was removed by centrifugation. This process was repeated till supernatent became faintly coloured.

3.4.1.3 Purification of humic acid:

Purification of humic acid was done by following dialysis method (Schnitzer and Gupta, 1965).

Humic acid fraction was redissolved in 0.1N NaOH and centrifuged 3-4 times to make it free from chlorides and then reprecipitated with 1N HCl. This material was shaken with HF + HCl (0.5 mL 48 per cent HF + 0.5 mL conc. HCl + 99 mL of water) and dialysed in dialysis bag for several days with CO_2 free distilled water till it was made free of fluorides and chlorides. The dialysed humic acid was dried at room temperature and the purified humic acid, thus, obtained was used for further analysis.

SCHEMATIC DIAGRAM I



3.5 CHARACTERISATION OF HUMIC ACIDS

3.5.1 Oxygen containing functional groups

3.5.1.1 Total acidity:

Total acidity in humic acid was determined by $Ba(OH)_2$ method. Wright and Schnitzer (1961) applied this method for humic substances. In the modified procedure of Schnitzer and Gupta (1965), the sample is allowed to react with an excess of $Ba(OH)_2$. The unreacted $Ba(OH)_2$ could be determined by back titration with standard acid.

$$Ba(OH)_2 + 2\ HA \text{ — } BaA_2 + 2\ H.OH \quad \text{——} \quad (I)$$

One hundred milligrams of humic acid along with blank was taken in separate stoppered flasks and 20 mL of 0.2N $Ba(OH)_2$ was added. The flasks were shaken for 24 h at room temperature. The suspension was filtered and the residue was washed with CO_2 free distilled water. The filtrate and washings were titrated against 0.5N HCl to pH 8.4 potentiometrically. Identical blanks were maintained simultaneously.

$$\text{Total acidity (me g}^{-1}\text{)} = \frac{\text{(TV for blank - TV for sample)} \times 0.5\ \text{N} \times 1000}{\text{wt. of sample (mg)}}$$

3.5.1.2 Carboxyl groups:

The method adopted is based on the liberation of acetic acid when acids are treated with calcium acetate and its titration with standard 0.1N NaOH (Schnitzer and Khan, 1972).

$$2R - COOH + (CH_3COO)_2\,Ca \longrightarrow (R\,COO)_2\,Ca + 2\,CH_3COOH \text{ —— (II)}$$

To one hundred milligrams of humic acid in a stoppered flask, 10 mL of 1 N $(CH_3COO)_2$ Ca and 40 mL of CO_2 free distilled water were added. Blank was set up simultaneously. After shaking at room temperature for 24 h, the suspension was filtered and the residue was washed with CO_2 free distilled water. The filtrate and washings were titrated potentiometrically with standard 0.1N NaOH to pH 9.8.

$$\text{COOH groups (me g}^{-1}\text{)} = \frac{\text{(TV for sample - TV for blank) x Normality of base (0.1 N) x 1000}}{\text{Wt. of sample (mg)}}$$

3.5.1.3 Phenolic OH groups:

The phenolic-OH groups were calculated as the difference between total acidity and COOH acidity.

$$\underset{(\text{me g}^{-1})}{\text{Phenolic-OH groups}} = \underset{(\text{me g}^{-1})}{\text{(total acidity)}} - \underset{(\text{me g}^{-1})}{\text{(-COOH acidity)}}$$

3.5.2 Spectral characteristics

3.5.2.1 E_4/E_6 ratios:

Thirty milligrams of humic acid were dissolved in 100 mL of std. 0.1N NaOH (Kononova, 1966) and the optical densities were measured at 465 and 665 nm on a spectronic-21-UVD-UV-visible spectrophotometer. The ratios of optical densities at 465 and 665 nm were calculated and were expressed as E_4/E_6 ratios.

3.5.2.2 UV-Spectra of humic acids:

Spectral characteristics of HA were evaluated in UV-range of 200 to 300 nm. For this purpose, the solution containing 10 mg HA mL^{-1} of distilled water was prepared and the absorbance was recorded on a spectronic 21-UVD-UV-visible spectrophotometer.

3.6 POTENTIOMETRIC TITRATIONS

One hundred milligrams of humic acid were dissolved in 25 mL of distilled water and was titrated against 0.1 N NaOH potentiometrically on digital pH meter (Digisun-DI-707).

3.7 CONDUCTOMETRIC TITRATIONS

One hundred milligrams of humic acid were dissolved in 25 mL of distilled water and was titrated against standard 0.1N NaOH conductometrically on a EC meter (Digisun-DI-909) using conductivity cell.

3.8 METHODS TO CHARACTERISE COMPLEXES OF HUMIC ACID WITH METAL IONS

The potentiometric titrations, absorption spectroscopic methods and stability constants worked out were used to characterise the interactions of humic acids with Cu (II), Mn (II), Zn (II) and Fe (II).

3.8.1 Potentiometric methods

Potentiometric methods are based on the fact that during complex formation, metal ion displaces one (or) more weak acidic protons from complexing agent (ligand) (Chaberek and Martell, 1959).

$$M^{n+} + HA \longrightarrow MA^{n-1} + H^{+} \quad \text{——} \quad (III)$$

From ligands containing large number of acidic groups such as humic acid, several H^+ ions are dissociated in the course of complex formation, usually involving a drop in the pH of solution. The greater the tendency for metal to combine with a complexing agent, the more will be the drop in pH.

The procedure followed for potentiometric titration of humic acid both in presence and in absence of metal ions is described below.

Humic acids obtained from selected soil samples were dissolved in 0.1N NaOH to give a final concentration of 5 mg of sample per mL of 0.1N NaOH. In 100 mL beakers, 10 mL of above solutions were taken to which 25 mL of 0.1N KCl were added in each case. The pH of sample was adjusted to 3.0 by the addition of 0.1N NaOH or 0.1N HCl, after which the volume of solutions in each case was made upto 50 mL with 0.1N KCl. The NaOH was added in increments of 0.05 mL and the change in pH of the system was recorded by digital pH meter.

For titration in the presence of metal ions, 4 mL of 0.05N of appropriate metal ions [Zn(II), Cu(II), Mn(II) and Fe(II)] were added to the sample to supply 100 umoles of metal ions. Similarly, 100 umoles of metal ions were also titrated alone for comparison.

3.8.2 Absorption spectroscopic methods

Absorption spectrophotometric technique was originally developed by Job (1928) for determination of complex combination between metal ion and complexing agent. The formation of complex is represented by the equation

$$A + nB \longrightarrow AB_n \qquad \text{———} \qquad \text{(IV)}$$

where,

A = Metal ion
B = the ligand

To determine 'n', the solutions of A and B of the same concentration are mixed in varying proportions and their absorbance was measured in the visible range from 380 to 560 nm.

The differences (Y) between each absorbance formed and the corresponding absorbance that would have resulted in no reaction would have occurred on mixing solutions of humic acid and the metal ion is plotted against mole fraction of each of the component (X). The plot will show a maximum at the ratio of metal to ligands, which corresponds to the formation of the metal complex. This method has been used for studying complex formation between humic acid and metal ion [Zn(II); Cu(II); Mn(II) and Fe(II)].

The following procedure was adopted for characterising the complexes of humic acids with metal ions (MacCarthy and Mark, 1976) with slight modifications.

Six aliquots of 0, 4, 8, 12, 16 and 20 mL of $1x10^{-3}$ M humic acid varying from 0 to 20 mL i.e in 4 mL increments aliquots were pipetted in a series of 50 mL beakers. It was followed by addition of $1x10^{-3}$ M metal ion [Zn(II), Mn(II), Cu(II) and Fe(II)) varying from 20 to 0 mL in increment of 4 mL to the same series of beakers and the pH was adjusted to 7.0. The solutions were then transferred into 25 mL volumetric flasks and the volume was made up.

A standard curve was prepared with six aliquots of 0, 4, 8, 12, 16 and 20 mL of $1x10^{-3}$ M humic acid pipetted into a series of 50 mL beakers and the pH was adjusted to 7.0. The volume was made upto 25 mL in volumetric flasks.

The absorbance of each solution was then measured at 400 nm for Cu(II), Zn(II), Fe(II) and 420 nm for Mn(II) on a spectronic 21-UVD-UV-Spectrophotometer.

3.9 DETERMINATION OF STABILITY CONSTANTS OF METAL-HA COMPLEXES

If a metal ion M reacts with X moles of complexing agent A, to form a metal complex MA_x.

$$M + XA \longrightarrow MA_x$$

the stability constant is given by

$$K = \frac{(MA_x)}{(M)\,(A)^x} \qquad \text{———} \qquad (V)$$

Among different methods to determine stability constants, ion exchange equilibrium method was adopted. This method is based on the complexation of metal ion between the complexing agent and the cation exchange resin.

The stability constant is obtained from the following equation.

$$\text{Log}\left(\frac{\lambda_o}{\lambda} - 1\right) = \log K + x \log (A) \qquad \text{——} \ (VI)$$

When log K is the intercept and x is the slope of plot of

$$\log\left(\frac{\lambda_o}{\lambda} - 1\right) \text{ vs } \log (A).$$

Log K for each step was calculated from equation (5).

λ & λ_0 are distribution constants of metal ion in the presence and absence of A respectively.

K = stability constant

x = number of moles of A which combines with one mole of metal ion

A = conc. of HA (moles/L)

In the present case the weight of one mole of HA was taken as 1000 g.

λ was obtained from the following equation.

$$\lambda = \frac{\alpha V}{(100 - \alpha) \times g} \qquad \text{———} \qquad (VII)$$

α = % of metal bound to exchange resin

$(100 - \alpha)$ = % of total metal remaining in solution

V = volume of solution

g = wt. of exchange resin

λ_0 was calculated in the same manner as but in the absence of A. Volume for these studies was kept at 100 mL and one gram of amberlite IR-120 resin was used in these studies.

3.9.1 Procedure

From the stock solution, 6 to 24 mg of HA were pipetted out into flask and diluted approximately to 40 mL with distilled water. To each of these flasks, 5 mL of 1N KCl were added followed by 10 mL of appropriate metal [Fe(II), Cu(II), Mn(II) and Zn(II)] chloride solutions containing 20 μg of metal/mL. The pH was adjusted to 7. The volume was made upto 50 mL with distilled water. In the glass stoppered flask, 1 g of potassium saturated amberlite IR-120 resin was taken. The solutions of metal chlorides, humic acid and KCl were transferred to flask and volume was made upto 100 mL. The flask was shaken at 30°C for one hour. The resin was decanted and metal ions remaining in the solution were determined by atomic absorption spectrophotometry (spectr AA-20).

Identical blanks were also carried out in the absence of HA to determine λ_o.

3.10 POT CULTURE STUDIES

Two separate pot culture experiments were conducted during *kharif*, 1999 under greenhouse conditions to study the relative efficacy of different zinc carriers in supplementing zinc nutrition. A zinc deficient Alfisol collected from Students' farm, College of Agriculture, Rajendranagar, was used in this study. The soil had a pH of 7.3. It was low in organic carbon and available N, medium in available P_2O_5, high in available K_2O and deficient in DTPA extractable zinc (0.65 μg g^{-1} soil). Four kgs of soil was filled in earthern pots of dimensions of 9"x9".

For both the experiments, zinc humate was prepared by complexing zinc with HA extracted from Alfisol.

3.10.1 Preparation of complex of humic acid with zinc

The complex was prepared by using x-value i.e., the number of moles of HA required to complex with one mole of Zn, from the stability constant data obtained at pH 7.0 and 30°C temperature. The value of x was found to be approximately 1.5 for humic acid. In this and other calculations, the value of molecular weight used was 1000 for humic acid. It was arrived at based on elemental composition and functional group analysis data (Nagamadhuri, 1996; Sujana Reddy, 1997).

Amounts of $ZnSO_4$ corresponding to 2.5 and 5.0 ppm zinc were weighed in beakers containing the required amount of HA for complexation. The contents of the beaker were diluted with water and pH of the solution was adjusted to 7.0.

3.10.2 Details of experiments

In the first experiment, rice (var. Tellahamsa) was used as test crop. A uniform fertilizer dose @ 120 kg N + 60 kg P_2O_5 + 30 kg K_2O/ha was applied to each pot. Nitrogen was applied in the form of urea in two equal splits viz., one as basal and another as top dressing at maximum tillering stage (35 DAT), whereas P in the form of SSP and K in the form of MOP were applied as basal. The rice seedlings of 25 days old were transplanted in four hills @ two seedlings hill^{-1}. The soil was kept under submergence till harvest of rice crop at flowering stage.

In the second experiment, maize (var. DHM-105) was grown as the test crop. Four hills pot^{-1} were maintained by direct dibbling of maize seeds. Recommended dose of N @ 120 kg, P_2O_5 @ 60 kg and K_2O @ 30 kg ha^{-1} was applied to each pot. Nitrogen was applied in the form of urea in two equal splits one as basal and another at knee heigh stage (35 DAS), whereas P in the form of SSP and K in the form of MOP were applied as basal. Thinning and gap filling were taken up 8 days after sowing so as to maintain one seedling hill^{-1}.

The following treatments were tried in a complete randomised design with each treatment replicated thrice in case of both rice and maize experiments:

Treatment	**Source**	**Zn level(ppm)**
T_1	Control	0
T_2	Humic acid*	—
T_3	$ZnSO_4$	2.5
T_4	$ZnSO_4$	5.0
T_5	Zn-humate	2.5
T_6	Zn-humate	5.0
T_7	Zn-chelate	2.5
T_8	Zn-chelate	5.0

* represents the concentration of HA equivalent to its amount used in 5 ppm of Zn-humate complexes.

Plants were grown until flowering stage and then harvested. Plant samples were shade dried for 3-4 days, then oven dried at 65°C to a constant weight and dry matter production (g pot^{-1}) was recorded. The DTPA extractable zinc content in soils collected from each of the pots was determined (Lindsay and Norvell, 1978). The zinc content in plant was determined by digesting with triacid mixture (Conc. HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:4:1) and using atomic absorption spectrophotometer. The uptake of zinc by rice and maize was calculated by using the formula.

$$\underset{(\mu g\ pot^{-1})}{\text{Zn uptake}} = \underset{(\mu g\ g^{-1})}{\text{Zinc content}} \times \underset{(g\ pot^{-1})}{\text{dry matter production}}$$

3.11 STATISTICAL ANALYSIS

The data obtained from pot culture experiments was subjected to statistical analysis for CRD by following standard procedures (Rao, 1983). The critical difference was used to evaluate the treatmental effects.

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RESULTS

CHAPTER IV

RESULTS

The results obtained from studies carried out in this investigation are presented under the following heads.

4.1 ORGANIC CARBON AND ORGANIC MATTER STATUS OF RESEARCH FARMS

Directorate of Rice Research has 16 ha of land under cultivaton. On this farm rice crop is grown. The organic carbon contents of soils of Directorate of Rice Research (Table 2) varied from 0.36 (plot-B_{10}) to 1.15 per cent (plot-D_8). Out of 68 samples analysed, 14, 35 and 19 samples belonged to low, medium and high categories, respectively.

The National Research Centre for sorghum has 41.9 ha of land under cultivation. Predominantly sorghum crop is grown on this farm. The organic carbon contents of fields of National Research Centre for sorghum (Table 3) varied from 0.23 (Plot-D_1) to 1.04 per cent (GTC plot). Out of 25 samples analysed, 7, 14 and 4 samples fell under low, medium, high categories respectively.

The following soils were selected for isolation, fractionation and characterisation of humic substances. For convenience of presentation the following legends are used.

Table 2: Organic carbon and organic matter contents of soils of Directorate of Rice Research, Rajendranagar, Hyderabad

Plot No.	Content (%)	
	Organic carbon	Organic matter
A1	0.69	1.18
A2	0.59	1.01
A3	0.64	1.10
A4	0.67	1.15
A5	0.48	0.82
A6	0.52	0.89
A7	0.46	0.79
B1	0.54	0.92
B2	0.56	0.96
B3	0.76	1.30
B4	0.83	1.42
B5	0.63	1.08
B6	0.77	1.32
B7	0.60	1.03
B10	0.41	0.70
C1	0.73	1.25
C2	0.78	1.34
C3	0.49	0.84
C4	0.71	1.22
C5	0.82	1.41
C6	0.74	1.27
C7	1.02	1.75
D1	0.68	1.16
D2	0.78	1.34
D3	0.81	1.39
D4	0.65	1.11
D5	0.76	1.30
D6	0.84	1.44
D7	0.82	1.40
D8	1.15	1.97
D9	0.76	1.30
E1	0.53	0.91
E2	0.49	0.84

Contd..

Plot No.	Content (%)	
	Organic carbon	Organic matter
E3	0.45	0.77
E4	0.38	0.65
E5	0.77	1.32
E6	0.56	0.96
E7	0.60	1.03
E8	0.54	0.92
E9	0.54	0.92
E10	0.61	1.04
F1	0.69	1.18
F2	0.64	1.10
F3	0.76	1.30
F4	0.73	1.25
F5	0.92	1.58
F6	0.81	1.39
F7	0.78	1.34
F8	0.74	1.27
F9	0.74	1.27
F10	0.92	1.58
F11	0.71	1.22
F12	0.68	1.16
G1	0.59	1.01
G2	0.61	1.64
G3	0.71	1.22
G4	0.56	0.96
G5	0.39	0.28
G6	0.47	0.80
G7	0.36	0.61
G8	0.45	0.77
H1	0.48	0.82
H2	0.39	0.67
H3	0.46	0.79
H4	0.51	0.87
H5	0.54	0.92
H6	0.61	1.04
H7	0.63	1.08

Table 3: Organic carbon and organic matter contents of soils of National Research Centre for Sorghum, Rajendranagar, Hyderabad

Plot No.	Content (%)	
	Organic carbon	Organic matter
A1	0.64	1.10
A2	0.36	0.61
A3	0.27	0.46
A4	0.58	0.99
A5	0.58	0.99
A6	0.39	0.67
A7	0.70	1.21
B1	0.68	1.16
B2	0.76	1.30
B3	0.52	0.89
B4	0.64	1.10
B5	0.71	1.22
B6	0.42	0.72
B7	0.39	0.67
B8	0.61	1.04
C1	0.77	1.32
C2	0.67	1.15
C3	0.69	1.18
C4	0.73	1.25
Rabi	0.86	1.47
Agronomy	0.52	0.89
GTC	1.04	1.78
D1	0.23	0.38
D2	0.53	0.91
D3	0.30	0.51

Student's Farm, College of Agriculture, Rajendranagar

A_1 - Alfisol (0-15 cm)
A_2 - Alfisol (15-30 cm)

Agricultural Research Institute, Rajendranagar

V_1 - Vertisol (0-15 cm)
V_2 - Vertisol (15-30 cm)

4.2 PHYSICO-CHEMICAL PROPERTIES, ORGANIC MATTER CONTENTS AND TEXTURE OF SOILS USED FOR FRACTIONATION STUDIES

The salient properties of soils used for fractionation studies are presented in Table 4.

In the Alfisol of Students farm, the organic carbon content was 0.51 per cent in surface layer and 0.35 per cent in sub-surface layer.

In the Vertisol of Agricultural Research Institute, the organic carbon content was 0.92 per cent in surface horizon and 0.84 per cent in sub-surface horizon.

Vertisol contained higher amount of organic carbon as compared to Alfisol. In both soils, sub-surface layer had lower content of organic carbon than that of surface layer.

4.3 NITROGEN CONTENTS OF SOILS

Total and available N contents of soils used in the study are presented in Table 5.

The content of total nitrogen in Alfisol of Student's farm was 6.1×10^{-2} and 3.02×10^{-2} per cent in surface and sub-surface layers respectively.

The content of total nitrogen in the Vertisol of Agricultural Research Institute was 7.2×10^{-2} and 6.3×10^{-2} per cent in surface and sub-surface layers, respectively.

Regarding the total nitrogen contents in Vertisol and Alfisol it was observed that the Vertisol had higher amounts of total nitrogen than that of Alfisol. In both the soils, sub-surface layer had lower amount of total nitrogen than surface soil.

In the Alfisol of Student's farm, the available nitrogen content was 10.89×10^{-3} per cent in surface and 6.76×10^{-3} per cent in sub-surface layer.

In the Vertisol of Agricultural Research Insitute the available nitrogen content was 27.1 and 8.9 per cent in surface and sub-surface layers respectively.

The available nitrogen content of Vertisol was more than that of Alfisol. In both these soils, sub-surface layer had lower amount of available nitrogen than the surface soil.

Table 4: Physico-chemical properties, organic matter contents and texture of soils used for fractionation studies

Soil type	Depth (cm)	pH	EC (dSm^{-1})	Organic carbon (%)	Organic matter (%)	Texture
A_1	0-15	7.77	0.307	0.51	0.87	Sandy loam
A_2	15-30	8.00	0.263	0.35	0.60	Sandy loam
V_1	0-15	8.10	0.456	0.92	1.58	Clayey
V_2	15-30	8.19	0.501	0.84	1.44	Clayey

Table 5: Nitrogen contents and C/N ratios of soils used under study

Soil type	Depth (cm)	Total N (%) ($X \times 10^{-2}$)	Available N (%) ($X \times 10^{-3}$)	C/N ratio
A_1	0-15	6.10	10.89	8.36
A_2	15-30	3.02	6.76	11.58
V_1	0-15	7.20	27.10	12.78
V_2	15-30	6.30	8.90	13.33

In the Alfisol of Student's farm, the C/N ratios were 8.36 and 11.58 in surface and sub-surface layers, respectively.

In the Vertisol of Agricultural Research Institute,the C/N ratios were 12.78 and 13.32 in surface and sub-surface layers, respectively.

Vertisol had higher C/N ratio than the Alfisol and this ratios increased with depths in both soils.

4.4 CHARACTERIZATION OF HUMIC ACIDS OBTAINED FROM ALFISOL AND VERTISOL

During the fractionation of humic substances, humic acid fraction was extracted, purified and characterised. For the reasons beyond control, sufficient amounts of fulvic acid fraction could not be obtained. Hence only humic acid fractions are presented.

4.4.1 Functional group contents

Functional group analysis provides information about the type of reactive sites on humic acid. The data on contents of total acidity, carboxyl, phenolic-OH groups of humic acid are presented in Table 6.

In the humic acid obtained from Alfisol of Student's farm, the total acidity (me g^{-1}) was 3.82 in surface layer while it was 4.82 sub-surface layer.

The content of total acidity in the humic acid obtained from Vertisol of Agricultural Research Institute was 4.45 and 5.47 me g^{-1} in surface and sub-surface layers respectively.

Table 6: Functional group [Total acidity, carboxyl and phenolic-OH]contents and E_4/E_6 ratios of humic acids obtained from an Alfisol and a Vertisol

Soil type	Depth	Content (me g^{-1})			E_4/E_6(cm) ratios
		Total acidity	Carboxyl groups	Phenolic-OH	
A_1	0-15	3.82	2.13	1.69	4.08
A_2	15-30	4.82	3.43	1.39	4.19
V_1	0-15	4.45	2.41	2.04	4.46
V_2	15-30	5.47	4.06	1.41	4.89

In the humic acid obtained from Alfisol of Student's farm, the carboxyl group contents (me g^{-1}) were 2.13 in surface layer while it was 3.43 in sub-surface layer.

In the humic acid obtained from Vertisol of Agricultural Research Institute, the carboxyl groups ranged from 2.41 (surface layer) and 4.06 me g^{-1} (sub-surface layer).

The phenolic groups in the humic acid obtained from surface and sub-surface layers of Alfisol of Student's farm were 1.69 and 1.39 me g^{-1}, respectively.

In the humic acid obtained from Vertisol contained phenolic-OH groups (me g^{-1}) to the extent of 2.04 and 1.41 in surface and sub-surface layers, respectively.

The contents of total acidity, carboxyl groups and phenolic groups were high in humic acid obtained from Vertisol as compared to humic acid obtained from Alfisol. The total acidity and carboxyl group contents increased with depth and phenolic-OH groups decreased with depth.

4.4.2 Contribution of functional groups to total acidity

The contents of carboxyl and phenolic-OH groups (expressed as per cent of total acidity) were calculated and are presented in Table 7.

In the humic acid obtained from Alfisol, COOH groups contributed to 55.75 and 74.22 per cent of total acidity in surface and sub-surface layers, respectively.

Table 7: Content of carboxyl and phenolic-OH groups (expressed as % of total acidity) of humic acids obtained from an Alfisol and a Vertisol

Soil type	Depth (cm)	Content (% of total acidity)	
		Carboxyl groups	Phenolic-OH groups
A_1	0-15	55.75	44.24
A_2	15-30	74.22	25.77
V_1	0-15	54.15	45.84
V_2	15-30	58.71	41.28

able 7: Content of carboxyl and phenolic-OH groups (expressed as % of total acidity) of humic acids obtained from an Alfisol and a Vertisol

Soil type	Depth (cm)	Content (% of total acidity)	
		Carboxyl groups	Phenolic-OH groups
A_1	0-15	55.75	44.24
A_2	15-30	74.22	25.77
V_1	0-15	54.15	45.84
V_2	15-30	58.71	41.28

In the humic acid obtained from Vertisol, the COOH groups accounted for 54.15 and 58.71 per cent of total acidity in surface and sub-surface layers, respectively.

In the humic acid obtained from Alfisol, the phenolic-OH groups accounted for 44.24 and 25.77 per cent of total acidity in surface and sub-surface layers respectively.

In the humic acid obtained from Vertisol, the phenolic-OH groups contributed to 45.84 and 41.28 per cent of total acidity in surface and sub-surface layers, respectively.

4.5 SPECTRAL PROPERTIES

4.5.1 UV spectra

The ultraviolet spectra of humic acids were featureless with a decreasing optical density and increasing wavelength (Fig.1). The UV spectra of diverse origin were similar in nature inspite of differences in their sources.

4.5.2 E_4/E_6 ratio

In the humic acid obtained from Alfisol of Student's farm the E_4/E_6 ratios were 4.08 and 4.19 in surface and sub-surface layers, respectively.

Humic acid obtained from surface and sub-surface layers of Vertisol of Agricultural Research Institute, the E_4/E_6 ratios were 4.46 and 4.89, respectively.

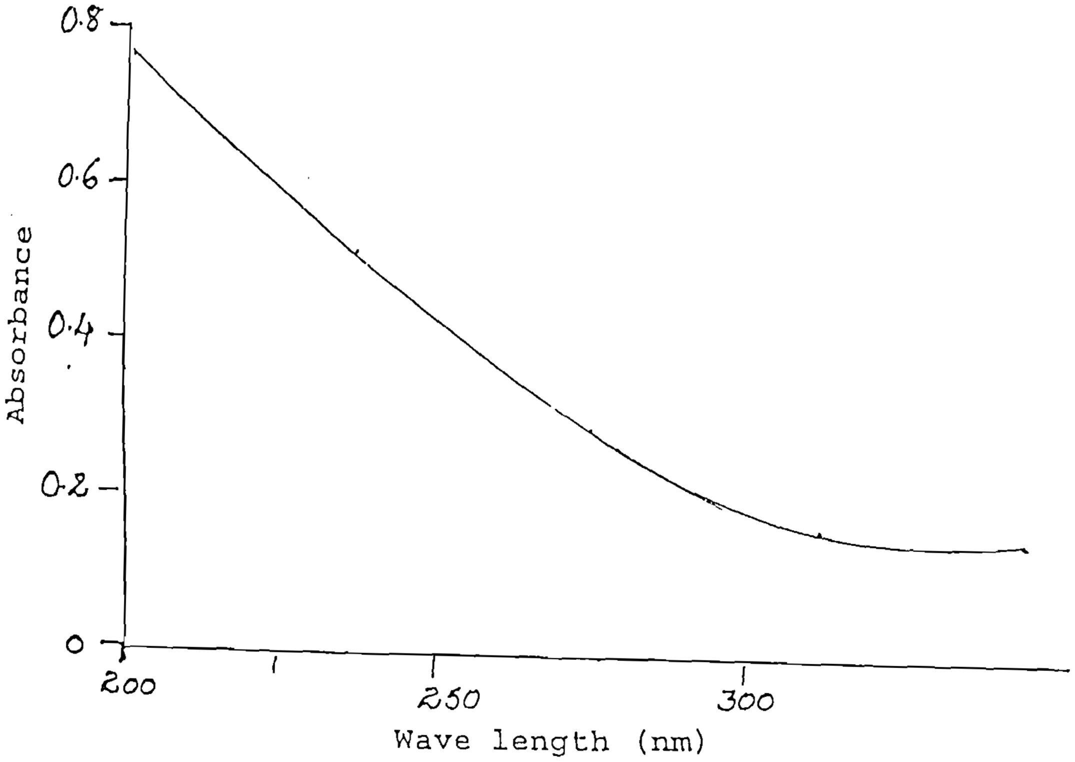


Fig.1: UV SPECTRUM OF HUMIC ACID

Humic acid obtained from Vertisol showed higher E_4/E_6 ratio as compared to humic acid obtained from Alfisol. In both these soils, humic acid obtained from subsurface layer exhibited higher E_4/E_6 ratio than the humic acid obtained from surface layer.

4.6 POTENTIOMETRIC TITRATIONS

The potentiometric titration curves of humic acids with std.0.1N NaOH are shown in Fig.2. There was a gradual increase in pH with the addition of base, thus, indicating a high buffering capacity of humic acids. This shows that humic acids behaved as weak acid polyelectrolytes.

4.7 CONDUCTOMETRIC TITRATIONS

Conductometric titration curves of humic acids with std. 0.1 NaOH are depicted in Fig.3. From the curve, it can be seen that with the addition of NaOH, conductance increased very slowly in the beginning followed by a sharp increase.

4.8 CHARACTERISATION OF METAL COMPLEXES WITH HUMIC ACIDS

4.8.1 Potentiometric methods

The magnitude of pH drop on addition of metal ion to aqueous solution of humic acid is indicated by potentiometric titrations.This information along with the buffer effects obtained in titration curves were taken as indicator of complex formation. Potentiometric titrations are simple,

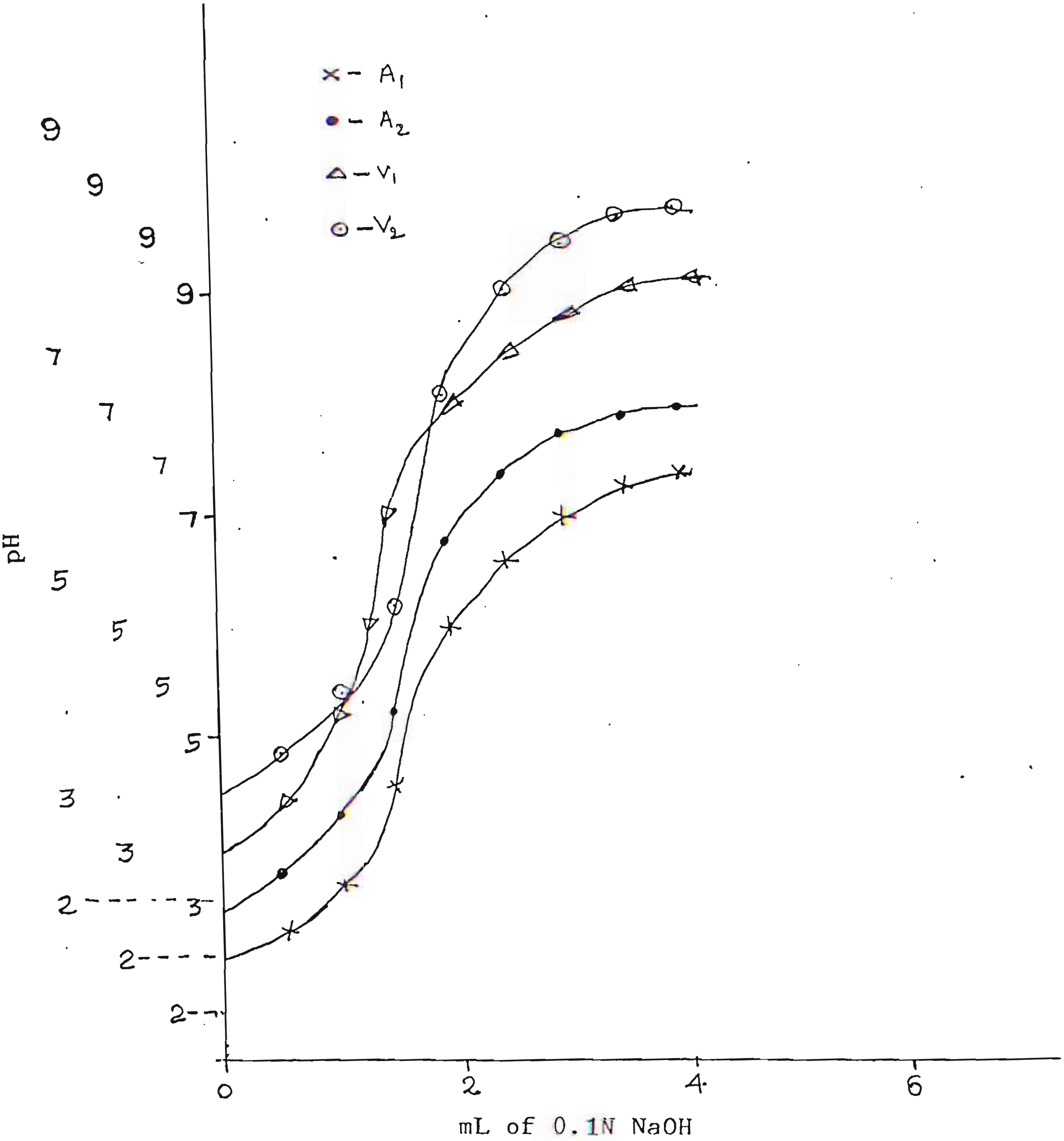


Fig.2: Potentiometric titration curves of humic acids (0.1N NaOH)

rapid and provide information on relative tendencies of metals to form complexes. Potentiometric titrations were carried out in the presence and absence of metal ions.

4.8.1.1 Titration of metal ions with alkali:

The titration curves of Cu(II); Zn(II), Mn(II) and Fe(II) ions are presented in Fig.4. The inflections in the titration curves indicated the formation of metal hydroxides. The inflections in the curves at pH 5.3 for Cu (II); at pH 8.7 for Mn (II); at pH 8.4 for Fe (II) and two inflections in case of Zn (II) at pH 7.5 and 9.6 was observed due to the formation of the zinc hydroxide ($Zn(OH)_2$) and sodium zincate, respectively.

4.8.1.2 Titration of humic acids and metal humates:

Titration curves of humic acids and corresponding metal humates are shown in Fig.5a to 5d. While no inflection was observed in the titration curves of humic acid a close look at the titration curves of metal-humates indicated that the formation of metal hydroxides was suppressed when metal ions were added to humic acid and the absence of inflection was taken as an index of complex formation.

The titration curves were almost similar for all humic acids obtained from Alfisol and Vertisol in the presence of metal ions (Fig.5a to 5d). However, displacement in curve of humic acid in presence of metal ion was wider at higher pH and reverse was true at lower pH. The reduction in pH on titration of humic acid in the presence of various metal ions followed the

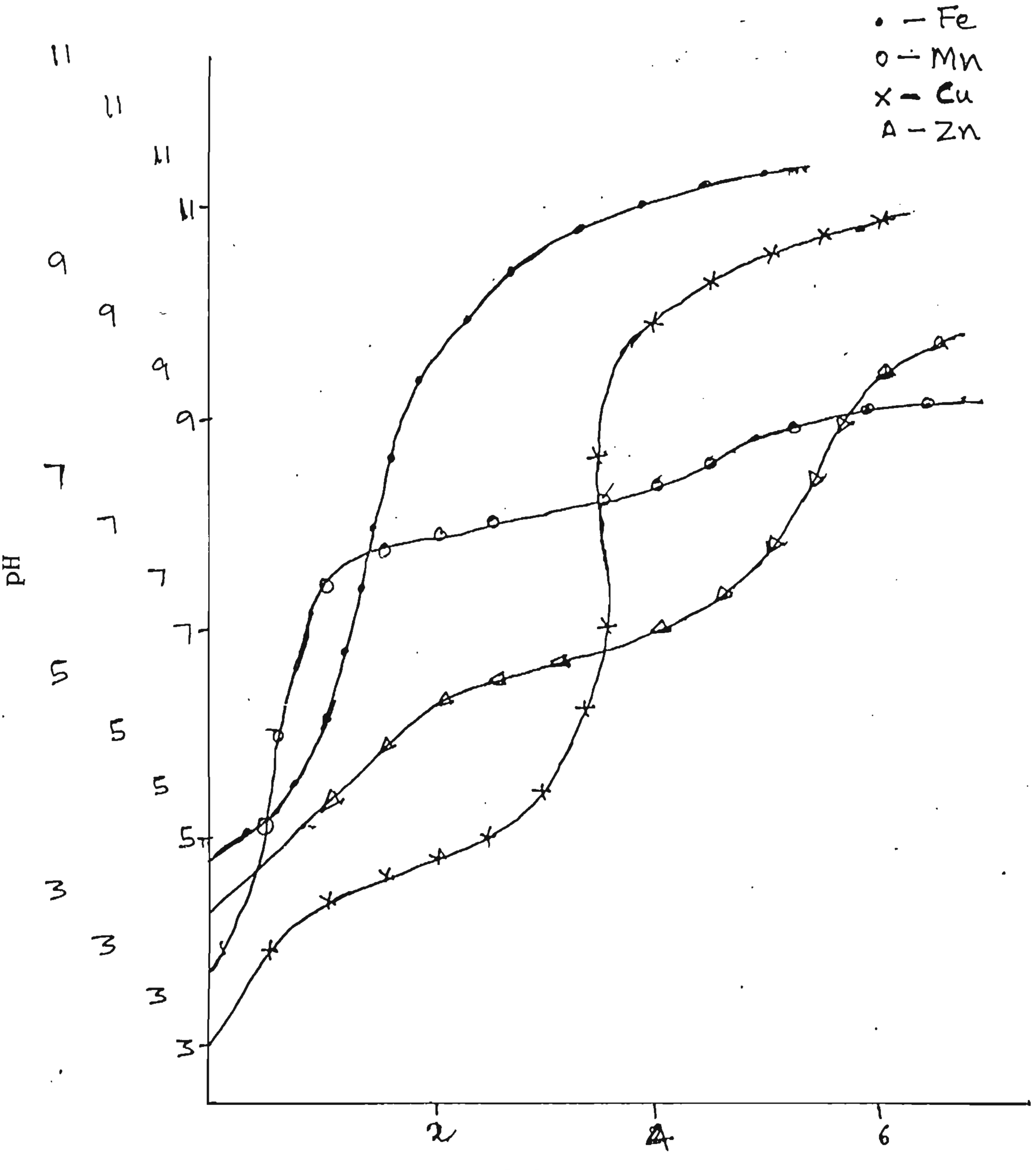


Fig.4: Potentiometric titration curves of metal ions (100 um)

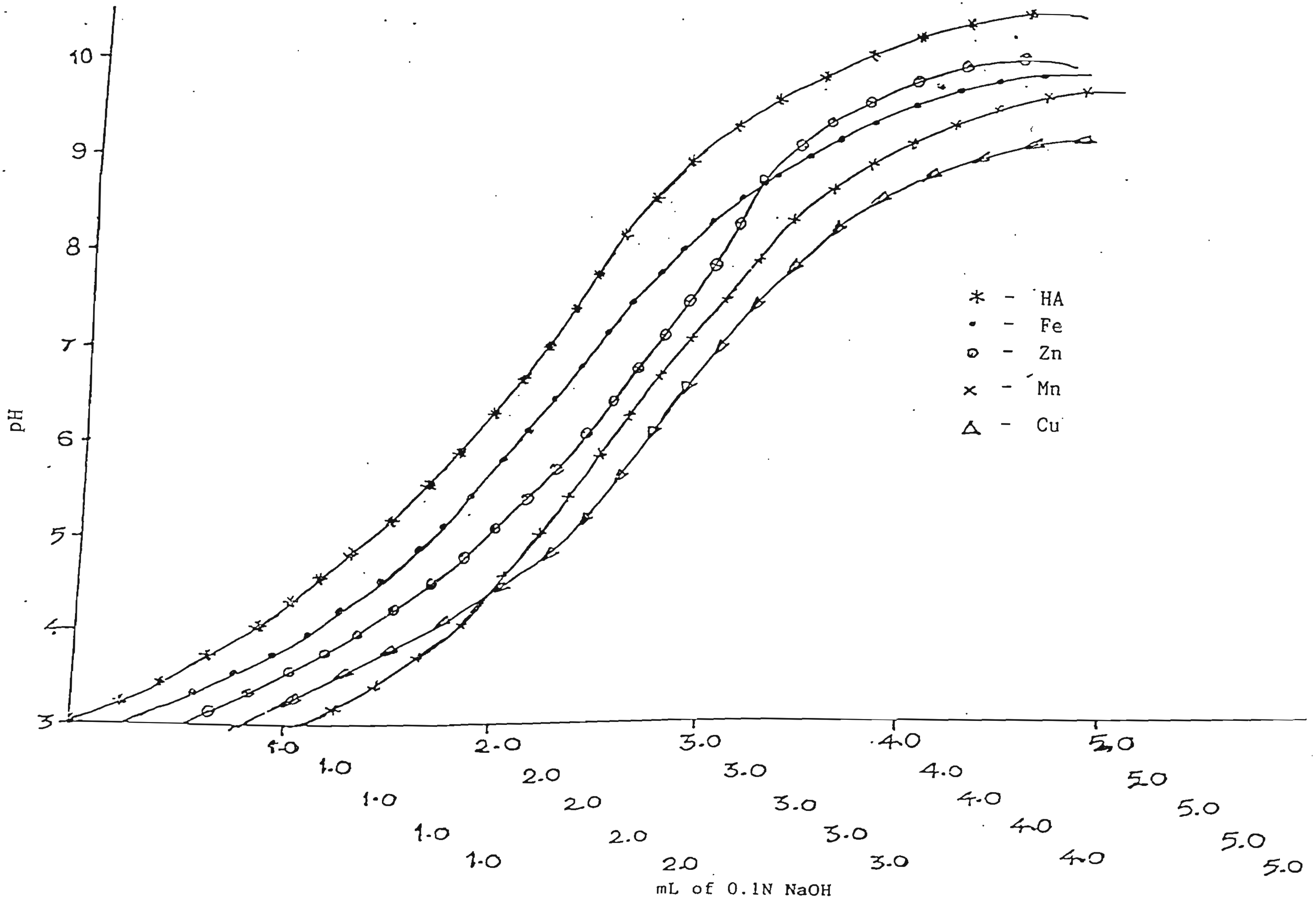


Fig 5a: POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID (A_1) AND METAL HUMATES

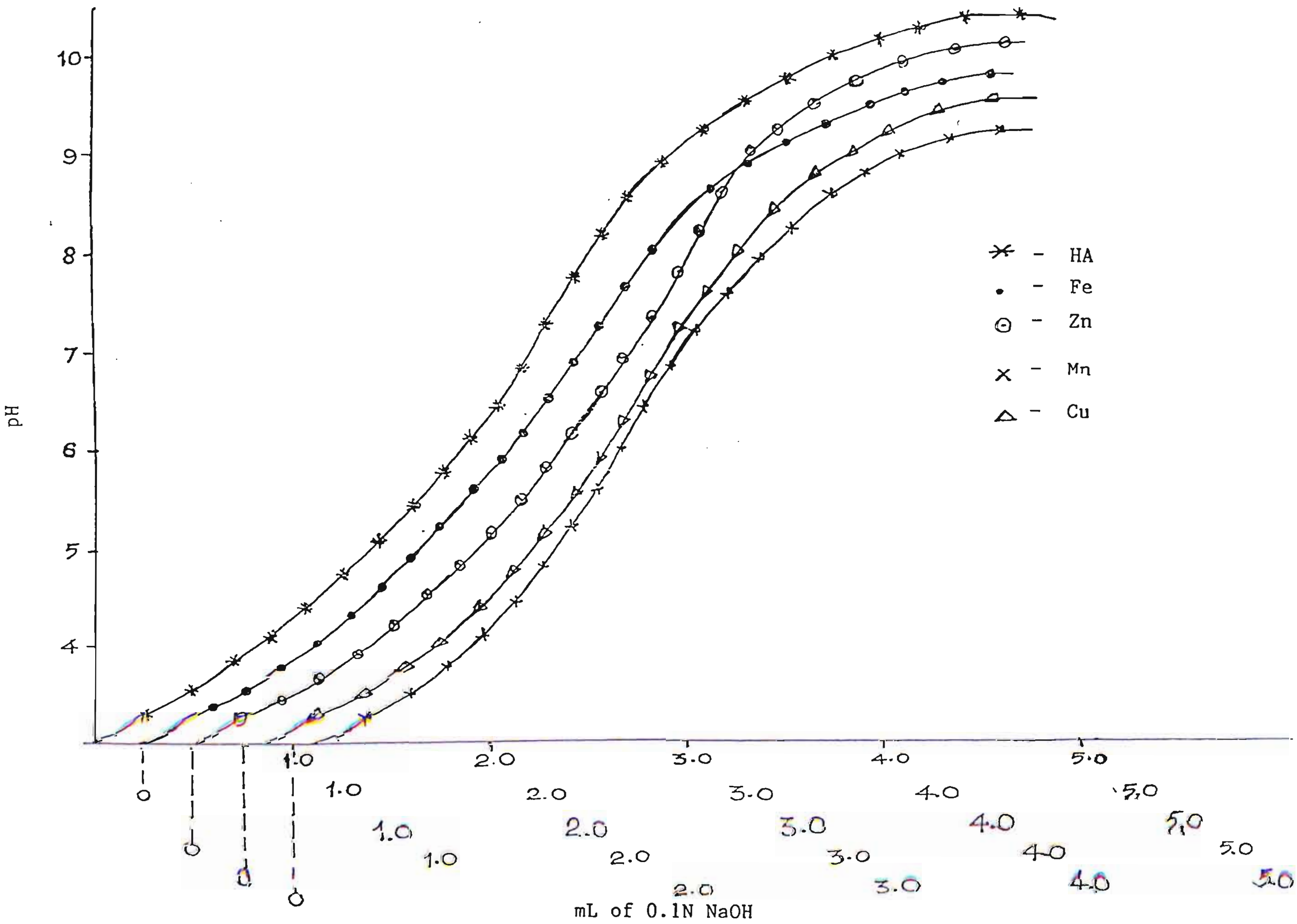


Fig.5b: POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID (A_2) AND METAL HUMATES

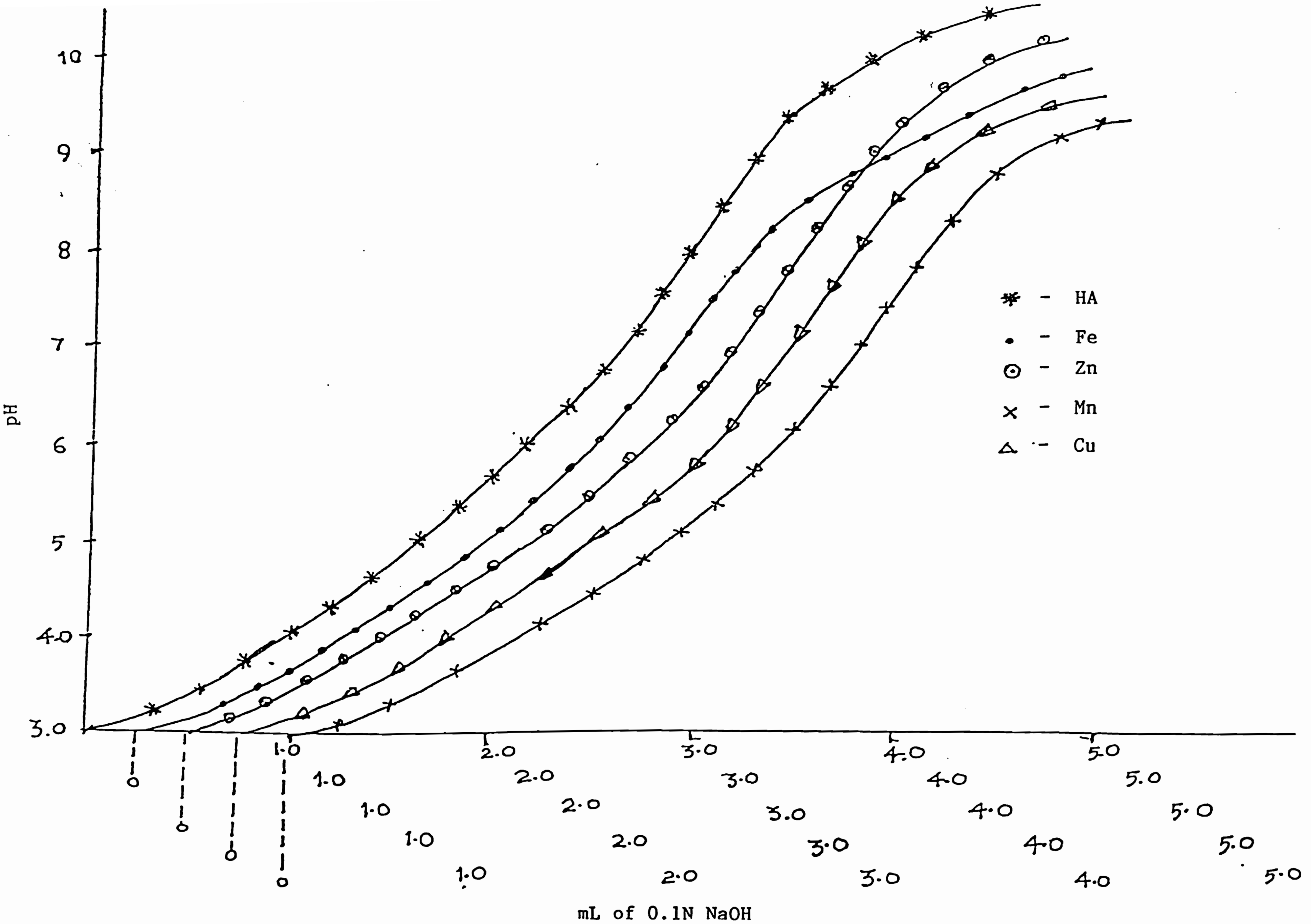


Fig.5c: POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID (V_1) AND METAL HUMATES

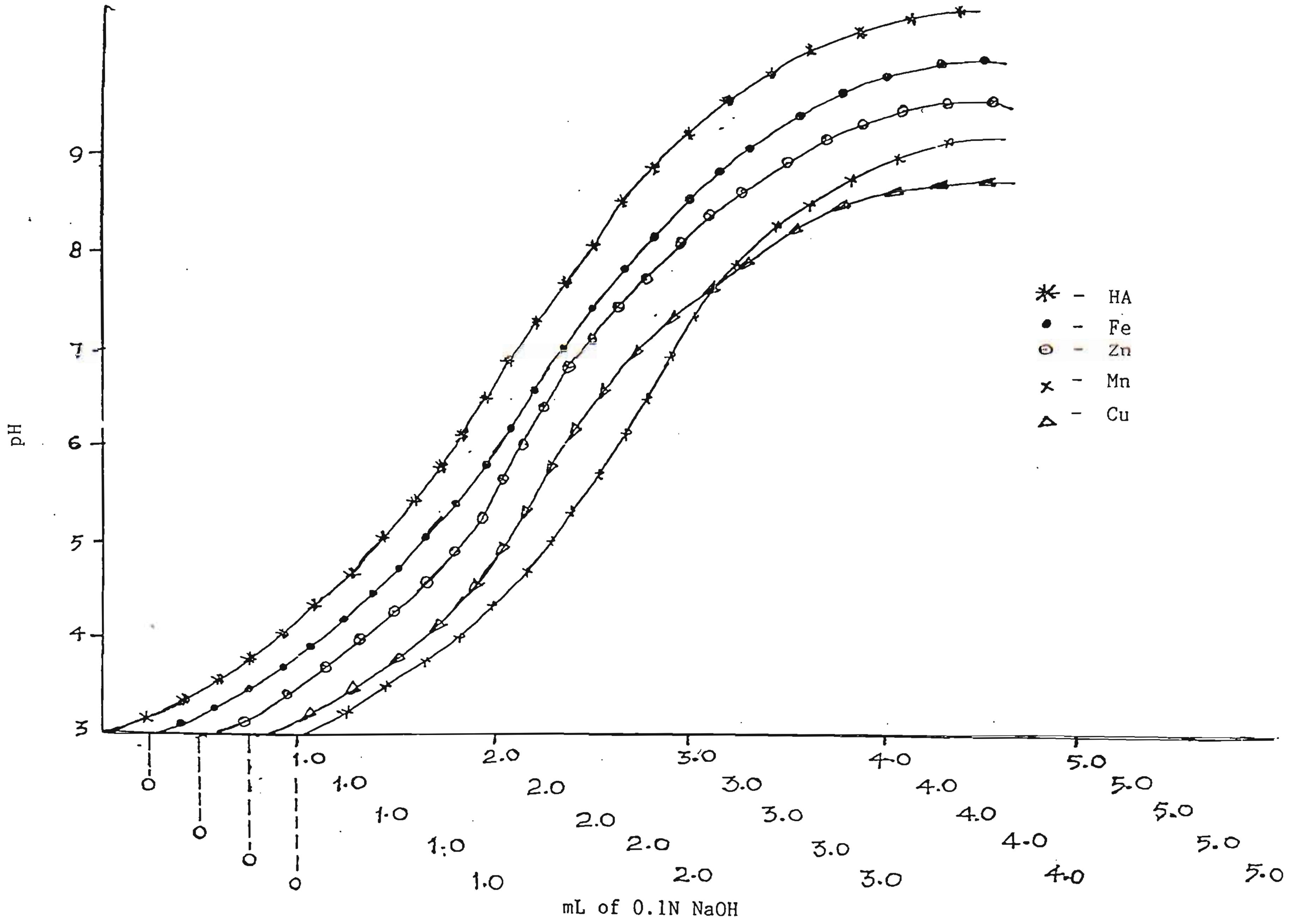


Fig.5d: POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID (V_2) AND METAL HUMATES

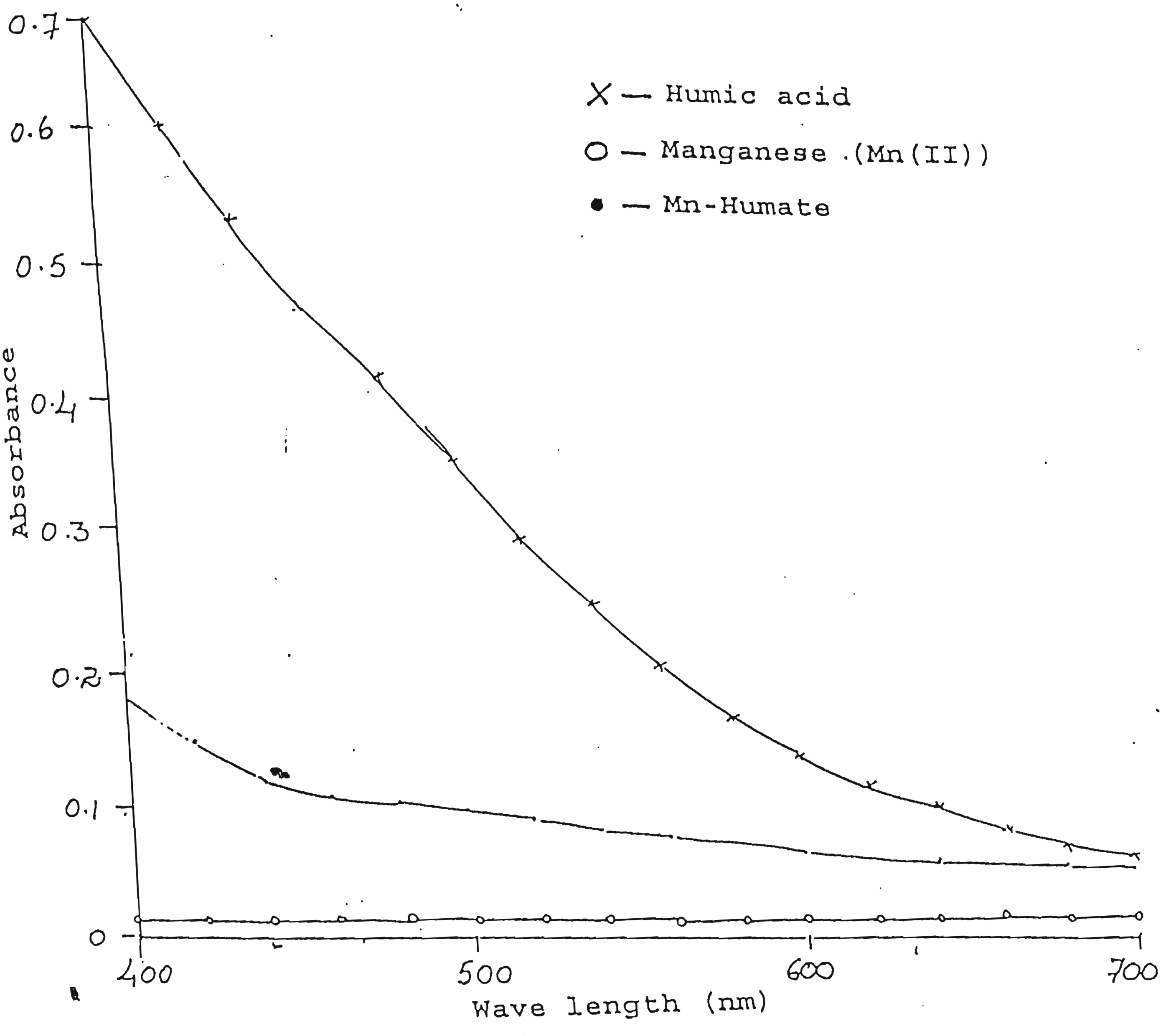


Fig.6 a: ABSORPTION SPECTRA OF METAL HUMATES, HUMIC ACID AND METAL IONS

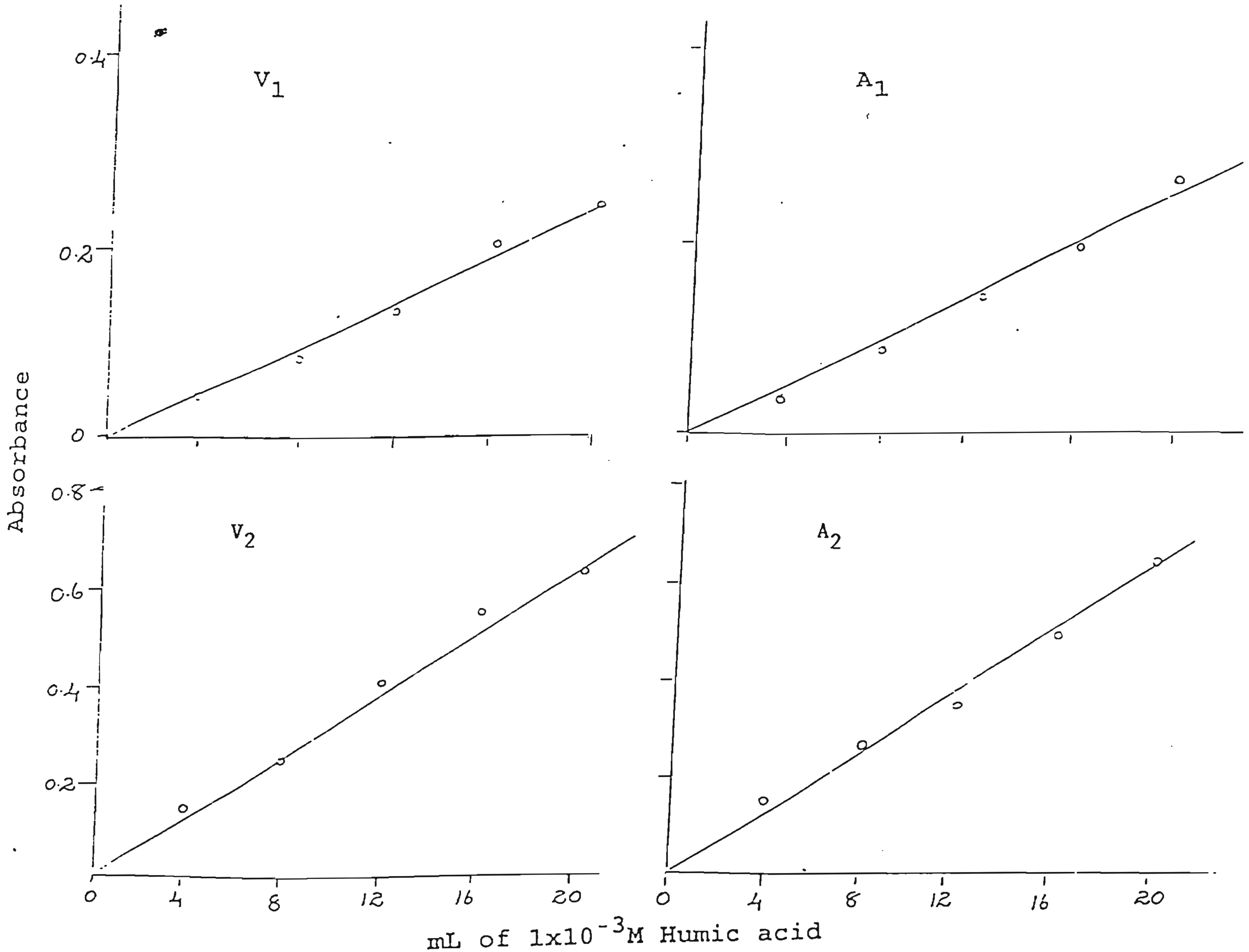


Fig.7: STANDARD CURVES OF HUMIC ACIDS

4.8.2.2 Job's plot:

Based on spectroscopic measurement, the function y,

Y = Absorbance calculated assuming no reaction - Absorbance recorded with metal humate

was plotted as a function of X (X=mole fraction of one of the components). From Job's plot for metal humates presented in Fig.8a and 8b, it is clearly evident that maximum absorbance occurred at X=0.6 for Cu(II), Zn(II), Mn(II) and Fe(II) with humic acids obtained from various sources.

4.9 STABILITY CONSTANTS OF METAL - HA COMPLEXES

The numerical value of stability constants, referred to as log k, is of significance in predicting the solubility and movement of micronutrients in soils. Stability constants were determined for Cu(II), Fe(II), Zn(II), and Mn(II) metal ion complexes with HA at pH 7.0 and at 30^0C.

In the ion exchange method used in this study, the following important parameters were evaluated.

1) **Log K** : Provides an indication of affinity of metal ion to the complexing agent

2) **x-value**: The number of moles of HA that reacted with one mole of metal ion

λ_0 and λ : Represent distribution constants of metal ions in the absence and presence of HA

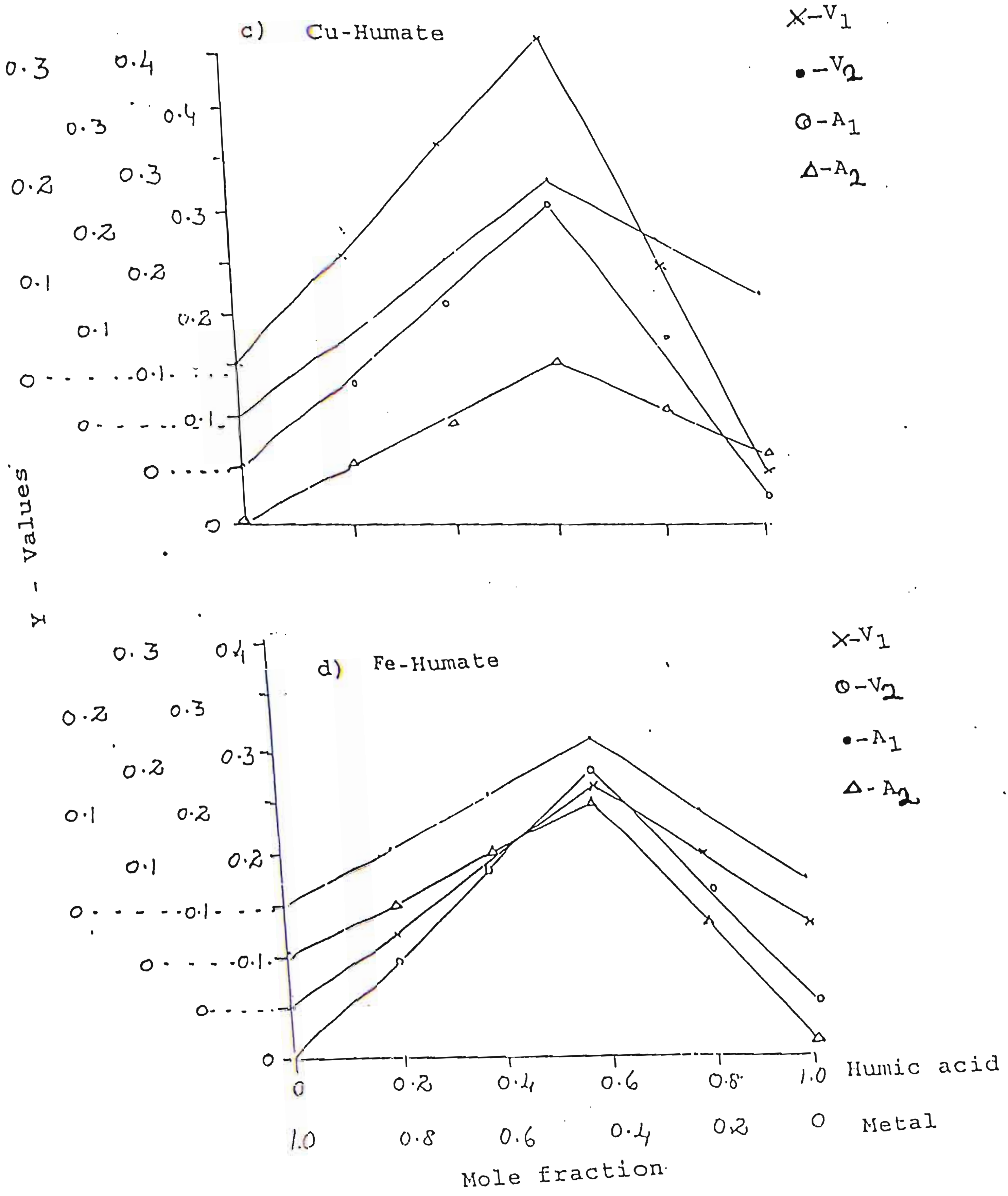


Fig 3 a: JOB'S PLOTS OF METAL HUMATES

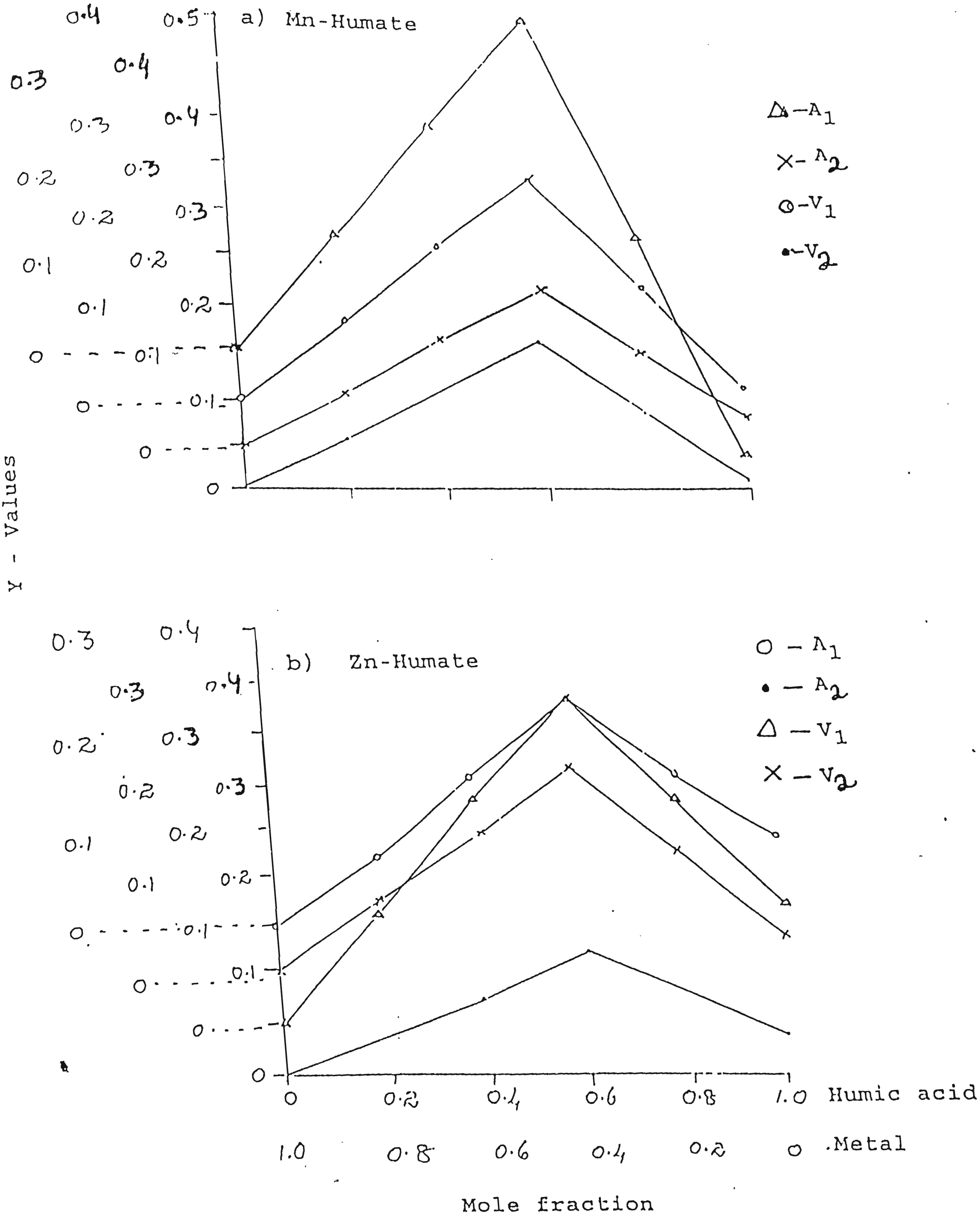


Fig. 8b: JOB'S PLOTS OF METAL HUMATES

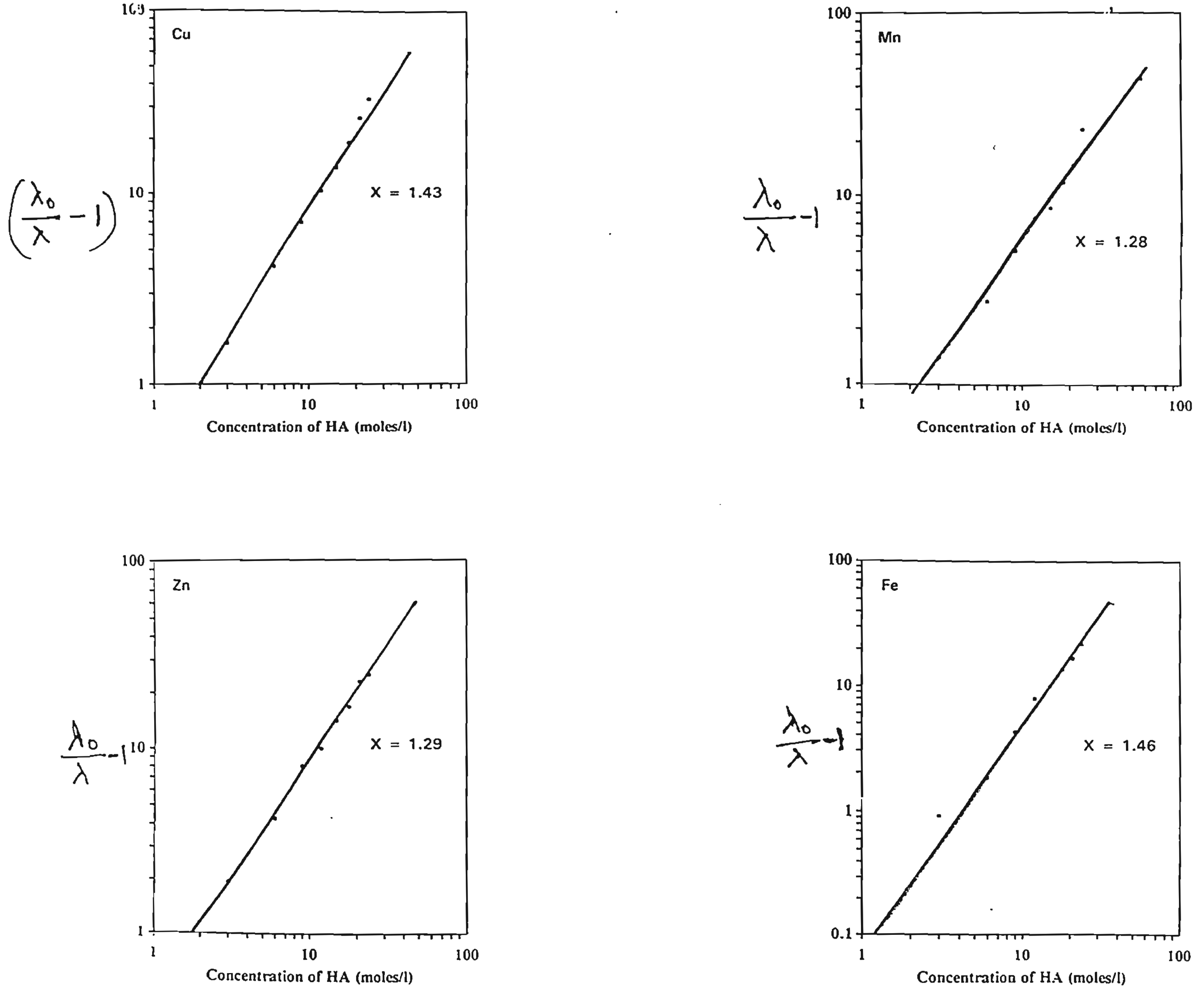


Fig.9a: Stability constants of metal humic acid (A_1) complexes

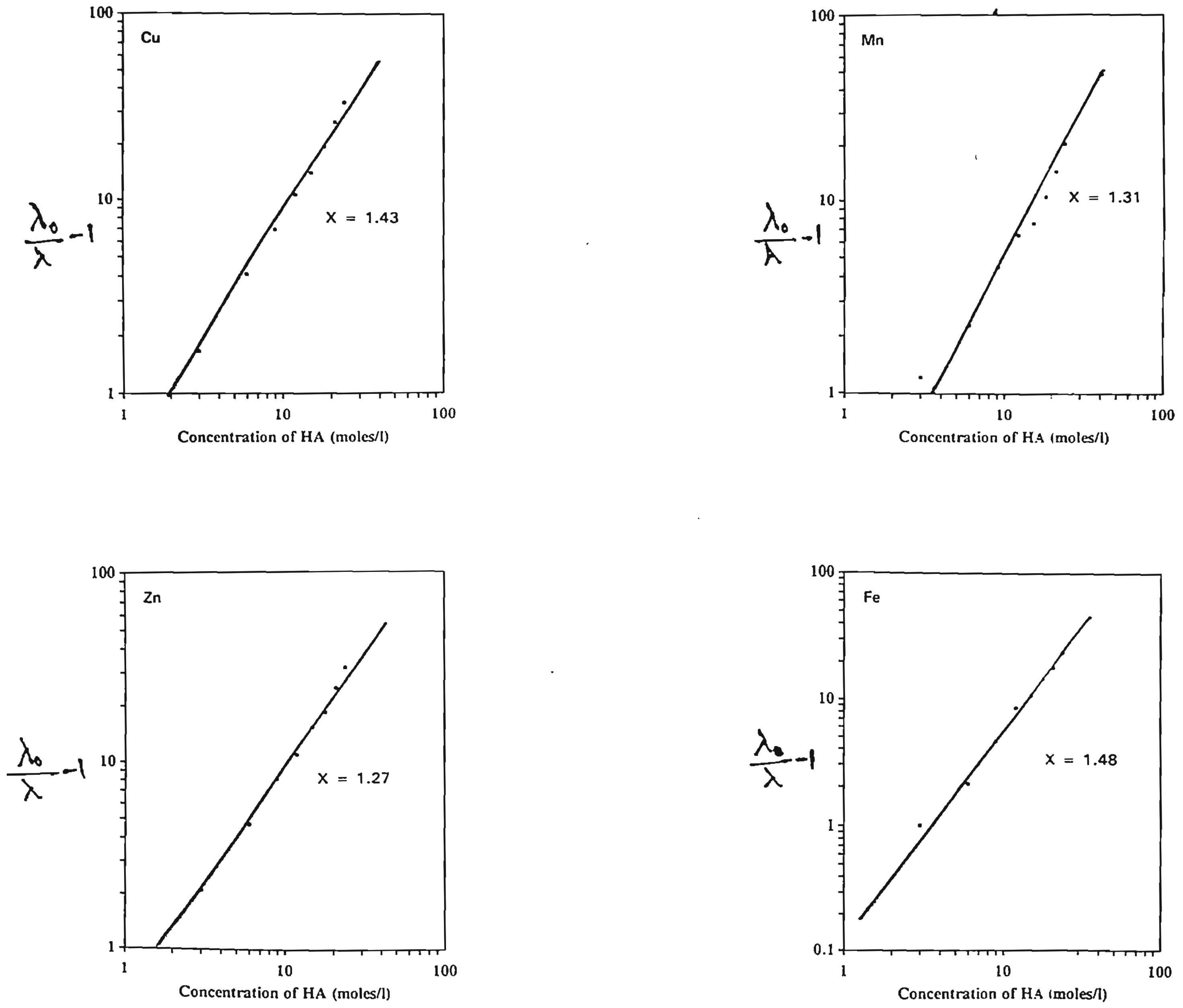


Fig.9b: Stability constants of metal-humic acid (A_2) complexes

4.9.1 Complexation behaviour of humic acid fraction with metal ions

As indicated above the number of moles of HA that reacted with one mole of metal ion in the formation of complex is referred to as x-values. These 'x' values were obtained from the slope of straight line by plotting $(\frac{\lambda_0}{\lambda} - 1)$ Vs conc. of HA in log - log graph and are presented in Table 8 and are depicted in Fig.9a to 9d. The values of distribution constants i.e., & are presented in Appendix.

For the humic acid obtained from surface layer of Vertisol, the x values were 1.51, 1.40, 1.34 and 1.33 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively. In case of humic acid obtained from sub-surface layer, these values were 1.52, 1.39, 1.34 and 1.35 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively.

For the humic acid obtained from surface layer of Alfisol, the x values were 1.43, 1.46, 1.29 and 1.28 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively. With regard to humic acid obtained from sub-surface layer, these values were 1.43, 1.48, 1.27 and 1.31 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively.

In general, the HA-metal ion complexes showed almost similar x-values at pH 7.0 and at temperature of 30^{0}C. Relatively higher x-values were observed for Cu(II) - HA complexes than other complexes.

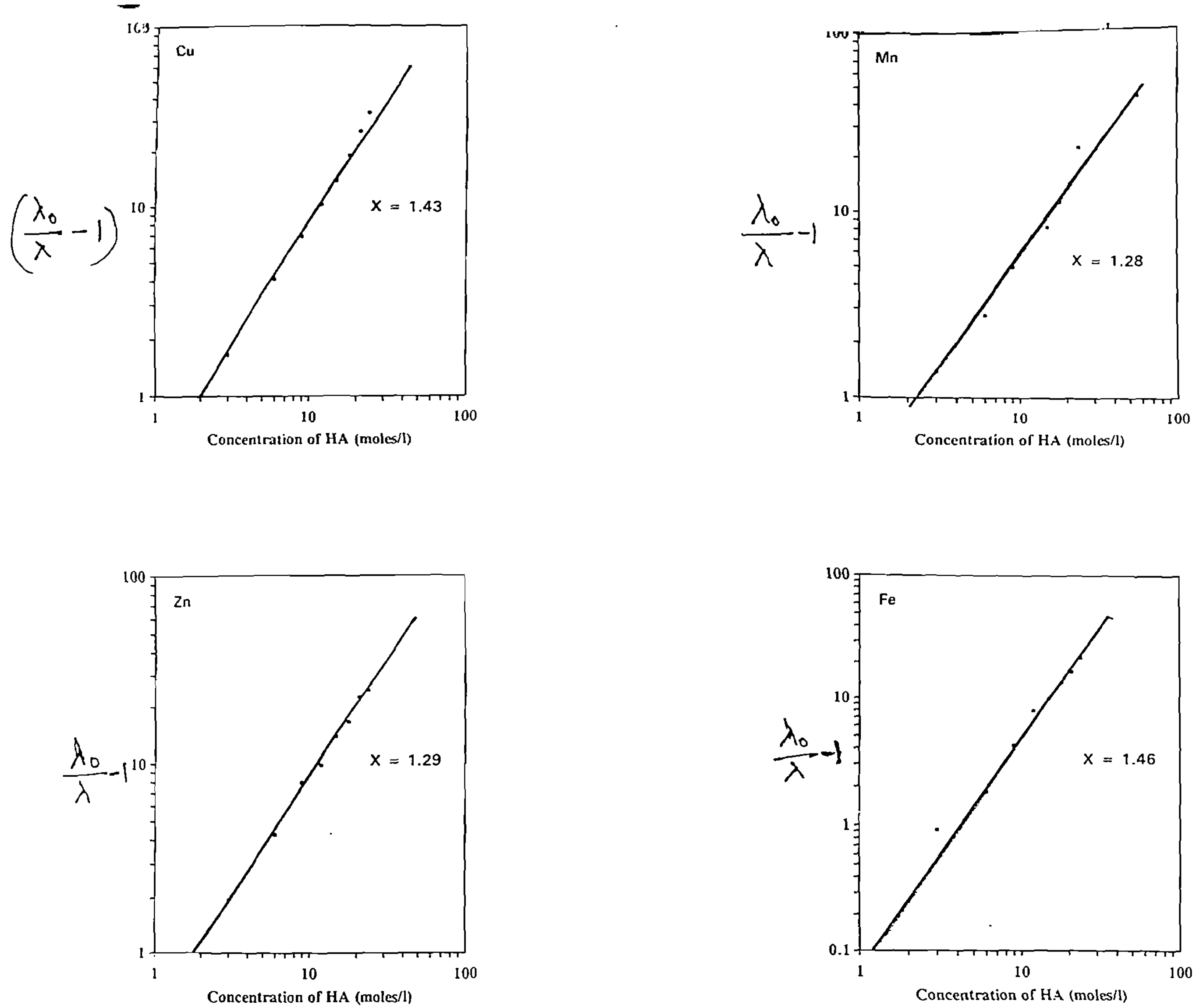


Fig.9a: Stability constants of metal humic acid (A_1) complexes

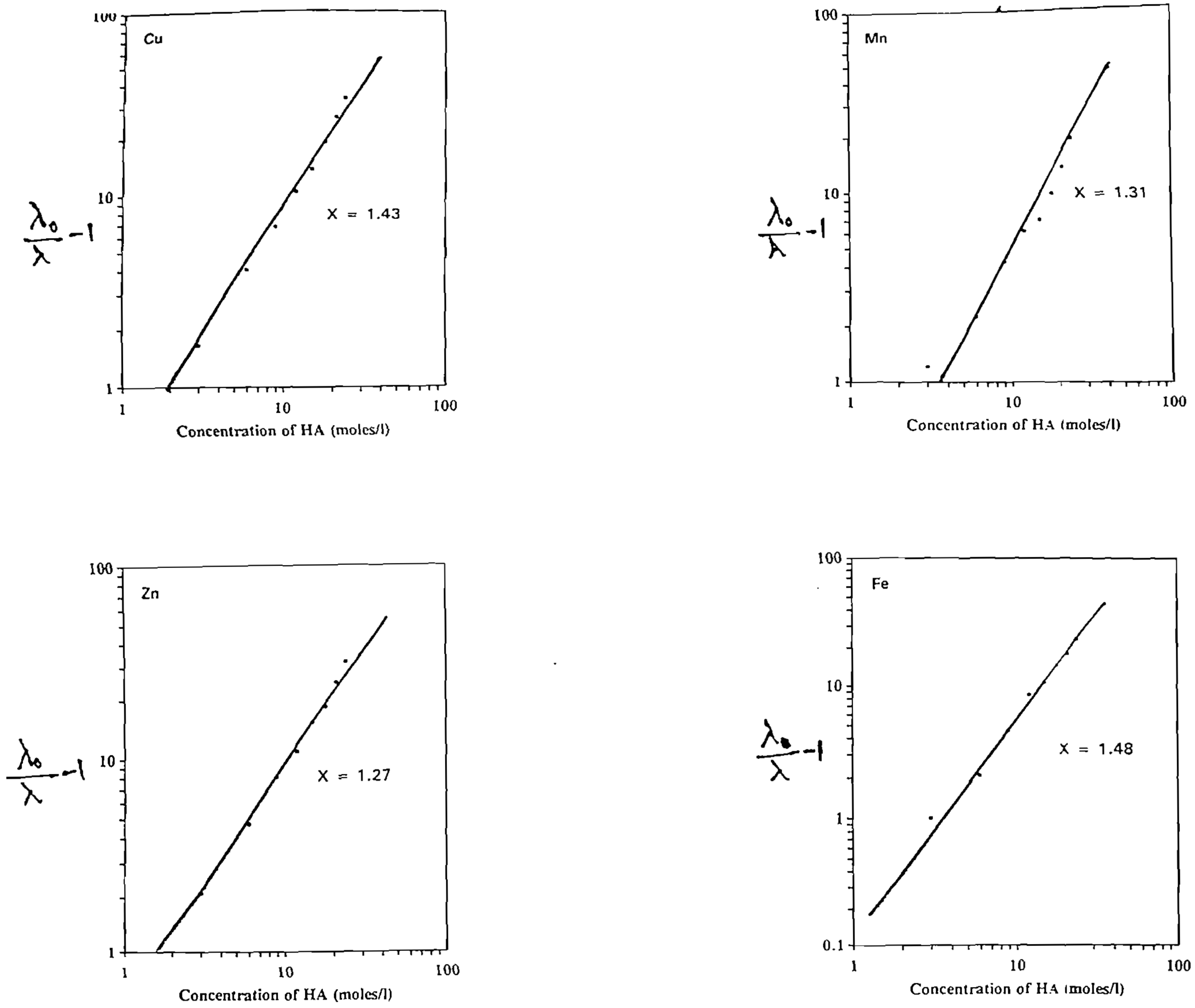


Fig.9b: Stability constants of metal-humic acid (A_2) complexes

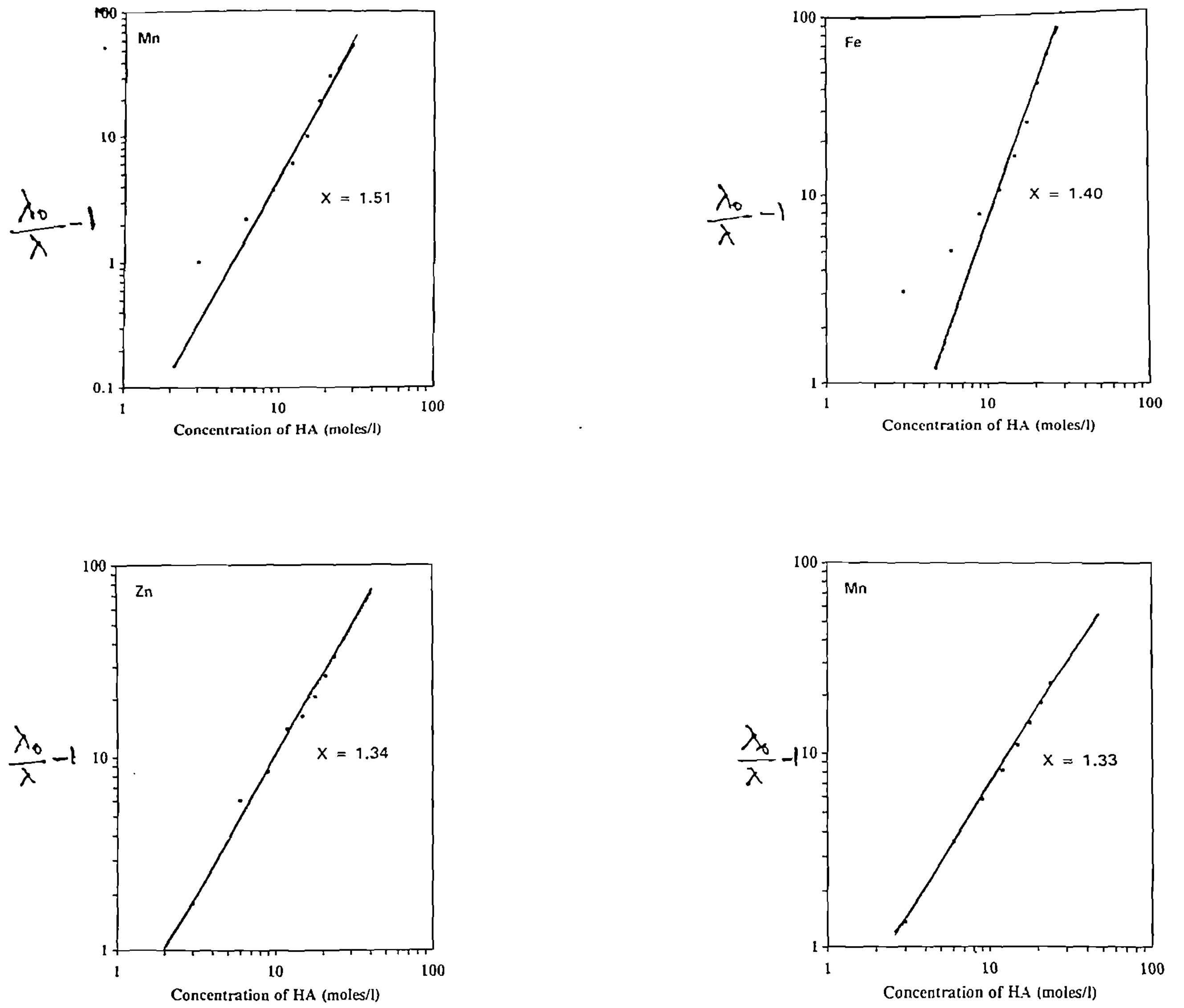


Fig.9c: Stability constants of metal humic acid (V_1) complexes

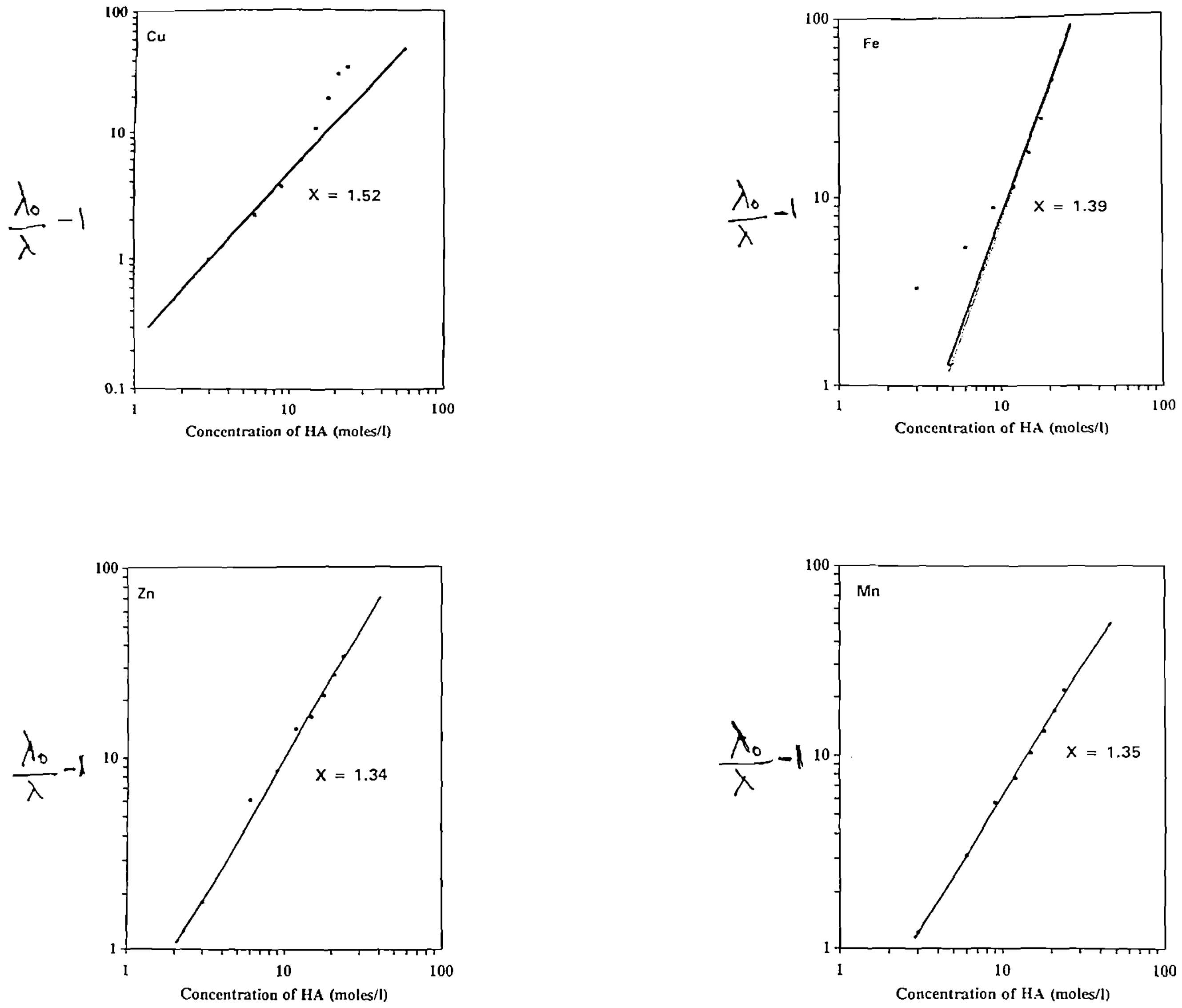


Fig.9d: Stability constants of metal humic acid (V_2) complexes

4.9.2 Differential ability of HA fraction to form complexes with metal ions

The data on ability of metal ions [Cu(II), Fe(II), Zn(II) and Mn(II)] to form complexes with humic acids (1/x values) are presented in the Table 9.

For Vertisol, the 1/x values for humic acid complexed with Cu(II), Fe(II), Zn(II) and Mn(II) were 0.66, 0.71, 0.74 and 0.75 in case of surface layer and 0.65, 0.71, 0.74 and 0.74 in case of sub-surface layer respectively.

For Alfisol, the 1/x values for humic acid complexed with Cu(II), Fe(II), Zn(II) and Mn(II) were 0.69, 0.68, 0.77 and 0.78 in the surface layer, and 0.69, 0.67, 0.78 and 0.76 in the sub-surface layer, respectively.

Generally,low values were obtained for Cu(II) - HA complexes, which indicate better ability of humic acid to form complex with Cu(II).

4.9.3 Stability constants of HA - metal complexes

The log K (stability constant) values of HA metal complexes were calculated from following equation:

$$\mathrm{Log}\left(\frac{\lambda_0}{\lambda}\right) = \log K + x \log (\quad A) \qquad \text{——} \qquad (VI)$$

For each concentration of HA, the mean values of log K were computed for individual metal ions. These values are presented in Tables 10 to 13.

Table 8: Moles of HA that reacted with one mole of metal ion [Cu(II), Fe(II), Zn(II) and Mn(II)] in formation of complex (x values)

Source of humic acid	Metal ions			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
A_1	1.43	1.46	1.29	1.28
A_2	1.43	1.48	1.27	1.31
V_1	1.51	1.40	1.34	1.33
V_2	1.52	1.39	1.34	1.35

Table 9: Ability of metal ions [Cu(II), Fe(II), Zn(II) and Mn(II)] to form complexes with humic acids (1/x values)

Source of humic acid	Metal ions			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
A_1	0.69	0.68	0.77	0.78
A_2	0.69	0.67	0.78	0.76
V_1	0.66	0.71	0.74	0.75
V_2	0.65	0.71	0.74	0.74

Table 10: Stability constants (Log K values) of HA-metal [Cu(II), Fe(II), Zn(II) and Mn(II)] complexes of HA obtained from Alfisol (A_1)

Humic acid ML^{-1}	Log K			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
0				
3 x 10^{-5}	6.65	6.56	6.07	5.90
6 x 10^{-5}	6.69	6.42	6.12	5.85
9 x 10^{-5}	6.63	6.53	6.11	5.88
12 x 10^{-5}	6.63	6.62	6.04	5.94
15 x 10^{-5}	6.62	6.57	6.06	5.82
18 x 10^{-5}	6.64	6.60	6.03	5.86
21 x 10^{-5}	6.69	6.58	6.08	5.88
24 x 10^{-5}	6.71	6.61	6.04	6.00
Mean	6.65	6.56	6.06	5.89

Table 11: Stability constants (Log K values) of HA-metal [Cu(II), Fe(II), Zn(II) and Mn(II)] complexes of HA obtained from Alfisol (A_2)

Humic acid ML^{-1}	Log K			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
0				
3 x 10^{-5}	6.68	6.69	6.09	6.04
6 x 10^{-5}	6.73	6.56	6.05	5.99
9 x 10^{-5}	6.67	6.65	6.06	5.97
12 x 10^{-5}	6.67	6.73	6.09	5.97
15 x 10^{-5}	6.65	6.68	6.05	5.99
18 x 10^{-5}	6.68	6.70	6.05	6.05
21 x 10^{-5}	6.72	6.69	6.08	6.00
24 x 10^{-5}	6.74	6.71	6.11	6.07
Mean	6.69	6.67	6.07	6.01

Table 12: Stability constants (Log K values) of HA-metal [Cu(II), Fe(II), Zn(II) and Mn(II)] complexes of HA obtained from Vertisol (V$_1$)

Humic acid ML^{-1}	Log K			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
0				
3 x 10^{-5}	6.83	6.63	6.32	6.15
6 x 10^{-5}	6.73	6.84	6.44	6.17
9 x 10^{-5}	6.69	6.59	6.35	6.14
12 x 10^{-5}	6.71	6.73	6.40	6.12
15 x 10^{-5}	6.78	6.58	6.34	6.13
18 x 10^{-5}	6.95	6.66	6.33	6.14
21 x 10^{-5}	7.04	6.78	6.35	6.15
24 x 10^{-5}	7.01	6.86	6.38	6.18
Mean	6.84	6.67	6.35	6.14

Table 13: Stability constants (Log K values) of HA-metal [Cu(II), Fe(II), Zn(II) and Mn(II)] complexes of HA obtained from Vertisol (V$_2$)

Humic acid ML^{-1}	Log K			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
0				
3 x 10^{-5}	6.87	6.65	6.36	6.22
6 x 10^{-5}	6.76	6.85	6.45	6.22
9 x 10^{-5}	6.72	6.61	6.36	6.24
12 x 10^{-5}	6.75	6.55	6.40	6.20
15 x 10^{-5}	6.85	6.60	6.34	6.20
18 x 10^{-5}	6.98	6.67	6.34	6.20
21 x 10^{-5}	7.08	6.80	6.33	6.22
24 x 10^{-5}	7.05	6.87	6.39	6.25
Mean	6.88	6.69	6.37	6.21

For the humic acid obtained from surface layer of Vertisol, the mean log K values were 6.84, 6.67, 6.35 and 6.14 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively. Whereas in case of humic acid obtained from sub-surface layer, these values were 6.88, 6.69, 6.37 and 6.21 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively.

The mean log K values of humic acid obtained from surface layer of Alfisol were 6.65, 6.56, 6.06 and 5.89 for Cu(II), Fe(II), Zn(II) and Mn(II),respectively. With regard to humic acid obtained from sub-surface layer, these values were 6.69, 6.67, 6.07 and 6.01 for Cu(II), Fe(II), Zn(II) and Mn(II), respectively.

Thus, it was observed that the order of stabilities of humic acid complexes and four divalent metal ions at pH 7 were Cu(II) > Fe(II) > Zn(II) > Mn(II). Similar orders were followed for both surface and sub-surface layers of Vertisol and Alfisol and these log K values increased with depth. Vertisol had higher log K values than Alfisol.

4.9.4 Free energy change during complexation

Mean values of standard free energy change ($-\Delta Gr^0$) for the complexation of metal ions with humic acids were calculated from the following formula

$$\Delta Gr^0 = -RT \ln K \qquad \text{———} \qquad (VIII)$$

K = stability constant

R = gas constant = 1.987 cal degree^{-1} mole^{-1}.

ΔGr^0 = standard free energy change in Kcal mole^{-1}

t = Absolute temperature

The data on free energy change ($-\Delta Gr^0$) are presented in Table 14.

The humic acid obtained from Vertisol had free energy (ΔGr^0) values of 9.48, 9.24, 8.80 and 8.51 in the surface layer and 9.53, 9.27, 8.83 and 8.61 in sub-surface layer for Cu(II), Fe(II), Zn(II) and Mn(II), respectively.

The humic acid obtained from Alfisol had free energy (ΔGr^0) values of 9.22, 9.09, 8.40 and 8.16 in the surface layer and 9.28, 9.24, 8.41 and 8.27 in sub-surface layer for Cu(II), Fe(II), Zn(II) and Mn(II) respectively. The ($-\Delta Gr^0$) values for Cu(II)-HA complexes were higher than for other metal - complexes.

4.10 AVAILABILITY OF ZINC FROM ZINC-HUMATE COMPLEXES TO RICE AND MAIZE

Two seperate pot culture experiments were conducted in the greenhouse to evaluate the availability of Zn-complexes of HA to rice and maize grown as test crops. Effects of these complexes were compared with $ZnSO_4$ on parameters viz., dry matter yield, zinc content and uptake besides the zinc content in post harvest soil samples.

Table 14: Standard free energy change($-\Delta Gr^o$) values for the complexation reactions of metal ions [Cu(II), Fe(II), Zn(II) and Mn(II)] with humic acid fractions

Source of humic acid	Metal ions			
	Cu(II)	Fe(II)	Zn(II)	Mn(II)
A_1	9.22	9.09	8.40	8.16
A_2	9.28	9.24	8.41	8.27
V_1	9.48	9.24	8.80	8.51
V_2	9.53	9.27	8.83	8.61

4.10.1 Rice

4.10.1.1 Dry matter production:

The data on dry matter production of rice as influenced by different sources and levels of Zinc are presented in Table 15 and are shown in Fig. 10a.

A close perusal of data reveals that Zinc application significantly increased the dry matter production. The treatments T_3 and T_4 resulted in significantly higher dry matter production than with other treatments. Between the levels, application of zinc at 2.5 ppm increased the dry matter production significantly over control while the increase was very small at 5.0 ppm level. The relative effectiveness of Zinc sources in increasing dry matter production followed the order : $ZnSo_4$ > Zn-humate > Zn-chelate.

The photograph showing the difference in plant growth as a result of application of zinc through various treatments are presented in plates 1 to 3.

4.10.1.2 Zinc content and uptake

The data on zinc content and uptake at flowering as influenced by different sources and levels of zinc are presented in Table 15.

A close look at the data indicates that the treatmental effects on zinc content and uptake were significant. The minimum zinc content of rice plants was 11.75 $\mu g\ g^{-1}$ when zinc was not applied while it increased significantly due to treatmental effects. Among the levels, application of zinc at 2.5 ppm

Table 15: Effect of sources and levels of zinc on dry matter production, plant zinc content, zinc uptake and zinc content in post harvest soil samples of rice

Treatment	Dry matter production (g pot^{-1})	Plant zinc content (µg g^{-1})	Zinc uptake (µg pot^{-1})	Zinc content in post harvest soil samples (µg g^{-1})
T_1 Control	7.58	11.75	89.32	0.47
T_2 Only HA	9.95	14.44	144.17	0.59
T_3 $ZnSO_4$-2.5 ppm	20.74	22.08	458.93	3.56
T_4 $ZnSO_4$-5 ppm	21.13	22.34	472.10	3.61
T_5 Zn humate-2.5 ppm	18.28	19.88	363.75	3.03
T_6 Zn humate-5 ppm	18.76	20.27	379.98	3.04
T_7 Zn-chelate-2.5 ppm	17.82	19.32	343.14	2.56
T_8 Zn-chelate-5 ppm	18.44	19.62	361.68	2.65
S.Ed.	0.83	0.76	23.03	0.23
CD (0.05)	1.77	1.62	48.83	0.48

Plate 1: Response of rice to zinc carriers (Zn-humate, Zn-chelate and $ZnSO_4$) at 2.5 ppm level of zinc

Plate 2: Response of rice to zinc carriers (Zn-humate, Zn-chelate and $ZnSO_4$) at 5 ppm level of zinc

Plate 3: Response of rice to only humic acid

gave maximum zinc concentration and it differed from 5 ppm level significantly.

The data on zinc content indicated a considerable increase with the application of zinc fertilizer. Application of the zinc complexes either as zinc humates (or) zinc chelates resulted in lower zinc uptake and the values were inferior to $ZnSO_4$ though they were showing increase with increasing level of its application through these sources.

4.10.1.3 Zinc content in post harvest soil samples:

The data on available zinc content after harvest of rice crop are presented in Table 15. The data indicated that the available zinc content was maximum in T_3 and T_4 as compared to other treatments. Between the two organic sources of zinc, Zn-humate was better to supply zinc to rice than zinc chelate.

4.10.2 Maize

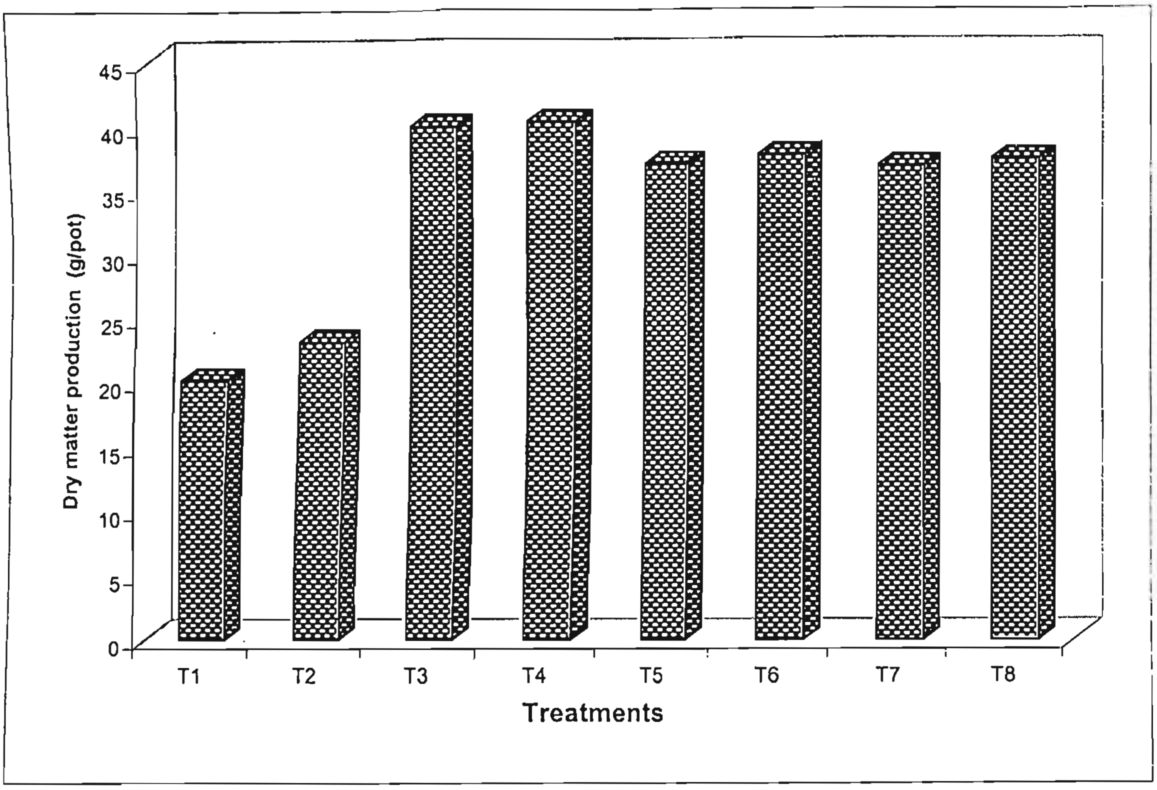
4.10.2.1 Dry matter production:

The data on dry matter production of maize as influenced by different sources and levels of zinc are presented in Table 16 and are shown in Fig.10b.

A close perusal of data reveals that zinc application significantly increased the dry matter production. The treatments $T_3$and T_4 resulted in significantly higher dry matter production than the other treatments. Among

Table 16: Effect of sources and levels of zinc on dry matter production, plant zinc content, zinc uptake and zinc content in post harvest soil samples of maize

Treatment	Dry matter production (g pot^{-1})	Plant zinc content (μg g^{-1})	Zinc uptake (μg pot^{-1})	Zinc content in post harvest soil samples (μg g^{-1})
T_1 Control	20.13	10.82	218.39	0.42
T_2 Only HA	23.11	13.56	289.73	0.61
T_3 $ZnSO_4$-2.5 ppm	39.85	21.11	840.80	3.44
T_4 $ZnSO_4$-5 ppm	40.23	21.50	866.39	3.62
T_5 Zn humate-2.5 ppm	36.84	19.86	732.17	2.64
T_6 Zn humate-5 ppm	37.55	20.18	758.37	2.95
T_7 Zn-chelate-2.5 ppm	36.58	19.27	705.25	2.46
T_8 Zn-chelate-5 ppm	37.14	19.43	723.15	2.60
S.Ed.	0.87	0.57	33.18	0.28
CD (0.05)	1.85	1.22	70.34	0.59



T_1 : Control
T_2 : Only HA
T_3 : $ZnSO_4$ - 2.5 ppm
T_4 : $ZnSO_4$ - 5 ppm
T_5 : Zn humate - 2.5 ppm
T_6 : Zn humate - 5 ppm
T_7 : Zn chelate - 2.5 ppm
T_8 : Zn chelate - 5 ppm

Fig. 10b: Effect of sources and levels of zinc on dry matter production of maize the levels, application of zinc at 2.5 ppm increased dry matter production significantly over control while the increase was slight at 5.0 ppm level. The relative effectiveness of zinc sources in increasing dry matter production followed the order: $ZnSO_4$ > Zn-humate > Zn-chelate.

4.10.2.2 Zinc content and uptake:

The data on zinc content and uptake of maize at flowering as influenced by different sources and levels of zinc are presented in Table 16. A close look at the data indicates that the treatmental differences were significant with respect to zinc content and uptake. The lowest zinc content of maize plants (10.82 $\mu g\ g^{-1}$) was observed when zinc was not applied. Among the levels, 2.5 ppm zinc gave maximum increase in zinc concentration followed by 5 ppm level.

The data on zinc uptake indicated that it increased considerably due to application of zinc fertilizer. In comparison to control, application of zinc complexes either as zinc humate or zinc chelate resulted in higher zinc uptake though it was inferior to zinc sulphate. However, the values were showing increase with increase in level of application of zinc through any of the sources used.

4.10.2.3 Zinc content in post harvest soil samples:

The data on available zinc content after harvest of maize crop are presented in Table 16. The data indicated that the soil zinc content was highest in treatments T_3 and T_4 among all the treatments tried. The organic sources of zinc i.e., zinc humate and Zn-chelate applied at 2.5 ppm and 5 ppm levels were on par with each other with respect to soil zinc content though they were superior to T_1 and T_2. However, control with the lowest available zinc content was inferior to all other treatments.

...

DISCUSSION

CHAPTER V

DISCUSSION

The soils of ANGRAU, Rajendranagar campus, Hyderabad are mainly of semi-arid tropical in origin and contain low to moderate amounts of organic matter. These soils have been brought under intensive cultivation during the past 3-4 decades. Since the soil organic matter constitutes a dynamic system, it is subject to continual changes during the process of its decomposition. The land use patterns altered and transformed the ecological conditions of soils with respect to their organic matter content. Earlier, these soils were under natural vegetation with thick forest trees under impeded drainage conditions. In the farm of Directorate of Rice Research, where the regular research work related to rice is being carried out, the soils are continuously subjected to submergence and hence, are in reduced conditions. In National Research Centre for Sorghum, where the research work related to sorghum crop under dry/irrigated dry conditions is being carried out, the soils are under oxidised conditions. Thus, continuous cultivation under these moisture regimes might have altered several of the characteristics of soils besides the organic matter content. Hence, it is felt ideal to prepare the latest map indicating the organic carbon status of soils of fields belonging to these two research institutes.

The organic carbon content of soils of Directorate of Rice Research varied from a minimum of 0.36 per cent to a maximum of 1.15 per cent (Table 2). Out of the 68 samples analysed, 20.6, 51.5 and 27.9 per cent soils were grouped under low, medium and high categories, respectively as per the ratings suggested by Jackson (1967). Similarly in the soils of National Research Centre for Sorghum, the organic carbon content ranged from 0.23 to 1.04 per cent (Table 3). Out of 25 samples analysed, 28 per cent fell under low, 56 per cent under medium and 16 per cent belonged to high categories.

The maps indicating the organic carbon content of Directorate of Rice Research and National Research Centre for Sorghum are dipicted in Figs. 11 and 12.

The soils of the farm field selected for fractionation studies were an Alfisol from Students' farm and a Vertisol from Agricultural Research Institute. These were neutral to slightly alkaline in reaction and have been subjected to continuous cultivation. These soils had a lower contents of total nitrogen. These results support the results of Nagamadhuri (1996) and Sujana Reddy (1997) that the soils under continuous cultivation had a lower total N content than similar soils under natural vegetation. Chaudhury and Saha (1973) also reported a wide variation in the total N content in surface and subsurface layers of different soils of West Bengal. Further, soils selected under study showed higher amounts of available nitrogen (Table 5). This could be attributed to the accumulation of N from the added fertilizer sources. These soils have been put to use for the past 40-50 years and were put to intensive cultivation. The use of inorganic fertilizers for increased crop production in these soils might have caused an increase in the available N content of both surface and sub-surface layers. Hence, it is possible to conclude that bulk of available N content was contributed from inorganic

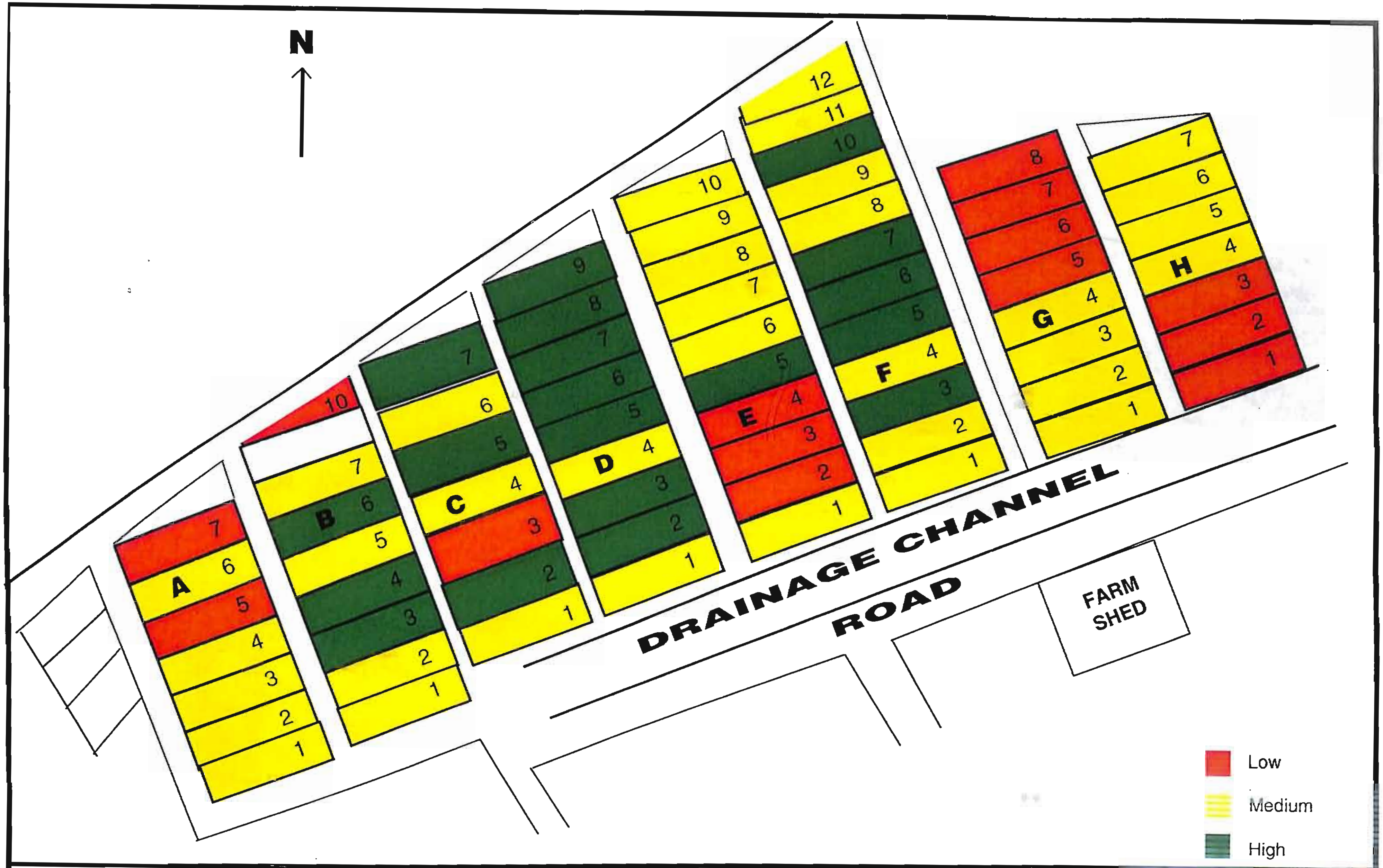


Fig 11: Map indicating organic carbon status of soils of Directorate of Rice Research, Rajendranagar, Hyderabad.

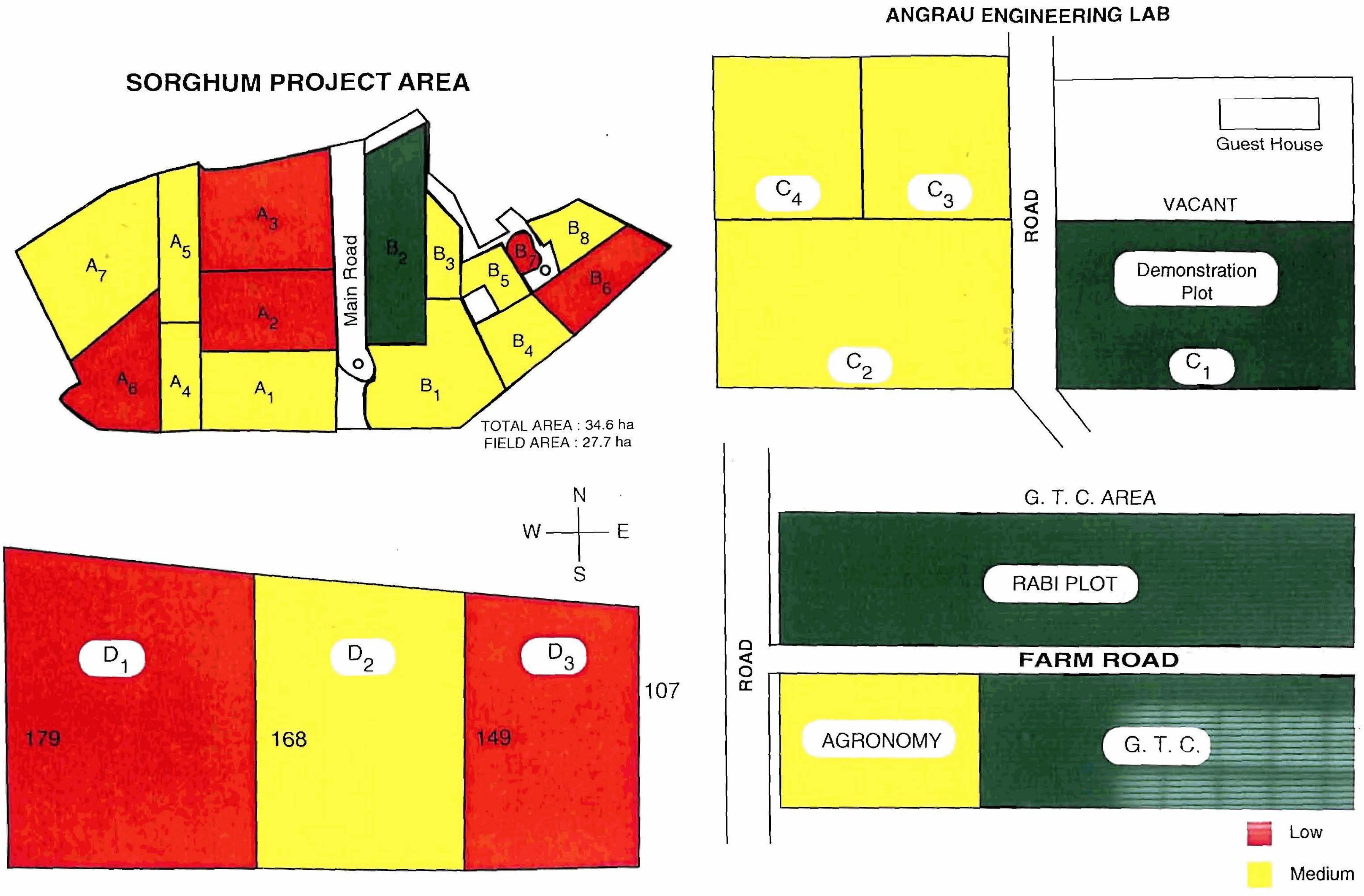


Fig.2: Map indicating organic carbon status of soils of National Research Centre for Sorghum.

sources. Similar observations were made by Rasmussen *et al.* (1980), Janzen (1987), Campbell and Zentner (1991), Sujana Reddy (1997), Nagamadhuri *et al.* (1998). They reported that the addition of N fertilizers on regular basis for many years led to an increase in nitrogen content of soils. Tiessen and Stewart (1983) reported that the mineralization of organic matter occurred as a result of continuous cultivation and it contributed to the increase in available nitrogen status of soils. The C/N ratios of these soils were low as compared to C/N ratio of similar soils under natural vegetation. Janzen *et al.* (1992) studied the contents of organic matter and nitrogen in soils under long term crop rotation and fertilization treatments and observed that the differences in organic matter contents were due to variable agronomic and fertilizer practices adopted and variations in the rates of substrate decomposition. Veroney *et al.* (1981) established the fact that large amounts of organic matter were lost during long term cultivation. Changes in organic matter content and C/N ratio are also directly related to the amounts of organic manure added (Rasmussen *et al.*, 1980).

5.1 COMPOSITION OF SOIL HUMUS

The humic acid fractions extracted from the selected soils were analysed for their functional groups. The functional group analysis provides information about the occurrence of major functional groups in humic acid and are thus, an index of their reactivity. The contents of oxygen containing functional groups (Table 6) obtained from the humic acids extracted from soils are similar to those obtained by Banerjee and Mukherjee (1972), Zende and Raman (1978), Challa and Raman (1984), Sujana Reddy (1997) and

Nagamadhuri *et al.* (1998). The lower contents of oxygen containing functional groups in soils (as reported here) as compared to the high contents of oxygen containing functional groups in similar soils under natural vegetation could be attributed to the inherent difference in chemical composition and molecular weights of humic substances (Dkhar *et al.*, 1986; Sujana Reddy, 1997; Nagamadhuri *et al.*, 1998). The higher contents of total acidity and -COOH groups in the sub-surface layer as compared to the surface layer indicate their higher potential for interaction with metals and clays. The easier movement as well as the rapid decomposition of -COOH of aliphatic compounds as compared to aromatic compounds tended to increase the relative proportion of phenolic-OH groups in the surface layer.

The insignificant variations in the content of functional groups of humic acid fraction extracted from these soils suggested that the humic acid is relatively more resistant to changes and this can also be attributed to its insolubulity in acidic pH. Further,the highly complex structure of humic acid might have accounted for this (Zende and Raman, 1978; Stevenson, 1982).

The elemental analysis carried out by Nagamadhuri (1996) and Sujana Reddy (1997) along with the functional group analysis of humic acids were used in deriving the molecular weight and the average molecular weight of humic acid, which was assumed to be around 1000. These values are at best only tentative, covering limited information but do not show the exact relationship of elemental analysis and functional groups to the unreactive matrix of humic acids.

Potentiometric and conductometric titrations have been used to characterise the acidic functional groups in humic acid. The potentiometric titration curves of humic acids (Fig.2) were sigmoidal in nature indicating weak acidic character of HA. Similar results were obtained by Mukhopadhyay *et al.* (1982), Sharma and Gupta (1987), Nagamadhuri *et al.* (1998), Sujana Reddy *et al.* (1999). The conductometric titration curves (Fig.3) of humic acid showed a slow increase in the initial stage followed by a steep increase at later stages.

Measurement of absorbance in different regions of electromagnetic spectrum has been used for qualitative and quantitative investigations on humic substances (Stevenson, 1982). The ratio of optical densities at 465 and 665 nm is often used for characterisation of humic substances (Schnitzer and Khan, 1972; Stevenson, 1982). This ratio referred to as E_4/E_6 ratio and is independent of concentration of humic acid but varies with humic material extracted from different soil types (Schnitzer and Khan, 1972; Stevenson, 1991; and Nagamadhuri, 1996). It is believed that the magnitude of E_4/E_6 ratio is related to the degree of condensation of aromatic carbon network; the low ratio being indicative of high degree of condensation of aromatic humic acid (Chen *et al.*, 1977; Stevenson, 1982). The optical densities of humic acids obtained from sub-surface layer were higher than those obtained from surface layers. It could be due to higher degree of aromaticity in the carbon atoms of the humic acid of sub-surface layer than that is occurring in the top layer.

The absorbance of light in UV range is due to the presence of multiple bonds and due to unshared electron pair in the organic molecule. These groups which confer colour to the humic substances, are called chromophores. The typical chromophores known to occur in humic acid are C=C and C=O groups (Stevenson, 1982). The UV spectra of one of the humic acids obtained in this study is presented in Fig.1. The humic acid from other soils and depths followed similar pattern. The UV spectra of humic acids were featureless with a decrease in optical density with an increase in wave length. It is interesting to observe that the humic acids of diverse origin were similar inspite of differences in their composition. The lack of absorbance in UV range could be due to the fact that the humic substances are considered to be an intermediate state of development between lignin and coal (Schnitzer and Khan, 1972; Schnitzer, 1978; Stevenson, 1982).

5.2 CHARACTERISATION OF METAL-HUMIC ACID COMPLEXES

5.2.1 Potentiometric methods

Potentiometric measurements have been used extensively to provide information about the contribution of metal complexes. According to Chaberek and Martell (1959), the pH effect is as much a property of metal complex as its absorption intensity. Potentiometric measurements have been claimed to be convenient way to study metal-HA interactions (Chakravarti *et al.*, 1984). The titration of metal ion with alkali is presented in Fig.4. The formation of metal hydroxide was indicated by the inflection in titration curves at pH 5.3 for Cu(II) at 7.5 and 9.6 for Zn(II), at 8.4 for Fe(II) and

8.7 for Mn(II). These results are similar to those reported for Cu(II) (Schnitzer and Skinner, 1963; Stevenson, 1977), Zn(II) (Meelu and Randhawa, 1971; Zende and Raman, 1978), Fe(II) (Relan *et al.*, 1986) and Mn(II) (Khan, 1969; Schnitzer and Khan, 1972). The pH drop on addition of metal ions to humic acid followed the order: Cu(II) > Zn(II) > Fe(II) > Mn(II), which was dependent on the nature of metal ion. The decrease in pH might be due to displacement of proton from the acidic groups in humic acid. A possibility also exists for the formation of hydroxy complex as a result of dissolution (or) transfer of molecules bound to the metal ion at high pH (Stevenson, 1976).

The titration curves presented in Figs. 5a to 5d are almost similar for humic acids obtained both from Alfisol and Vertisol. However, the displacements in curves of humic acids in presence of metal ions were wider at high pH while reverse was true at lower pH. It can be explained that the formation of metal complex involves displacement of H^+ ions from the ligand according to the following scheme.

$M^{n+} + HA \longrightarrow MA^{n-1} + H^+$ ——— (IX)

$MA^{n-1} + HA \longrightarrow MA^{n-2} + H^+$ ——— (X)

$M^{n+} + HmA \longrightarrow MA^{n-m} + mH^+$ ——— (XI)

Humic acid contains large number of acidic functional groups and several H^+ ions are displaced during complexation leading to a drop in pH of the solution. This pH drop is taken as a quantitative indication of complex formation. Absence of such inflection in the presence of HA (Fig.5a to 5d) indicates that the metal ion formed complex with these fractions (Meelu and Randhawa, 1971; Zende and Raman, 1978; Challa and Raman, 1984; Sujana Reddy *et al.*, 1998). The magnitude of pH drop on addition of metal ion to humic acid followed the order Cu(II) > Zn(II) > Fe(II) > Mn(II) at pH 7.4-7.7, which was dependent upon the nature of the metal ion. The decrease in pH might be due to displacement of protons from acidic functional groups of humic acid (Relan *et al.*, 1986) and at pH greater than 7.8, the order followed was Zn(II) > Cu(II), then followed the Irving - William series (Pb > Cu > Ni > Co > Zn > Cd > Fe > Mn > Mg) confirming the results of Khanna and Stevenson (1962), Banerjee and Mukherjee (1972), Shah *et al.* (1976), and Challa and Raman (1984). The potentiometric titration of Cu(II) indicated a higher tendency of Cu for complexation than other metal ions and confirming the fact that greater the tendency of a metal ion to combine with complexing agent, greater is the drop in pH (Martell and Calvin, 1952).

The higher stability of Cu(II) and Zn(II) humic acid complexes could be attributed to the coordinate covalent bonding. Since Cu(II) is a transition metal ion belonging to the first transition series with $3d^9$ configuration and with partly filled d orbitals, it can accept, π electrons from organic molecules (electron donors), thus, forming stable complexes with humic acid. In many of the situations, the transition metal ions with low oxidation state and the associated ligand molecule possessing vacant 'd' orbital of ligands accept the electrons from filled metals to form bonds, thus the bond is further strengthened. In case of Zn(II), even though it is a transition metal, due to its $3d^{10}$ configuration, there is little affinity for this metal ion for complexation either through octahedral (d_2sp^3 hybridisation) or through square planar (d_2sp^2 hybridisation) complexation. Thus, the complexation might be due to π acidity, and π complexes are weak with low stabilities. Similar observations also hold good for Mn(II) and Fe(II). The number of moles of humic acid that reacted with one mole of metal ion determines the availability of these ionic nutrients to plants. Hence, the availability of Cu(II) when it is complexed with humic acid will be low as compared to Zn(II), Fe(II) and Mn(II). These findings confirm the observation of Zende and Raman (1978), Challa and Raman (1984) and Nand Ram and Raman (1988).

It is noteworthy that none of the metal ions precipitated during the course of titration and addition of all metal ions to humic acid solution decreased the pH sometimes slightly. These experiments demonstrated that one mole of humic acid could form a water soluble complex with at least one mole of each of free metal ions. This does not exclude the possibility of formation of higher complexes.

5.2.2 Absorption spectroscopic methods

The absorption spectroscopic methods are based on variation in optical densities of solutions containing different ratios of metal ions and complexing agent while simultaneously maintaining a constant total concentration of reactants. This method has been used for studying the complex formation between humic substances and metal ions, by several workers (Schnitzer and Hansen, 1970; Schnitzer and Khan, 1972; Geisy *et* *al.*, 1986; Stevenson and Chen, 1991) applied continuous distribution models to calculate the apparent stability constants of Cu(II) complex by humic acid. Sujana Reddy *et al.* (1999) used absorption spectroscopic methods to characterize the interaction between metal ion viz., Cu(II), Zn(II), Mn(II) and Fe(II) and humic acids obtained from different soils of ANGRAU, Rajendranagar campus.

The absorption spectra of solutions of humic acids (Fig.6a) in the visible range were featureless showing no maximum or minimum and the absorbance decreased as the wavelength increased. Increased absorbance at shorter wavelength (λ) was attributed to the increased mobilities of electrons of aromatic carbon nuclei (Schnitzer and Khan, 1972). At a constant total concentration of humic acid and metal ion and when these are brought together. The absorbance of resulting metal humate was low as compared to the corresponding concentration of humic acid alone (Fig.6a & b). The wavelength of maximum absorbance (λmax) was determined as the difference between the absorbance of humic acid and metal humate, since both the humic acid and metal ion were coloured and were responsible for absorbance, the absorbance due to metal humate was subtracted from the absorbance of humic acid at each concentration, as suggested by Martell and Calvin (1952) to get the values of y. Then, y was plotted as the function of x (x=mole fraction of one of the component) for obtaining job's plot. A close perusal of job's plot (Figs.8a & b) suggests that the inflection in the difference of absorbance of humic acid and metal humate (y) occurred at 60% of concentration of humic acid and 40% of concentration of metal ion. This

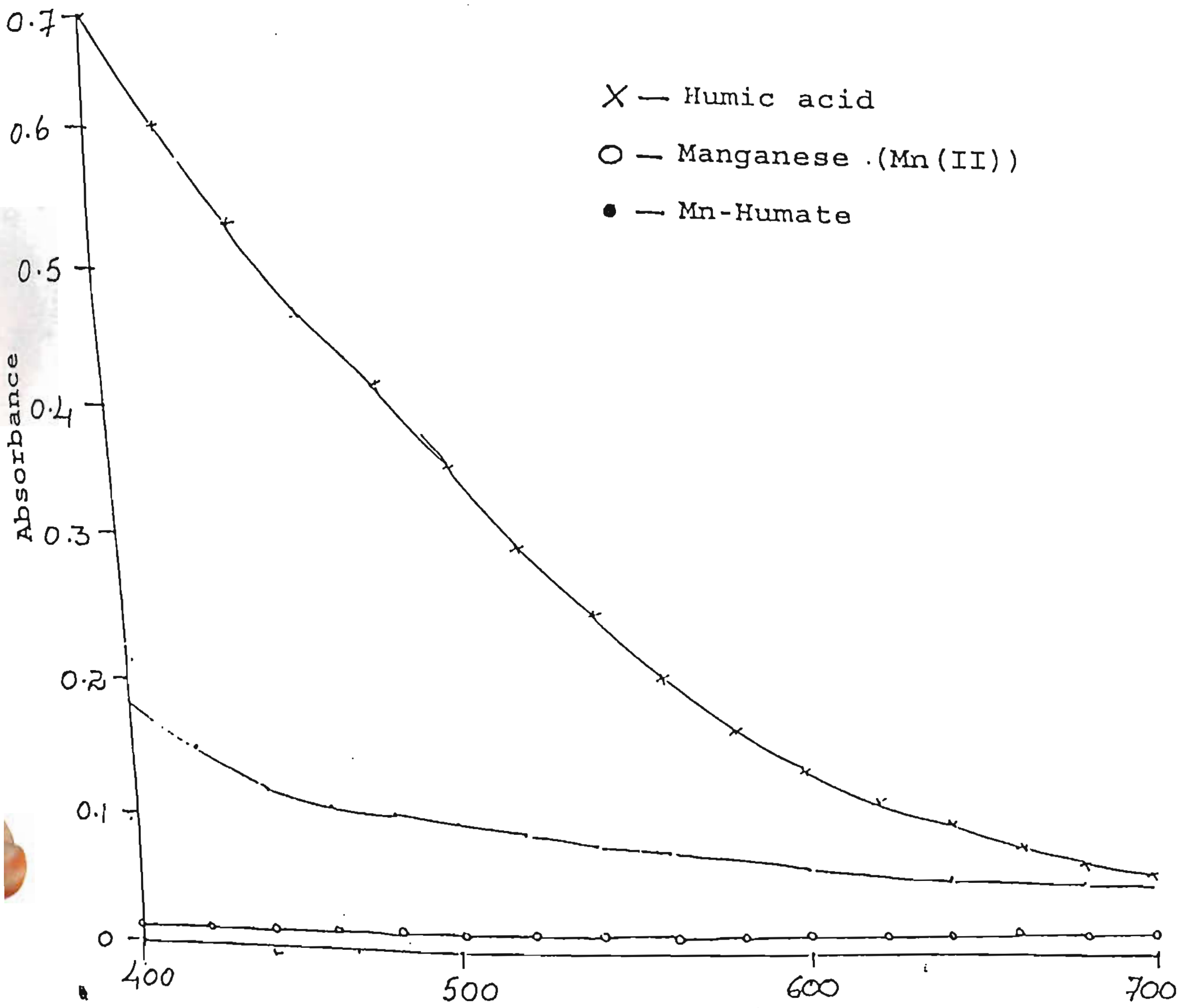


Fig.6 a: ABSORPTION SPECTRA OF METAL HUMATES, HUMIC ACID AND METAL IONS

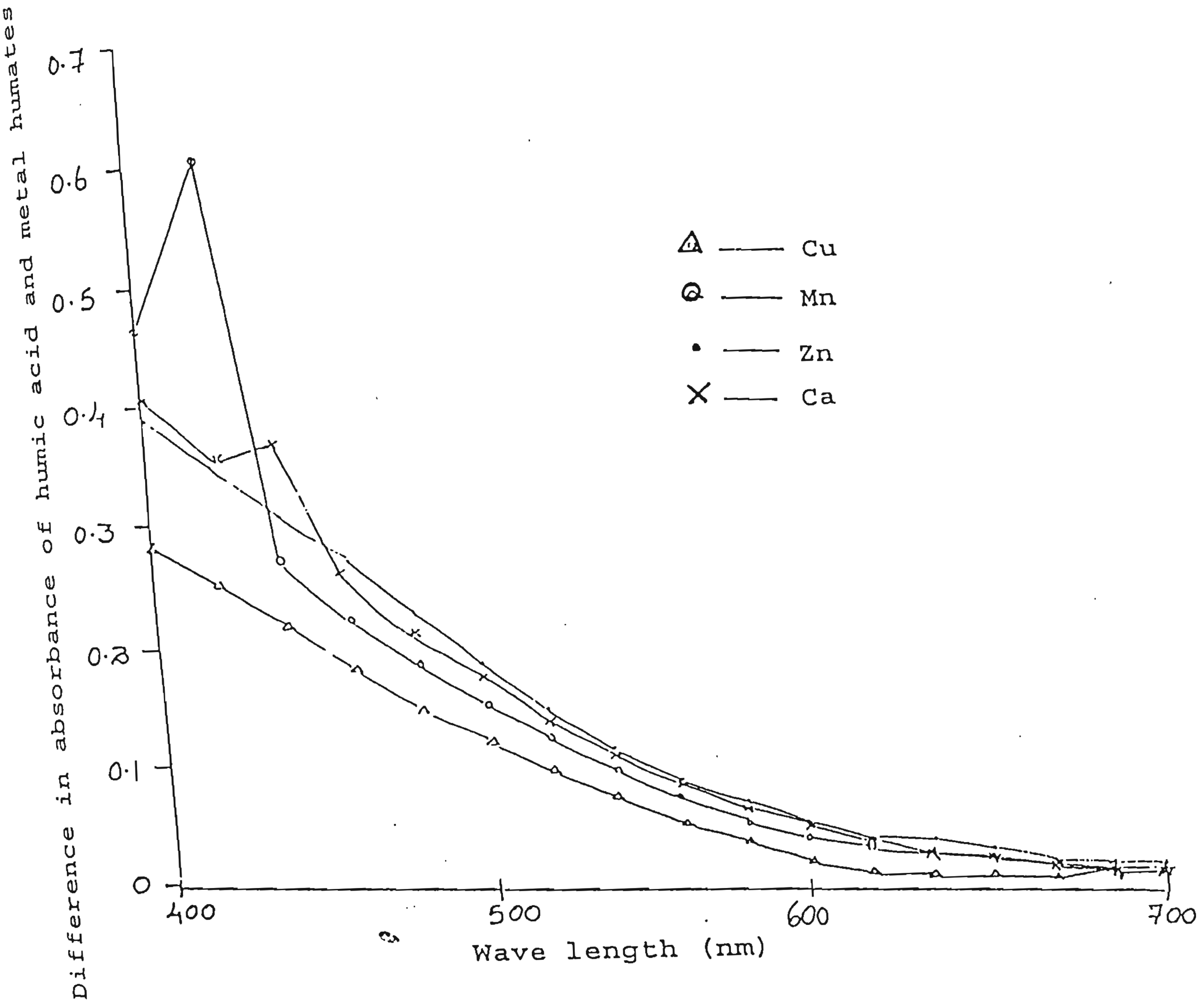


Fig.6b: Absorption spectra of metal humates, humic acid and metal ions

suggests that three moles of metal viz., Zn(II); Cu(II); Mn(II) and Fe(II) reacted with two moles of humic acid leading to the formation of 1.5:1 complex.

5.3 STABILITY CONSTANTS OF METAL-HA COMPLEXES

The complexation behaviour of humic acids with metal ions depends on the quality and quantity of functional groups present in humic acids and also on the type of metal ion. The extent of dissociation of functional groups on humic acids also determine the binding capacity of humic acid with metal ion. In the complex formation, the complexing agent acts as electron donor, while metal ion acts as an electron acceptor, in which metal either may bridge the clay and organic radical as in clay-metal-organic complexes or may directly link with the functional groups of humic acid leading to the formation of mettallo-organic complexes (Mortland, 1970; Stevenson, 1982). The data on number of moles of humic acid that reacted with one mole of metal ion (x-values) at pH 7.0 and at a temperature of 30°C (Table 8) showed higher values than those reported at pH 3.5 and 5.0 by other workers (Schnitzer and Skinner, 1966; Levesque, 1969; Challa and Raman, 1984; Nand Ram and Raman, 1988). It indicates the variation in degree of ionisation of functional groups at higher pH levels. The reactivity of functional group of humic acid would thus change with change in pH. From the data it is also clear that x-values did not differ for different metal ions. It is worth mentioning here that these values were determined at such a pH wherein humic acid remains in solution with dissociation of all the functional groups. In general slightly higher x-values were observed in the present investigation which was carried out at pH 7.0. This could be attributed to increased ionisation of functional groups leading to the more number of moles of ligand for complexing with one mole of metal ion. The x-values of Cu-HA complexes were higher than the other metal complexes. The number of moles of humic acid that reacted with one mole of metal ion (x-value) determines the availability of nutrients (metal ions) to plants. Hence, at comparable concentrations, the availability of copper when it is complexed with humic acid will be low as compared with the other metal ions (Challa and Raman, 1984).

In all the cases, x-values obtained were not whole numbers. This indicated the formation of polynuclear complexes (Randhawa and Broadbent, 1965). The x-values for all the metal ion-HA complexes were approximately a unit value which suggests the formation of 1:1.5 metal-HA complexes.

Stability constants are numerical expressions of stabilities of metal-HA complexes. These constants would not only help understand better to the role of these complexes in soil system, but also enable to predict their behaviour (Schnitzer and Skinner, 1967). It was observed that the stability constants (Log K values) were high at pH 7.0 and temperature of 30°C used in the present study and followed the order Cu (II) > Fe (II) > Zn (II) > Mn (II), indicating that the metal ion is bonded with a large number of active sites, thereby, increasing the stability of complex formed. Several workers have reported higher values for stability constants of humic substances with metal complexes at high pH level (Randhawa and Bradbent, 1965; Meelu and Randhawa, 1971; Dijk, 1971; Dhillon *et al.*, 1975; Zende and Raman, 1978; Challa and Raman, 1984) indicating the increased ionization of functional groups.

Further, the log K values of humic acid obtained from subsurface layer were higher than those of humic acid obtained from surface layer. This could be due to higher total acidity and carboxyl groups in the lower layers. Also the log K values for Vertisol were higher than those of Alfisol. From this it is clear that the log K values were directly proportional to amount of oxygen containing functional groups which are present in large numbers in the former than the latter. More number of functional groups will lead to high log K values. The carboxylic groups seem to predominate over the phenolic OH groups in case of their ability to form stable complexes. The stabilities of metal complexes followed the order Cu (II) > Fe (II) > Zn (II) > Mn (II). Similar order of stabilities was reported by Challa and Raman (1984); and Nand Ram and Raman (1988).

The higher stability of Cu (II)-HA complexes can be attributed to the coordinate covalent bond between the complexing agent and the metal ion. Cu (II), being a transitional metal ion with $3d^9$ configuration and partly filled d-orbitals, can accept the π-electrons from the complexing agent and, thus, forms stable complexes with humic acid (Zende and Raman, 1978; Stevenson, 1982; Nand Ram and Raman, 1988). The ligand molecules possess vacant orbitals and accept the electrons from the filled in metal orbitals to form π bonding and σ bonding. The bond is formed due to donation of lone pair of electrons. This type of bonding makes the metal ligand complex much stronger (Schnitzer and Skinner, 1967; Stevenson, 1982; Challa and Raman, 1984; Nand Ram and Raman, 1988; Stevenson, 1991). In case of Zn (II), even though it is also a transititional metal ion, but zinc complexes have lower stabilities than Cu (II) complexes due to the fact that with $3d^{10}$ configuration, it has completely filled d orbitals. Hence, there is no attraction of organic molecule either through octahedral (d_2Sp^3 hybridization) (or) through square planar (dSp^2 hybridization). These organo-metal complexes might have been formed through π bonding and the π complexes, thus, formed are weak with low stabilities.

The standard free energy change (ΔGr^o) for the complexation of metal ions with humic acid was computed from the respective values of their stability constants using the equation 8. The negative values of ΔGr^o (Table 14) suggested that the complexation reaction between metal ion and humic acid was spontaneous (Dkhar *et al.*, 1985; Nand Ram and Raman, 1988). The larger negative ΔGr^o values for Cu (II) - HA complexes suggested that the complexes formed between humic acid and Cu (II) are thermodynamically more stable than the other metal ion complexes under study. The higher thermodynamic stability of Cu-HA complexes can be attributed to the existance of coordinate covalent bonds between the complexing agent (HA) and the Cu (II) ions, possibly due to the formation of σ bond by donation of lone pair of electrons from the complexing agents to Cu (II) having $3d^9$ configuration. On the other hand, Zn^{+2} with $3d^{10}$ configuration has no attraction for the organic ligands either through octahedral (d_2SP_3 hybridization) or square planar (dSP_2 hybridization) and the Zn-complexes could be formed only through π bonding, which is weaker than σ bond leading to their lesser stability (Irving and William, 1948; Nand Ram and Raman, 1988).

5.4 AVAILABILITY OF ZINC FROM ZINC-HUMATE COMPLEXES TO RICE AND MAIZE

The advent of high yielding varieties of crops and increased intensity of cultivation coupled with imbalanced fertilizer application have resulted in deficiencies of micronutrients particularly that of zinc and there is a need to search for the new carrier of zinc, which can help in increasing yields through balanced fertilisation. Two separate pot culture experiments were conducted with zinc humic acid complex as a source comparing it with zinc chelate and $ZnSO_4$. The complex of Zn was prepared in such a way that complete complexation of added metal ion was assured. To this end, the ratio of HA to metal ion was manifested such that the metal ion forms the complex without free (or) non complexed ion. This ratio was obtained from the extent of stability constant determination (x value).

The results obtained on drymatter production of rice (Table 15) and maize (Table 16) indicated that $ZnSO_4$ produced higher than zinc humates. This can be attributed to the increased solubility of Zn from $ZnSO_4$ than from zinc humate rendering its more availability to crop plants. In both the experiments, the levels of Zn did not differ significantly from each other. A dose of 2.5 ppm of zinc was found to be optimum for rice and maize under greenhouse conditions through these sources. The percentage increase in dry matter production over control was 141.16, 135.09 and 173.61 in rice and

83.01, 81.71 and 97.96 in maize due to Zn-humate, Zn-chelate and $ZnSO_4$ at 2.5 ppm level.

The results further showed that mere addition of humic acid alone gave higher dry matter production than control indicating that humic acid acts as a supplier and regulator of plant nutrients. Some of the polyphenols which serve as respiratory catalysts high chlorophyll and stimulate nutrient uptake and growth rates (Linehan, 1978; Tan and Nopamornbodi, 1979; Milap Chand *et al.*, 1980; Singh *et al.*, 1980; Rauthan and Schnitzer, 1981; Gupta and Deb, 1985; Visser, 1985; Piccolo *et al.*, 1992; Young and Chen, 1997; Pandeya *et al.*, 1998). Both the content of zinc and its uptake (Tables 15 and 16) increased as a result of zinc application (Subramanyam and Mehta, 1974) to these crops. Higher zinc content in post harvest soil samples was observed with $ZnSO_4$ than other treatments.

Based on the above discussion, the following conclusions could be drawn:

1) The organic carbon content of soils of Directorate of Rice Research ranged from 0.36 per cent to 1.15 per cent. Out of 68 samples analysed, 20.6 per cent were grouped under low, 51.5 per cent under medium and 27.9 per cent under high categories.

2) In the soils of National Research Centre for Sorghum, the organic carbon content ranged from 0.23 to 1.04 per cent. Out of 25 samples analysed, 28 per cent fell under low, 56 per cent under medium and 16 per cent under high categories.

3) Soils under study contained higher amount of available nitrogen. The use of inorganic fertilizers for increased crop production in these soils caused an increase in the available N content.

4) Humic acid obtained from sub-surface layer of an Alfisol and a Vertisol contained higher contents of total acidity and carboxyl groups than the surface layer.

5) The potentiometric titration curves of humic acids were sigmoidal in nature, attesting the high buffering capacity associated with them. The UV-spectra of humic acids were featureless showing a decreasing optical density with increasing wavelength.

6) The potentiometric titration curves of metal humates indicated that the formation of metal hydroxide was suppressed when metal ions were added to humic acid and the titration curves of all humic acids were the same irrespective of their sources. The reduction in pH on titration of humic acid in the presence of various metal ions followed the order: Cu(II) > Zn(II) > Fe(II) > Mn(II) at pH 7.5 and Zn(II) > Cu(II) at pH above 7.8.

7) Job's plot of humic acids indicated the formation of 1.5:1 complexes with Cu(II), Fe(II), Zn(II) and Mn(II).

8) The number of moles of humic acid that reacted with one mole of metal ion (x-value) varied with type of metal ion and with type of organic fraction. These values suggested the formation of 1.5:1 HA-metal complexes.

9) The lower $1/_x$ values were obtained for Cu(II)-HA complexes, which indicate better ability of humic acid to form complex with Cu(II).

10) The order of stabilities of humic acid complexes and four divalent metal ions at pH 7 were Cu(II) > Fe(II) > Zn(II) > Mn(II). The log K values for humic acid obtained from subsurface layer were higher than those of surface layer and these values obtained for humic acid extracted from Vertisol were higher than those of the humic acid extracted from Alfisol.

11) The negative values of ΔGr^0 suggested that the complexation reaction between metal ion and humic acid was spontaneous.

12) The percentage increase in dry matter production over control was 141.16, 135.09 and 173.11 in rice and 83.01, 81.71 and 97.96 in maize due to Zn-humate, Zn-chelate and $ZnSO_4$ at 2.5 ppm level.

13) The effectiveness of different zinc sources followed the order: $ZnSO_4$ > Zn-humate > Zn-chelate with respect to dry matter production, plant zinc content and uptake besides the zinc content in post harvest soil samples.

14) The dose of 2.5 ppm zinc was found to be optimum for rice and maize crops grown under pot culture conditions.

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SUMMARY

CHAPTER VI

SUMMARY

An investigation entitled **"Interactions between metal ions and humic fractions extracted from an Alfisol and a Vertisol in relation to their plant availability"** was carried out with a view to characterise the nature of organic matter, the complexes formed by interaction of humic fractions with Cu(II), Zn(II), Fe(II) and Mn(II) and to evaluate the relative effectiveness of zinc carriers in zinc nutrition of rice and maize. The organic carbon mapping of soils of the fields belonging to research farms under the control of Directorate of Rice Research and National Research Centre for Sorghum located at Rajendranagar was also taken up and they were characterised into low, medium and high categories based on the accepted ratings.

The soils of Directorate of Rice Research and National Research Centre for Sorghum, Rajendranagar campus were evaluated for their status with respect to organic carbon and organic matter contents.

The organic matter was isolated, fractionated and purified by following Tyurin's method in selected Alfisol and Vertisol from two depths (0-15 cm and 15-30 cm). The humic acid fraction was characterised by functional group analysis, spectral properties and potentiometric and conductometric titrations.

The interactions between selected humic acids and metal ions (Cu(II), Zn(II), Fe(II) and Mn(II) were evaluated by potentiometric titrations and absorption spectroscopic methods besides working out stability constants.

For evaluating the relative effectiveness of zinc humates as compared to other zinc sources, rice and maize crops were grown on Alfisol under pot culture conditions.

The results obtained are summarised as follows:

1. The organic carbon content of soils of Directorate of Rice Research ranged from a minimum of 0.36 per cent to a maximum of 1.15 per cent. Out of 68 samples analysed, 20.6 per cent were grouped under low, 51.5 per cent under medium and 27.9 per cent under high categories.

2. In the soils of National Research Centre for Sorghum, the organic carbon content ranged from 0.23 to 1.04 per cent. Out of 25 samples analysed, 28 per cent fell under low, 56 per cent under medium and 16 per cent under high categories.

3. Vertisol contained higher amount of organic carbon as compared to Alfisol. In both the soils, sub-surface layers had lower content of organic carbon than that of surface layer.

4. The available nitrogen contents were higher in these soils due to continuous application of inorganic fertilizers.

5. The total nitrogen, available nitrogen and C/N ratios were higher in Vertisol than that of Alfisol. Total nitrogen and available nitrogen in both the soils showed a decrease with increase in depth. The C/N ratios also increased with increase in depth of soil.

6. The contents of total acidity, carboxyl groups and phenolic-OH groups were high in humic acid extracted from Vertisol as compared to humic acid extracted from Alfisol. The total acidity and carboxyl group contents increased with depth and phenolic-OH groups decreased with depth.

7. The potentiometric titration curves of humic acids were sigmoidal in shape indicating the high buffering capacity of these materials. The UV-spectra of humic acids were featureless showing a decreasing optical density with an increasing wavelength.

8. The potentiometric titration curves of metal humates showed no inflection indicating that the formation of metal hydroxides was suppressed when metal ions are added to humic acid indicating the formation of a complex. The titration curves of all humic acids are of similar nature irrespective of their sources. The reduction in pH on titration of humic acid in presence of various metal ions followed the order: Cu(II) > Zn(II) > Fe(II) > Mn(II) at a pH 7.5 and Zn(II) > Cu(II) at above pH 7.8.

9. Job's plot of humic acids indicated the formation of 1.5:1 complexes with Cu(II), Zn(II), Mn(II) and Fe(II).

10. The number of moles of humic acid that reacted with one mole of metal ion (x-value) varied with type of metal ion and with type of organic fraction. These values suggested the formation of 1.5:1 HA-metal complexes.

11. The lower 1/x values were obtained for Cu(II)-HA complexes, which indicated better ability of humic acid to form complex with Cu(II).

12. The order of stabilities of humic acid complexes formed with four divalent metal ions at pH 7 were Cu(II) > Fe(II) > Zn(II) > Mn(II). The log K values for humic acid obtained from subsurface layer were higher than those of humic acid obtained from surface layer complex. The values obtained for humic acid extracted from Vertisol were higher than those of the humic acid extracted from Alfisol.

13. The negative values of ΔGr^0 suggested that the complexation reaction between metal ion and humic acid was spontaneous.

14. The percentage increase in dry matter production over control was 141.16, 135.09 and 173.61 in rice and 83.01, 81.71 and 97.96 in maize due to application of zinc through Zn-humate, Zn-chelate and $ZnSO_4$ at 2.5 ppm level.

15. The effectiveness of differnet zinc sources followed the order $ZnSO_4$ > Zn-humate > Zn-chelate with respect to dry matter production, plant zinc content and uptake besides the zinc content in post harvest soil samples.

16. The dose of 2.5 ppm zinc was found to be optimum for rice and maize crops grown under pot culture conditions.

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*Original not seen

Note: This literature cited is as per the ANGRAU guidelines.

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APPENDIX

Distribution constants of HA-metal [Cu(II) and Mn(II)] complexes of humic acid obtained from Alfisol (A_1)

Cu(II)					Mn(II)				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	95.61	2177.90			0	96.28	2588.17		
3×10^{-5}	89.11		818.27	1.66	3×10^{-5}	91.50		1076.47	1.40
6×10^{-5}	80.95		424.93	4.12	6×10^{-5}	87.21		681.86	2.79
9×10^{-5}	73.11		271.88	7.01	9×10^{-5}	81.11		429.38	5.02
12×10^{-5}	65.24		190.42	10.43	12×10^{-5}	75.34		305.51	7.47
15×10^{-5}	59.06		144.25	14.09	15×10^{-5}	73.26		273.97	8.44
18×10^{-5}	51.56		106.44	19.42	18×10^{-5}	67.18		204.69	11.64
21×10^{-5}	44.11		78.92	26.59	21×10^{-5}	60.34		165.53	14.63
24×10^{-5}	38.41		62.36	33.92	24×10^{-5}	51.83		107.59	23.05

Distribution constants of HAmetal [Zn(II) and Fe(II)] complexes of humic acid obtained from Alfisol (A_1)

Zn(II)					Fe(II)				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	97.95	4753.65			0	94.61	1755.28		
3×10^{-5}	94.12		1600.68	1.96	3×10^{-5}	90.11		911.12	0.92
6×10^{-5}	90.00		900.00	4.28	6×10^{-5}	86.15		622.02	1.82
9×10^{-5}	84.15		530.91	7.95	9×10^{-5}	76.96		334.02	4.25
12×10^{-5}	81.45		439.08	9.82	12×10^{-5}	66.15		195.42	7.98
15×10^{-5}	76.21		320.34	13.83	15×10^{-5}	61.35		158.73	10.05
18×10^{-5}	73.11		271.88	16.48	18×10^{-5}	54.26		118.62	13.79
21×10^{-5}	66.82		201.38	22.60	21×10^{-5}	49.42		97.70	16.96
24×10^{-5}	61.45		185.76	24.59	24×10^{-5}	42.98		75.37	22.28

Distribution constants of HA-metal [Cu(II) and Mn(II)] complexes of humic acid obtained from Alfisol (A_2)

Cu(II)					**Mn(II)**				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	95.67	2209.46			0	95.86	2315.45		
3×10^{-5}	89.21		826.78	1.67	3×10^{-5}	91.25		1042.85	1.22
6×10^{-5}	81.35		436.19	4.06	6×10^{-5}	87.21		681.86	2.29
9×10^{-5}	75.32		277.64	6.95	9×10^{-5}	80.95		424.93	4.44
12×10^{-5}	65.34		188.51	10.72	12×10^{-5}	75.62		310.17	6.46
15×10^{-5}	59.40		146.30	14.10	15×10^{-5}	73.21		273.27	7.47
18×10^{-5}	51.63		106.73	19.70	18×10^{-5}	67.11		204.04	10.34
21×10^{-5}	44.25		79.37	26.83	21×10^{-5}	60.25		151.57	14.27
24×10^{-5}	38.52		62.65	34.27	24×10^{-5}	52.12		108.85	20.27

Distribution constants of HA-metal [Zn(II) and Fe(II)] complexes of humic acid obtained from Alfisol (A_2)

Zn(II)					**Fe(II)**				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	98.11	5191.00			0	94.97	1888.07		
3×10^{-5}	94.32		1660.56	2.12	3×10^{-5}	90.24		934.83	1.01
6×10^{-5}	90.05		905.02	4.73	6×10^{-5}	85.90		609.21	2.099
9×10^{-5}	85.11		571.59	8.08	9×10^{-5}	77.00		334.78	4.63
12×10^{-5}	81.46		439.31	10.81	12×10^{-5}	66.17		195.59	8.65
15×10^{-5}	76.24		320.87	15.17	15×10^{-5}	61.50		159.74	10.81
18×10^{-5}	72.91		269.13	18.28	18×10^{-5}	54.29		118.77	14.89
21×10^{-5}	66.85		201.65	24.74	21×10^{-5}	49.53		98.13	18.24
24×10^{-5}	61.26		158.13	31.82	24×10^{-5}	43.11		75.77	23.91

Distribution constants of HA-metal [Cu(II) and Fe(II)] complexes of humic acid obtained from Vertisol (V_1)

Cu(II)					**Fe(II)**				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	97.16	3421.12			0	98.05	5028.20		
3×10^{-5}	94.25		1686.04	1.02	3×10^{-5}	92.43		1221.00	3.11
6×10^{-5}	91.36		1044.11	2.27	6×10^{-5}	89.11		818.27	5.14
9×10^{-5}	87.54		702.56	3.86	9×10^{-5}	84.22		553.71	8.08
12×10^{-5}	82.51		471.75	6.25	12×10^{-5}	80.91		423.83	10.86
15×10^{-5}	75.34		305.51	10.19	15×10^{-5}	74.06		285.50	16.61
18×10^{-5}	62.12		163.99	19.86	18×10^{-5}	65.40		189.01	25.60
21×10^{-5}	51.40		105.76	31.34	21×10^{-5}	53.71		116.02	42.33
24×10^{-5}	48.23		93.16	35.72	24×10^{-5}	44.82		81.22	60.90

Distribution constants of HA-metal [Zn(II) and Mn(II)] complexes of humic acid obtained from Vertisol (V_1)

Zn(II)					Mn(II)				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	97.45	3821.56			0	96.75	2976.92		
3×10^{-5}	93.16		1361.98	1.80	3×10^{-5}	92.63		1256.85	1.36
6×10^{-5}	84.51		545.57	6.00	6×10^{-5}	86.71		652.44	3.56
9×10^{-5}	80.23		405.81	8.41	9×10^{-5}	81.39		437.34	5.80
12×10^{-5}	71.96		256.63	13.87	12×10^{-5}	76.50		325.53	8.14
15×10^{-5}	69.13		223.93	16.06	15×10^{-5}	71.26		247.94	11.00
18×10^{-5}	64.11		178.62	20.39	18×10^{-5}	65.94		193.59	14.37
21×10^{-5}	58.06		138.43	26.60	21×10^{-5}	60.83		155.29	18.17
24×10^{-5}	52.35		109.86	33.78	24×10^{-5}	55.16		123.01	23.20

Distribution constants of HA-metal [Cu(II) and Fe(II)] complexes of humic acid obtained from Vertisol (V_2)

Cu(II)					**Fe(II)**				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	97.14	3396.50			0	98.71	5393.98		
3 x 10^{-5}	94.41		1688.90	1.01	3 x 10^{-5}	92.53		1238.69	3.35
6 x 10^{-5}	91.25		1042.85	2.25	6 x 10^{-5}	89.18		824.21	5.54
9 x 10^{-5}	87.61		707.10	3.80	9 x 10^{-5}	84.32		537.75	9.03
12 x 10^{-5}	82.53		472.40	6.18	12 x 10^{-5}	80.95		424.93	11.69
15 x 10^{-5}	75.35		305.67	11.11	15 x 10^{-5}	74.11		286.24	17.84
18 x 10^{-5}	62.17		164.34	19.67	18 x 10^{-5}	65.43		189.26	27.50
21 x 10^{-5}	51.50		106.18	30.98	21 x 10^{-5}	53.72		116.07	45.47
24 x 10^{-5}	48.24		93.19	35.44	24 x 10^{-5}	44.86		81.35	65.30

Distribution constants of HA-metal [Zn(II) and Mn(II)] complexes of humic acid obtained from Vertisol (V_2)

Zn(II)					Mn(II)				
Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	Conc. of HA (ML^{-1})	α	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$
0	97.48	3868.25			0	96.55	2798.55		
3×10^{-5}	93.20		1370.58	1.82	3×10^{-5}	92.63		1256.85	1.22
6×10^{-5}	84.55		547.24	6.06	6×10^{-5}	87.12		676.39	3.13
9×10^{-5}	80.26		406.58	8.51	9×10^{-5}	80.56		414.40	5.75
12×10^{-5}	72.15		259.06	13.93	12×10^{-5}	76.34		322.65	7.67
15×10^{-5}	69.32		225.94	16.12	15×10^{-5}	71.25		247.82	10.29
18×10^{-5}	63.84		176.54	20.91	18×10^{-5}	66.13		195.24	13.33
21×10^{-5}	58.11		138.72	26.88	21×10^{-5}	60.75		154.77	17.08
24×10^{-5}	52.40		110.08	34.14	24×10^{-5}	54.96		122.02	21.93