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Spectroscopic and conductometric study of complexes formed by humic and fulvic acid with metal ions

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Abstract
The present study was carried out at the laboratory, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar. The interactions of metal ions with humic substances in 1:1.5 molar ratio metal to ligand are investigated by spectroscopic technique with absorbance at 400 nm for Fe (II), Cu (II) and Zn (II) and 420 nm for Mn (II). The complexes formed by humic and fulvic acid with metal ions was studied by molar ratio method and job's plot method. The results revealed that from Job's plot for metal humates maximum absorbance occurred at X = 0.4, 0.4, 0.4 and 0.2 for Fe (II), Cu (II), Mn (II) and Zn (II) with humic acid and from the Job's plot for metal fulvates maximum absorbance occurred at X = 0.8 for Mn (II) with fulvic acid respectively. In case of conductometric titrations humic acid conductance increased very slowly in the beginning and then followed by a steep rise and for fulvic acid the conductance decrease initially due to neutralisation of strong functional group with further addition of NaOH, a constant or slight increase is observed.

Keywords: Humic acid (HA), Fulvic acid (FA), Metal ions, Spectroscopic study, Job's plot, Conductometric titrations

Introduction
Soil consists of inorganic components, organic matter and living organisms. The inorganic component consists of the minerals and clay. The organic matter (OM) present in soils is a mixture of products formed at decomposition stages, resulting from chemical and biological degradation of plant and animal residues and synthetic activity of microorganisms. The soil organic matter has two components, humified (humic acid, fulvic acid and humin) and non-humified (carbohydrates, lipids, amino acids, nucleic acids and lignin). Humic substances are the major constituents of soils, and generally known as biologically refractory degradation components (Chefetz *et al*., 2002) [2]. The humic substances are dark coloured amorphous polymers. They are formed in the environment with biomass constituents (Stevenson, 1982) [16]. It is considered that humic substances initially lose those components which are easily decomposed, and thereafter condensation-polymerisation and oxidative reactions takes place which results in a more stable structure (Kumada, 1987) [7]. This process corresponds to the progression of humification, and it is identified on the basis of the degree of darkening of colour of humic acids, alkali-soluble and acid insoluble fraction (Watanabe and Takada 2006) [21]. These substances are usually fractionated on the basis of their solubility characteristics and the pH value of solvents as well.

Humic acid (HA) and fulvic acid (FA) make up an important part of soil organic matter and their binding capacity affects the fate of metal ions (especially micronutrients). These humic substances play an important role in mobility of nutrients (Srilatha, 2001 and Stevenson, 1994) [14&17]. Soil organic matter fractions are capable of forming complexes with metal ions and the ability of these humic substances to form stable complexes with metal ions can be accounted for their high content of oxygen containing functional groups *viz,* carboxylic, phenolic aliphatic and alcoholic –OH groups. The complex formation reactions between metal ions and humic substances are helpful in understanding the problems of plant nutrition (Stevenson *et al.,* 1993) [18]. During complexation, numerous compounds including humic acid (HA) and fulvic acid (FA) are involved which control the distribution and supply of micronutrients to plants and interact with metal ions through their functional groups forming metal complexes of varying stabilities.

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The spectroscopic studies of humic compounds have been the focus of researchers in the recent past. The humic-fulvic acids have been extensively characterized by UV-VIS spectroscopy. Schnitzer (1977) [13] studied that HAs and FAs extracted from soils formed under different geographic and pedologic environments had similar analytical characteristics and chemical structures. The colour of humic substances is an important physical property, which is used to characterize humic fractions of soil (Flaig *et al.,* 1975; Kononova 1966; Schnitzer 1971; and Tan and Van Schuylenborgh 1961) [3, 6, 12&20]. The colour ratio is used as an index for the rate of light absorption in the visible range. Absorption of humic acid in the ultraviolet range is based on the aromatic groups that contribute to the molecular structure, *i.e.* the sum of phenyl propane units, and on several chromophoric structural elements. Although some differences exist in the UV-VIS spectra of the absorption curves emphasizes their characteristic nature, that is, whether they are aliphatic or aromatic. The loss of aromatic structures of humic acid results in significant changes in the UV-VIS spectrum of these substances.

Material and methods
The present study was carried out at the laboratory to characterize the complex formation of humic fractions with metal ions at Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar in the year 2016-17.

Methods for characterising complexes of humic acid/ fulvic acid with metal ions
Absorption spectroscopic methods were used to characterize the interactions of humic acid and fulvic acids with Cu (II), Mn (II), Zn (II) and Fe (II).

Absorption spectroscopic methods
Absorption spectroscopic technique developed by Job (1928) [4] was used for determination of complexes of HA and FA. The formation of complex is represented by the equation.

$$A + nB \longrightarrow ABn \longrightarrow \quad (I)$$
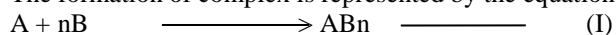
Where,
A = metal ion
B = the ligand
To determine "n", the solutions of A and B of the same concentration are mixed in varying proportions and their absorbance was measured in the visible range from 380-560 nm. The differences (y) between each absorbance formed and the corresponding absorbance where no reaction would have occurred on mixing solutions of humic acid/fulvic acid and 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 ligand, which corresponds to the formation of the metal complex. This method has been used for studying complex formation between humic acid/fulvic acid and metal ion Zn (II), Cu (II), Mn (II), and Fe (II)).
The following procedure was adopted for characterizing the complexes of humic acid/ fulvic acids with metal ions (Mac Carthy and Mark, 1976) [8] with few modifications:
Six aliquots in 4 ml increments i.e., 0, 4, 8, 12, 16 and 20 ml of 1 x 10⁻³ M humic acid/fulvic acid varying from 0 to 20 ml were pipetted in a series of 50 ml beakers. It was followed by addition of 1 x 10⁻³ M metal ion Zn (II), Mn (II), Cu (II) and Fe (II) varying from 20 to 0 ml of 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.
From the data obtained by Srilatha (2001) [14] and Ramalakshmi (2011) [9] based on elemental composition, the molecular weight of humic acid was arrived at 1000g and that of Fulvic acid at 700g.
A standard curve was prepared with six aliquots of 0, 4, 8, 12, 16 and 20 ml of 1 x 10⁻³ M humic/fulvic acid pipetted into a series of 50 ml beakers and the pH of which was adjusted to 7.0. The volume was made up to 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 UV-spectrophotometer (Rayleigh UV - 9200).

Conductometric titrations
One hundred milligrams of HA/FA was dissolved in 25 ml of distilled water and was titrated against standard 0.1*N* NaOH conductometrically on an EC meter (Elico CM 180) using conductivity cell.

Results and discussion
Absorption spectroscopic methods
The molar compositions of humic acid, fulvic acid and metal complexes were determined by Job's method at pH 7.0.

Absorption spectra of humic acid and metal humates
Absorption curves of 1 x 10⁻³ M humic acid, metal ions and mixture of humic acid and metal ion Mn (II) between 380 to 560 nm are presented in (Fig 1). The absorbance curves for all the metal ions, and the corresponding metal humates followed similar pattern. Absorbance's for the metal ions were 0.01 (or) less at the concentration used in the study over a wider range of wavelength examined. The absorbance of humic acid-metal complex was lower than that of humic acid alone with the greatest difference observed at 400 nm for Zn (II); Cu (II) and Fe (II) and 420 nm for Mn (II). The absorbance was higher at lower wavelengths and it decreased with increasing in wavelength. The plot of absorbance vs. concentration of humic acid is depicted in (Fig 2). It followed a straight line, thus obeying the Beer's law. This plot was used for calculating 'y' values in Job's plot at pH 7.0. Since the absorbance values for metal ions alone Zn (II), Cu (II), Mn (II) and Fe (II) even at the high concentration used in the studies were less than 0.01, they were ignored in calculating 'y' values.

Absorption spectra of fulvic acid and metal fulvates
Absorbance curves of 1 x 10⁻³ M fulvic acid, metal ion and the mixture of fulvic acid and metal ion Mn (II) were scanned between 380-560 nm and are presented in (Fig 3). Absorbance values for all the metal ions were 0.01 (or) less at the concentration used in the present investigation over a wide range of wavelengths examined. The absorbance of fulvic acid –metal complex was lower than that of fulvic acid alone with the greater difference observed at 400 nm for Zn, Cu and Fe and at 420 nm for Mn. The absorbance was high at lower wavelength and it decreased with increase in the wavelength.
The plot of absorbance vs. concentration of fulvic acid is presented in (Fig 4). It showed a straight line, thus obeying the Beer's law. This plot was used for calculating 'y' values in Job's plot at pH 7.0. Similar to the humic acid the absorbance values of metal ions Zn (II), Cu (II), Mn (II) and Fe (II) were neglected in calculating the 'y' values since the absorbance values were less than 0.005.

Job's plot

Based on spectroscopic measurement, the function Y,

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

"Y" was plotted as a function of 'X' (X =mole fraction of one of the components). From Job's plot for metal humates presented in (Figs 5, 6, 7 and 8) it was clearly evident that maximum absorbance occurred at X = 0.4, 0.4, 0.4 and 0.2 for Fe (II), Cu (II), Mn (II) and Zn (II) with humic acid respectively.

As explained for humic acids, the 'Y' values have been plotted as a function of 'X'. The Job's plots for metal fulvate complexes are presented in (Fig 9). From the Job's plot it was observed that maximum absorbance occurred at X = 0.8 for Mn (II) fulvic acid.

According to this mechanism, a proton is displaced from the acidic group of the humic acid at low pH. At high pH, protons are dissociated from the water molecules covalently bonded to the metal ion and hydroxo complexes are formed. Kawaguchi and Kyuma (1959) [5] also believe that hydroxo complexes are formed which become soluble as more base is negative charge. As more bases are added the negative charge is restored and the complex is peptized and thus there is no such maximum absorbance point 'X' in case of FA-Zn (II), FA-Fe (II) and FA-Cu (II) was observed.

Conductometric titrations

Humic acid and fulvic acids were conductometrically titrated against 0.1 N NaOH. It could be observed from the curves (Fig 10) that in case of humic acid the conductance increased very slowly in the beginning and then followed by a steep rise. The slow increase in conductance during initial suggested that humic acids behave as weak acids which could be due to a slow increase in conductance on addition of NaOH to neutralize its acidity. These values are supported by functional group analysis. The weak acidic character of humic acid was also earlier reported by Sailaja (1999) [11] and Sujana Reddy and Rao (2000) [19].

Contrary to this titration curves of fulvic acid indicated that the conductance decrease initially due to neutralisation of strong functional group with further addition of NaOH, a constant or slight increase and again increased abruptly. The decrease in conductance was due to decrease in concentration of H_2O ions and final increase in conductance could be attributed to free Na^+ ions. Higher conductance of fulvic acid as compared to humic acid might be due to the difference in purification of these fractions, as the humic acid dialysed was almost free of ions that resulted in lower conductance. These results are in conformity with those of (Adhikari et al., 1972; Ramalakshmi et al., 2013 and Srilatha et al., 2013) [1, 10&15].
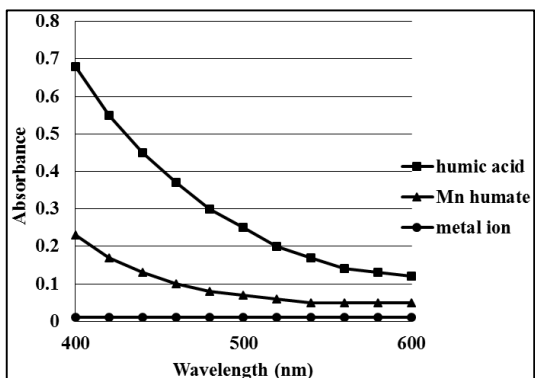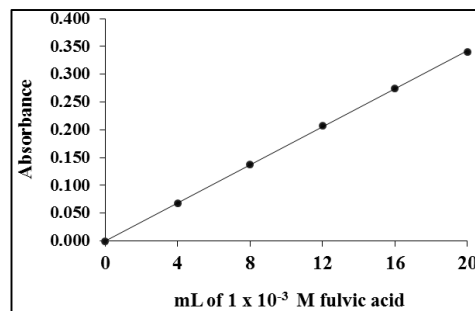

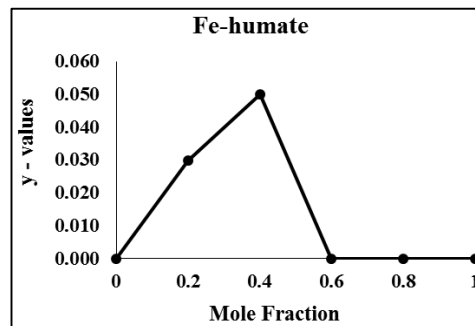**Fig 1:** Absorption spectra of humic acid, metal humates and metal ions
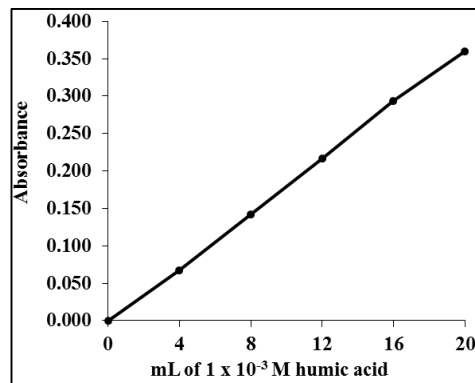
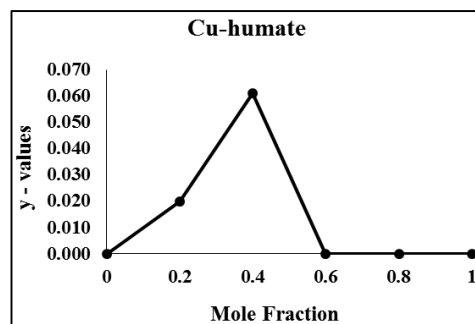**Fig 2:** Standard curve of humic acid
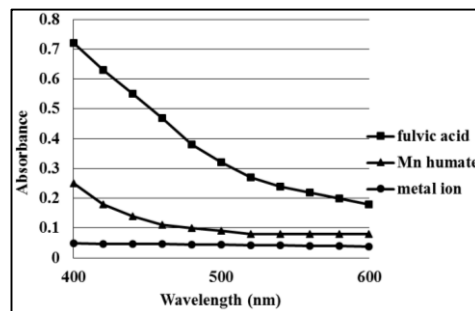
Fig 3: Absorption spectra of fulvic acid, metal fulvates and metal ions

Fig 4: Standard curve of fulvic acid

Fig 5: Job's plot of metal humate
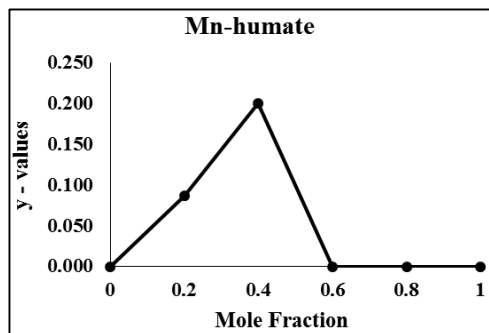

Fig 6: Job's plot of metal humate

Mn-humate

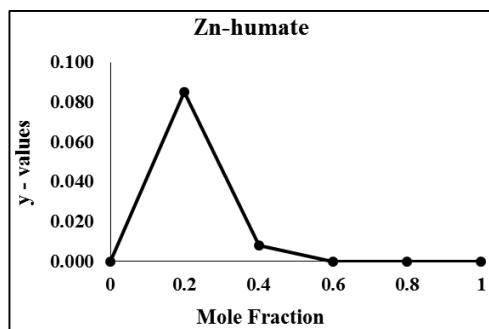**Fig 7:** Job's plot of metal humate

Zn-humate

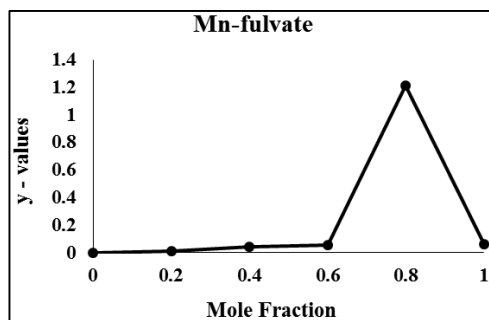**Fig 8:** Job's plot of metal humate

Mn-fulvate

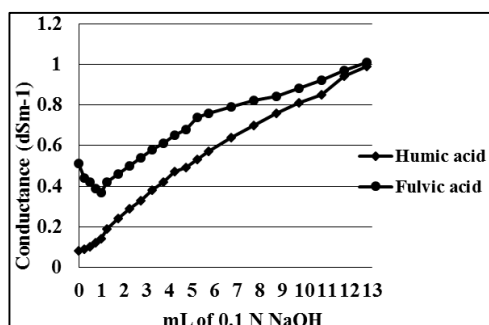**Fig 9:** Job's plot of metal fulvate

Fig 10: Conductometric titration curves of humic and fulvic acid

Conclusions

The present findings concluded that from Job's plot for metal humates maximum absorbance occurred at X = 0.4, 0.4, 0.4 and 0.2 for Fe (II), Cu (II), Mn (II) and Zn (II) with humic acid and from the Job's plot for metal fulvate maximum absorbance occurred at X = 0.8 for Mn (II) with fulvic acid. These 'X' value indicates the point at which metal ion forms complex with ligand (humic or fulvic acid). At high pH, protons are dissociated from the water molecules covalently bonded to the metal ion and hydroxo complexes are formed. Kawaguchi and Kyuma (1959) also believe that hydroxo complexes are formed which become soluble as more base is negative charge. As more bases are added the negative charge

is restored and the complex is peptized and thus there is no such maximum absorbance point 'X' in case of metal fulvates FA-Zn (II), FA-Fe (II) and FA-Cu (II). Higher conductance of fulvic acid as compared to humic acid might be due to the difference in purification of these fractions, as the humic acid dialysed was almost free of ions that resulted in lower conductance.

References
1. Adhikari M, Chakravarthy G, Hazra GC. Fulvic acid metal complexes. Journal of the Indian Society of Soil Science. 1972; 20(3):311-321.
2. Chefetz B, Tarchitzky J, Deshmukh AP, Hatcher PG, Chen Y. Structural characterization of soil organic matter and humic acids in particle size fractions of an agricultural soil. Soil Science Society of America Journal. 2002; 66:129-141.
3. Flaig W, Beutelspacher H, Rietz E. Chemical composition and physical properties of humic substances. In Soil Components, Vol. 1 Inorganic Components (J.E. Gieseking, Ed.), Springer-Verlag, New York, 1975, 1-211.
4. Job P. Research Sur La formation de complexes minivux in solution at Sur leeur stability. Annals of Chemistry. (Series 10). 1928; 9:113-203.
5. Kawaguchi K, Kyuma K. Soil plant food. 1959; 5:54.
6. Kononova MM. Soil Organic Matter. Press, 1966, 400-410.
7. Kumada K. Chemistry of Soil Organic Matter. Elsevier, Amsterdam, 1987.
8. Mac Carthy P, Mark Jr HB. An evolution of job's method of continuous variation as applied to organic matter. Metal ion interaction. Soil Science Society of America Journal. 1976; 40:26-276.
9. Ramalakshmi ChS. Vermicomposting for effective waste management and its evaluation under INM rice-pulse cropping system. Ph.D. (Ag.) thesis submitted to Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad, 2011.
10. Ramalakshmi Ch S, Rao PC, Sreelatha T, Padmaja G, Madhavi M. Nitrogen use efficiency and production efficiency of rice under integrated nutrient management. Journal of Rice Research. 2013; 4(1&1):42-59.
11. Sailaja M. Interactions between metal ions and humic fractions extracted from an Alfisol and Vertisol in relation to their plant availability, M.Sc. (Ag.) thesis submitted to Acharya NG Ranga Agricultural University, Hyderabad, 1999.
12. Schnitzer M. Characterization of humic constituents by spectroscopy. In Soil Biochemistry (A.D. McLaren and J. Skujins, Eds.), Marcel Dekker, New York. 1971; 2:60-95.
13. Schnitzer M. Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. In Soil Organic Matter Studies. International Atomic Energy Agency, Vienna, II, 1977, 117-132.
14. Srilatha M. Characterization of humic substances and their interactions with metal ions in relation to their availability to rice. M.Sc. (Ag.) thesis submitted to Acharya N.G. Ranga Agricultural University, Rajendranagar and Hyderabad, 2001.
15. Srilatha M, Rao PC, Sharma SHK, Padmaja G. Physico - chemical characterization of humic substances under long – term application of fertilizers and manures in rice – rice

cropping sequence in an Inceptisol. International Journal of Advanced Research. 2013; 1(10):343-348.

16. Stevenson FJ. Extraction, fractionation, and general chemical composition of soil organic matter. In Humus Chemistry: Genesis, Composition, Reactions (F.J. Stevenson, Ed.). John Wiley and Sons, New York, 1982, 26-54.

17. Stevenson FJ. Humus Chemistry: Genesis, Composition, Reaction, Second Edition, John Wiley and Sons, New York, 1994.

18. Stevenson FJ, Lanath FA, Brar MS. Stability constants of Cu (II)-humate complexes: comparison of select models. Soil Science. 1993; 155(2):77-91.

19. Sujana Reddy K, Rao PC. Characterization of humic-metal ion interactions in alfisols and vertisols by potentiometric methods. Journal of the Indian Society of Soil Science. 2000; 48(3):596-598.

20. Tan KH, Van Schuylenborgh J. On the organic matter in tropical soils. Nertherland Journal of Agricultural Sciences. 1961; 9:174-180.

21. Watanabe A, Takada H. Structural stability and natural ^{13}C abundance of humic acids in buried volcanic ash soils. Soil Science and Plant Nutrition. 2006; 52:145-152.