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Complexation Behavior of Humic and Fulvic Acids with Metal Ions and their Assessment by Stability Constants

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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 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 Fe (II), Cu (II), Mn (II) and Zn (II) metal ion complexes with humic and fulvic acid at pH 7.0 and at 30^0C. The results revealed that for humic acid the log K values were 5.84, 5.97, 5.94 and 5.84 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. In case of fulvic acid the log K values were 5.93, 5.96, 6.01 and 5.89 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively.

Key words: *Humic acid (HA), Fulvic acid (FA), Metal ions, Stability constants.*

INTRODUCTION

Important constituents of the soil organic matter are humic substances including humic acid (HA), fulvic acid (FA) and humin. The term 'humic substances' is intended to designate that class of organic matter in or extracted from decayed or decaying biomatter of soil, sediment or natural water, which does not fall in to any of the discrete classes of organic substances. The maximum binding capacity (MBC) of a given organic substrate to complex metals depends on the total number of (strong and weak) binding sites, which is related to the number of functional groups. The total acidity, i.e. the number of carboxylic acids and phenolic hydroxyl groups, is often used as a measure for the binding capacity[3&15]. A second parameter that determines the binding of a given metal to an organic ligand, and consequently its behavior in (soil) solution, is the binding strength, generally expressed as the (overall) stability constant (K_0).

The stability constants of humic fractions-metal complexes are one of the important parameters in determining the thermodynamic stability of the metal complexes and their effectiveness in augmenting or decreasing the availability of metal cations to plants[6,7,12&13].

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 humic and fulvic acid at pH 7.0 and at 30^0C. Among different methods to determine stability constants, ion exchange equilibrium method was adopted, which is based on the complexation of metal ion between the complexing agent and the cation exchange resin. 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/FA 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/FA respectively.

The complexation behaviour of humic and fulvic acid fractions depends up on the quality and quantity of functional groups of these fractions and also on the type of metal ion. In the complex formation, the complexing agent acts as electron donor, while metal ion acts as an electron acceptor, in which the metal either may bridge the clay and organic radical as in clay-metal-organic complexes or may directly establish link with the functional groups of humic acid leading to the formation of metallo-organic complexes[13]. Stability constants are numerical expressions of stabilities of metal-HA and metal-FA complexes. These constants not only help to understand better role of these complexes in soil system, but also enable to predict their behaviour[10].

MATERIAL AND METHODS

The present study was carried out at the laboratory for Complexation of humic and fulvic acids with metal ions and their assessment by stability constants at Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar in the year 2016-17.

DETERMINATION OF STABILITY CONSTANTS OF METAL-HA / METAL-FA COMPLEXES

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

$$M + XA \longrightarrow MA_x$$

Then the stability constant is given by

$$K = \frac{(MA_x)}{(M)(A)^x} \qquad (I)$$

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

Then the stability constant was obtained from the following equation:

$$\mathrm{Log}\left(\frac{\lambda_0}{\lambda} - 1\right) = \log K + x \log (A) \qquad (II)$$

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

$$\log\left(\frac{\lambda_0}{\lambda} - 1\right) \text{ vs } \log (A)$$

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

λ and 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/FA (moles L^{-1})

In this experiment, the weights of one mole of HA and FA were taken as 1000 g and 700 g, respectively.

λ obtained from the following equation

$$\lambda = \frac{\alpha \, V}{(100-\alpha)\,Xg} \quad \text{(III)}$$

Where,

α = % metal bound to exchange resin

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

V = Volume of solution

g = Weight of exchange resin

λ_0 was calculated in the same manner 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.

Procedure

From the stock solution, aliquots containing 6 to 24 mg of HA/FA were pipetted out into flask and diluted approximately to 40 ml with distilled water. To these flasks, 5 ml of 1 *N* 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^{-1}. The pH was adjusted to 7.0, finally, the volume was made up to 50 ml with distilled water. One gram of potassium saturated amberlite IR-120 resin was taken in a glass stoppered flask, to which the solutions of metal chlorides, HA/FA and KCl were transferred and the volume was made up to 100 ml. Then 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 spectrophotometer (200 Series AA). Identical blank was also carried out in the absence of HA/FA to determine λ_0.

RESULTS AND DISCUSSION

Complexation behaviour of humic acid and fulvic acid fraction with metal ions

As indicated above, the number of moles of HA and FA that reacted with one mole of metal ion in the formation of complex is referred to as x-value. These 'x' values were obtained from the slope of straight line by plotting (λ_0/λ-1) vs. conc. of HA and FA in log –log graph are presented in Table 1 and are depicted in Figures 1 and 2 for humic acid and fulvic acid respectively.

For humic acid 'x' values were 1.00, 1.02, 1.01 and 1.00 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. In case of fulvic acid 'X' values were 1.02, 1.02, 1.03 and 1.01 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. In general, humic acid and fulvic acid metal ion complexes showed almost similar x-values with relatively higher x-values for cu-humate complex than other complexes.

The data on number of moles of humic acid and fulvic acid that reacted with one mole of metal ion (x-values) showed higher values at pH 7.0 and at a temperature of 30^0C than those reported by several workers[2,4&5] indicating variation in the degree of ionization of functional groups at higher pH levels. From the data it is also clear that these values were determined at such pH where in both humic and fulvic acids remain in solution with dissociation of all the functional groups. Slightly higher values were obtained in the present investigation, which was carried out at pH 7.0. This could be attributed to increased ionization of functional groups leading to 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/fulvic acid that

reacted with one mole of metal ion (x-values) determined the availability of these metal ions to plants. Hence, at comparable concentrations the availability of copper when it is complexed with humic and fulvic acid will be low as compared with the other metal ions[9&16].

In all the cases, x-values were not the whole numbers. This indicates the formation of polynuclear complexes. The x-values for all the metal-HA and metal-FA complexes were approximately of unit value and suggested the formation of 1:1.5 metal-HA and metal-FA complexes[12].

Differential ability of HA and FA fractions to form complexes with metal ions

The data on ability of metal ions [Fe (II), Cu (II), Mn (II) and Zn (II)] to form complexes with humic acids and fulvic acids (1/x values) are presented in Table 2.

For humic acid the 1/x values are 1.00, 0.98, 0.99 and 1.00 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. In case of fulvic acid the 1/x values are 0.98, 0.98, 0.97 and 0.99 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. The lower 1/x values were obtained for both Cu (II)-HA and Cu (II)-FA complexes, which indicated that better ability of these humic fractions to form complex with Cu (II).

Stability constants of HA and FA metal complexes

The log K (stability constants) values of HA metal complexes were calculated from the following equation.

$$Log (\lambda_0/\lambda - 1) = \log K + x \log (A)$$

For each concentration of HA and FA, the log K values were computed for individual metal ions and their mean log K values were also calculated. These values for humic acid are presented in Table 3 and for fulvic acid in the Table 4 respectively.

For humic acid the log K values were 5.84, 5.97, 5.94 and 5.84 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. In case of fulvic acid the log K values were 5.93, 5.96, 6.01 and 5.89 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively.

The complexation behaviour of humic and fulvic acid fractions depends up on the quality and quantity of functional groups of these fractions and also on the type of metal ion. In the complex formation, the complexing agent acts as electron donor, while metal ion acts as an electron acceptor, in which the metal either may bridge the clay and organic radical as in clay-metal-organic complexes or may directly establish link with the functional groups of humic acid leading to the formation of metallo-organic complexes[13].

The stability constants of metal-HA followed the order Cu (II) > Mn (II) > Zn (II) = Fe (II) while it was Mn (II) > Cu (II) > Fe (II) > Zn (II) for metal-FA, indicating that the metal ion is bonded with a large number of active sites, thereby increasing the stability of complex formed. Among HA & FA log K values were higher with metal-FA than metal-HA. This could be due to high total acidity and carboxyl groups content in fulvic acid than humic acid. Several workers reported higher values for stability constants of humic acid with metal complexes at high pH level indicating the increased ionization of functional groups[1&9]. More the number of functional groups higher log K values[11].

Free energy change during complexation

Mean values of standard free energy change (ΔGr^0) for the complexation of metal ions with humic acids and fulvic acids were calculated from the following formula:

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

K = stability constant
R = Gas constant = 1.987 cal degree^{-1} mole^{-1}
ΔGr^0 = Standard free energy change in K cal mole^{-1}
T = Absolute temperature

The data on free energy change (ΔGr^0) are presented in Table 5 for humic acid and Table 6 for fulvic acid. The humic acid had the free energy ΔGr^0 values of 9.56, 9.68, 9.66 and 9.56 for Fe (II), Cu (II), Mn (II) and Zn (II) and fulvic acid had the free energy ΔGr^0 values of 9.65, 9.68, 9.72 and 9.61 for Fe (II), Cu (II), Mn (II) and Zn (II) respectively. From the data of ΔGr^0 values, it is observed that the extent of free energy was differed and related to the stabilities of complexes formed. Cu (II)

humate and Mn (II) fulvate had higher values than the other complexes.

The higher stability of Cu (II)-HA complex could 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 p-electrons from the complexing agent and, thus, forms stable complexes with humic acid and fulvic acid[5,8,9&17]. The ligand molecule possesses vacant orbitals and accepts the electrons from the filled in metal orbitals to form p bonding and s bonding. The bond is formed due to donation of lone pair of electrons. This type of bonding makes the metal ligand complex much stronger[1,5,10,13&14]. In case of Zn (II), though it is also a transitional metal ion, 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 organic metal complexes might have been formed through p bonding and the p complexes, formed that the acids are weak with low stabilities.

The standard free energy change (ΔGr^0) for the complexation of metal ions with humic acid and fulvic acid was computed from the respective values of their stability constants using the equation VIII. The negative values of ΔGr^0 suggested that the spontaneous reaction showing greater possibility of the complex formed[2&5]. The larger negative ΔGr^0 values for Cu (II)-humate, Mn (II)-fulvate are thermodynamically more stable than the other metal ion complexes used in study. The higher thermodynamic stability of Cu-HA complex could be attributed to existence of coordinate covalent bonds between the complexing agents (HA/FA) and the Cu (II) ions, possibly due to the formation of s bonds by the donation of lone pair of electrons from the complexing agents to Cu (II) having $3d^9$ configuration. These results are in accordance with[2].

Table 1: Moles of HA and FA reacted with one mole of metal ion [Fe (II), Cu (II), Mn (II) and Zn (II) in formation of complex (x values)]

Source	Metal ions			
	Fe (II)	**Cu (II)**	**Mn (II)**	**Zn (II)**
Humic Acid	1.00	1.02	1.01	1.00
Fulvic Acid	1.02	1.02	1.03	1.01

Table 2: Ability of metal ions [Fe (II), Cu (II), Mn (II) and Zn (II)] to form complexes with humic acids (1/x values)

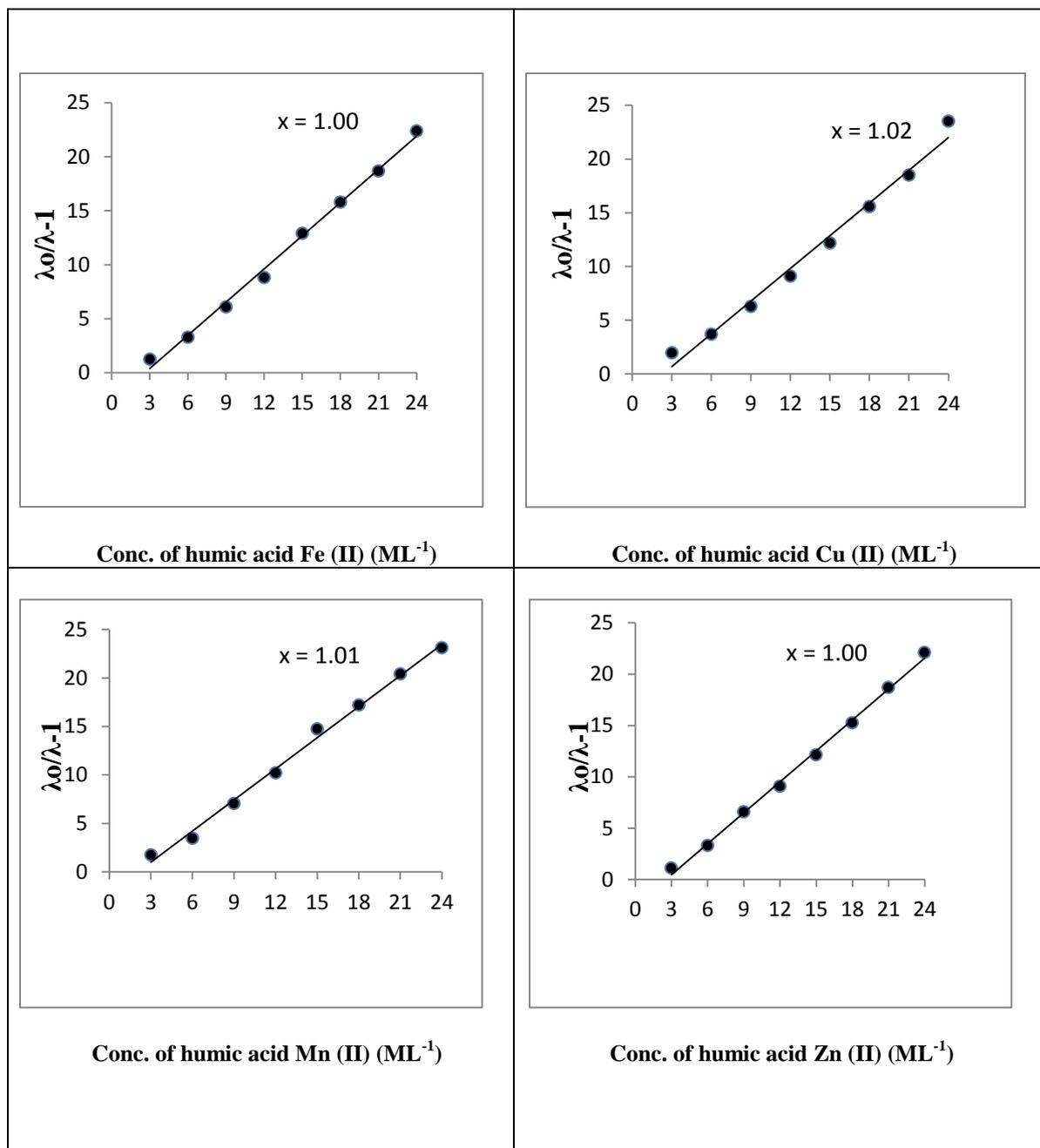| Source	Metal ions			
	Fe (II)	**Cu (II)**	**Mn (II)**	**Zn (II)**
Humic Acid	1.00	0.98	0.99	1.00
Fulvic Acid	0.98	0.98	0.97	0.99

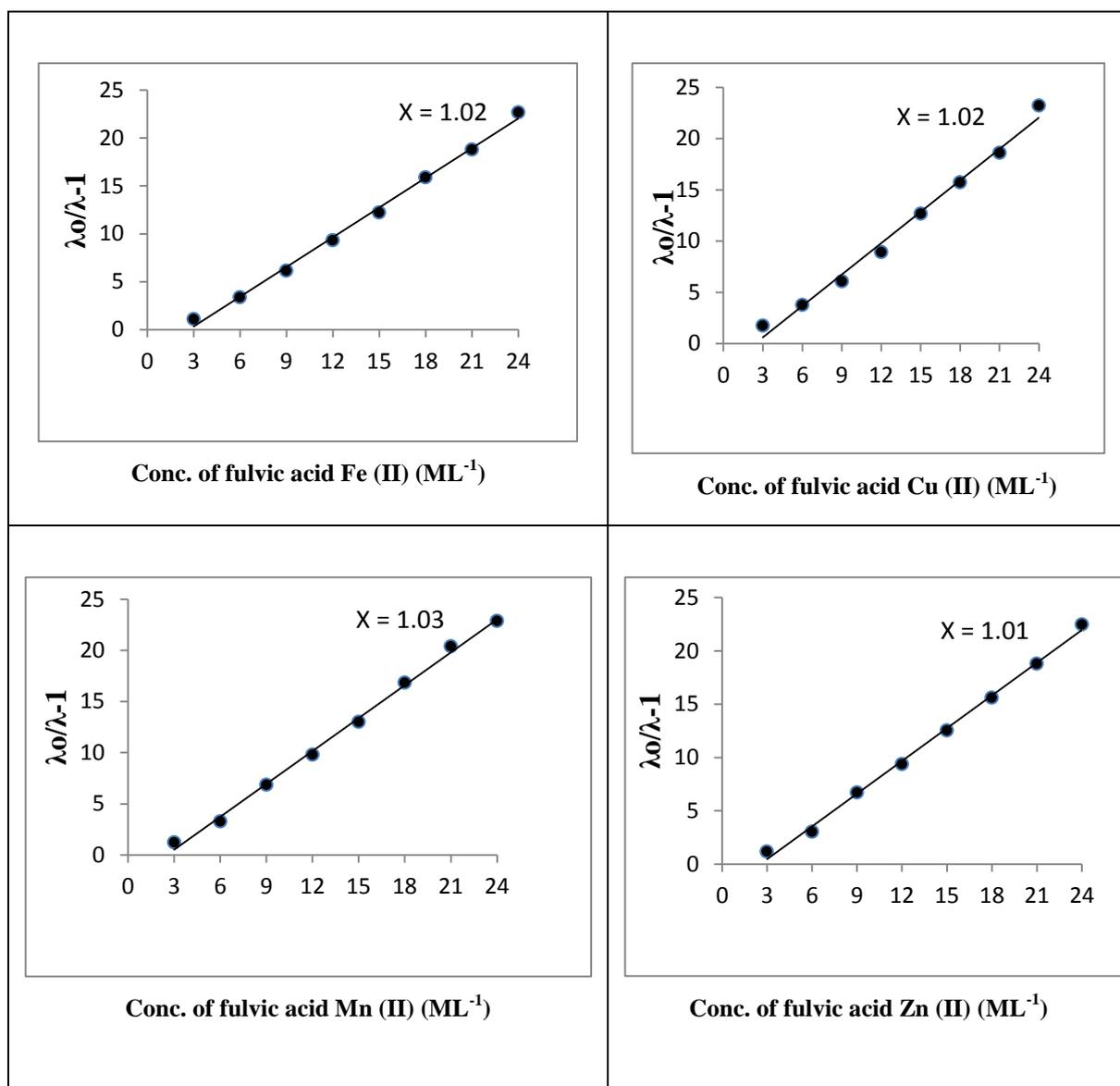**Fig. 1: Stability constants of metal -humic acid complexes**

Fig. 2: Stability constants of metal -fulvic acid complexes

Table 3: Stability constants (Log K values) of HA-metal [Fe (II), Cu (II), Mn (II) and Zn (II)] complexes

Humic Acid ML⁻¹	Log K			
	Fe (II)	**Cu (II)**	**Mn (II)**	**Zn (II)**
0				
3 X 10⁻⁶	5.61	5.92	5.80	5.56
6 X 10⁻⁶	5.73	5.88	5.80	5.73
9 X 10⁻⁶	5.82	5.93	5.93	5.85
12 X 10⁻⁶	5.86	5.96	5.96	5.87
15 X 10⁻⁶	5.93	5.99	6.02	5.90
18 X 10⁻⁶	5.93	6.02	6.01	5.92
21X 10⁻⁶	5.94	6.02	6.01	5.94
24 X 10⁻⁶	5.96	6.07	6.01	5.95
Mean	**5.84**	**5.97**	**5.94**	**5.84**

Table 4: Stability constants (Log K values) of FA-metal [Fe (II), Cu (II), Mn (II) and Zn (II)] complexes

Fulvic Acid ML^{-1}	Log K			
	Fe (II)	Cu (II)	Mn (II)	Zn (II)
0				
3 X 10^{-6}	5.67	5.87	5.76	5.63
6 X 10^{-6}	5.84	5.89	5.88	5.75
9 X 10^{-6}	5.92	5.92	6.02	5.91
12 X 10^{-6}	5.97	5.96	6.05	5.93
15 X 10^{-6}	5.99	6.01	6.07	5.95
18 X 10^{-6}	6.03	6.02	6.10	5.97
21X 10^{-6}	6.03	6.02	6.11	5.98
24 X 10^{-6}	6.05	6.06	6.09	6.00
Mean	**5.93**	**5.96**	**6.01**	**5.89**

Table 5: Standard free energy change (-ΔGr^0) values for the complexation reactions of metal ions [Fe (II), Cu (II), Mn (II) and Zn (II)] with humic acid fractions

Source	Metal ions			
	Fe (II)	Cu (II)	Mn (II)	Zn (II)
Humic acid	9.56	9.68	9.66	9.56

Table 6: Standard free energy change (-ΔGr^0) values for the complexation reactions of metal ions [Fe (II), Cu (II), Mn (II) and Zn (II)] with fulvic acid fractions

Source	Metal ions			
	Fe (II)	Cu (II)	Mn (II)	Zn (II)
Fulvic acid	9.65	9.68	9.72	9.61

CONCLUSIONS

In general, humic acid and fulvic acid metal ion complexes showed almost similar x-values with relatively higher x-values for cu-humate complex than other complexes indicating variation in the degree of ionization of functional groups at higher pH levels. This could be attributed to increased ionization of functional groups leading to more number of moles of ligand for complexing with one mole of metal ion. The lower 1/x values which indicated that better ability of these humic fractions to form complexes. The complexation behaviour of humic and fulvic acid fractions depends up on the quality and quantity of functional groups of these fractions and also on the type of metal ion. The log K values were higher for fulvic acid compared to humic acid indicates more the number of functional groups more log K values. The higher negative values of ΔGr^0 suggested that the spontaneous reaction showing greater possibility of the complex formed between humic fractions and metal ions.

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