



## Evaluation of pKa Values of Soil Humic Acids and their Complexation Properties

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors SP, TS and DS collected samples and performed experiments. Author DB calculated the stability constants and authors PPS and MKB designed, analyzed and interpreted data and wrote the manuscript.

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### ABSTRACT

A study has been undertaken to determine the acidic, and metal complexing properties of humic acid extracted from tea garden soils of different depths. The dissociation constant of humic acid has been evaluated from the pH titration curves. The pKa values obtained in this study almost agree well with the data reported for humic acids from natural sources. The presence of carboxylic and/or hydroxyl groups in the humic acids, under study, have been confirmed. Metal complexation properties of the extracted humic acids have been studied with three metal ions (Zn<sup>2+</sup>, Ni<sup>2+</sup>, and Cu<sup>2+</sup>). The stability constants of the metal humic acid complexes have also been determined. One pKa value for humic acid extracted from the surface soil (pKa = 2.50 and the 30 cm depth soil (pKa = 5.2) has been obtained whereas two pKa values (3.1 and 7.5) for humic acid from the 15 cm depth soil have been found. These acids contain carboxylic and/or hydroxylic acid groups. Stability constants of metal-humic acid complexes (log K) do not differ much for humic acids from the surface soil (1.92, 2.09 and 2.07) and the 15 cm depth soil (2.01, 2.31 and 1.72) for Zn<sup>2+</sup>, Cu<sup>2+</sup> and Ni<sup>2+</sup> respectively. This work will contribute some knowledge to the nature of organic matter in tea garden soil.

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## 1. INTRODUCTION

The properties of soil organic matter are diverse and depend on its source and diagenetic state. Most of the organic matter in natural water and soil consists of complex heterogeneous, brown or yellow acidic polymers collectively known as humic matter [1]. Most of the organic matter in ancient sediments is made up of post-depositional altered humic substances namely kerogen and coal. Humic matter is a relatively stable by-product of the microbial decomposition of a great variety of biochemical compounds derived from the dead cells and extracellular products of various organisms chiefly land plants and aquatic algae [2].

Humic acid exhibits buffering capacity over a wide pH range. This is one of the unique properties of humic substances [1]. Buffering capacity of humic acid is of considerable practical importance because most plants grow best within a narrow pH range. However, the exact contribution of humic acid to the buffering capacity of the soil is not precisely known but this behaviour of the acid is remarkable. In short, soils that are rich in humic substance are well buffered.

Humic acid behaves as a negatively charged colloid due to the ionization of the acidic carboxyl and hydroxyl groups [3]. Sol state of such colloids represent principally an equilibrium state reached spontaneously by dissolution of dry colloid by appropriate solvent. The shape of the molecule in solution is influenced by pH and also the presence of neutral salts. In neutral or slightly alkaline medium, the molecules are in an expanded state due to mutual repulsion of charged acidic groups. On the other hand, in presence of a neutral electrolyte and also low pH, contraction occurs due to charge reduction and the molecule becomes more spherical shape. If the pH is very low and at quite high concentrations, molecular aggregates are formed [1].

Since humic acid carries net negative charges, it exhibits mobility in the presence of an applied electric field. The electronegative groups may also be present that can participate in redox reactions. Various redox reactions between transition metal ions and humic acid are well known. For example, iron commonly exhibits two valence states – ferrous and ferric states. It has

been reported that iron is preferentially uptake by humic acid [4]. Two important factors have been considered to influence the preferential uptake of iron by humic acid: (a) The change of degree of ionization of humic acid is determined by its redox state. The redox potential of humic acid is +0.70v in aqueous solution while that of the ferric-ferrous system is known to be +0.77v [5]. This similarity between the two systems implies rapid exchange of charge between humic acid and iron. (b) Ionic potential (valence state of a cation divided by its ionic radius) is an important parameter to elucidate the coordination as well as retention abilities of metal ions. Pauli [6] has reported the interaction of various cations with humic acid considering the ionic potential. Among most of the elements, ferric iron has quite high ionic potential and thus the retention of iron by humic acid is quite significant. Therefore, metal-humic acid complexes have been studied by largely because these complexes have considerable agricultural and industrial interests.

Metals are natural components in soil. Heavy metals are conventionally defined as elements with atomic number greater than 20. For growth of plants, some heavy metals are necessary because these act as micronutrients. Generally Mn, Co, Ni, Cu and Zn are available in soil besides some toxic metals such as Hg, Pb and Cd.

When humic acid comes in contact with the soluble metal ions, metal-humic acid complexes are formed. This organic substance contains acidic groups, as electron donating groups, act as ligands which are responsible for the formation of complex compounds. The formation of metal complexes is highly pH dependent. A competition for the humic acid ligand between metal ion and the proton is possible for binding.

It has already been mentioned above that humic acid is a heterogeneous substance commonly contains carbon, hydrogen, nitrogen, sulphur and oxygen. Therefore, this organic substance can form varieties of complex compounds; these are precisely considered as chelate formation. The number of protons removed during the formation of chelate compounds can be determined from a pH titration of a mixture containing humic acid and metal ion in solution.

In this paper, our main aims are to know the elemental composition of humic acids at different

soil depths, to know the pH values at which equivalence points of humic acids are obtained, to determine the pKa values of humic acids, to understand the pH-titration of humic acid in presence of metal ion for the determination of stability constants of the metal-humic acid complexes.

## 2. MATERIALS AND METHODS

### 2.1 Soil Samples

Soil samples from a tea garden under Titabar subdivision, Jorhat district, Assam was used for this study. The samples were collected from the surface, below 15 cm depth and below 30 cm depth. The three soil samples were air dried. The dried samples were ground and then sieved well. These were stored in polythene bags.

### 2.2 Extraction of Humic Materials from Soil

100 g of each air dried soil sample was taken in a 1000 ml beaker and to it 0.1 M 300 ml NaOH solution was added slowly with occasional stirring. The materials in the beaker were kept overnight at room temperature (26°C). The supernatant liquid was siphoned out and collected in a 1000 ml beaker. The alkaline extraction was repeated additionally two times and in those cases, the soluble humic material was also collected as before. The brown coloured soluble humic material was then centrifuged to remove any clay material present. This process was repeated twice. The clear soluble humic material was stored in a plastic container.

### 2.3 Precipitation of Humic Acid

The clear soluble brown humic material was acidified by slowly adding 6M HCl until stable pH 1.0 was attained. The material was kept overnight for complete precipitation. The precipitate was separated with the help of a centrifuge and washed with distilled water till chloride ion gave negative test. The wet material was collected and then kept in a freeze. This wet brown material was humic acid.

For the preparation of dry humic acid, wet organic matter was taken in a petre disk and it was kept in an electric oven. The temperature of the oven was maintained at 60°C and the organic matter was heated for two hours. The dry

humic acid was ground in a mortar and finally kept in a plastic bottle.

### 2.4 Analysis of Humic Acid

Some important analysis were carried out to understand the nature and type of the humic acids extracted from surface, below 15cm depth and below 30 cm depth soil samples. The analysis carried out are - moisture, ash, carbon, hydrogen, nitrogen, sulphur and oxygen content [7,8].

### 2.5 pH - Titration of Humic Acid

The titration of humic acid with sodium hydroxide solution is an acid-base titration, more precisely, weak acid-strong base titration. While determining the equivalence point of an acid-base reaction by titration method, generally an indicator is used, which by changing its colour gives the end point of the titration. The heterogeneous nature of humic acid which is also brown in colour, the equivalence point is determined from the pH titration curve. From the pH titration of the unknown acid, it is possible to determine the concentration and the dissociation constant (pKa) of the humic acid.

0.1 g (on dry basis) of wet humic acid each of different depths soil was taken in a beaker and to it 20 ml distilled water was added. To this, 1M 5 ml KCl solution was added and finally made the volume of the solution to 50 ml by adding more distilled water. Measurement of pH was made with glass electrode and calomel reference electrode with the aid of a digital pH meter having a sensitivity of + 0.01 pH. The cell of the meter was dipped into the humic solution taken in the beaker. Titrations were performed against standard NaOH (0.0096 M) solution with continuous stirring at room temperature (26°C). The stable pH value was recorded after each addition of 2 ml of the alkali. Titrations were done for humic acids obtained from surface, 15 cm depth and 30 cm depth of tea garden soil samples.

### 2.6 Similar pH Titrations were done for Dry form of these Humic Acids

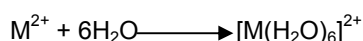
In a pH titration plot, the pH of an equivalence point is determined by finding the mid point where a significant rise in pH is observed. Also the pKa value of a humic acid is determined as the half point of the volume required for the

equivalence point; the corresponding point in the pH axis gives the pKa value of that particular humic acid. The pKa is the pH at which the molecule is 50% protonated. Measured pKa values depend on the ionic strength of the solution under investigation [9].

## 2.7 pH-Titration of Metal Salt Solution

The procedure is that first, pH titration of humic acid is done alone. The result of this titration gives the dissociation constant of the humic acid. Secondly, a pH titration is carried out in which humic acid is titrated under identical conditions in the presence of metal ions. NaOH is generally used as titrant. Finally, the stability constant(s) for the metal-humic acid complex can be determined. The pH titration of humic acid in presence of a metal ion in solution has been carried out for individual metal ion. The titration curves of humic acids in absence and presence of metal ion have been given to know the pH at which the complex formation starts. During pH titration, the available concentration of humic acid in absence and presence of metal ion is different. This results in distinct variation of pH titration curves of humic acid alone and that in the presence of metal ion. From the pH titrations, the concentration of humic acid (in absence and presence of metal ion) and that of the metal-humic acid complex can be evaluated; the concentrations of sodium ion and metal ions are known. These values are necessary for the determination of stability constant of metal-humic acid complex.

When a metal salt is dissolved in water, it forms aquo complex



To the aqueous solution of the metal salt, when humic acid is added, some of the aquo ligands are substituted by humic acid (HA) to form metal-humic acid complex. To depict the interaction here, it is convenient if the charge on metal ion and number of ligands are not considered.



The equilibrium constant (K) from the above reaction is

$$K = [M(HA)] [H_2O] / [M(H_2O)] [HA]$$

In aqueous solution, the concentration of water can be regarded as constant, the equilibrium constant, K is

$$K = [M(HA)] / [M(H_2O)] [HA]$$

K is known as the stability constant for [M(HA)]. The reaction between a pure known ligand and a metal ion may occur stepwise and therefore, stepwise stability constant can be obtained. The higher the value of stability constant for a complex, the greater will be its stability. Generally, stability constants are quoted as logK.

For titrations of various metal salt solutions, each solution (0.01M, 2 ml) of either ZnSO<sub>4</sub>.H<sub>2</sub>O, CuSO<sub>4</sub>.5H<sub>2</sub>O, or NiSO<sub>4</sub>.7H<sub>2</sub>O, (all AR grade) was taken in a beaker. To the metal salt solution, 1M, 5 ml KCl solution was added. Then distilled water was added to make the final volume to 50 ml. Titrations were done as before with 0.0096 M NaOH solution; the pH value obtained after each addition of 2 ml of alkali was recorded.

## 2.8 pH- Titration of Humic Acid in Presence of Metal Ion

0.1 g (on dry basis) of wet humic acid was taken in a beaker and to it 20 ml distilled water was added. Then 1M 5 ml KCl solution followed by a metal salt solution (0.01 M 2 ml) were added to the beaker containing humic acid. Finally the volume of the solution was made to 50 ml by adding more distilled water.

Titrations were done as before using NaOH solution (0.0096 M) and the values of pH were recorded.

## 3. RESULTS AND DISCUSSION

### 3.1 Elemental Analysis of Humic Acids

The proximate and ultimate analysis of humic acids, under study, are given in Table 1. The value of oxygen content was calculated by difference. It is observed from the Table 1 that the moisture contents in humic acids do not differ much as the values are in the range 5.54-7.53%. the values gradually decrease on increasing depth of the soil. On the other hand, the ash contents in the humic acids significantly differ much. Highest ash content is observed in the humic acid obtained from 15 cm depth soil and lowest in the humic acid obtained from the surface soil. The ash contains mainly metal oxides and some amount of silica [10]. The elements in the ash are bonded with the organic matter which are not leachable in both alkali and acid treatments used for extraction of humate from the soil and precipitation of humic acid, respectively.

The volatile matter present in the humic acid are predominantly low molecular weight compounds. The values of volatile matter obtained in humic acids, under study, are in the range 34.64 – 39.74% signifying occurrence of almost similar amounts of volatile matters in these humic acids. The fixed carbon content in the humic acids is relatively higher than those of volatile matters and is in the range 47.88 – 54.22%.

The carbon contents in all the humic acids extracted from different soil samples (Table 1) are relatively lower than those reported by other workers [7,8]. However, hydrogen, nitrogen and sulphur contents in humic acids, under study, are within the range as reported by previous researchers [7,8]. The oxygen percentage in the three humic acids are significantly high.

Based on the percentage composition of the elements (Table 1), empirical formulae of humic acids extracted from different samples of tea garden soils are calculated as:  $C_{70}H_{138}N_6SO_{260}$  for humic acid from surface soil,  $C_{123}H_{164}N_{10}SO_{277}$  for humic acid from 15 cm depth soil, and  $C_{100}H_{148}N_8SO_{254}$  for humic acid from 30 cm depth soil. The C/N ratio for surface, 15cm depth and 30cm depth soil humic acids have been found to be 10.0, 10.5, and 8.1 respectively. These values are in quite similar to the reported data suggesting good quality of organic matter [11].

### 3.2 Equivalence Points and Dissociation Constants of Humic Acids

In our work, the pH titration has been carried out using a strong base, NaOH. The change in pH that occurred during the titration of humic acid with NaOH solution was monitored. A large number of pH values were recorded after each 2 ml addition of the alkali solution; these are represented in Fig. 1; the plots have shown a curve. The humic acids extracted from surface soil, 15 cm depth soil and 30 cm depth soil show distinct different type of curves (Fig. 1). Moreover, the shapes of the pH titration curves for humic acids are not alike. At the equivalence point, a sudden change in the pH has occurred as the solution goes to strongly basic.

Humic acid from the surface soil sample required highest volume of alkali solution to attain the equivalence point. The humic acid of the 15 cm depth sample requires lesser amount of alkali solution while the humic acid of the 30 cm depth samples requires further less amount of the alkali

solution for attaining the equivalence points. In case of the humic acid from 15 cm depth soil sample, two equivalent points may be possible as the nature of the curve shows two breaks.

At the beginning, humic acid was taken in the wet form which is weakly ionized. As the titration proceeds, some  $H^+$  ions are removed from the acid and substituted by  $Na^+$  ions; the  $H^+$  ions are neutralized to form  $H_2O$ . Therefore, the concentration of the  $H^+$  ions gradually decreases. At the equivalence point, the neutralization is complete and finally sodium humate would remain in the solution. However, on further addition of NaOH solution, the  $OH^-$  ion concentrations are rapidly increased.

The pH values at which equivalence points of humic acids from surface soil, 15 cm depth soil and 30 cm depth soil samples and their corresponding volume of alkali needed are presented in Table 2. Moreover, pKa values of the three different humic acids are also given in the Table 2.

It appears that humic acid obtained from the various soil samples are not all alike as their pKa values and also the pH values of the equivalence points differ. This variation is due to presence of acidic functional groups as well as on electronic factors. It is known that the overall acidity is factored as: (a) A lower pKa value, normally around 4.2, represents the carboxylic distribution and (b) A high pKa value, normally around 9.1 attributes the phenolic distribution. In our study, quite high pKa value is not found and, therefore, the contribution of the carboxylic acid is dominant. The pKa values for humic acid from the surface soil has been found to be 2.5 (Table 2). In case of humic acid from 15 cm depth soil, two pKa values at 3.1 and 7.5 are found (Table 2). Only one pKa value of 5.2 is found in case of humic acid from 30 cm depth soil (Table 2). The nature and type of humic acid from 15 cm depth soil is considerably different from those of other two humic acids as the former humic acid shows two pKa values.

The dissociation constants obtained in this study for different humic acids are compared with reported data. In Table 3, the pKa values of humic acid from some natural sources and some aromatic acids are given [12-17].

The pKa values obtained in this study almost agree well with the data reported for humic acids from natural sources. Moreover, it is apparent

that the low pKa value of humic acids from surface soil and 15 cm depth soils, attributes ortho position with respect to a carboxyl group is occupied either by a hydroxyl or another carboxyl group as the pKa values of these standard acids (i.e., O-hydroxybenzoic acid and O-phthalic acid) coincide with the humic acids, under study. However, the pKa value (of 7.5) obtained in the humic acid from 15 cm depth soil (Table 2) may be correlated with the contribution of phenolic groups. Also it may be inferred that the pKa value of humic acid from the 30 cm depth soil is similar to that of the p-hydroxy benzoic acid; the pKa value of 4.96 has been reported for soil humic acid [14].

A question is: Why do pKa values differ in different humic acids extracted from different soil samples? In a tea garden soil, the content of the organic matter is quite high at the surface soil because of enough deposition and decomposition of plant materials at the top soil. At the surface soil, humification process is

continuous resulting in formation of more acidic groups. It is important that humification is a major geochemical phenomenon of great importance to biosphere [18,19]. It occurs under a wide range of environmental condition – aerobic and anaerobic. Humifying and decomposing activities of microorganism are very intense at the distance below the surface; but with increasing depth of burial, microbial activity is gradually superseded by slow, physiochemical alternation or maturation of humus [1]. Maturation is the result of mild heat and pressure; it is possible that interaction with mineral surfaces and formation of metal complexes are also involved. Temperature is probably the most crucial variable in this process. Thus, with increasing depth of the tea garden soil, maturation of humic acid and contribution of metal ions (in complex formation) are quite obvious. This results in gradual decrease of acidic groups with increasing depth of the soil and for this fact, it quite probable that consumption of alkali while titration with humic acid decreases with increasing depth of soils (Fig. 1).

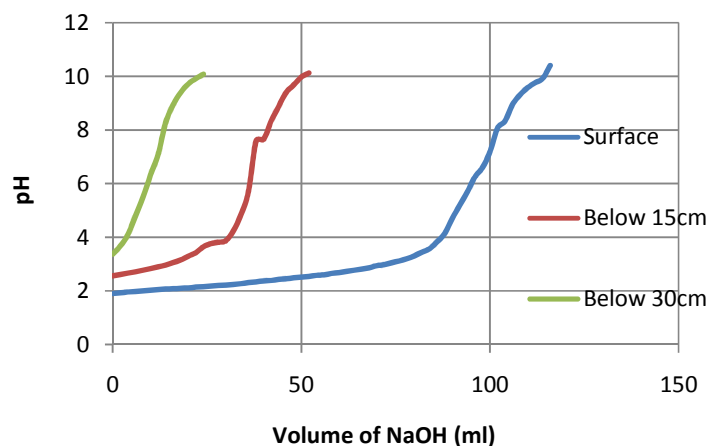


Fig. 1. pH titration curves for humic acids extracted from surface soil, 15 cm depth soil and 30 cm depth soil

Table 1. Proximate and ultimate analysis of humic acids obtained from tea garden soils

| Humic acid from | Proximate analysis |         |                     |                  | Ultimate analysis* |       |       |       |                     |
|-----------------|--------------------|---------|---------------------|------------------|--------------------|-------|-------|-------|---------------------|
|                 | Moisture (%)       | Ash (%) | Volatile matter (%) | Fixed carbon (%) | C (%)              | H (%) | N (%) | S (%) | O (%) by difference |
| Surface soil    | 7.53               | 3.61    | 34.64               | 54.22            | 15.99              | 2.62  | 1.59  | 0.61  | 79.19               |
| 15 cm depth     | 6.62               | 12.58   | 35.43               | 45.37            | 23.49              | 2.62  | 2.24  | 0.63  | 71.02               |
| 30 cm depth     | 5.54               | 6.84    | 39.74               | 47.88            | 21.68              | 2.67  | 1.91  | 0.58  | 73.16               |

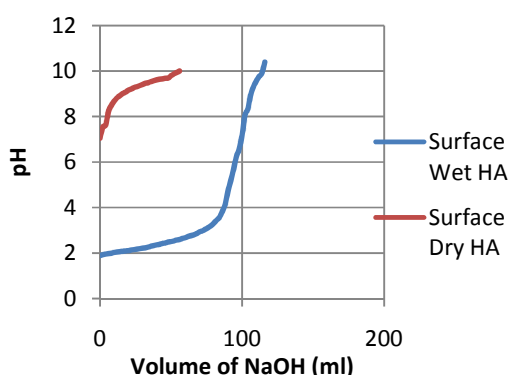
\*Moisture and ash free basis

**Table 2. pH and volume of alkali required for equivalence point and pKa values of humic acids**

| Humic acid from  | For attaining equivalence point |                                | pKa |
|------------------|---------------------------------|--------------------------------|-----|
|                  | pH                              | Volume of alkali required (mL) |     |
| Surface soil     | 6.2                             | 96.4                           | 2.5 |
| 15 cm depth soil | 6.2                             | 34.0                           | 3.1 |
|                  | 8.7                             | 44.0                           | 7.5 |
| 30 cm depth soil | 7.4                             | 15.0                           | 5.2 |

**Table 3. Dissociation constants of humic acid from some natural sources and some aromatic acids**

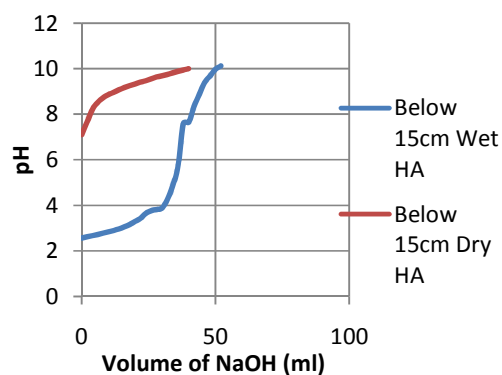
| Sl. no. | Acid                     | pKa       | Reference |
|---------|--------------------------|-----------|-----------|
| 1       | Humic acid from soil     | 4.50      | 12        |
| 2       | Humic acid from soil     | 8.50      | 13        |
| 3       | Humic acid from soil     | 4.96      | 14        |
| 4       | Humic acid from sediment | 4.0 & 9.0 | 15        |
| 5       | Humic acid from lignite  | 2.80      | 16        |
| 6       | o-hydroxy benzoic acid   | 2.97      | 17        |
| 7       | p-hydroxy benzoic acid   | 4.48      | 17        |
| 8       | m-hydroxy benzoic acid   | 4.06      | 17        |
| 9       | Benzoic acid             | 4.19      | 17        |
| 10      | o-phthalic acid          | 2.89      | 17        |
| 11      | p-phthalic acid          | 3.51      | 17        |
| 12      | m-phthalic acid          | 3.54      | 17        |

**Fig. 2. pH titration curves for dry and wet humic acids from surface soil**

A comparison of dry and wet humic acids have also been carried out to understand their behavior. pH titration curves for dry and wet humic acids from surface soil, 15 cm depth soil and 30 cm depth soil are presented in Figs 2-4, respectively. Different pattern of the curves for dry and wet humic acids for all the samples are observed.

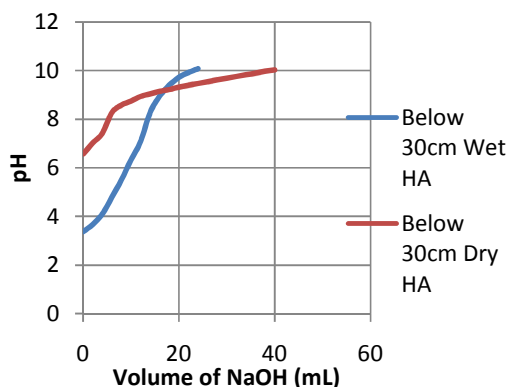
When wet humic acid is dried, some configurational change of the structure of the organic matter is possible. During drying, contraction of humic acid takes place with the elimination of water. This leads to shrinkage

which is completely irreversible. Consequently, the acidic sites which are readily available for interaction with alkali, are considerably reduced. The pH titration curves for dry and wet humic acids for all the three samples are quite different. Rapid rise of pH and less consumption of alkali in case of dry humic acids further reveals that availability of acidic sites in the dry organic matter is quite low.

**Fig. 3. pH titration curves for dry and wet humic acids from 15 cm depth soil**

An important observation noticed in the Fig. 4 is that the pH titration curves for dry and wet humic acids cross at pH about 9.2. This behavior is not found in Figs. 2 and 3 where the curves are far

apart. Crossing of curves for dry and wet forms at pH about 9.2 in case humic acids obtained from 30 cm depth soil sample (Fig. 4) may be due to the phenolic groups which probably did not alter during drying and as a result, same volume of alkali has been consumed at that pH.

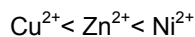


**Fig. 4. pH titration curves for dry and wet humic acids from 30 cm depth soil**

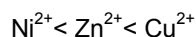
### 3.3 Metal Complexation of Humic Acids

The pH largely affects the ionization of acidic groups resulting in number of binding sites available for complex formation. Thus the values of stability constant would be different at different pH. Moreover, some configurational changes in the organic substance are also possible due to complex formation. Although there are some limitations of stability constant measurements for metal-humic acid complex but we have attempted to calculate the stability constant of metal-humic acid complex for humic acids obtained from the surface soil and 15 cm depth soil here.

In our study, we have considered three metal ions ( $Zn^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$ ) which are commonly occurring in soil as micronutrient for the plants. In the first step of our experiment, pH titration of these metal ions individually have been carried out with NaOH as the titrant. The pH titration curves are shown in Fig. 5. The formation of metallic hydroxide is indicated by the inflection in the titration curves. At pH 10, the volume of the alkali required for the hydrolysis of the metal ions is in order



The increase in pH is in the following order.

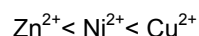


The pH titration curves for humic acid extracted from surface soil in presence of  $Zn^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$ , individually are presented in Figs. 6-8. Similarly, the pH titration curves for humic acid extracted from 15 cm depth soil in presence of  $Zn^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$ , separately are presented in Figs. 9-11.

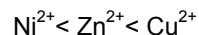
The formation of metal humic acid complex starts at low pH. It can be determined by knowing the pH at which the pH titration curves of humic acid alone and humic acid in presence of metal ion differs. For humic acids extracted from the surface soil and 15 cm depth soil, the starting pH at which the formation of metal humic acid complexes for three metal ions are given in Table 4. The specific values of pH, recorded at which pH titration curves of humic acid alone and humic acid in presence of metal ion differs, for humic acid from the surface soil is observed (Table 4). In case of humic acid from 15 cm depth soil, pH titration curve of humic acid alone and humic acid in presence of  $Cu^{2+}$  ion differ but in presence of other two metal ions ( $Zn^{2+}$  and  $Ni^{2+}$ ), both the curves do not touch at any point of pH.

The stability constant values are given in Table 5. The values are similar; however, the order of stability of the complexes are;

For humic acid from surface soil:



For humic acid from 15 cm depth soil:

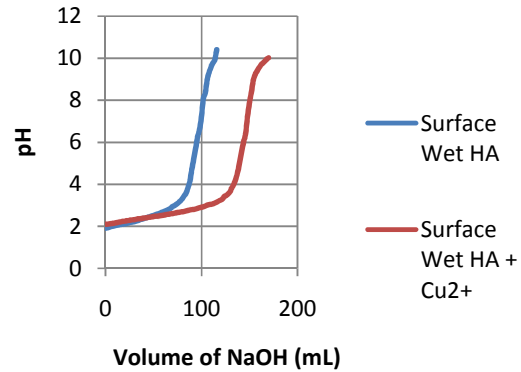


The pH titration curves for wet humic acid from surface soil and 15 cm depth soil in presence of  $Zn^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$  ion (Figs. 12 and 13) have shown clearly the variation of the meta-humic acid complex formation. Since nitrogen, oxygen and sulphur are prominent electron pair donating elements in humic acid, their total percentage varies in these acids revealing that the extent of metal-humic acid formation will not be the same, thus strongly supporting our results of variation of the stability constants ( $\log K$ ) of metal-humic acid complexes.

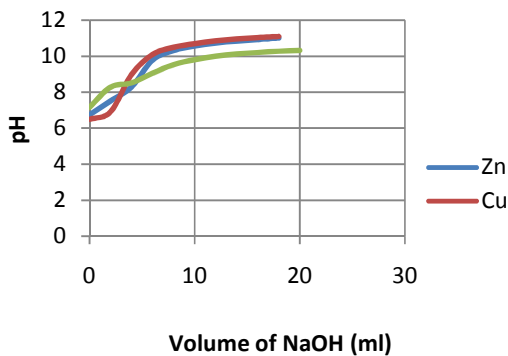


**Table 4. pH and volume of alkali needed for metal-humic acid complex formation**

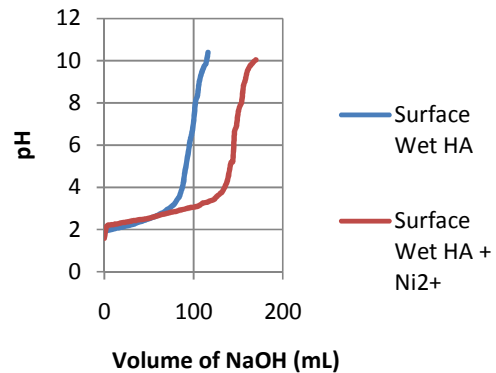
| Humic acid from  | Metal ion        | Metal humic acid complex formation starts at |                                |
|------------------|------------------|--|--------------------------------|
|                  |                  | pH   | Volume of alkali required (mL) |
| Surface soil     | Zn <sup>2+</sup> | 2.4  | 42.0                           |
|                  | Cu <sup>2+</sup> | 2.6  | 56.0                           |
|                  | Ni <sup>2+</sup> | 2.4  | 42.0                           |
| 15 cm depth soil | Zn <sup>2+</sup> | 3.5  | 25.0                           |
|                  | Cu <sup>2+</sup> | 3.8  | 26.0                           |
|                  | Ni <sup>2+</sup> | 3.2  | 22.0                           |



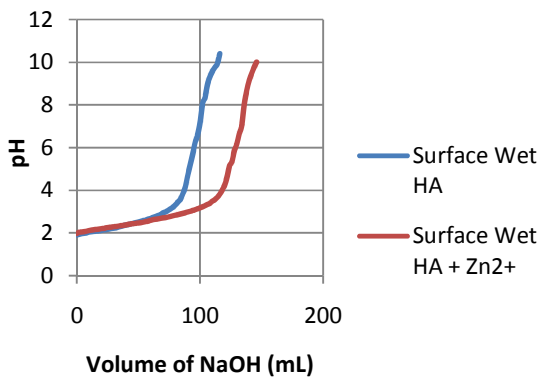
**Fig. 7. pH titration curves for wet humic acid from surface soil in absence and presence of Cu<sup>2+</sup> ion**



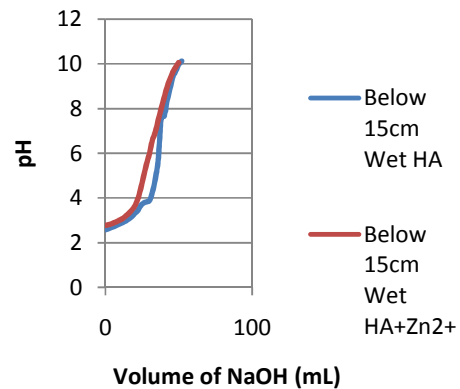
**Fig. 5. pH titration curves for Zn<sup>2+</sup>, Cu<sup>2+</sup> and Ni<sup>2+</sup> salt solutions**



**Fig. 8. pH titration curves for wet humic acid from surface soil in absence and presence of Ni<sup>2+</sup> ion**



**Fig. 6. pH titration curves for wet humic acid from surface soil in absence and presence of Zn<sup>2+</sup> ion**



**Fig. 9. pH titration curves for wet humic acid from 15 cm depth soil in absence and presence of Zn<sup>2+</sup> ion**

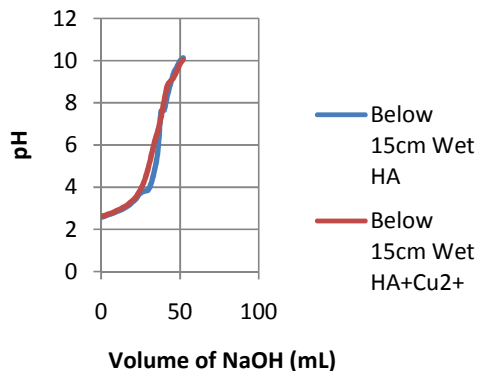


Fig. 10. pH titration curves for wet humic acid from 15 cm depth soil in absence and presence of Cu<sup>2+</sup> ion

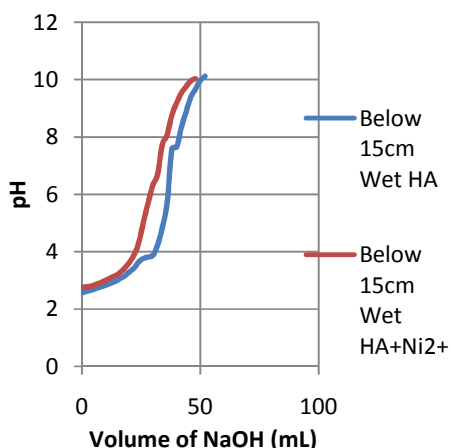


Fig. 11. pH titration curves for wet humic acid from 15 cm depth soil in absence and presence of Ni<sup>2+</sup> ion

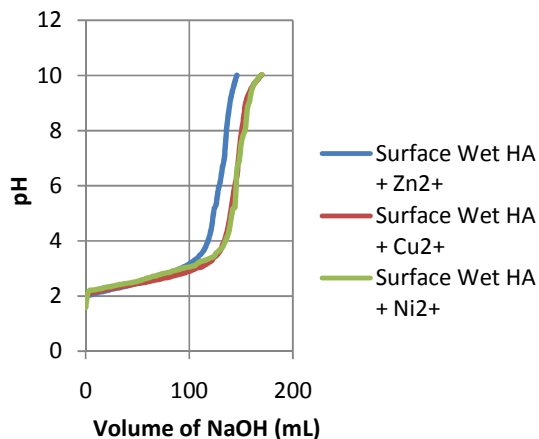


Fig. 12. pH titration curves for wet humic acid from surface soil in presence of Zn<sup>2+</sup>, Cu<sup>2+</sup> and Ni<sup>2+</sup> ion

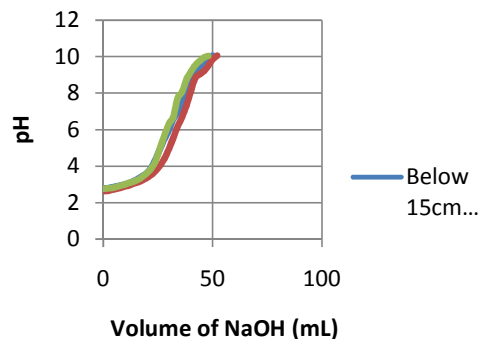


Fig. 13. pH titration curves for wet humic acid from 15 cm depth soil in presence of Zn<sup>2+</sup>, Cu<sup>2+</sup> and Ni<sup>2+</sup> ion

Table 5. Stability constants (log K) of metal-humic acid complexes

| Humic acid from  | Metal ion        | Stability constant of metal-humic acid complex (logK) |
|------------------|------------------|---|
| Surface soil     | Zn <sup>2+</sup> | 1.92  |
|                  | Cu <sup>2+</sup> | 2.09  |
|                  | Ni <sup>2+</sup> | 2.07  |
| 15 cm depth soil | Zn <sup>2+</sup> | 2.01  |
|                  | Cu <sup>2+</sup> | 2.31  |
|                  | Ni <sup>2+</sup> | 1.72  |

#### 4. CONCLUSION

It may be concluded that the acidic nature of the humic acids obtained from different depth tea garden soils are not alike as these have different pKa values suggesting variation of the content of carboxylic and hydroxylic groups in these organic matters. Further due to different content of electron pair donating elements (N,O and S) in these organic acids, the stability constant have been found to vary. This work reveals a better understanding of nutrient supply by the organic matter in different depth soils to the tea plants.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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