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## Stability of organo-zinc complex in application of inorganic and organic nutrients to rice (*Oryza sativa*) growing soils of West Bengal (India)

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### Abstract

Humic and fulvic acids play a significant role in complexing metal ions in the soil-matrix. A two years field experiment was carried out under an acidic, sandy loam soil conditions, to study the effect of farm yard manure (FYM) and green manure (GM) and inorganic (N-P-K-Zn) nutrient sources on soil humic and fulvic acid characteristics, organo-Zn stability constant, Zn-uptake and yield of puddle rice in an acidic sandy loam soil. The fulvic acids showed higher total acidity and intrinsic viscosity than humic acids, and the carboxylic groups were higher than phenolic groups in all the humic and fulvic acid samples extracted from the experimental plots. The wide variation in  $\Delta E_4/E_6$  characteristics for humic acids was reflected by a higher degree of condensation of aromatic rings. The value of stability constant ( $\log K$ ) was affected by different inorganic and organic (FYM and GM) inputs and varied from 1.32 to 2.45 for humic acids, and from 1.20 to 2.09 for fulvic acids. The organo-zinc stability influenced the Zn uptake by rice and the residual status of Zn in soils. Considering all treatments over the years, integrated application of both the inorganic (NPK 50% & Zn) and organic (50% N through FYM) was found as the most suitable in terms of rice yield.

**Keywords:** humic acids, fulvic acids, zinc, organo-metal complex, submerged rice

### Introduction

Humic and fulvic acids are the important components of soil organic matter (Christensen *et al.* 1998) [8] which regulates the soil physical and chemical characteristics (Schinitzer, 1992) [24]. Ability of these acids (HA/FA) to form complex metal ions provides an additional benefit into the soil system (Stevenson, 1982) [31]. Humic acids form aqueous complex with micronutrients in a limited extent (Aiken *et al.* 1985) [1]; Bama and Selvakimari, 2001) [5] and it also bind heavy metals into soil colloidal surfaces, which is not easily leached (Spark *et al.* 1997) [30], thereby promotes heavy metal (e.g. Cu and Zn) sorption to soil minerals. Complexes of fulvic acids with metals are generally more soluble as compared to humic acids, possibly due to lower molecular weight and higher solubility of fulvic acids in water (Stevenson and Ardakani, 1972 [32]; Tan, 2014) [35]. The solubility of metal-humic complexes is usually expressed in terms of solubility constant; and depending on the solubility constants of the complex compounds, they can be soluble or insoluble in water. Carboxylic (-COOH), phenolic (-OH), amine (-NH<sub>2</sub>), and carbonyl (C=O) are the important functional groups of these substances and active in retaining /complexing metal ions (Cu<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Fe<sup>2+</sup> etc.) (Datta *et al.* 2001) [9]; although carboxylic (COOH) and phenolic (OH) groups are most abundant in humic substances.

Zn deficiency is a widespread soil micronutrient disorder for the major parts of India. Introduction of the high yielding varieties, crop intensification and thereby continuous removal of native soil zinc have further aggravated its deficiency especially in rice growing soils. Availability of the applied inorganic zinc in soil varied and it depends on its mobility, diffusion rate, natural processes in soil solution and precipitation (Diatloff *et al.* 1998) [11]; McLean and Bledsoe 2014) [18]. Acidic soils of West Bengal are deficient in zinc (Ghosh *et al.* 1992) [13]; Singh 2001) [28] due to the high content of Fe-P, leading to the formation of Zn-P complex (Ali, 1992 [2]; Austruy *et al.* 2014) [3] in the soil system. Metallic cation binding ability of organic compounds (HA/FA) may play important role to maintain available Zn status at optimum level in this puddled rice growing highly weathered acidic soils.

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However, very little information is available about the impact of organic nutrient sources on formation and stability of organo-Zn complexes in these soils. Therefore, a field study was undertaken to evaluate the impact of farm yard manure and green manure addition on changes in characteristics of the humic/ fulvic acid substances and stability of organo-Zn complexes in a highly weathered puddled rice growing acidic soil. The effect of organic and inorganic nutrient management in this acidic soil on rice yield, Zn uptake and the status of the residual soil zinc have also been studied.

## Materials and Methods

### Location and soil characteristics of experimental site

The field experiment with rice (submerged) was conducted at Nagrakata, District Jalpaiguri of State West Bengal, India (26°52'11.2"N latitude and 88°54'36.7"E longitude) during *kharif* season for two consecutive years. The soil of the experimental site was sandy loam in texture, with pH 4.97, organic carbon 21.8 g kg<sup>-1</sup>, bulk density 1.26 (g cc<sup>-1</sup>), available nitrogen 49.55 kg ha<sup>-1</sup>, phosphorus 30.98 kg ha<sup>-1</sup>, potassium 243.24 kg ha<sup>-1</sup> and DTPA extractable zinc 0.52 mg kg<sup>-1</sup> soil.

### Experimental design and treatments

The experiment was laid out in randomized block design with eight treatment combinations [T<sub>1</sub>-Control, T<sub>2</sub>-50% recommended doses (RD) of NPK through inorganic fertilizer + Zn, T<sub>3</sub>-75% RD of NPK through inorganic fertilizer + Zn, T<sub>4</sub>-100% RD of NPK through inorganic fertilizer + Zn, T<sub>5</sub>-50% RD of NPK through inorganic fertilizer + 50% RD of N through organic sources (i.e, Farm Yard Manure) + Zn, T<sub>6</sub>-75% RD of NPK through inorganic fertilizer + 25% RD of N through organic source (i.e, Farm Yard Manure) + Zn, T<sub>7</sub>-50% RD of NPK through inorganic fertilizer + 50% RD of N through organic source (i.e, Green Manure) + Zn, T<sub>8</sub>-75% RD of through inorganic fertilizer NPK + 25% RD of N through organic (i.e, Green Manure) + Zn,] having three replications for rice (Cv. Swarna). The size of the each experimental plot was 5m x 3m. The proper agronomic packages of practices were followed till the harvest of the crop. The N, P and K nutrients of rice were supplied through Urea (46% N) as N source, Single Super Phosphate (SSP-16% P<sub>2</sub>O<sub>5</sub>) as P source and Muriate of Potash (MOP-60% K<sub>2</sub>O) as K source of fertilizers respectively, while the zinc was supplemented through Zinc Sulphate (21% Zn) for rice. The organic inputs like farm yard manure (FYM) and *exsitu* green manure (GM) were incorporated 7 days before transplanting of rice.

### Soil and plant sample analysis

Soil samples were collected (0-15cm) from each plot, dried, processed and analyzed. The DTPA-extractable zinc from soils were measured by using the extracts containing 0.005 molar DTPA (diethylene triamine penta acetic acid), 0.01M calcium chloride, and 0.1 M tri-ethanol amine buffered at pH 7.3 (Lindsay and Norvell, 1972)<sup>[17]</sup>, and estimated by Atomic Absorption Spectrophotometer (AAnalyst 200). The plant samples were collected at harvest, dried, processed and analyzed to estimate the Zn uptake by rice.

### Isolation and purification of humic and fulvic acids

Collected air dried soil samples from different treatments were shaken with 0.5 N sodium carbonate in a Winchester bottle and kept overnight. The supernatant was siphoned off and acidified to a pH of 2.0-3.4 with HCl to precipitate humic acid and kept overnight for complete precipitation. This

process was repeated for dissolution and re-precipitation of humic acid. The supernatant was siphoned off and the precipitation was dialyzed by using dialysis paper till it was free from chloride. The supernatant was adjusted to pH 8.0 and barium chloride was added to precipitate the fulvic acid as Ba-fulvate. The Ba-fulvate was dialyzed till it was free from chloride. Then it was passed through a hydrogen saturated resin column to obtain pure fulvic acid (Kononova *et al.* 1969)<sup>[16]</sup>. The zinc-HA/FA complexation equilibria were explored by resorting to an Ion-Exchange Method (Schnitzer and Skinner, 1966)<sup>[26]</sup>.

The different humic acid samples were denoted as follows

Humic acids	Collection site
HAT <sub>1</sub>	Humic acid extracted from the soils treated with T <sub>1</sub>
HAT <sub>2</sub>	Humic acid extracted from the soils treated with T <sub>2</sub>
HAT <sub>3</sub>	Humic acid extracted from the soils treated with T <sub>3</sub>
HAT <sub>4</sub>	Humic acid extracted from the soils treated with T <sub>4</sub>
HAT <sub>5</sub>	Humic acid extracted from the soils treated with T <sub>5</sub>
HAT <sub>6</sub>	Humic acid extracted from the soils treated with T <sub>6</sub>
HAT <sub>7</sub>	Humic acid extracted from the soils treated with T <sub>7</sub>
HAT <sub>8</sub>	Humic acid extracted from the soils treated with T <sub>8</sub>

The different fulvic acid samples were denoted as follows

Fulvic acid	Collection site
FAT <sub>1</sub>	Fulvic acid extracted from the soils treated with T <sub>1</sub>
FAT <sub>2</sub>	Fulvic acid extracted from the soils treated with T <sub>2</sub>
FAT <sub>3</sub>	Fulvic acid extracted from the soils treated with T <sub>3</sub>
FAT <sub>4</sub>	Fulvic acid extracted from the soils treated with T <sub>4</sub>
FAT <sub>5</sub>	Fulvic acid extracted from the soils treated with T <sub>5</sub>
FAT <sub>6</sub>	Fulvic acid extracted from the soils treated with T <sub>6</sub>
FAT <sub>7</sub>	Fulvic acid extracted from the soils treated with T <sub>7</sub>
FAT <sub>8</sub>	Fulvic acid extracted from the soils treated with T <sub>8</sub>

### Determination of humic and fulvic acids (HA & FA) characteristics

Carboxylic (COOH) and phenolic (OH) groups of HA & FA by potentiometric titration

Samples of equal volumes of HA/FA suspension in a set of plastic bottles, different amounts of standard NaOH (0.01N) solution, *viz.*, 0 (no alkali), 2 drops, 4 drops, 6 drops, etc. of NaOH (0.01N) solution were added. The suspensions were stirred well with a glass rod and the corresponding pH was measured with the help of a pH meter. The suspensions were then kept overnight to ensure equilibrium and in the next day the pH was measured again. The carboxylic and phenolic (OH) group contents of each acid were recorded from the acidity equivalents of the first and the final inflexion points of the titration curves (Schnitzer and Khan, 1972)<sup>[25]</sup>.

Visible spectrophotometric measurement (E<sub>4</sub>/E<sub>6</sub>) of HA & FA. An optical densities at 465 nm and 665 nm for the dilute solutions of humic/fulvic acid (Na-form) were noted with the help of a Systronics model (Model: 104) of visible spectrophotometer at two different pH value, *viz.* 7.0 and 9.0. Viscometric measurement to compute molecular weight of HA & FA

Prior to the viscosity experiment, all the carboxyl groups to each HA/FA sample were rendered in the carboxylate form by the addition of requisite amount of alkali (NaOH) corresponding to the first inflexion point of the potentiometric titration curves.

The viscosities of the HA/FA suspensions were measured by the use of an Ostwald's Viscometer in the usual way, involving a prior knowledge about  $\eta_0$  (water) and  $\rho_0$  (density of water) at the experimental temperature. For each sample,

viscosities were measured at five different concentrations and for each case the ambient temperature of the laboratory was recorded. The mean flow time at each concentration was recorded. The densities of suspensions were also measured by the use of a specific gravity bottle. The specific viscosity ( $\eta_{sp}$  =  $\eta_{real} - I = \eta/\eta_0 - I$ ) at various concentrations for each HA/FA sample was thus found out and the corresponding graph, showing the plots of  $\eta_{sp} / \sqrt{C}$  vs.  $\sqrt{C}$  was constructed. The A and B coefficients of the *Jones-Dole's* equation (1929) were obtained from the intercept and slope of the best-fit linear plot as above, according to the relation,

$$\left( \frac{\eta}{\eta_0} - 1 \right) / \sqrt{C} = \frac{\eta_{sp}}{\sqrt{C}} = A + B\sqrt{C} \quad (1)$$

Where,  $\eta$  and  $\eta_0$  denoted the respective viscosities at the same temperature of the HA/FA suspension, having a percent concentration 'C', and water, while A and B were empirical constants sensitive to the interaction between the disperse phase particles and that between the disperse phase and dispersion medium, respectively. The  $B_{\text{expt.}}$  values of the humate anion were then obtained by subtracting the literature value of  $B_{\text{Na}^+}$  from  $B_{\text{expt.}}$  value of sodium humate, i.e.,  $B_{\text{expt.}} = B_{\text{Na-HA}^-} - B_{\text{Na}^+}$ , in view of the additivity of such  $B_{\text{ion}}$  values (Sanyal and Mandal, 1983) [23].

The  $B_{\text{expt.}}$  values were approximated to the respective intrinsic viscosities,  $[\eta]$  (Chen and Schnitzer, 1976), which were used to compute the viscosity-average molecular weight ( $M$ ) of the humic/fulvic substance, by setting  $K = 7.3 \times 10^{-4}$  and  $\alpha = 0.65$  in the modified Staudinger's equation as given below:

$$[\eta] = KM^\alpha \quad (2)$$

Determination of stability constant of organo-Zn complexes of humic and fulvic acids

The complexation equilibria between zinc and humic/fulvic substances was studied by following the standard techniques and the stability constants of the zinc-humic/fulvic complexes formed were evaluated by the method developed by Schnitzer and Skinner (1966) [26].

Aliquots of 0 to 10 ml of HA/FA solution were pipetted into 50 ml volumetric flasks and diluted to approximately 30 ml with distilled water. To each flask 5 ml of 1N NaCl was added, followed by 6 ml of an aqueous solution of  $ZnSO_4$  containing 250 mg Zn L<sup>-1</sup>. The pH was then adjusted to 5.0 by the addition of a few drops of 0.1 N NaOH or 0.1 N HCl solution as the case may be. Finally, the volume of each flask was made up to 50 ml with distilled water.

About 0.5 g of H<sup>+</sup> saturated cation exchange resin, along with 50 ml of zinc/HA or zinc/FA solution, was taken into 250 ml conical flask with glass stopper and shaken for 1 hour. Tests indicated that equilibrium was reached within this period of time. The exchange resin was then removed by filtration and the filtrate plus washing were taken to dryness on a water bath, and then were digested with 5: 1 of HNO<sub>3</sub> - H<sub>2</sub>SO<sub>4</sub>. Each digest was diluted with distilled water to a volume of 100 ml. The concentrations of the zinc in these aliquots were then measured by the atomic absorption spectrophotometer (Model: AAnalyst 200).

The logarithm of the stability constant of the zinc-humate/fulvate complex (log K) was determined from the following relationship.

$$\log \left( \frac{\lambda_0}{\lambda} - 1 \right) = \log K + x \log [HA] \quad (3)$$

Where,

$\lambda_0$  = Distribution constant in absence of HA/FA

$\lambda$  = Distribution constant in presence of HA/FA

K = Stability constant of zinc/HA or FA complex

x = Number of moles of HA/FA which combine with one mole of zinc

[HA] = Concentration of HA/FA in mole per liter

$\lambda_0$  was determined from the following expression:

$$\lambda_0 = \frac{\alpha_0 V}{(100 - \alpha_0)g} \quad (4)$$

Where,

$\alpha_0$  = percent of total metal bound to exchange resin

(100 -  $\alpha_0$ ) = percent of total metal remaining in solution

V = volume of solution (100 ml)

g = weight of exchange resin (0.5 g)

$\lambda$  was measured in the same manner as for  $\lambda_0$  in the presence of different HA/FA concentrations, where, 'x' can be obtained separately for zinc from the slope of the best-fit linear plot of  $\log (\lambda_0/\lambda - 1)$  vs. different HA/FA concentrations and the corresponding logK value from the intercept.

### Statistical analysis

A analysis of variance (ANOVA) was performed to elucidate the different humic and fulvic acids properties. All the statistical analysis of the data was carried out in accordance with the procedure suggested by Gomez and Gomez (1984) [15]. Analysis of variance (ANOVA) for comparison of means was performed using Microsoft Excel 2007 (Microsoft Corp., USA) and SPSS 16.0 (SPSS Inc., Chicago, USA) Windows version packages. Unless otherwise stated, the level of significance referred to in the results is  $p < 0.05$ .

## Results and Discussion

### Characteristics of humic and fulvic acids

#### Functional groups

The potentiometric characterization of humic and fulvic acids extracted from the different organically and inorganically treated soils showed that the carboxylic (-COOH) acidity was greater than phenolic (OH) group acidity (Table 1) in all the treatments. The similar observation was earlier reported by Mukhopadhyay and Sanyal (2004) [21]. Total acidity varied from 0.72 to 1.23 meq g<sup>-1</sup> for humic acids and 1.51 to 2.31 meq g<sup>-1</sup> for fulvic acids. Total acidity was relatively higher in the fulvic acid samples than the corresponding humic acid samples (Table 1). The treatment T8 showed the highest values of carboxylic (-COOH), phenolic (OH) and total acidity for humic acid fraction; and phenolic (OH) group acidity for fulvic acid fraction. The highest values of carboxylic (-COOH) and total acidity for fulvic acid fraction was recorded under T7. Compared to the control and inorganic treatments (i.e. T1 to T4), significantly higher content of both carboxylic and phenolic acidity in fulvic acid fraction was recorded in the green manured plots (T7 and T8). The study thus indicated that the application of green manure

as a nutrient source increased the total acidity characteristics of HAs/FAs in rice soil.

**Table 1:** Functional group analysis (i.e., content of carboxylic–COOH and phenolic –OH groups) of humic acid (HA) /fulvic acid (FA) samples through potentiometric titration

HA Sample	Amount of COOH group /meq. g <sup>-1</sup>	Amount of OH group /meq. g <sup>-1</sup>	Total acidity / meq.g <sup>-1</sup>	FA Sample	Amount of COOH group /meq. g <sup>-1</sup>	Amount of OH group /meq. g <sup>-1</sup>	Total acidity meq. g <sup>-1</sup>
HAT <sub>1</sub>	0.510	0.290	0.800	FAT <sub>1</sub>	1.04	0.47	1.51
HAT <sub>2</sub>	0.520	0.280	0.800	FAT <sub>2</sub>	1.24	0.61	1.85
HAT <sub>3</sub>	0.680	0.350	1.030	FAT <sub>3</sub>	1.03	0.66	1.69
HAT <sub>4</sub>	0.560	0.330	0.890	FAT <sub>4</sub>	1.03	0.58	1.61
HAT <sub>5</sub>	0.470	0.250	0.720	FAT <sub>5</sub>	1.11	0.88	1.99
HAT <sub>6</sub>	0.520	0.390	0.910	FAT <sub>6</sub>	1.23	0.88	2.11
HAT <sub>7</sub>	0.580	0.240	0.820	FAT <sub>7</sub>	1.53	0.78	2.31
HAT <sub>8</sub>	0.710	0.520	1.230	FAT <sub>8</sub>	1.33	0.89	2.22
SE	0.002	0.114		SE	0.021	0.014	
CD (P<0.05)	0.007 (0.01)	0.035 (0.03)		CD (P<0.05)	0.064 (0.06)	0.044 (0.04)	

### Δ pH inflexions

The pH values corresponding to the first and final inflexion points, estimated through potentiometric titration curves for the individual humic and fulvic acid samples are given in table 2. A pH difference, namely, ΔpH = pH<sub>¾</sub> – pH<sub>¼</sub>, was recorded at, pH<sub>¼</sub> and pH<sub>¾</sub> refer to pH values corresponding to the one-fourth and the three-fourths of the pH at the final inflexion points. The ΔpH values were influenced by the application of inorganic and organic inputs and varied from 3.69 to 5.33 for humic acid fractions and from 4.17 to 7.22 for

fulvic acids fractions among the different treatments. According to the criteria for Sturrock (1968), all the humic and fulvic acids extracted from the experimental rice plots were found to be polyprotic in nature. The ΔpH values of >0.954 (Sinha and Bhattacharya 2011) [29] indicated that the given HA and FA fractions were capable to accept or lose more than a single proton per molecule during the reactions. The results of the study concluded that organic and inorganic management did not alter the native polyprotic nature of the HAs/FAs.

**Table 2:** Estimation of ΔpH values of humic acid/fulvic acid through potentiometric titration to assess the samples nature

HA Sample	pH at first inflexion point (pH <sub>1</sub> )	pH at final inflexion point (pH <sub>2</sub> )	ΔpH = pH <sub>¾</sub> – pH <sub>¼</sub> (pH = pH <sub>2</sub> )	Conclusion	FA Sample	pH at first inflexion point (pH <sub>1</sub> )	pH at final inflexion point (pH <sub>2</sub> )	ΔpH = pH <sub>¾</sub> – pH <sub>¼</sub> (pH = pH <sub>2</sub> )	Conclusion
HAT <sub>1</sub>	4.56	9.56	5.00	Polyprotic	FAT <sub>1</sub>	4.12	8.29	4.17	Polyprotic
HAT <sub>2</sub>	5.26	8.95	3.69	Polyprotic	FAT <sub>2</sub>	3.25	8.25	5.00	Polyprotic
HAT <sub>3</sub>	4.56	9.89	5.33	Polyprotic	FAT <sub>3</sub>	3.25	9.89	6.64	Polyprotic
HAT <sub>4</sub>	5.12	9.75	4.63	Polyprotic	FAT <sub>4</sub>	3.14	8.95	5.81	Polyprotic
HAT <sub>5</sub>	4.96	9.67	4.71	Polyprotic	FAT <sub>5</sub>	2.89	9.56	6.67	Polyprotic
HAT <sub>6</sub>	4.59	9.93	5.34	Polyprotic	FAT <sub>6</sub>	3.21	9.25	6.04	Polyprotic
HAT <sub>7</sub>	4.92	8.96	4.04	Polyprotic	FAT <sub>7</sub>	2.69	9.91	7.22	Polyprotic
HAT <sub>8</sub>	5.92	9.88	3.96	Polyprotic	FAT <sub>8</sub>	2.69	9.22	6.53	Polyprotic
SE	0.037	0.05			SE	0.018	0.033		
CD (P<0.05)	0.113	0.15			CD (P<0.05)	0.053	0.101		

### E<sub>4</sub> / E<sub>6</sub> ratio

Spectrophotometric characterization through the E<sub>4</sub>/E<sub>6</sub> ratio of the extracted HA/FA samples provides an index of aliphatic/aromatic balance (Busato *et al.* 2010) [6]. The aromatic moiety (i.e. absorbance at 665 nm) is the most hydrophobic part and tends to hide itself in the innermost core of the coil, leaving the aliphatic moiety (i.e. absorbance at 465 nm) forming the outer periphery of the coil with a greater absorbance (Datta *et al.* 2001) [9]. The value of the Δ E<sub>4</sub>/E<sub>6</sub> was found positive for most of the humic and fulvic acid samples but the treatments HAT<sub>6</sub> (i.e., humic acid extracted from the soil treated with FYM) and FAT<sub>7</sub> (i.e., fulvic acid extracted from the soil treated with GM) were displayed negative magnitude with values of -0.24 and -0.71 respectively (Table 3), which might helps to extensive decoiling of the higher aromatic moiety (Ghosh *et al.* 2012) [14] after managed soil inorganic and organically. Relatively higher Δ(E<sub>4</sub>/E<sub>6</sub>) ratio of humic acids and fulvic acids at pH 7.0 than at pH 9.0 indicated that low degree of aromatic

condensation and a large proportion of aliphatic structure similarly lower Δ(E<sub>4</sub>/E<sub>6</sub>) ratio indicated a high degree high degree of aromatic condensation for HAT<sub>6</sub> and FAT<sub>7</sub> (Fong *et al.*, 2006) [12].

### Viscosity and molecular weight

The intrinsic viscosity [η] and computed average molecular weight (M) of HA and FA fractions under different treatments are given in table 4. The highest molecular weight (4215 M) among the humic acids was obtained in HAT<sub>5</sub> (i.e., humic acid extracted from the soil treated with FYM) and the lowest (489 M) in HAT<sub>1</sub> (control). The molecular weight of the HAs extracted from different treatments decreased in the order of HAT<sub>5</sub>>HAT<sub>6</sub>>HAT<sub>7</sub>>HAT<sub>2</sub>>HAT<sub>3</sub>>HAT<sub>8</sub>>HAT<sub>4</sub>>HAT<sub>1</sub>. The highest molecular weight for fulvic acids was also obtained in FAT<sub>5</sub> (3212 M) and lowest in FAT<sub>1</sub> (2100 M). The molecular weight of FAs decreased in the order of FAT<sub>5</sub>>FAT<sub>6</sub>>FAT<sub>8</sub>>FAT<sub>3</sub>>FAT<sub>7</sub>>FAT<sub>4</sub>>FAT<sub>2</sub>>FAT<sub>1</sub>.

The humic acid samples had relatively greater molecular weight than the fulvic acid samples, which might be due to the advanced stage of polymerization and poly-condensation (Debnath *et al.* 2013) [10]. The lower molecular weights of HA/FA samples might be due to the oxidative degradation of higher molecular groups and destruction of cementing or

easily mineralizable components such as carbohydrate (Martin *et al.* 1998) [19]. The experimental data reflects that application of organic manures through FYM (T5 and T6) increased molecular weight for both the HA and FA substances.

**Table 3:** Spectrophotometric (E<sub>4</sub> and E<sub>6</sub>) characteristics analysis of humic /fulvic acid samples at two different pH and  $\Delta(E_4/E_6)$  values of the humic/fulvic acid samples

HA Sample	E <sub>4</sub> /E <sub>6</sub> at pH		$\Delta(E_4/E_6) = (E_4/E_6 \text{ at pH } 7.0 - \text{pH } 9.0)$	FA Sample	E <sub>4</sub> /E <sub>6</sub> at pH		$\Delta(E_4/E_6) = (E_4/E_6 \text{ at pH } 7.0 - \text{pH } 9.0)$
	7.0	9.0			7.0	9.0	
HAT <sub>1</sub>	10.25	2.33	7.92	FAT <sub>1</sub>	9.43	6.43	3.00
HAT <sub>2</sub>	4.74	0.65	4.09	FAT <sub>2</sub>	6.11	2.57	3.54
HAT <sub>3</sub>	3.56	1.81	1.75	FAT <sub>3</sub>	7.00	2.44	4.56
HAT <sub>4</sub>	6.24	5.87	0.37	FAT <sub>4</sub>	5.33	2.38	2.95
HAT <sub>5</sub>	9.88	4.56	5.32	FAT <sub>5</sub>	5.50	2.17	3.33
HAT <sub>6</sub>	3.87	4.11	-0.24	FAT <sub>6</sub>	6.73	6.22	0.51
HAT <sub>7</sub>	9.56	7.73	1.83	FAT <sub>7</sub>	3.29	4.00	-0.71
HAT <sub>8</sub>	1.91	1.84	0.07	FAT <sub>8</sub>	6.88	5.20	1.68
SE	0.05	0.03		SE	0.05	0.08	
CD ( <i>P</i> <0.05)	0.15	0.10		CD ( <i>P</i> <0.05)	0.14	0.25	

**Table 4:** Estimation of viscosity[ $\eta$ ], and mean molecular weight of different humic/fulvic acid samples through viscometric analysis.

HA Sample	[ $\eta$ ] / 100 ml/g	Molecular weight (M) of humic acid	FA Sample	[ $\eta$ ] / 100 ml/g	Molecular weight (M) of fulvic acid
HAT <sub>1</sub>	0.810	489	FAT <sub>1</sub>	0.831	2100
HAT <sub>2</sub>	0.823	3103	FAT <sub>2</sub>	0.840	2201
HAT <sub>3</sub>	0.819	2814	FAT <sub>3</sub>	0.868	2312
HAT <sub>4</sub>	0.811	2389	FAT <sub>4</sub>	0.840	2212
HAT <sub>5</sub>	0.837	4215	FAT <sub>5</sub>	0.956	3212
HAT <sub>6</sub>	0.830	4125	FAT <sub>6</sub>	0.918	3085
HAT <sub>7</sub>	0.826	3312	FAT <sub>7</sub>	0.846	2245
HAT <sub>8</sub>	0.815	2547	FAT <sub>8</sub>	0.874	2512
SE	0.007	3.44	SE	0.004	4.315
CD ( <i>P</i> <0.05)	0.002	10.43	CD ( <i>P</i> <0.05)	0.013	13.088

### Stability constant at pH 5.0

Number of moles (x) of humic acid that combined with 1 mole of zinc as estimated by ion-exchange equilibrium method, the x value of humic acids varied from 1.00 to 1.84 (Table 5) in between treatments. The highest value of x (1.84) was recorded under the treatment of HAT<sub>4</sub>. The variation in x values among the different treatments does not follow similar trends for metal organic matter complexes after managing the soil organically or inorganically. Similarly 'x' value of fulvic acids did not proportionally influenced by the management practices. The value of 'x' varied from 0.63 to 2.69 (Table 5) for fulvic acids.

The ability of complexation can be expressed in terms of conditional stability constant (log K). At pH 5.0, the log K values (Table 5) of Zn-humic complexes significantly varied from 1.32 (HAT<sub>3</sub>) to 2.45 (HAT<sub>7</sub>), the highest value being received from the green manure treated soils. The log K values were quite comparable to those described earlier for heavy metal-humic complexes (Stevenson 1977<sup>[33]</sup>; Mukhopadhyay and Sanyal 2004) <sup>[21]</sup>, but were relatively higher than the values observed for stability constants of organo-metallic complexes formed by root exudates containing low molecular weight organic acids (Mench *et al.* 1988) <sup>[20]</sup>.

At pH 5.0, the log K values of Zn-fulvic complexes varied from 1.20 to 2.09, which was relatively lower than humic acids but highest value being obtained with FAT<sub>7</sub> [i.e., fulvic acid extracted from the soils treated with green manure], which indicated that the application of green manure in soil as organic sources also increase the stability for both zinc-humic

and fulvic acid complexes. The values of log K of fulvic acids were quite comparable to those reported earlier for heavy metal-humic complexes (Stevenson, 1977) <sup>[33]</sup>. The log K value for the present zinc-HA/FA complex showed the following order;

HAT<sub>7</sub>>HAT<sub>5</sub>>HAT<sub>1</sub>>HAT<sub>6</sub>>HAT<sub>8</sub>>HAT<sub>2</sub>>HAT<sub>4</sub>>HAT<sub>3</sub>, and FAT<sub>7</sub>>FAT<sub>5</sub>>FAT<sub>8</sub>>FAT<sub>1</sub>>FAT<sub>6</sub>>FAT<sub>4</sub>>FAT<sub>2</sub>>FAT<sub>3</sub>

The trend of log K value of humic and fulvic acids indicated that the application of higher doses of organic manures (50% of the RD) led to significant increase in log K value. Comparatively, the lower the stability constant of fulvic acids than the humic acids might be due to its higher hydrophobic (coiled) content of moieties rendering less reactivity with zinc. However, the exposed decoiled aromatic moieties of the humic acids having the higher molecular weight would bind heavy metals more strongly than do the lower molecular weight acids (Mukhopadhyay and Sanyal 2004) <sup>[21]</sup>. Considering all the facts combined application of inorganic and organic nutrient source influenced positively on the value of stability constant (log K) with Zn.

### Treatment wise variation in Zn uptake, residual Zn status and rice yield

The mean yield of rice (Table 6) at different treatment combinations reflected the significant yield differences, where, the minimum yield (2.53 t ha<sup>-1</sup>) was at the treatment T<sub>1</sub> (i.e. untreated control plots) and maximum yield with T<sub>6</sub> (6.27 t ha<sup>-1</sup>), where both the inorganic (NPK 75% & Zn) and organic (25% N through FYM) were applied as an integrated manner. Relatively less yield in green manure treated plots

might be due to lower availability of other nutrients except N. Wide variation in zinc uptake is observed in different treatments by rice, with mean value ranged from 0.06 to 0.21 kg ha<sup>-1</sup> and comparatively lower Zn uptake was observed in

solely inorganically managed plots. Zinc fertilization along with or without organic inputs significantly increased the residual zinc status with ranged from 0.49 to 4.47 kg ha<sup>-1</sup>, and built up in soil Zn was observed in organically treated plots.

**Table 5:** Estimation of the stability constants of zinc-humic/fulvic acid complexes at pH 5.0

HA Sample	x	log K	FA Sample	x	log K
HAT <sub>1</sub>	1.48	1.96	FAT <sub>1</sub>	0.81	1.80
HAT <sub>2</sub>	1.06	1.77	FAT <sub>2</sub>	1.53	1.63
HAT <sub>3</sub>	1.31	1.32	FAT <sub>3</sub>	1.19	1.20
HAT <sub>4</sub>	1.84	1.56	FAT <sub>4</sub>	1.15	1.67
HAT <sub>5</sub>	1.52	2.06	FAT <sub>5</sub>	1.21	1.84
HAT <sub>6</sub>	1.08	1.90	FAT <sub>6</sub>	2.69	1.78
HAT <sub>7</sub>	1.72	2.45	FAT <sub>7</sub>	1.83	2.09
HAT <sub>8</sub>	1.00	1.82	FAT <sub>8</sub>	0.63	1.81
SE	0.03	0.02	SE	0.032	0.03
CD ( <i>P</i> <0.05)	0.09	0.07	CD ( <i>P</i> <0.05)	0.10	0.09

**Table 6:** Yield (t ha<sup>-1</sup>), Zn-uptake (kg ha<sup>-1</sup>) and soil residual Zn status of rice

Treatment	Yield (t ha <sup>-1</sup> )			Zn uptake (kg ha <sup>-1</sup> )			Residual Zn (kg ha <sup>-1</sup> )		
	1 <sup>st</sup> year	2 <sup>nd</sup> year	Mean	1 <sup>st</sup> year	2 <sup>nd</sup> year	Mean	1 <sup>st</sup> year	2 <sup>nd</sup> year	Mean
T <sub>1</sub>	2.37	2.68	2.53	0.06	0.07	0.06	0.50	0.48	0.49
T <sub>2</sub>	2.96	2.93	2.95	0.14	0.15	0.15	3.00	3.35	3.18
T <sub>3</sub>	3.60	3.57	3.59	0.15	0.14	0.15	2.54	3.60	3.07
T <sub>4</sub>	5.50	5.35	5.43	0.16	0.14	0.15	3.15	3.46	3.31
T <sub>5</sub>	5.93	5.87	5.90	0.21	0.21	0.21	3.78	5.16	4.47
T <sub>6</sub>	6.52	6.01	6.27	0.21	0.20	0.21	3.27	5.16	4.21
T <sub>7</sub>	4.04	4.31	4.18	0.20	0.21	0.21	2.47	4.23	3.35
T <sub>8</sub>	3.80	4.35	4.08	0.20	0.22	0.21	2.16	4.16	3.16
SE	0.67	0.634		0.019	0.018		0.148	0.127	
CD ( <i>P</i> <0.05)	0.90	0.86		0.07	0.08		0.32	0.20	

#### Correlation coefficient of the characteristics of humic acids

Pearson correlation coefficient (Table 7) among the humic acids characteristics indicate that the spectrophotometric characterization i.e.,  $\Delta$  ( $E_4/E_6$ ) of humic acid were negatively correlated with total acidity ( $r = -0.626$ ), Zn uptake ( $r = -0.642$ ), residual Zn ( $r = -0.577$ ) and rice yield ( $r = -0.502$ ). Molecular weight of humic acids was also positively and significantly correlated with viscosity ( $r = 0.882$ ) which indicated that molecular weight of humic acid is directly dependent on viscosity. Zn uptake, residual Zn and rice yield is also influenced by both the molecular weight and viscosity. A positive significant correlation was also observed between soil organic carbon and x value ( $r = 0.64^*$ ) of humic acid, indicated that the number of moles (x value) of humic acid in combined with heavy metals is regulated by SOC.

#### Correlation coefficient of the characteristics of fulvic acids

Correlation coefficients (Table 8) of the characteristics of the

fulvic acids indicated that the total acidity of fulvic acid was negatively and significantly correlated with  $E_4/E_6$  ( $r = -0.759^*$ ) but positively and significantly correlated with Zn uptake ( $r = -0.873^{**}$ ) at 1% level of significance. The uptake of Zn ( $0.836^{**}$ ) by rice and residual soil Zn ( $0.708^*$ ) was also positively and significantly influenced by the  $\Delta$ pH of fulvic acid at 1% level of significance. As humic acid a strong positive significant relationship was also observed between molecular weight and viscosity ( $r = 0.980^{**}$ ) for fulvic acids at 1% level of significance. But the stability constant (log K) of fulvic acids had significantly negative correlation with the viscosity ( $-0.783^*$ ) at 1% and 5% level of significance respectively. The yield of rice had positive significant relationship with both molecular weight ( $r = 0.790^*$ ) and viscosity ( $r = 0.732^*$ ) at 5% level of significance. Positive and significant relationship was also observed between the zinc uptake and molecular weight of fulvic acids ( $r = 0.708^*$ ) at 5% level of significance.

**Table 7:** Correlation matrix of the characteristics of humic acids extracted from the different organically and inorganically managed soils

Properties	Organic carbon	Total acidity	$\Delta$ pH	$\Delta E_4/E_6$	$[\eta]$	Molecular weight	x	log K
Total acidity	0.38							
$\Delta$ pH	-0.41	-0.046						
$\Delta E_4/E_6$	-0.53	-0.626	0.026					
$[\eta]$	0.05	-0.412	0.36	-0.012				
Molecular weight	0.42	-0.119	-0.035	-0.447	0.882			
x	0.64*	-0.480	0.100	0.160	-0.128	-0.172		
log K	-0.08	-0.430	-0.359	0.236	0.430	0.184	0.229	
Zn Uptake	0.42	0.229	-0.235	-0.642	0.676	0.865	-0.174	0.314
Residual Zn	0.26	0.008	-0.063	-0.577	0.751	0.963	-0.100	0.054
Yield	0.11	-0.079	0.310	-0.502	0.558	0.712	0.165	0.102

\*\* , \* Correlation is significant at the 0.01 level and 0.05 level respectively (2-tailed)

**Table 8:** Correlation matrix of the characteristics of fulvic acids extracted from the different organically and inorganically managed soils

	Organic carbon	Total acidity	$\Delta\text{pH}$	$\Delta\text{E}_4/\text{E}_6$	$[\eta]$	Molecular weight	x	log K
Total acidity	0.20							
$\Delta\text{pH}$	0.63*	0.691						
$\Delta\text{E}_4/\text{E}_6$	0.11	-0.759*	-0.376					
$[\eta]$	-0.16	0.385	0.447	-0.310				
Molecular weight	-0.26	0.434	0.388	-0.155	0.980**			
x	-0.52	0.394	0.199	-0.531	0.297	0.415		
log K	-0.02	0.576	0.113	-0.783*	0.086	0.178	0.181	
Zn Uptake	0.42	0.873**	0.836**	-0.518	0.618	0.639	0.422	0.320
Residual Zn	0.26	0.582	0.708*	-0.209	0.699	0.708*	0.505	0.054
Yield	0.11	0.358	0.499	-0.310	0.732*	0.790*	0.497	0.219

\*\* , \* Correlation is significant at the 0.01 level and 0.05 level respectively (2-tailed)

## Conclusions

The results on the characterization of the extracted humic/fulvic acids from the treated soils showed the nature of condensation of those HA/FA samples corresponding to the different inorganic and organic inputs to the soils. The zinc-HA/FA complexation equilibria are expected to elucidate the stability of these complexes affecting the retention /release of zinc in soil matrix which has considerable bearing on the ability of the native organic fractions to regulate the zinc uptake for the best yield of rice.

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