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Physicochemical characterization of humic acids extracted from vermicompost and its impact on growth of *Oryza sativa*

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ABSTRACT

The research work focusing the suitable method for extracting humic acid from manures at farmer's field. The results reveal that the humic acid extracted by KOH and NaOH have a loosely honeycomb structure characterized by a predominance of flakes and fragments adhering on the surface and fractions exhibited a distinct physical appearance, being close-grained and lumpy. Chemical analysis shows that the predominant components are Mg, Fe, Na, K, Ca, and P in humic acids. Furthermore the EDS spectra also show the presence of Mg, Fe, Na, K, Ca, and P in the humic acid extracted with KOH and additional minerals Al, Si, S and Cl in the humic acid. The HAE3 Humic acid (KOH used for extraction) was showed higher seed germination (88%) and vigour index (31.51%) in paddy (*Oryza sativa*).

KEYWORDS

Humic acids;
SEM analysis;
EDAX analysis;
Humic substances.

INTRODUCTION

Humic acid (HA) is a stable major component of humic substances (HS) formed from decomposed soil or natural organic matter mainly includes plant residues (lignin) from soil, algae and microorganisms from aquatic source[26]. Humus forming process is called Humification and the degree and quality of formed humus from organic matter decomposition is primarily determined by presence of ratio of H/C, O/C and N/C atomic ratios; acidic functional group contents, especially carboxylic and phenolic groups which are the major functional group in humic acid[16]; molecular weight and spectroscopic parameters[14,15,29]. HA core structure consists of alkyl and aromatic units in cross links with oxygen and nitrogen groups[16], during composting process more amount of aromatic compounds from polysaccharides are incorporated in humic acid core structure called humification[19]. The recent extensive study on humic substances with modern instruments (like HPSEC, ESI-MS) says that small and heterogeneous humic molecules (as mentioned above) self assemble in core structure formation and this supramolecular conformations are stabilized by involvement of weak forces includes Van der Waals, π- π and CH- π bonds and hydrogen bonds[3]. Formation of humic acid aggregates and its strong interactions are depend upon the richness of O-containing groups and bonding capacity between them through charge transfer and hydrogen bonding[20,35]. Soil humic acid is complex structure, soluble in alkaline pH due to ionization of acid functional group in HA structure but gets precipitated in acidic pH because of protonation in carboxyl group from acidic environment[34]. In alkaline pH, the negative charge or ionized part of

humic acid structure (carboxyl and phenolic OH) involve in complexation reactions with metal ions like Mg, Ca[31] leads to increase in HA molecular weight and adsorption process[8], e.g., removal of humic acid from water by adsorbing capacity of HA through protonation at low pH (3) with activated carbon coated with iron and also by increased HA concentration[10]. The important functions of HA in environment are regulation of carbon cycle, releasing nutrients from compacted soil and makes its availability to plants through cation exchange capacity of HA[9,23].

Many studies were done on humic acid functional for structure determination and molecular weight analysis, but all these findings says about hypothesis of humic acid structural arrangement. Few studies are carried out which are very useful to see actual humic acid topography/textural understanding in various conditions including pH and presence of reactive ions. The success of this research is highly shared with other supporting data from EDX value, FTIR, NMR, CP-MAS. In this study HA extracted from vermicompost by different extraction methods are used for textural studies through SEM and advantages of the extraction method is compared with Energy Dispersive X-ray Analysis (EDAX) values. This is helpful for preparation of humic acid solution in required concentration for field application as fertilizer along with necessary supplements.

MATERIALS AND METHODS

Sample collection

Organic manure of vermicompost sample was collected from Shri AMM Murugappa Chettiar Research Centre, Chennai, India which was used for extraction of Humic Acid (HA).

Humic acid extraction

The humic acid was extract from vermicompost by five different method such as IHSS method (HAE1), rapid method (HAE2), modified of rapid method (HAE3)[32], boiling method (HAE4) and modified boiling method (HAE5)[25].

Modified of rapid method (HAE3)

Two hundred gram (dry weight basis) manure samples were placed into 5 liter conical flask and 2000 mL of 0.1 M KOH solution was added and the bottles were tighten with cotton plug. The remaining were followed as same as in the method HAE2[32].

Modified of boiling method (HAE5)

Here 200 g of manure was taken into a conical flask and mixed only with distilled water for HA extraction and the procedure was followed as in the method HAE4 to get the HA in precipitated form.

Physico-chemical analysis of humic acids

Wet HA extracts were analyzed for its physico-chemical parameters in conventional methods including pH, Electro Conductivity (EC), Nitrogen (N), Phosphorus (P), Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn) described by Tandon[37].

SEM and EDAX analysis of humic acids

From all the extraction methods dried Humic Acid (HAE1 to HAE5) at 40^0C and two commercial product such as Himedia humic acid (HAC6) and China humate (HAC7) were sent to IIT Madras for SEM observation and obtaining EDAX values. The sample was weighted (0.2 g) and prepared in flat and smooth especially for quantitative and qualitative determination. All humic acid samples were analyzed for their microstructure and elemental compositions under SEM (FEI Quanta FEG 200-High Resolution).

Seed germination humic acid extracted by different method

Whatman No.1 filter paper adopted a modified roll towel method as per ISTA[11] with four replications each @ 20 seeds of the Paddy (white ponni). The rolls were then dipped in different dilutions (30, 50, 70 and 100mg/100ml) of humic acid (HAE1 to HAE5) along with control where the towel was kept in distilled water. The germination set up was maintained at 30 ± 2^0C and 75 ± 3 % Relative Humidity (RH). After the germination period of 15 days the germination test was evaluated as normal and abnormal seedlings and as hard and dead seeds as per ISTA[11]. Then based on the normal seedling the germination percentage, root length, shoot length and vigour index were calculated[6].

1) T1 - Control (Distilled water)
2) T2-T5 - HAE1 humic acid used in different concentration (30, 50, 70, 100mg in $100ml^{-1}$)
3) T6-T9 - HAE2 humic acid used in different con-

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centration (30, 50, 70, 100mg100ml^{-1})

4) T10-T13 - HAE3 humic acid used in different concentration (30, 50, 70, 100mg100ml^{-1})
5) T14-17 - HAE4 humic acid used in different concentration (30, 50, 70, 100mg100ml^{-1})
6) T18-T21 - HAE5 humic acid used in different concentration (30, 50, 70, 100mg100ml^{-1})

RESULTS AND DISCUSSION

In this study oven dried HA aggregates were observed under SEM, its nutrients content were estimated through EDAX and its physico-chemical parameters were analyzed in order to know the differences between extraction methods for a single sample (vermicompost) and to select a possible best method for HA extraction in large scale for field trial application.

HA extraction by boiling methods having less quantity of HA and high quantity of the elements than other extraction methods, it indicates the improper HA extraction by boiling but the remaining methods for HA extraction showed maximum yield from the manure with lesser nutrient contamination (TABLE 1). Humic substances change their structure depending on pH and the type of metals present. The oxidized sites on the molecule are saturated with ions like aluminum and potassium relatively easily, which is readily exchanged for all major micronutrient ions in the soil[2,28]. Physicochemical properties of different humic acids revealed presence of significantly higher amount of major micronutrients in HAE3 and HAE1 than the commercial humic acids (TABLE 1), This is helpful to prepare HA formulations with other necessary supplements in desired ratio for successful absorption of nutrients by plants and helps to prevent excess or wastage applications of these nutrients.

SEM images of all the HA extracts show aggregated pattern of humic molecules due to drying (Figure 1 to Figure 7). In general HA is a heterogeneous organic compound and the core structure mainly composed of hydrophobic and hydrophilic molecules particularly carboxyl and phenolic groups, these molecules' ratio play a role in charge determination of HA structure also leads to weak behavior of HA in acidic condition[1].

Negre *et al.*[21], said that elimination of water during freezing of HA increases the interaction between humic molecules, related to this concept, a study was carried out by Saab *et al.*[27], Pontie *et al.*[22], and Zara *et al.*[17], revealed that aggregation of HA in acidic pH was through reduction of negative charges in HA structures, weak electrostatic forces and hydrogen bonding between humic molecules leads to macromolecular configuration; and also said that flexible structure of HA in alkaline pH is due to ionization of functional group getting increased which causes strong electrostatic repulsion and break down of weak electrostatic forces leads to dispersed configuration. In this study also the same condition had occurred in oven dried HAs, the images of studied HAs showed highly compacted precipitated structure especially in Figures 1, 6 and 7 with some extent of regular forms, in case of Figure 2, 3 and 4 the HA precipitates showed uneven and highly heterogeneity in their structure due to particle distribution, this is supported by EDAX values, reveal that more or less all the particles are present in these three extracts with simi-

TABLE 1 : Physicochemical properties of humic acid extracted by different method

Sample	OC	pH	EC	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Yield (g/ 200g)
HAE 1	27.68± 0.80	7.02 ±0.20	5.58 ±0.16	2.77 ±0.08	0.77 ±0.03	0.55 ±0.02	0.62 ±0.02	0.60 ±0.02	0.30 ±0.01	2319.02 ±76.03	338.00 ±9.76	76.00 ±2.19	17.00 ±0.49	109.32
HAE 2	44.68 ±1.29	7.67 ±0.22	3.71 ±0.11	1.82 ±0.05	0.41 ±0.01	0.67 ±0.02	0.89 ±0.02	0.72 ±0.02	0.29 ±0.01	2403.00 ±69.37	242.00 ±6.99	79.00 ±2.28	12.00 ±0.35	83.075
HAE 3	32.53 ±0.94	7.38 ±0.21	5.06 ±0.14	2.35 ±0.07	0.50 ±0.01	1.27 ±0.03	0.90 ±0.03	0.77 ±0.02	0.31 ±0.01	1996.00 ±57.62	226.00 ±6.52	32.00 ±0.92	6.00 ±0.17	85.67
HAE 4	38.32 ±1.11	7.23 ±0.21	4.32 ±0.13	0.70 ±0.02	1.60 ±0.05	0.28 ±0.01	0.54 ±0.02	0.09 ±0.00	1.06 ±0.03	988.00 ±28.52	40.00 ±1.15	65.00 ±1.88	3.00 ±0.09	46.23
HAE 5	28.21 ±0.81	7.76 ±0.23	3.45 ±0.10	0.62 ±0.02	0.89 ±0.02	0.19 ±0.01	0.44 ±0.01	0.12 ±0.01	0.98 ±0.03	789.00 ±22.78	32.00 ±0.92	43.72 ±1.43	2.40 ±0.07	32.66
HAC 6	42.92 ±1.24	6.23 ±0.18	3.54 ±0.10	1.32 ±0.04	0.07 ±0.00	0.05 ±0.00	0.48 ±0.01	0.75 ±0.02	0.21 ±0.01	26.99 ±0.78	89.00 ±2.57	48.00 ±1.39	39.00 ±1.13	
HAC 7	55.10 ±1.59	5.82 ±0.17	4.23 ±0.12	0.70 ±0.02	0.04 ±0.00	0.06 ±0.00	0.06 ±0.00	0.13 ±0.01	0.37 ±0.01	17.76 ±0.51	5.00 ±0.14	2.00 ±0.06	2.00 ±0.06	

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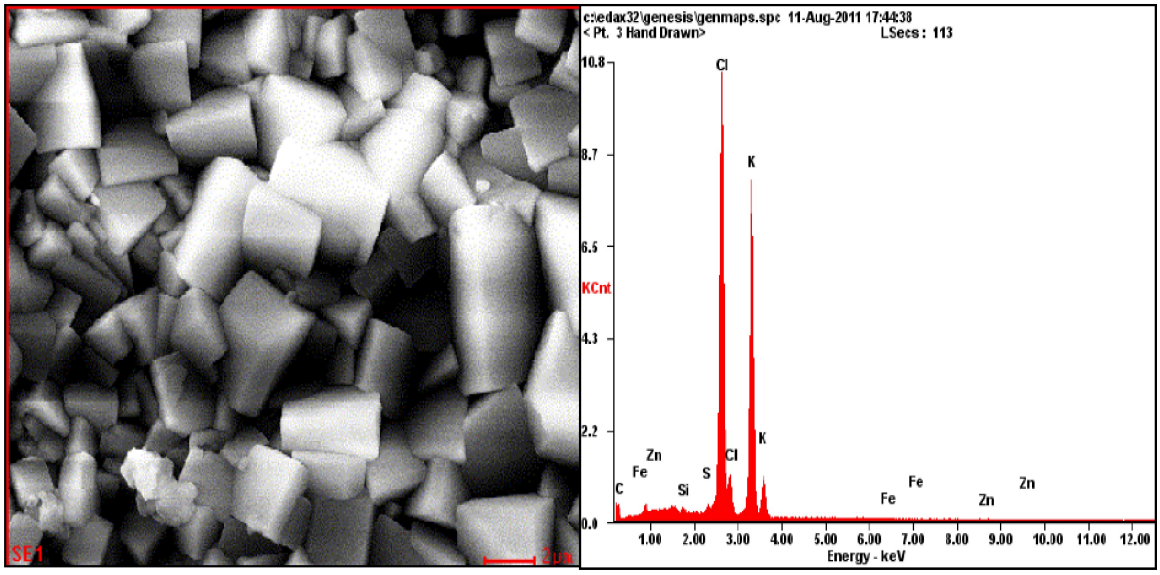

Figure 1 : SEM image and EDAX data of HAE1 extracted by IHSS method

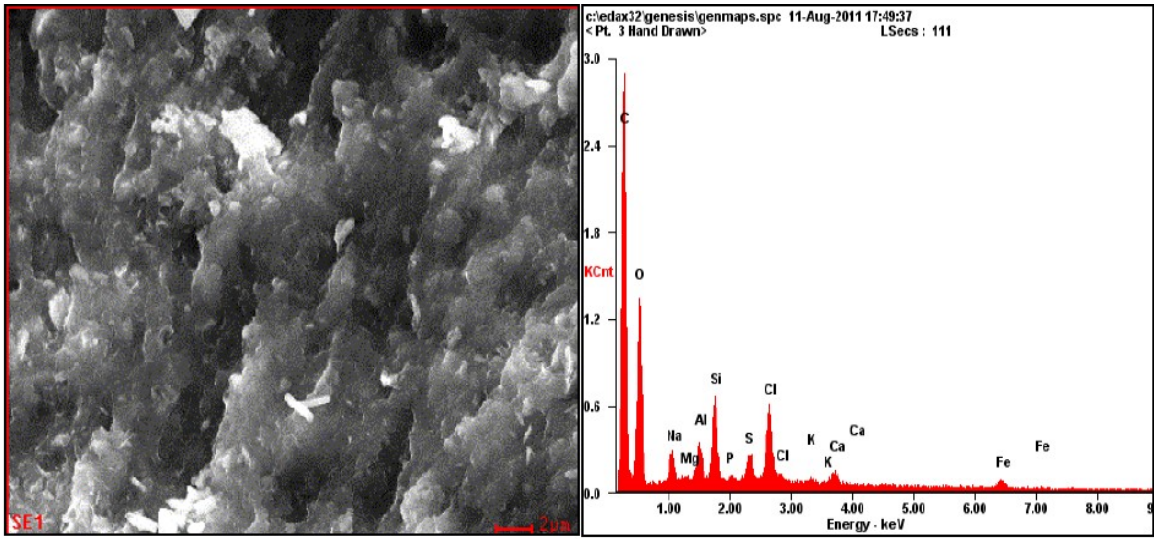

Figure 2 : SEM image and EDAX data of HAE2 extracted by rapid method

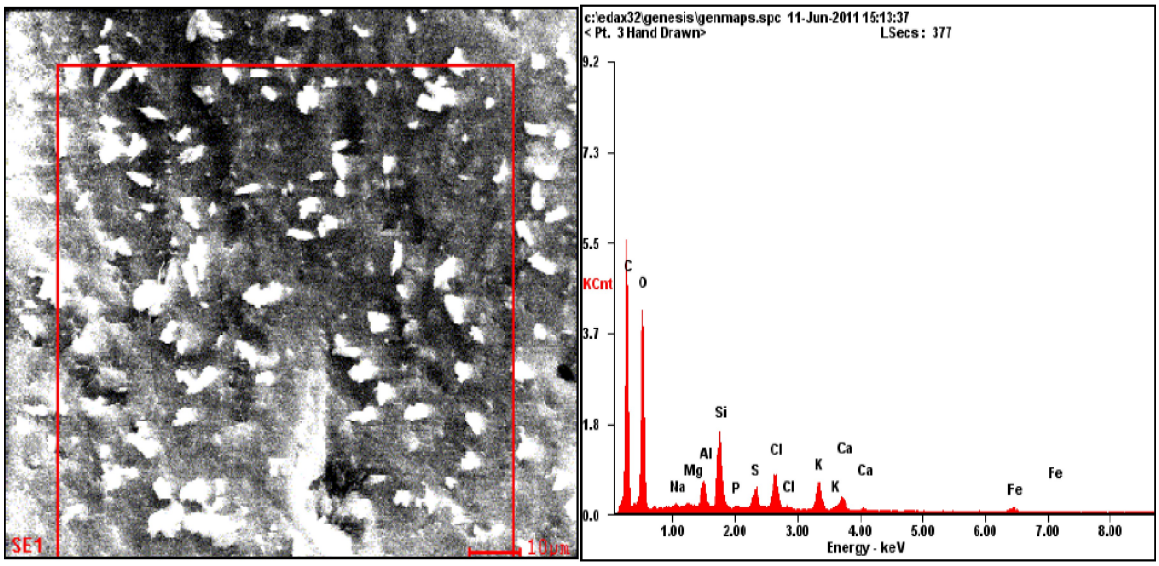

Figure 3 : SEM image and EDAX data of HAE3 extracted by modified rapid method

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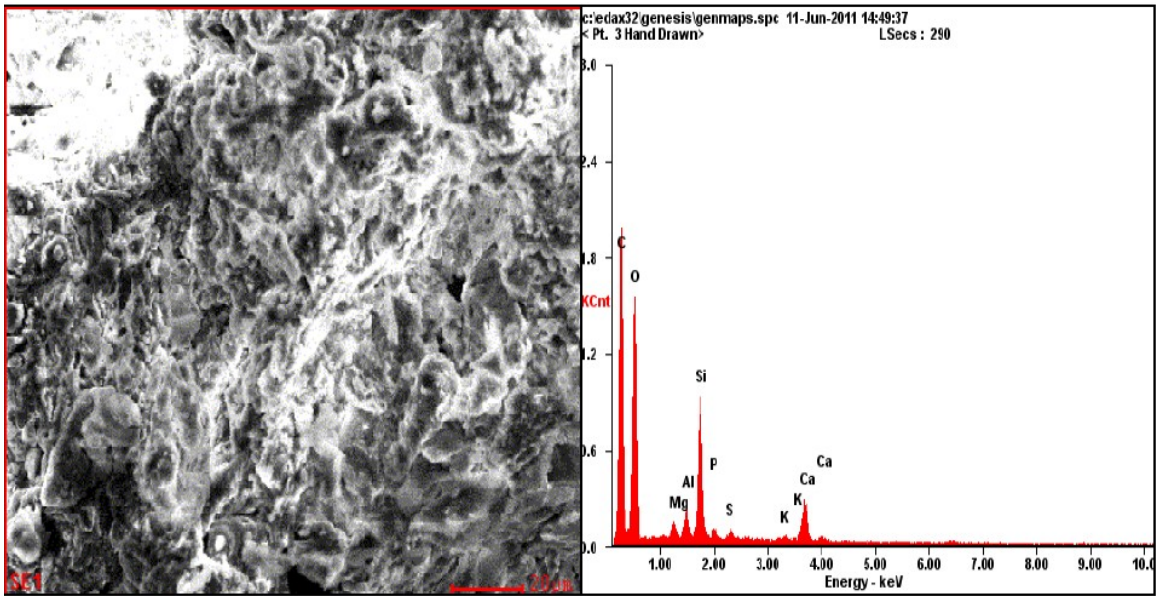

Figure 4 : SEM image and EDAX data of HAE4 extracted by boiling method

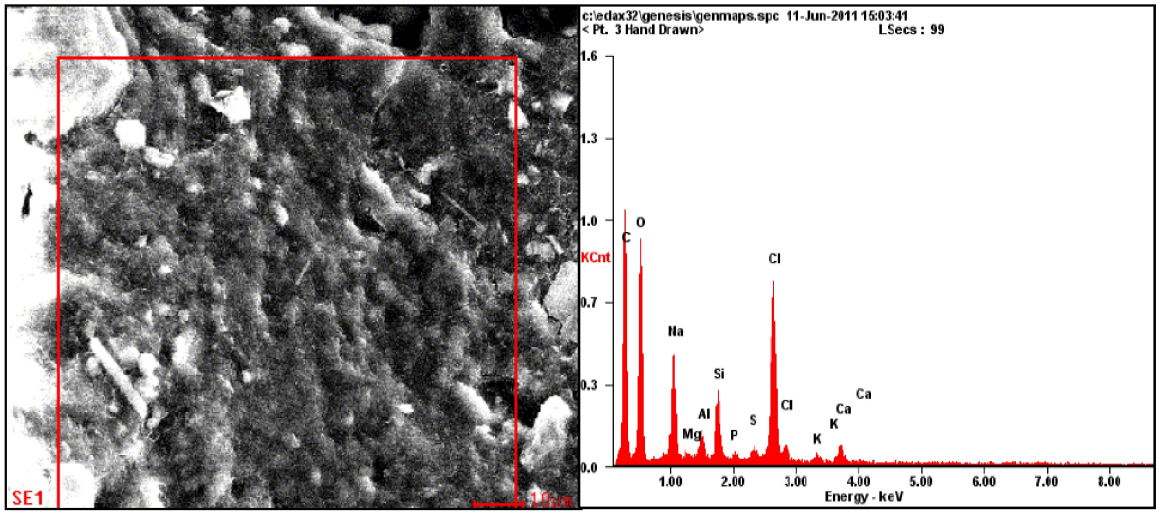

Figure 5 : SEM image and EDAX data of HAE5 extracted by modified boiling method

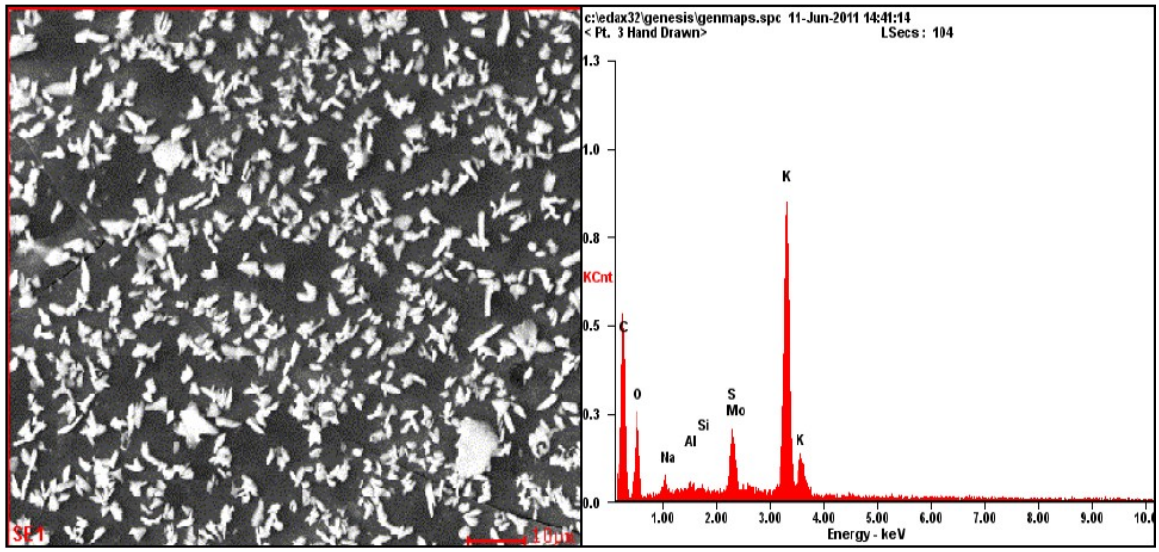

Figure 6 : SEM image and EDAX data of HAC6

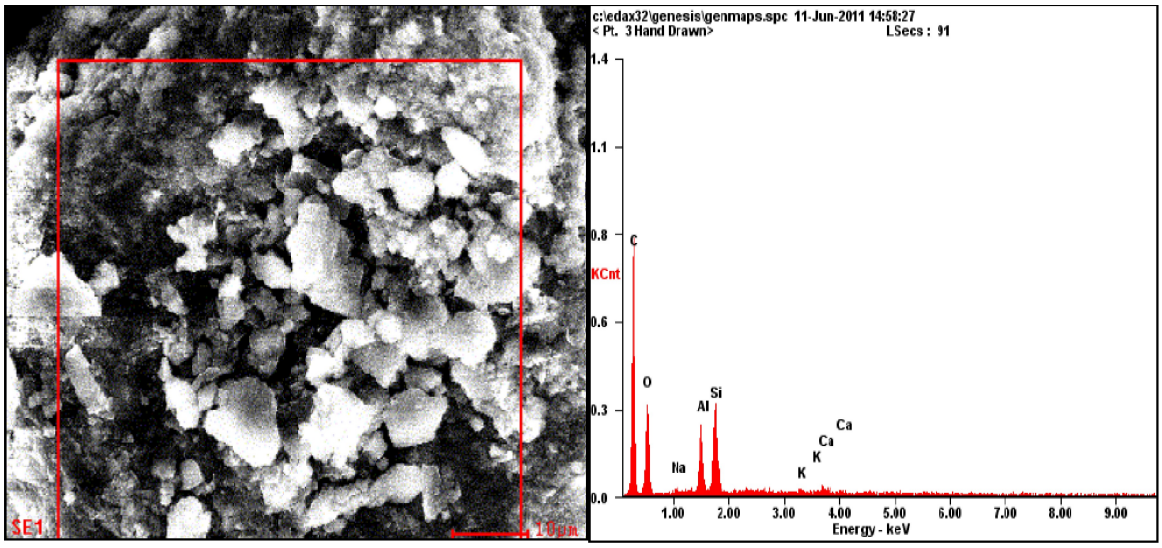

Figure 7 : SEM image and EDAX data of HAC7

lar atomic weight but their distribution is lower in HA extracts by IHSS, and commercial HAs, indicate the purity of HA extracts (TABLE 2). The study of Giovanela *et al.*[20] says that rich O containing groups in humic structure tends to form strong interactions by charge transfer and hydrogen bonding, this point is helpful to explain the heterogeneity structure of HA in this study with the results of EDAX analysis showing that high atomic weight of O in almost all the extracts which leads to precipitation of HA along with other nutrients. This concept is further confirmed by a study of Bratskaya *et al.*[33], and said that while reducing the pH HA lost its colloidal stability, and during this precipitation metals adsorbed in HA also gets precipitate, this technique is used in metal recovery process and in gold micro and nanoparticles production[12]. This is make to know recovery of nutrient concentration in HA structure during acidification and to calculate the dilution ratio of HA with water for field application. In case of Figure 5 and EDAX values shows moderate relationship value with others, and showed that water in boiling state also can extract some amount of HA.

EDAX values of other important particles (are Si, P, Mg, Fe, Ca) are present in HA extracted by modi-

TABLE 2 : Atomic weight (%) of particles present in precipitated humic Acids (HA)

SAMPLE	HAE1		HAE2		HAE3		HAE4		HAE5		HAC6		HAC7	
ELEMENT	Wt %	At%	Wt%	At%	Wt %	At %	Wt %	At %	Wt %	At %	Wt %	At %	Wt %	At %
CK	20.4	44.4	64.7	74.2	57.25	67.07	56.47	67.55	55.51	65.08	48.68	64.93	68.35	76.65
OK	-	-	24.4	21	32.51	28.59	27.33	24.54	34.36	30.25	23.41	23.44	22.53	18.97
NaK	-	-	1.33	0.79	0.08	0.05	4.68	2.92	-	-	1.02	0.71	0.44	0.26
AlK	-	-	1.05	0.54	0.97	0.51	0.8	0.43	1.07	0.56	0.37	0.22	3.24	1.62
SiK	0.23	0.21	2.35	1.15	2.84	1.42	2.06	1.05	4.92	2.47	0.27	0.16	4.7	2.25
SK	0.61	0.5	0.94	0.4	0.94	0.41	0.39	0.18	0.35	0.15	2.49	1.24	-	-
KK	42.1	28.1	0.31	0.11	1.76	0.63	0.3	0.11	0.32	0.11	21.97	9	0.19	0.07
PK	-	-	0.12	0.05	0.06	0.03	0.17	0.08	0.31	0.14	-	-	-	-
MgK	-	-	0.16	0.09	0.11	0.06	0.14	0.09	0.58	0.33	-	-	-	-
CaK	-	-	0.65	0.22	0.84	0.3	0.89	0.32	2.58	0.91	-	-	0.56	0.19
ClK	36.1	26.6	2.75	1.07	1.82	0.72	6.77	2.74	-	-	-	-	-	-
FeK	0.29	0.13	1.28	0.32	0.82	0.21	-	-	-	-	-	-	-	-
NiK	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ZnK	0.24	0.1	-	-	-	-	-	-	-	-	-	-	-	-
MoK	-	-	-	-	-	-	-	-	-	-	1.78	0.3	-	-

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fied rapid method and its modified method followed by other extracts of HA. All these are important for plant growth.

More than 240 auxin-like molecules have been described in Humic substance[5,24] which stimulate the plant and growth of plant. The effect of humic acid in germination and vigour index of paddy (*Oryza sativa*-white ponni) seed were examined. The highest germination percentage (90 %) was observed in 30 mg humic acid of HAE1, which was followed by humic acid of HAE3 (T10, T11, T12 - (88 %)). In case of vigour index the maximum percentage (31.51 %) of vigour index noticed in 30 mg humic acid of HAE3 (T11) and minimum vigour index percentage was found in distilled water (T1) (20.44 and 22.35 % respectively) (TABLE 3). This is in agreement with previous reports on the presence of intrinsic IAA-like molecules clustered within the HA supramolecular arrangement[18] which inducing the plant growth and seed germination. The assembling and disassembling behavior of HA molecular domains and their dynamic release of different molecular constituents[2,7] may then explain why humic compounds had been previously found to possess hormone-like activities similar to those of auxins, gibberellins, cytokinins and polyamines[30]. Malik and Azam[13] and Busato[36] showed that Humic Acids enhanced wheat root length by 321% and maize root.

TABLE 3 : Determination of seed germination and vigour index of Paddy

S.No	Treatments	Germination (%)	Root length (cm)	Shoot length(cm)	Vigour index (%)
1	T1	81.67	19.23±0.89	7.68±0.31	22.35
2	T2	90.00	23.50±1.39	7.80±0.52	28.17
3	T3	80.00	21.85±0.71	7.84±0.06	23.75
4	T4	83.00	22.74±0.83	8.21±0.71	25.80
5	T5	83.00	22.89±0.21	8.69±0.29	26.31
7	T6	88.00	25.81±0.25	8.50±0.17	30.30
8	T7	86.00	24.21±1.70	8.00±0.25	27.92
9	T8	83.00	23.54±1.08	8.06±0.42	26.09
10	T9	88.00	23.45±0.86	8.16±0.25	28.80
11	T10	88.00	24.03±1.75	8.58±0.32	31.51
12	T11	88.00	26.98±0.29	8.69±0.39	23.18
13	T12	71.00	24.08±0.42	8.20±0.19	26.17
14	T13	81.00	23.83±0.43	8.21±0.06	26.17
15	T14	85.00	22.94±1.11	8.94±0.29	27.09
16	T15	86.00	23.67±0.06	9.60±0.34	28.84
17	T16	80.00	23.06±0.09	9.91±0.52	26.23
18	T17	80.00	21.83±0.17	9.67±0.49	25.20
19	T18	81.00	24.74±1.66	8.23±0.24	26.93
20	T19	86.00	23.25±0.64	7.68±0.64	26.81
21	T20	78.00	23.52±1.29	7.88±0.28	24.60
22	T21	83.00	24.19±0.26	7.83±0.48	26.69

CONCLUSION

There are very few studies were conducted on HA for SEM analysis and to screen the quality of extracted HA for field application in large scale. In this study five methods were followed, and the resulting HAs were subjected for characterization through precipitation behavior of HA in five methods along with minimal loss of essential nutrients from its structure. SEM is highly efficient for this and the data from EDAX were highly supportive to analyze the particles in HA precipitates, this is useful to select the possible, economic and practical method HA extraction from vermicompost in large scale application along with required supplements. Here the concluded method is Rosliza *et al* method. Application of HA reduces the investment for fertilizer and helps profitable growth of plants.

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