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ANALYTICAL STUDY OF HUMIC ACID FROM VARIOUS SOURCES COMMONLY USED AS FERTILIZER: EMPHASIS ON HEAVY METAL CONTENT

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ABSTRACT

In recent years, new varieties of organic fertilizers such as vermi compost, sludge and sediment have been added as new sources of fertilizers, which have humic acid (HA) present in it. However, a systematic characterization of these substances and their comparison with cow dung has not been reported. This is essential to decide the relative merits and demerits of these substances as a fertilizer. The objective of this work was to extract and characterize the chemical compositions of various HA using various techniques such as UV-Vis, Fourier transform infrared spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), scanning electron microscopy (SEM), energy dispersive spectrometer (EDS), atomic absorption spectrophotometer (AAS) and X-ray diffractometer (XRD). Cow dung manure, vermi compost, sludge and lake sediment were used for this purpose. ^{1}H-NMR show that all HA samples were rich in aliphatic groups. EDS analysis found HA rich in carbon and oxygen and poor in nitrogen and presence of elements such as Na, Ca, K, Mg, Al, S, P, Cu, Fe, Cr, Co and Pb in various proportions. It was supported by AAS analysis where elements such as Na, K, Ca, Mg, Fe, Cu and Co were found in all HA samples in various concentrations. SEM images and XRD analysis showed some crystal forms found in all HA samples. These results elucidate that the chemical composition and the molecular structure of HA samples varies as per the origin and environment where decomposition has occurred. Present study also documented that the spectroscopic and imaging techniques can be successfully used to bring out the finer differences among HA extracted from different sources. It brings out the relative merits and demerits of vermi compost, sludge and lake sediment as compared to cow dung manure. This analysis showed that out of the four HA samples extracted from different sources except HA-SD can be used as fertilizer with caution to Al, Co and Cr content.

Keywords: alkaline extraction, fertilizer, humic acid, metal-crystal form, spectroscopic and imaging techniques.

1 INTRODUCTION

Manure and solid disposal have been used as a fertilizer, which have humic acid (HA) present in it. To use cow dung manure as a fertilizer is an age old practice in agriculture in many countries. In recent years, new varieties such as vermi compost, sludge and sediment have been added as new sources of fertilizers. It is obligatory to know the chemical composition of each for further use as fertilizer.

Organic compost is an end product of degraded organic matter (OM) that consists of transformed degradable compounds and dead microorganism tissues that are classified as humic substances (HS) [1, 2]. The extension of organic farming and sustainable agriculture has led to increasing applications of organic fertilizers such as HA, which is a fraction of HS. Increase in the organic content of soils improve aeration, soil structure, increase the water holding capacity of the soil [3] and has been found to reduce plant diseases more efficiently than commercial fertilizers and herbicides [4, 5]. OM must be characterized in order to predict and understand its behaviour and chemistry. Numerous studies have been attempted to make out its structure and chemical composition; however, due to heterogeneity of samples, there is no definitive structure for OM although many structural models have been proposed [6–8].

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Various studies about the characterization of HA extracted from manure, vermi compost, waste disposal and sediment have been carried out. Most of the work reported is about the content of organic elements (C, O, N, H) and E_4/E_6 (degree of aromaticity) of HA from particular source. Few of them have also reported some inorganic elements such as S and P present in HA [1, 8–16].

On this background, an objective study was planned to characterize HA from various sources in the same region using spectroscopy and imaging techniques. These included UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), atomic absorption spectrophotometer (AAS), X-ray diffractometer (XRD), energy dispersive spectrometer (EDS) and scanning electron microscopy (SEM). For this purpose, HA from four different sources was extracted using alkaline solution. These samples were selected to represent origin from the region in and around Pune city, India. These are cow dung manure (HA-CD), vermi compost (HA-VE), solid waste from 'domestic wastewater treatment plant' (sludge, HA-SL) and lake sediment (HA-SD).

The spectroscopic and imaging analysis show that HA from different sources have different chemical compositions and granular structure. Data such as E_{280}, E_2/E_3, inorganic content (heavy metals) and the crystal phases of it are also discussed, which have not been part of the most of the studies reported so far. The observation provides a systematic method to compare the constituents of different HA substances and their merits and demerits as compared to the HA obtained from cow dung manure.

2 MATERIALS AND METHODS

2.1 Sample collection

Cow dung manure was collected from organic manure manufacturing unit at Chinchward, Pune, India. After being air dried at room temperature, it was stored in a plastic bag for extraction. Vermi compost was supplied by Mangal-Raj Bio Tech, Sona Industries, Pune, India. It was used for HA extraction without any further treatment. Sludge was collected from 'Wastewater Treatment Plant', Kasarwadi, Pune, India. It was used for HA extraction without any further treatment. Sediment (0–5 cm depth) collected from banks of Pasan lake, Pune, India, was air dried at room temperature and stored before extraction.

2.2 Extraction and purification of HA

The HA was extracted with alkaline solution according to the protocol described elsewhere [9]. Briefly, the procedure consisted of (i) 10 g of cow dung manure continuously stirred with glass rod manually for 10 min in 100 mL of 0.1 N KOH at room temperature and then kept for 2 h; (ii) supernatant was filtered using Whatman No.1 filter paper and then centrifuged at 12,000 r.p.m. for 30 min, at 20°C; (iii) acidified with 6 N HCl to pH 1.0 and allowed to precipitate for 24 h at 4°C; supernatant was FA and the residue was HA; (iv) precipitated HA was recovered by centrifugation at 5000 r.p.m. for 30 min at 20°C, then dissolved in a small volume of 0.1 N KOH and again precipitated at pH 1.0 for 24 h at 4°C. This precipitation–dissolution procedure was repeated four times to eliminate FA; (v) after purification, the precipitated HA was dried at temperature 32–35°C in an oven for 14 days. Dried HA was kept in a sealed tube for characterization.

The HA extraction from vermi compost, sludge and sediment was carried out using the same procedure used for the cow dung manure.

2.3 Spectroscopic and imaging techniques

The UV-Vis absorption spectra of HA samples were recorded using a UV-Vis spectrophotometer, Perkin-Elmer, model-330. Around 2 mg of each HA sample was dissolved in 25 mL 0.05 N $NaHCO_3$ and spectra were recorded at wavelengths 240–700 nm range.

The infrared spectra of HA samples were recorded with a FTIR-8400 spectrophotometer (Shimadzu) using a pellet (pressed disk) technique. Around 0.5 mg of dried HA was grinded with 100 mg KBr (FTIR grade) till it became homogenous. After grinding, it was placed in a micro-disk. For the reference, a micro-disk of KBr was prepared. The spectra were recorded in the range 400–4000 cm^{-1}.

^{1}H-NMR was used to estimate the relative proton content from aromatic and aliphatic groups present in each sample. The spectra were recorded with a Fourier transform nuclear magnetic resonance spectroscopy, Varian Mercury YH-300, Germany. Around 5 mg of each sample was dissolved in 0.5 mL DMSO-d6 and spectra were recorded in δ range 0–10 ppm.

Granular structure and elemental analysis of samples were obtained using SEM/EDS (JEOL JSM-6360A, Japan) techniques. Each sample was prepared on the glass substrate and was coated with platinum for 45 s (about 20 nm thicknesses) under vacuum condition by auto fine coater machine (JEOL JFC-1600) and then examined with SEM/EDS.

Based on the EDS results, Na, K, Ca, Mg, Fe, Cu, Cr, Co and Pb analysis has been carried out using an AAS, Varian Spectra AA 220, Australia. All samples were prepared in 100 mL volumetric flasks. For each HA sample, around 3 mg of dried HA was dissolved in 10 mL of 0.05 N $NaHCO_3$ and then diluted with distilled water (DW) to make total volume of 50 mL. Each HA solution was treated with concentrated HNO_3:HCl, 3:1 and covered with a glass lid [1]. The samples were placed on a hot plate and heated to 85 ± 5°C for 10–15 min. They were then allowed to cool and 1 mL of concentrated HNO_3 was added. The lid was removed and then samples were heated again for 30 min. After cooling, the volume of the sample was adjusted to 100 mL by adding DW. The digested samples were then filtered by Whatman No. 41 filter paper and were kept in a glass bottle with lid for taking the required readings. The metal analysis for each sample has been repeated three times with the calibration of the system ($p < 0.05$) and the average values were taken.

X-ray diffraction of HA samples was recorded in powder form using a D8 Advance XRD Bruker AXS GmbH, Germany. Around 23 mg of each sample was exposed to X-rays ($\lambda = 1.54060$ Å; anode Cu) with 2θ angle varying within 5° and 80°. The applied voltage and current were 40 kV and 40 mA, respectively.

All the studies have been repeated three times independently with at least three observations each time.

3 RESULTS

3.1 The ratios, E_2/E_3 and E_4/E_6, and elemental analysis

The UV-Vis spectra of HA extracted in this study were relatively featureless, with the absorbance decreasing with increasing wavelength, Fig. 1. The spectra obtained were consistent with the HA spectra that have been reported by other investigators [2, 10, 17].

All the absorption spectra show similar pattern. A slight maximum could be indicated at approximately 275 nm, which is probably due to presence of quinone structure [2]. The absorbance of HA-SD is low compared to other samples. It indicates that less number of chromophore molecules, capable of absorbing electromagnetic radiation in the UV-Vis (240–700 nm) range, is present in HA-SD.

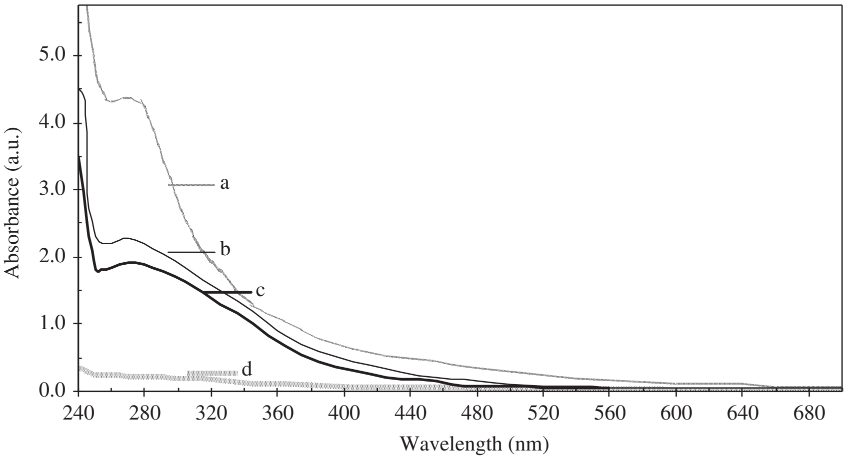


Figure 1: The UV-Vis spectra of HA extracted from four different sources: (a) HA-CD: HA extracted from cow dung manure; (b) HA-VE: HA extracted from vermi compost; (c) HA-SL: HA extracted from sludge and (d) HA-SD: HA extracted from sediment. Three independent studies were performed with similar outcome and the results from one representative experiment are shown.

Table 1 shows comparison of analytical data of HA studied in this work and data reported by other investigators [1, 8, 9, 18]. The data of HA extracted in the present study were compared with data reported earlier and are given in Table 1. It shows that all extracted samples are lower in percentage content of carbon as compared to the reported values and the difference is statistically significant ($p < 0.04$). However, difference in percentage content of oxygen is not significant as compared to the reported values. The nitrogen content was not detectable. The difference in the ratio of the percentage content of oxygen and carbon (O/C) and the ratios E_4/E_6 is not statistically significant.

As far as the comparison of each HA samples studied is concerned, the results from present study reported in Table 1 show that the difference of the ratio, E_4/E_6, is statistically significant ($p < 0.05$) compared to HA-CD value. It decreases in the order HA-CD > HA-VE > HA-SL > HA-SD. Decrement in this order is attributed to the lesser oxygen content in the samples [19]. These observations were confirmed by oxygen data obtained from EDS analysis. The ratio of oxygen decreases in the same order. Similar trend was obtained in the ratio, E_2/E_3. However, difference in the ratio, E_2/E_3, is not significant as compared to HA-CD.

The absorbance at 280 nm (E_{280}) has been used as an indicator of carbohydrate presence in the sample. The E_{280} values decrease in the order HA-CD > HA-VE > HA-SL > HA-SD and elucidate the increasing presence of carbohydrate in the samples [10, 20]. However, difference in E_{280} values, is not significant as compared to HA-CD value.

The EDS analysis in Table 1 shows that carbon decreases in the order HA-SD > HA-SL > HA-VE > HA-CD. The content of sulphur was found same in all samples, in the range of standard deviation. The content of oxygen and the ratio, O/C decreases in the order HA-CD > HA-VE > HA-SL ≈ HA-SD. The difference in the ratios, O/C are statistically significant ($p < 0.03$) as compared to HA-CD value. Whereas difference in the percentage content of carbon, oxygen and sulphur is not significant as compared to HA-CD value.

Table 1: Elemental analyses, atomic ratio and absorbance values of HA extracted in the present study and data reported by other workers.

	Percentage atomic content, At (%)							
Samples	C	O	N	S	O/C	E_{280}	E_2/E_3	E_4/E_6
HA-CD	20.08 ± 1.028	50.41 ± 3.076	nd	0.88 ± 0.057	2.51 ± 0.039	4.31 ± 0.120	3.68 ± 0.062	4.34 ± 0.073
HA-VE	30.68 ± 1.393	46.89 ± 3.771	nd	0.98 ± 0.062	1.3 ± 0.087	2.36 ± 0.064	3.24 ± 0.097	3.54 ± 0.114
HA-SL	43.14 ± 1.131	39.59 ± 1.994	nd	0.96 ± 0.014	0.92 ± 0.049	2.05 ± 0.078	2.88 ± 0.027	2.96 ± 0.051
HA-SD	45.29 ± 1.442	37.81 ± 1.329	nd	1.06 ± 0.092	0.84 ± 0.085	0.28 ± 0.035	2.43 ± 0.101	2.54 ± 0.083
HA[1]	52.63	37.39[4]	5.82	37.39[2]	0.52	na	na	1.92
IHSS HA[3]	57.50	33.80	4.10	na	0.40	na	na	3.70
HA[4]	56.20	36.30[4]	3.20	36.30[4]	0.50	na	na	4.80

E_{280}: absorbance at 280 nm in a.u.; E_2/E_3 and E_4/E_6: ratios of absorbance at 250, 365, 465 and 665 nm, respectively; nd: not detectable because the number was very small; na: not available.

[1] Average value of HA was extracted from various manure with the earthworm [1].

[2] %(O + S).

[3] HA from IHSS [8].

[4] Average values of several soil HA [18].

Table 2: The minerals found in four different HA samples using EDS.

	Percentage atomic content, At (%)			
Elements	HA-CD	HA-VE	HA-SL	HA-SD
Na	8.49 ± 0.183	5.49 ± 0.101	6.20 ± 0.163	4.29 ± 0.297
Ca	5.71 ± 0.195	3.90 ± 0.130	4.21 ± 0.028	2.79 ± 0.297
K	5.69 ± 0.164	4.77 ± 0.030	1.38 ± 0.039	2.92 ± 0.198
Mg	2.45 ± 0.070	1.52 ± 0.087	1.82 ± 0.085	1.20 ± 0.085
Al	2.25 ± 0.139	1.81 ± 0.090	1.23 ± 0.049	1.27 ± 0.071
S	0.88 ± 0.057	0.98 ± 0.062	0.96 ± 0.014	1.06 ± 0.092
P	1.22 ± 0.068	0.98 ± 0.035	0.69 ± 0.042	0.53 ± 0.028
Cu	0.05 ± 0.036	0.01 ± 0.005	0.17 ± 0.011	0.12 ± 0.021
Cr	–	–	0.12 ± 0.021	0.13 ± 0.011
Co	0.08 ± 0.015	0.03 ± 0.010	0.30 ± 0.007	0.16 ± 0.085
Fe	0.33 ± 0.014	0.27 ± 0.025	0.43 ± 0.028	0.57 ± 0.085
Pb	–	–	–	0.05 ± 0.014

The EDS analysis also shows the presence of minerals such as Na, Ca, K, Mg, Al, S, P, Cu, Co, Fe, Cr and Pb in all HA samples as listed in Table 2. The first five minerals i.e. Na, Ca, K, Mg and Al are around 1.23–8.49% and except Al, the highest content was found in HA-CD. The percentage content of S and P was around 0.10–2.73%. The percentage content of S was found same in all samples and P was higher in HA-CD. The last five metals i.e. Cu, Fe, Cr, Co and Pb are lower in percentage content (≤0.61%). Cr was found only in HA-SL (~0.12%) and HA-SD (~0.13%). Pb was found only in HA-SD (~0.05%). However, difference in the percentage content of Na, Ca, K, Mg, Al, S, P, Cu, Co and Fe is not significant as compared to HA-CD value.

1.1 AAS analysis

Table 3 shows the total quantity of metal elements found in each of the HA samples using AAS. It shows that the metals such as Na, K and Ca are present in larger quantity in all HA samples (~67 ppm) except for K in HA-CD (0.002 ppm) and Ca in HA-SL (0.093 ppm). The highest concentration of Na (87.24 ppm) was found in HA-CD. The concentration of Fe, Mg, Cu and Co was low (<0.473 ppm). Cr and Pb were not detectable as the concentrations were lower than the detection limits. The highest content of Ca was found in HA-CD. The highest content of K and Mg was found in HA-SD and the highest content of Fe was found in HA-SL. The concentration of Co was found approximately constant in all HA samples. This result is in agreement with the results obtained by EDS for the same elements. The concentration of Cu and Co was below the normal range in soils [1]. The difference in the concentrations of Na and Ca in other samples is statistically significant ($p < 0.005$) as compared to HA-CD value. However, difference in the concentration of K, Fe, Mg, Cu and Co is not significant as compared to HA-CD value.

1.2 Infrared spectra

The infrared spectra of HA are shown in Fig. 2. The analysis of the spectra was based on the studies of MacCarthy and Rice [7], Ouatmane et al. [9], Ghita et al. [12], Luciano et al. [13] and Stevenson

Table 3: Total quantity (ppm) of Na, K, Ca, Mg, Fe, Cu, Cr, Co and Pb in HA samples.

	Total quantity (ppm)								
Samples	Na	K	Ca	Fe	Mg	Cu	Co	Cr	Pb
HA-CD	87.238	0.002	16.921	0.163	0.034	0.018	0.023	nd	nd
HA-VE	60.426	0.871	0.445	0.323	0.003	0.005	0.032	nd	nd
HA-SL	61.170	1.783	0.093	0.473	0.002	0.199	0.028	nd	nd
HA-SD	63.405	2.262	1.261	0.146	0.119	0.031	0.029	nd	nd
Soil[a]	–	–	–	–	–	2–250	0.5–65	–	–

[a]Normal range for metals in soils [1]; nd: not detectable.

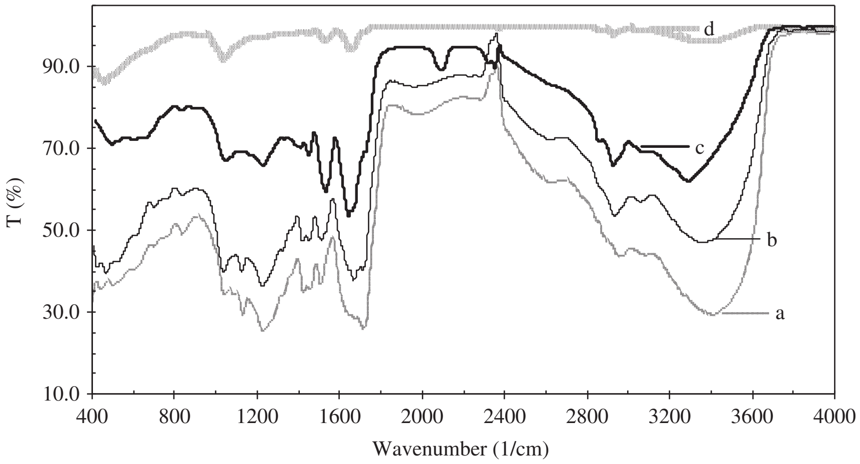


Figure 2: The infrared spectra of HA extracted from four different sources: (a) HA-CD: HA extracted from cow dung manure; (b) HA-VE: HA extracted from vermi compost; (c) HA-SL: HA extracted from sludge and (d) HA-SD: HA extracted from sediment. Three independent studies were performed with similar outcome and the results from one representative experiment are shown.

[21] relating to HA extracted from various sources. This gives the attribution of the main absorption bands presented in Table 4.

McDonnell et al. [14] have reported that a peak at around 2300 cm^{-1} was due to atmospheric CO_2 which was not adequately compensated for by the blank. In samples investigated in this study, similar peak has been detected.

A comparison of infrared spectra of HA (Fig. 2) shows that the spectra are qualitatively similar except one additional peak present at around 2095 cm^{-1} in HA-SL and two broad peaks at around 1990 and 2630 cm^{-1} in HA-CD and HA-VE, respectively. This indicates the presence of Si-H and S-H groups in HA-SL and HA-CD and HA-VE, respectively [22]. The observed differences in the intensity and the shape of the bands indicate that the chemical modifications have occurred, during the humification process of each sample [1, 9, 12, 23]. The shape of the band at around

Table 4: The main band present in infrared spectra of four different HA samples.

Wave number (cm^{-1})	Functional group
3300–3500	–OH stretch from –COOH and –COH [7, 21]
2800–3100	–CH stretch from –CH, –CH_2 and –CH_3 groups of aliphatic chains [1,5]
1700–1725	–C=O stretch from –COOH [7, 9, 12, 21]
1600–1650	aromatic –C=C vibration; –C=O stretch from H-bonded conjugated ketones; –COO– asymmetric stretch [7, 21]
1510–1550	aromatic –C=C stretch and/or N–H deformation and C=N stretch from secondary amides [9, 12]
1380–1400	–OH and –CO deformation from alcoholic and phenolic –OH; –COO– symmetric stretch [7, 9, 12, 21]
1200–1260	–CO stretch and –OH bending from –COOH [7, 9, 12, 21]
1020–1100	–CO stretch of polysaccharides; Si–O stretch from silicate impurities [7, 21]
900	–C=C aromatic [13]
800–817	–CH deformation of substituted aromatic groups [9, 12]

3300–3500 cm^{-1} in HA-CD, HA-VE and HA-SD is broader compared to that in HA-SL with the intensity decreasing in the order HA-CD > HA-VE > HA-SL > HA-SD. It elucidates that different types and number of hydroxyl groups (phenols, alcohols and carboxyl) are present in HA samples. The intensity of the bands at around 2925, 2868–2850, 1460–1440 and 1400–1380 cm^{-1} decreases in the order HA-CD > HA-VE > HA-SL > HA-SD. It elucidates that the presence of aliphatic group is more in HA-CD compared to other samples [7, 9, 12]. Similar observation was obtained for the aromatic groups around 1550–1510 and 1260–1200 cm^{-1} [12, 13].

The pattern of the bands in the 1740–1600 cm^{-1} is analogous to that found in the infrared spectra of various HA extracted from different types of vermi compost [1, 23, 24], which is characteristic of fresh HA. All spectra show two partially overlapped bands at around 1730 and 1645 cm^{-1} of moderate and strong intensity; these have been assigned to the presence of the functional groups such as O–C=O of ester and carboxylic acids or C=O of ketones, respectively. These functional groups as well as alcoholic and phenolic hydroxyls and carboxylic acids groups are considered as potential sites for binding with metallic species [1, 24].

3.2 ^{1}H-NMR study

^{1}H-NMR-based analysis was used to estimate the relative contents of aromatic and aliphatic protons present in the samples. The ^{1}H-NMR spectra (not shown) obtained show the peaks from 0 to 2.5 ppm region were assigned to the presence of the aliphatic protons in the HA samples. The sharp peak at around 0.9 ppm was attributed to the branched methyl proton; also the presence of carboxylate in HA samples. The peaks between 1.1 and 2.5 ppm can be explained by the presence of methylene and methyl protons, which are attached to electronegative groups, such as carboxyl group or an aromatic ring. The peaks from 6 to 8.5 ppm were attributed to the aromatic region of the protons. The sharp peak which appears at around 2.5 and 3.32 ppm can be attributed to the traces of DMSO-d6 [15, 25, 26].

The percentage atomic ratios of aromatic and aliphatic groups obtained by ^{1}H-NMR analyses of different extracted samples and data cited from references are listed in Table 5. It shows that the

Table 5: Distribution of hydrogen in different groups for four different samples was determined by ^{1}H-NMR.

Samples	Aromatic region	Aliphatic region
HA-CD	6.05 ± 0.251	16.40 ± 0.267
HA-VE	4.20 ± 0.025	22.80 ± 2.485
HA-SL	1.70 ± 0.141	24.96 ± 1.053
HA-SD	1.12 ± 0.113	17.55 ± 0.665
Dando[1]	9.90	43.80
Aldrich (purified)[2]	24.80	40.40

Integrated regions were settled as follows: aliphatic region (0.5–2.3 ppm) and aromatic region (6–8.5 ppm).

[1] Dando (soil HA standard of Japan brown forest soil).

[2] Aldrich HA in sodium salt form [15].

values of aromatic and aliphatic group for the samples studied are lower than the reported values ($p < 0.05$). The percentage content of aromatic groups is low (<6.5%). However, within the sample values for aromatic are more in HA-CD and HA-VE compared to HA-SL and HA-SD. The percentage content of aliphatic groups was found to be around 16–25%. It is slightly higher in HA-SL and HA-VE as compared to HA-CD and HA-SD.

3.3 XRDanalysis

The XRD analysis shows that in all HA samples, a broad peak at around 22.35–22.70° and some sharp peaks which can be attributed to the crystal forms were obtained as shown in Fig. 3a–d. The peaks revealed the presence of crystal forms in the HA samples (Table 6). $Na_2SiO_3.5H_2O$ was identified in all samples; $CaAl_8Fe_4O_{19}$ was identified in HA-CD, HA-SL and HA-SD; $Al\text{-}Si\text{-}O\text{-}OH\text{-}H_2O$ was identified in HA-VE, HA-SL and HA-SD and $KAlCO_4.H_2O$ was identified in HA-VE and HA-SD. Also different crystal forms are found in each sample as shown in Table 6. Some crystal forms found are similar with the crystal forms of soil, sediment and clay (i.e. quartz, aluminosilicate, kaolinite, hematite and anhydrate) [11, 27–31].

A small variation in the crystal types present in each sample may be due to difference in physicochemical environment and the concentration of each element present during the time when humification has occurred.

3.4 SEM observations

Figure 4 shows the granular structure of various HA that have been extracted from four different sources. In general, they show crystal-like forms aggregate along with various shapes. This result is in agreement with varies crystal forms that have been found in XRD analysis for each sample.

4 DISCUSSION

Percentage content of element, UV-Vis absorption spectra, infrared spectra, ^{1}H-NMR analyses, AAS and XRD analysis elucidate that the physical and chemical compositions of HA varies as per the

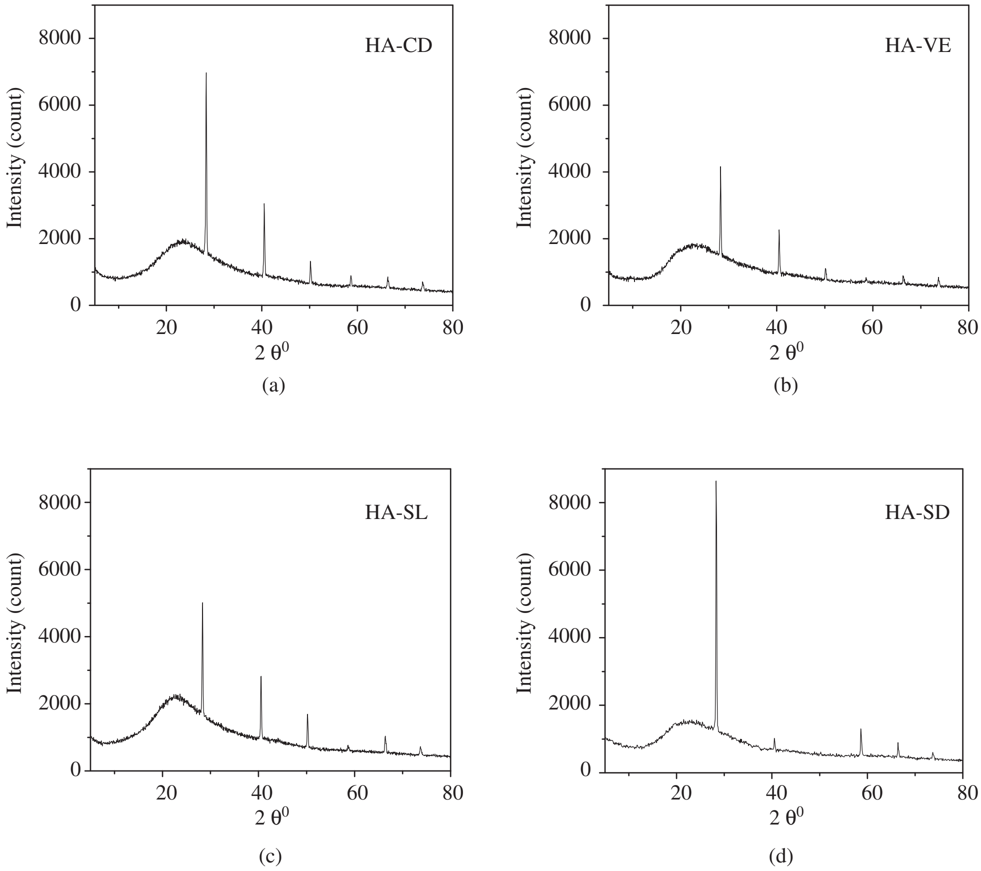


Figure 3: The XRD spectra of HA extracted from four different sources: (a) HA-CD: HA extracted from cow dung manure; (b) HA-VE: HA extracted from vermi compost; (c) HA-SL: HA extracted from sludge and (d) HA-SD: HA extracted from sediment. Three independent studies were performed with similar outcome and the results from one representative experiment are shown.

origin and environmental conditions. Moderate and strong intensities of infrared spectra in O–C=O (ester, carboxylic acids) or C=O (ketones) and N-H (amines, amides, alcoholic, phenolic hydroxyls and carboxylic acids) groups obtained in each HA sample possibly indicate high potential to bind to metallic species [1, 24, 32]. As heavy metals were found in all HA samples, presence of HA can increase the level of heavy metals in the environment. Presence in excess may cause a serious threat to human health, living resources and ecological systems as they are considered persistent, bioaccumulative and toxic substances [33–37].

On the other side, high organic elements and aliphatic groups present in HA samples are useful for agriculture. The aliphatic structures in soil OM contribute significantly to the increased sorption of organic pollutants. Therefore, the incorporation of compost or other OM into the soil may significantly affect the soil structure and may have significant benefits to agriculture and environmental sustainability.

Table 6: The crystal forms obtained in HA samples identified by XRD.

	Minerals identified in HA samples
HA-CD	$Na_2SiO_3.5H_2O$[1]
	$CaAl_8Fe_4O_{19}$[2]
	$K_{0.6}Al_2Si_4O_{10}(OH)_2.xH_2O$
	SiO_2
HA-VE	$Na_2SiO_3.5H_2O$[1]
	$K_{0.67}Na_{1.33}SO_4$
	$KAlCO_4.H_2O$[2]
	$Ca(Mg,Fe,Al)(Si,Al)_2O_6$
	$Al-Si-O-OH-H_2O$[2]
	Fe_2SiO_4
	Al_2SiO_5
	$K_2Ca_2(SO_4)_3$
HA-SL	$Na_2SiO_3.5H_2O$[1]
	$CaAl_8Fe_4O_{19}$[2]
	$Ca_4Al_8Si_{28}O_{72}.28H_2O$[2]
	$CaAl_{12}O_{19}$
	$CaK_3H(PO_4)_2$[2]
	$Al-Si-O-OH-H_2O$[2]
	$AlFeO_3$[2]
HA-SD	$Na_2SiO_3.5H_2O$[1]
	$CaAl_8Fe_4O_{19}$[2]
	$KAlCO_4.H_2O$[2]
	$KAlSiO_4.2H_2O$
	$Al-Si-O-OH-H_2O$[2]

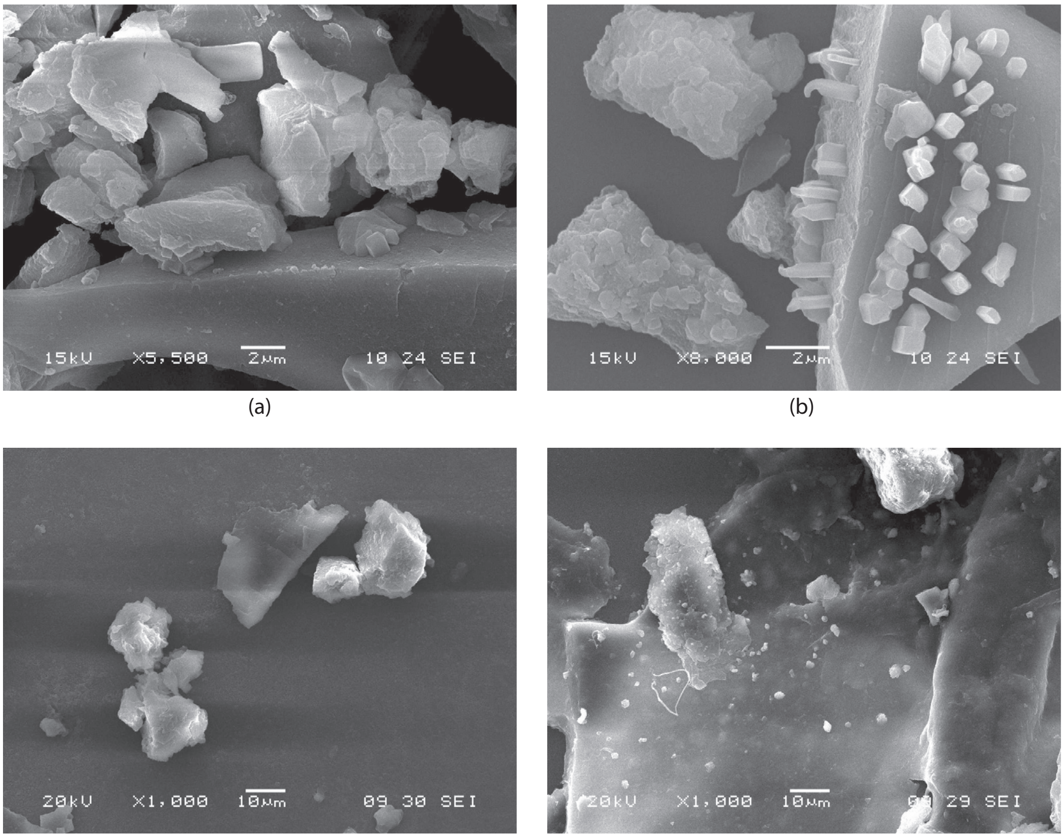
Note: Three independent studies were performed with similar outcome.

[1] The crystal form present in four samples.

[2] The crystal form present in some samples.

Many studies have been carried out to see the effect of HA on plant growth in general and its constituents in particular. It has been shown that the HS affect the activity of chlorophyllase [38]. The effect of foliar application of fulvic acid on water use, nutrient uptake and wheat yield has been reported [39]. Increase in stem height, diameter, leaf chlorophyll, size and production in tomato plants has also been studied [40, 41]. The effect of fulvic acid on green bean plants is to increase plant growth and bean weight at harvest [42]. HAs isolated from earthworm compost enhanced root elongation, lateral root emergence, and plasma membrane H^+-ATPase activity in maize roots [13]. Polyamines in HA affect radical growth of lettuce seedlings [43]. Anticlastogenic, antitoxic and sorption effects of HS on the mutagen maleic hydrazide are tested in leguminous plants [4]. Therefore, it becomes essential to characterize and evaluate HA when used as fertilizer.

The present work provides a detailed analytical information about the HA obtained from different sources commonly used as fertilizer. This scientific data may help to decide the suitability of HS, of which HA is one of the constituent, as fertilizer. Elements such as Al, Co and Pb (found only in HA-SD) are found in HA samples, which are not required by the plant. All HA samples are rich



(a) (b)

(c) (d)

Figure 4: The granular structure of HA samples from SEM: (a) HA-CD: HA extracted from cow dung manure; (b) HA-VE: HA extracted from vermi compost; (c) HA-SL: HA extracted from sludge and (d) HA-SD: HA extracted from sediment. Three independent studies were performed with similar outcome and the results from one representative experiment are shown.

enough in OM such as oxygen and carbon; macronutrients (N, P, K, Ca, Mg and S) and micronutrients (Cu and Fe), which are useful for growth and survival of plants. Based on this composition, all HA samples except HA-SD (content Pb ~0.05%) would serve the purpose as fertilizer with caution to Al, Co and Cr content. However, all HA samples are poor in nitrogen content and it needs to be supplied from other sources when HS in general and HA in particular are used as fertilizer.

The present data show that the various techniques can be successfully used to characterize HA in detailed manner. Such analysis would be useful to bring out fine differences among HA extracted from various sources.

5 CONCLUSION

HA from different sources commonly used as a fertilizer was extracted and characterized using various techniques. The elemental analysis and spectroscopy data show that HA exhibit different elemental compositions and spectroscopic characteristics. In general, all HA samples analysed are rich in carbon and oxygen and poor in nitrogen. EDS analysis showed the presence of elements such as Na, Ca, K, Mg, Al, S, P, Cu, Fe, Cr, Co and Pb. It was supported by AAS analysis where Na, K, Ca, Mg, Fe, Cu and Co were found in all HA samples in various concentrations. XRD analysis showed some crystal forms found similar to the crystal forms found in soil, sediment and clay. These results elucidate that the chemical composition and the molecular structure of HA samples varies as per the origin and environment where decomposition has occurred. Present study also documented that the spectroscopic and imaging techniques can be successfully used to bring out the finer differences among HA extracted from different sources. This analysis showed that out of the four HA samples extracted from different sources except HA-SD can be used as fertilizer with caution to Al, Co and Cr content. The used methods can be adopted to find out the suitability of sources as fertilizer and for further larger environmental studies.

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