HUMIC ACID ANALYSIS FOR COMPARISON OF SOIL SAMPLES: A FORENSIC PERSPECTIVE

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ABSTRACT: In the context of forensic examination of soil, the study of organic matter is important. Humic acid is a dark brown polymeric substance occurs in the organic matter of soil. From the forensic point of view, soil from different places *e.g*. field and gutter soils as physical evidence material is met with various criminal offences, such as murder, attempt of murder, rape and assaults. Many times, soil is adhered to the victims or the deceased or the accused clothes and even weapons. In this study, humic acid from gutter soil was extracted chemically and characterised by Infrared spectroscopy and thermal analysis. Gutter soil samples were taken from different regions of Mumbai. The results show that there was distinct difference in elemental composition (C, H, O and N) and thermal properties of humic acid isolated from gutter soil. In brief, careful soil analysis could provide another form of forensic information to aid in forensic investigation.

Keywords: Forensic science, gutter soil, humic acid.

Introduction

Humic acid is a result of bacterial and chemical degradation of plant tissues followed by certain secondary processes in the soil [1]. It is a ubiquitous brownish polymeric constituent of organic matter of the soil which is quite resistance to prolonged microbiological attack. The humic acids from same source show identical chemical and physical properties. The humic substances are generally characterised as weak acid carrying carboxyl (-COOH) and hydroxy (-OH) groups [2]. From the perspective of soil science, humic acid plays very important role in maintaining the acid base buffering ability of soils. They also hold mineral particles together in soil environment [3].

Forensic comparison of soil from different places could be an evidence material involved in criminal offences. In this context, the study of organic matter of soil (humic acid) is also important. A number of analytical techniques are available for this comparison based on microscopic, chromatographic and spectroscopic techniques. These included microscopic examination, density gradient [4-6] and thermal analysis [7-9]. Organic matter of soil *i.e*. humic acid was studied using different instrumental methods such as Laser Raman spectrometry [10], Infrared spectroscopy, Nuclear Magnetic Resonance spectroscopy [11-12] and differential scanning calorimentry [13].

Infrared spectroscopy is a useful technique for the study of humic substances. This technique gives useful information about spectral bands which occurs due to chemical reaction like methylation, acetylation, basification and reduction. These bands could be associated with carboxyl, phenolic, alcoholic and aromatic groups [14].

Forensic examination of soil collected from gutter plays an important role and have great evidential value. Many times gutter soil associated with criminal cases where weapons are thrown into gutter or in cases when soil is adhered to clothes of victim in murder cases. In this article, we proposed simple method for comparison of humic acids in gutter soils. In brief, gutter soil samples were collected from four different regions which are located in about 34 kilometers along north south direction of Mumbai and humic acid were subsequently extracted. Its characterisation and thermal study are reported in this article.

Materials and Methods

Sample collection

Gutter soil of about 1 kg from four different regions of Mumbai were collected. The gutter soil from one of the regions was divided into two parts. Humic acid from these gutter soil samples was extracted by acccording to chemical method of the International Humic Substances Society (IHSS).

Extraction of humic acid

For extraction, 500 g of soil samples collected from four different regions were respectively washed with 0.5 N hydrochloric acid followed by distilled

water, and subsequently dried at 60ºC. Then, each soil samples were mixed with 4 L of 0.5 N sodium hydroxide solution separately and stirred with magnetic stirrer. After 4 h, the samples were placed aside for 10 h to separate solid and liquid phases completely. After separation of the two phases, 5 N hydrochloric acid was slowly added to the liquid phase until the pH reached 1. The brown coloured precipitate of humic acid was obtained through centrifugation. The supernatant was decanted carefully and the humic acid residue was placed in drying oven at 90°C overnight.

FTIR Analysis and Thermal Study

FTIR spectra of the extracted humic acids were generated using potassium bromide (KBr) pellets. Solid samples were firstly milled with KBr to form a very fine powder, and then compressed into a thin pellet. FTIR spectra were collected in the wavenumber ranged between 4000 and 450 cm^{-1} at resolution 8 cm^{-1} using FTIR spectroscopy (Midac Corporation).

The percentages of carbon, nitrogen, oxygen and hydrogen were determined using EAGER-200 elemental analyser. Thermal study of these humic substances was carried out using simultaneous thermal analyser through differential thermal and thermo gravimetric analyses, where two curves were generated with varying temperatures from room temperature to 950°C.

Results and Discussion

One of the most important soil characteristic is the quality of soil organic matter. Soil organic matter were accessed by two methods, namely the ratio of humic to fulvic acids and by ratio of alkaline soil extract at wavelengths of 400 and 600 nm [15]. Humic acid extracted using a simple procedure adapted from the literature [16]. The humic acid extracted from gutter soil was shiny dark brown in colour. FTIR spectra of these samples are shown in Figure 1.

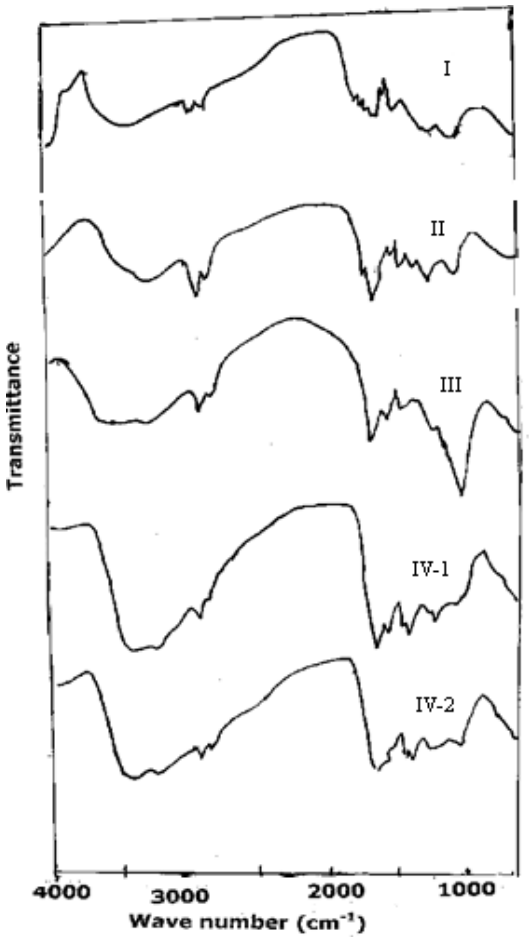

Figure 1: FTIR Spectra of Humic Acid extracted from sample region I to IV

From Figure 1, a strong peak was evident at 3412-3319 cm^{-1} due to hydrogen bonded –OH groups, free -OH groups and intramolecular bonded –OH groups, which could indicate the presence of humic acid. Another characteristically strong absorption peaks at 2984-2024 cm^{-1} due to aliphatic –CH-, -

CH_2-, and -CH_3 or carboxyl group. Also, there was absorption peak observed at 1675-1650 cm^{-1} due to C=O stretching of carboxylic acids, cyclic and acyclic aldehydes, ketones and quinones. The structural formula of humic acid is presented in the Figure 2.

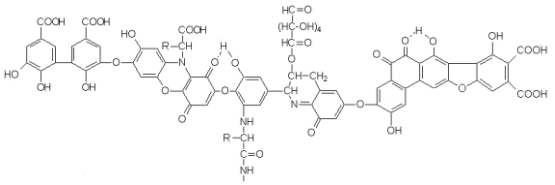

Figure 2: Structural formula of humic acid

Other peaks of aldehyde -C-H at 1460-1437 cm^{-1}, C-O stretching esters and phenols at 1236-1205 cm^{-1}, Si-O-Si vibration of silicates at 1039-1016 cm^{-1} were also seen in the IR spectra. From this FTIR study, it could suggest the presence of humic acid. However, no other difference was observed among the four samples collected from four different regions.

The percentage of carbon, nitrogen, hydrogen and oxygen of humic acid samples from four regions are shown in Table 1. As indicated in Table 1, carbon content varies from 17 to 53 %. The high percentage of carbon could be due to the highly polluted region while the low percentage of carbon could be from high society residential area. The percentage of nitrogen and hydrogen were varied from 2% to 8%. Generally, low percentage of nitrogen was in the form of heterocyclic structure. The oxygen in humic acid was distributed among carbonyl, phenolic and alcoholic hydroxyl functional groups. Thus, soil could be differentiated on the basis of percentage of nitrogen, hydrogen, carbon and oxygen.

Table 1: Elemental analysis of humic acid extracted from sample region I to IV

Sample No.	% of C	% of H	% of N	% of O
I	41.602	4.474	4.123	49.801
II	16.532	2.039	2.069	79.36
III	63.665	8.297	7.564	20.474
IV	55.519	6.236	7.149	31.096

Figures 3 and 4 show the DTA curves of humic acid samples from region II and region IV, respectively. These showed thermal activities between 400ºC to 600ºC for samples collected from regions II and IV. All these samples showed thermal activity by endothermic peaks. The humic acid sample from region IV showed two endothermic peaks at 450ºC and 540ºC, whereas sample from region II showed only one peak at 490ºC. These values indicated that degradation of these humic acid samples from different regions did not show similarity, an important feature to differentiate soil from different areas for forensic comparison.

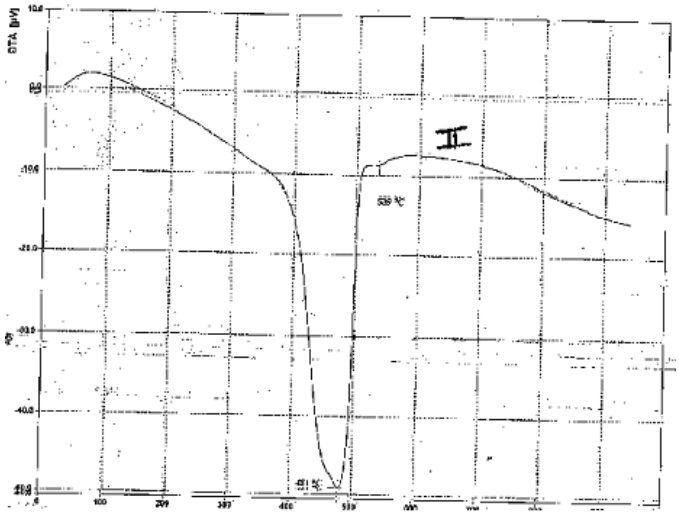

Figure 3: DTA curve of humic acid extracted from sample Region II

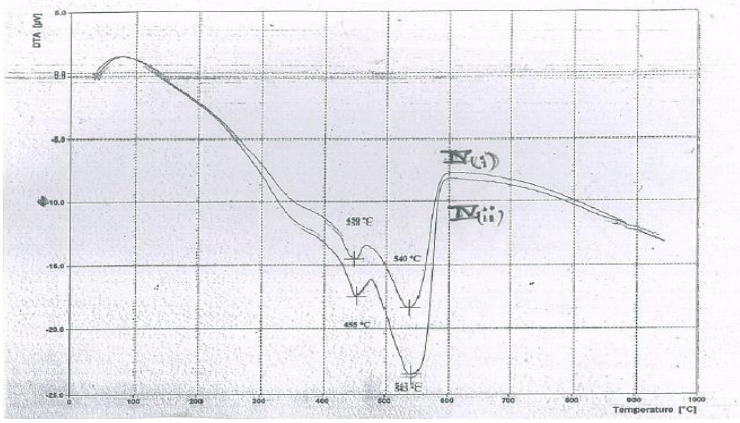

Figure 4: DTA curve of Humic Acid extracted from sample Region IV

Conclusion

Many times, soil samples could be compared as evidential material in many crime cases like murder, burglary, attempt to murder and rape. In these cases, humic acid from sufficient amount of soil can be extracted chemically and its characterisation can be done by FTIR spectroscopy. From the percentage of C, N, H and O and thermal analysis of humic acid of soil, it is possible for the forensic discrimination of soil samples originated from different areas.

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