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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

SUITABILITY OF ELECTRONIC NOSE AS A REFLECTIVE TOOL TO THE MEASUREMENT OF SOIL FERTILITY FACTORS

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ABSTRACT
Humic Acid (HA) and Fulvic Acid (FA) contribute prominently to soil fertility. They are aromatic in nature and hence can be determined by sensory array. This is apparently the first report on the employment of Electronic nose (E-nose) to detect HA and FA extracted from soil. The aim was to evaluate the E-nose sensor response to HA and FA chemically extracted from different agricultural soils. Humic acid and Fulvic acid were extracted from collected agricultural soils and the aroma was measured by E-nose from each of the seven soils. Their presence was confirmed by fluorescence spectroscopy. The Norm Aroma Index (NAI) was measured for: soil, soil after heat treatment, heat treated soil amended with extracted HA, heat treated soil amended with extracted FA, extracted HA and extracted FA. The NAI values were descending in the order: heat treated soil amended with extracted HA/FA, extracted HA/FA, soil (untreated) and heat treated soil. This indicates that HA and FA are detected by E-nose. It was also observed that the most sensitive sensors were 2, 3, 4, 8 and 5 for all agricultural soils tested. Out of the eight sensors in the sensory array of E-nose, above mentioned sensors consistently exhibited high response and these sensors when customized into a small unit may act as a soil fertility tester.

Key words: Electronic nose, Sensor array, Norm aroma index, Soil fertility, Fluorescence spectra, Humic acid, Fulvic acid

INTRODUCTION
Humic acid (HA) and Fulvic acid (FA) are essential for plant growth, development and plant productivity. Humic and Fulvic acids are components of humus and are produced during microbial decomposition of organic matter in the soil, a process called humification. "Humus is called the Soul of the Soil" [1]. The vegetative growth of plants (plant height, number of leaves and branches as well as fresh and dry weight of whole plant) was improved by HA spraying [2]. Humic acid promotes the root length [3, 4], and produce more fruits and flowers [5]. Humic and fulvic acids preparations were reported to increase the uptake of mineral elements [6].

Humic and fulvic acids possess odor that is characteristic and could be detected by E-nose. Electronic nose finds application in various fields like black tea quality evaluation, characterization of volatile aroma from jasmine flowers, peach quality evaluation, to assess the freshness of sardines, for early detection of microbial spoilage of milk-based products, tomato aroma profile for determining its maturity and the like [7, 8, 9, 10, 11, 12]. The aim of this study was to assess the E-nose sensor

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response to HA and FA extracted from different agricultural soils, as indicated by NAI. However, the sensitive sensors in the sensory array of E-nose were also to be identified with intent of customizing the instrument exclusively for soil fertility test through odor emission by HA and FA.

MATERIALS AND METHODS

Soil collection: Soil samples (S1 to S7) were collected at the rhizosphere proximity. The soil was dug to a depth of 10 cm from the surface. This region belongs to Horizon 'A' layer. The sources of soil samples for study are indicated in Table 1. The collected soils were powdered using pestle and mortar and passed through a 2 mm sieve to remove stones and leaves. The powdered soil samples were stored at room temperature until further analysis.

Table 1. Source of soil samples

Sample number	Soil sampling sites (Agricultural fields)	Standing crop/flower
S1	Veeranam, Salem district, Tamilnadu	Tapioca
S2	Veeranam, Salem district, Tamilnadu	Groundnut
S3	Mannarpalayam, Salem district, Tamilnadu	Jasmine
S4	Veeranam, Salem district, Tamilnadu	Sugarcane
S5	Channapatna, Ramanagara district, Karnataka	Ragi
S6	Channapatna, Ramanagara district, Karnataka	Coconut
S7	Kalahasti, Chittoor district, Andhra Pradesh	Paddy

Extraction of HA and FA from different agricultural soils

The sieved soils were used for extracting HA and FA by employing standard protocol as described by International Humic Substances Society (IHSS, USA), up till purification step [13]. 30 g of sieved soil was soaked in 300 ml of 0.1N sodium hydroxide solution as extractant (soil and extractant ratio, 1:10) in a 500 ml brown bottle. The suspension was stirred intermittently for 3 h and then allowed to settle overnight and then centrifuged at 10,000 rpm for 10 min at 4ºC. The supernatant liquid that had HA and FA were collected and the insoluble pellets were discarded. The supernatant liquid was acidified to pH<2 using 6 M hydrochloric acid. The HA being insoluble at pH<2, precipitated and the FA remained as clear supernatant at all pH levels. The HA pellet obtained was dissolved in 1-2 ml of 1 M NaOH and made up to a particular volume with distilled water. The HA and FA samples were stored at 4°C for fluorescence spectroscopic measurements and E-nose to determine Norm Aroma Index.

Confirmation of extracted HA and FA by Fluorescence spectroscopy

Preparation of standard solutions: Humic acid sodium salt (101254282; H16752-100G) was purchased from M/s. Sigma Aldrich. Humic acid standard solution was prepared by dissolving 5 mg of sodium salt of HA in 1000 ml distilled water. Working standard HA solutions with concentrations ranging from 0.5 to 5.0 μg ml^{-1} were prepared for fluorescence spectroscopic analysis. The standard FA was procured from the IHSS (2S103F - Pahokee Peat Fulvic acid Standard II). 42 mg of Pahokee

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Peat FA was dissolved in 1000 ml distilled water which was the standard FA solution. Working standard FA solutions with concentrations ranging from 3 to10 µg ml^{-1} were prepared for fluorescence spectroscopic analysis.

Extracted HA: 0.2 ml from each of soil extracted HA was diluted to 10 ml with distilled water and taken for Fluorescence spectroscopy measurement.

Extracted FA: The soil extracted FA obtained from extraction procedure was directly used for Fluorescence spectroscopy measurement. The Fluorescence study was done at Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bangalore.

Fluorescence spectroscopy was performed using a Varion Cary Eclipse Fluorescence Spectrophotometer. With the help of standard HA and FA, the excitation and emission wave length were fixed: Excitation wave length at 260 nm and Emission wave length between 350–650 nm [14]. The Fluorescence readings were obtained for HA and FA extracted from different agricultural soils.

NAI estimation by E-nose and sensor sensitivity determination

The E-nose sensory array and E-nose equipment was designed and developed by Centre for Development of Advanced Computing (C-DAC), Kolkata, India, for evaluating tea flavor (ENV UNIT). It is equipped with a set of eight metal oxide semiconductor sensors TGS-832, TGS-823, TGS-830, TGS-816, TGS-2600, TGS-2610, TGS-2611 and TGS-2620 of M/s. Figaro Engineering Inc. The sensors assembled in E-nose equipment are capable of detecting combustible gases, air contaminants, chlorofluorocarbons, methane and organic solvent vapors.

After confirmation of extracted HA and FA using Fluorescence spectroscopy, NAI of soil extracted HA and FA was determined using E-nose and also the sensitive sensors were identified. The design of experiment for seven different soils is shown in Table 2.

Table 2. Design of experiment for each of the agricultural soil

S. N.	NAI and Sensor readings obtained from
1	50 g Soil
2	50 g Heat Treated Soil (HTS)*
3	50 g HTS + 17 ml extracted HA of respective soil
4	50 g HTS + 17 ml extracted FA of respective soil
5	17 ml Extracted HA of respective soil
6	17 ml Extracted FA of respective soil
7	17 ml Standard HA
8	17 ml Standard FA

* HTS = Soil heated overnight in hot air oven at 100^{o}C; Soil and HTS (S. N. 1 and 2) were in quadruplicates and the remaining (S. N. 3 to 8) were in duplicates for all of which average NAI was taken.

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The conditions maintained in E-nose equipment were: Heating time = 45 sec; Headspace time = 30 sec; Sampling time = 50 sec and Purging time = 100 sec.

RESULTS AND DISCUSSION

Confirmation of extracted HA and FA from different agricultural soils by Fluorescence Spectroscopy

The extracted HA and FA from different agricultural soils were confirmed by Fluorescence spectroscopy and their readings are presented in Table 3 and Table 4 respectively. The fluorescence spectrum of standard HA was found at 521 nm and for standard FA was at 467 nm (Figure 1). The Fluorescence intensity for extracted HA was observed at wavelength 521.04 nm; and for extracted FA at a wavelength ranging from 428 – 458 nm (Figure 2).

Table 3. Fluorescence spectroscopic readings of HA extracted from different agricultural soils

Soil sample	Crop	Wavelength (nm)	Fluorescence Intensity (a.u.)*	HA concentration (mg/kg)
S1	Tapioca	521.04	245.544	150.469
S2	Ground nut	521.04	123.172	70.760
S3	Jasmine	521.04	232.332	520.275
S4	Sugarcane	521.04	119.124	70.505
S5	Ragi	521.04	403.175	450.357
S6	Coconut	521.04	785.121	880.326
S7	Paddy	521.04	139.497	150.693

*a.u. arbitrary units

Table 4. Fluorescence spectroscopic readings of FA extracted from different agricultural soils

Soil sample	Crop	Wavelength (nm)	Fluorescence Intensity (a.u.)*	FA concentration (mg/kg)
S1	Tapioca	458.03	76.679	130.802
S2	Ground nut	428.03	54.383	90.789
S3	Jasmine	458.93	56.733	100.212
S4	Sugarcane	456.96	50.990	90.178
S5	Ragi	453.03	40.072	70.213
S6	Coconut	456.96	66.068	110.892
S7	Paddy	458.03	43.982	70.917

*a.u. arbitrary units

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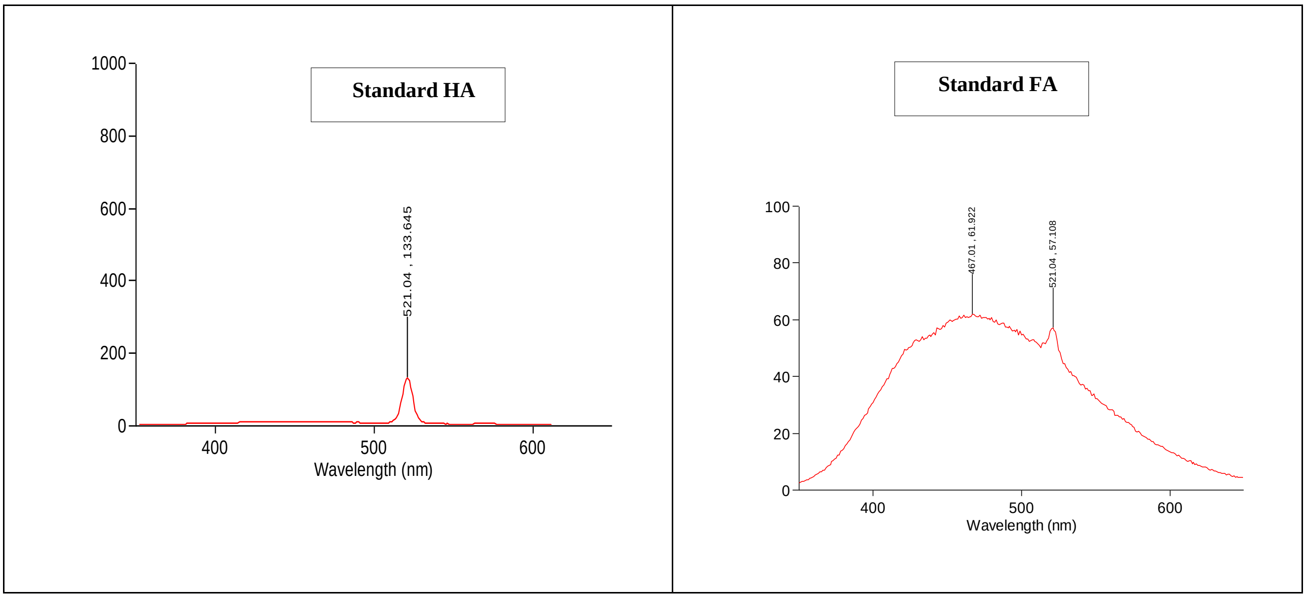

Figure 1. Fluorescence spectra for Standard HA and Standard FA

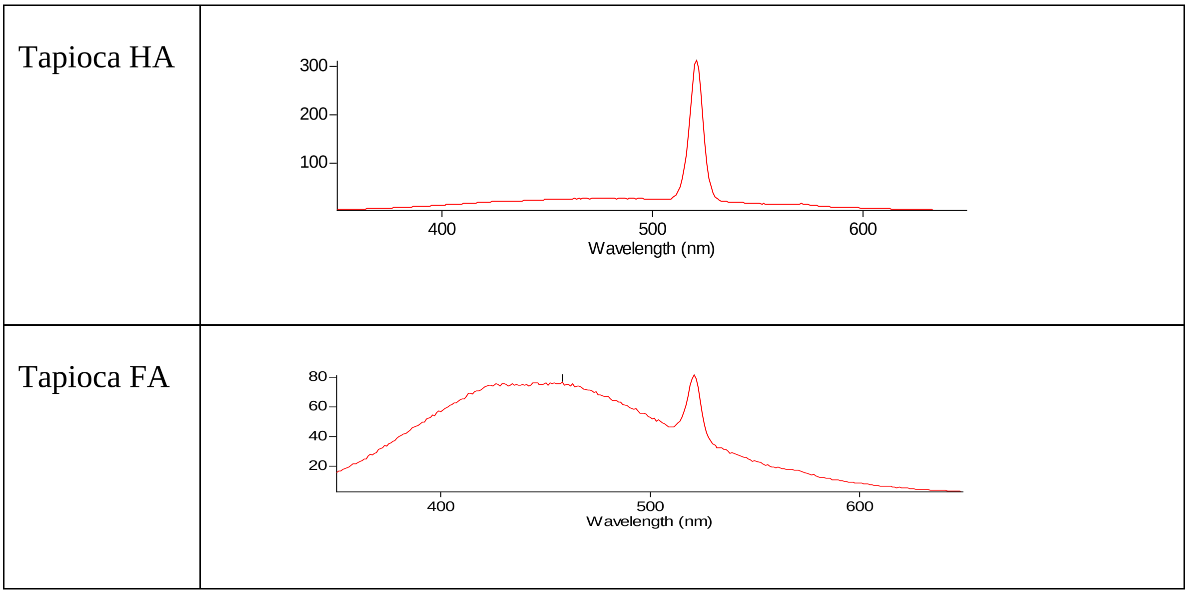

Figure 2. Fluorescence spectra for extracted HA and FA from S1 soil sample (Tapioca)

NAI estimation by E-nose

The NAI values were obtained for all the samples according to their treatment. In various agricultural soils (S1 to S7), observed NAI values and sensor readings are presented in Table 5.

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Table 5. NAI of different agricultural soils

Type of treatment	NAI						
	S1	S2	S3	S4	S5	S6	S7
Soil (untreated)	4.25	3.35	4.53	4.40	4.70	4.45	4.40
HTS	2.60	2.18	2.25	2.38	2.25	2.43	3.13
HTS + Extracted HA	7.25	8.85	10.20	10.15	8.75	6.10	5.90
HTS + Extracted FA	6.55	7.20	7.25	7.45	7.90	7.30	6.75
Extracted HA	4.65	4.35	6.00	12.65	4.90	6.10	5.32
Extracted FA	4.80	4.75	5.55	4.45	5.00	4.70	5.05

S1 Tapioca; S2 Groundnut; S3 Jasmine; S4 Sugarcane; S5 Ragi; S6 Coconut; S7 Paddy

For all the seven soils (S1 to S7), the following observations were made (Table 5): firstly, HTS gave the least NAI, as heating will remove the entire existing aroma. Secondly, heated soil amended with either extracted HA / FA was higher compared to the soil (untreated) which indicates that extracted HA and extracted FA do possess aroma and could be detected by E-nose; similarly monitoring of volatile organic compounds from composting of municipal solid waste was achieved by E-nose and GC-MS [15]. It is also possible to quantify humic substances like HA and FA using E-nose. Thirdly, NAI of extracted HA or extracted FA is slightly higher than the NAI of soil (untreated), which implies that, in soil HA or FA may be masked by other components and hence gives lesser NAI. With respect to recovery study of extracted HA, % of error spanned in the range of 0% to 92.06% and for extracted FA, 3.49% to 39.5%.

Generation of odor molecules while composting from food waste was measured by E-nose [16]. Onion bulb volatiles were affected due to differences in nitrogen, sulphur and soil types and were investigated with the help of E-nose [17].

Following observation of NAI values of different agricultural soils, sensor data for the soils were also noted as depicted in Table 6. Accordingly, a remarkable pattern was found in the result in terms of sensor response. The maximally responding sensors were found to be 2, 3, 4, and 8.

For all the seven soils, Sensors 2, 3, 4 and 8 showed high sensitivity followed by sensor 5. For standard HA and FA, the sensitive sensors were found to be 2, 3, 4, 5 and 8 (Table 7).

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Table 6. Sensor readings obtained for heated soil amended with extracted HA and FA of different agricultural soils

Soil	Expt.	Sensors							
		1	2	3	4	5	6	7	8
Tapioca	HTS + Extracted HA	0.603	2.281	1.452	0.965	0.661	0.101	0.687	1.639
	HTS + Extracted FA	0.603	2.281	1.620	1.000	0.815	0.101	0.779	1.756
Groundnut	HTS + Extracted HA	0.639	2.332	1.727	1.046	0.907	0.101	0.830	1.853
	HTS + Extracted FA	0.613	2.276	1.595	1.000	0.835	0.106	0.748	1.777
Jasmine	HTS + Extracted HA	0.598	2.287	1.549	0.995	0.784	0.101	0.712	1.731
	HTS + Extracted FA	0.603	2.266	1.544	0.985	0.738	0.106	0.712	1.716
Sugarcane	HTS + Extracted HA	0.613	2.292	1.697	1.020	0.881	0.101	0.773	1.853
	HTS + Extracted FA	0.588	2.271	1.488	0.975	0.728	0.106	0.692	1.655
Ragi	HTS + Extracted HA	0.664	2.276	1.875	1.081	1.070	0.101	1.084	1.940
	HTS + Extracted FA	0.593	2.271	1.544	0.985	0.758	0.106	0.723	1.711
Coconut	HTS + Extracted HA	0.623	2.067	2.099	1.036	1.198	0.106	1.217	2.083
	HTS + Extracted FA	0.557	2.195	1.396	0.929	0.631	0.101	0.641	1.568
Paddy	HTS + Extracted HA	0.567	2.205	1.401	0.949	0.661	0.101	0.661	1.568
	HTS + Extracted FA	0.532	2.185	1.274	0.898	0.564	0.101	0.600	1.435

Table 7. NAI and sensor datas of standard HA and FA

Expt.	NAI	1	2	3	4	5	6	7	8
Standard HA									
(a)	5.9	0.521	1.838	1.538	0.827	0.866	0.096	0.651	1.766
(b)	4.5	0.532	1.828	1.661	0.857	0.978	0.096	0.692	1.909
Standard FA									
(a)	5.7	0.511	1.787	1.620	0.837	0.917	0.106	0.681	1.868
(b)	5.5	0.501	1.817	1.477	0.806	0.804	0.106	0.620	1.715

It is possible to assemble the sensitive sensors for development of customized soil fertility tester specific for HA and FA. This instrument could be smaller that is hand held, cost effective and could be used by farmers themselves to test their soils.

CONCLUSION

Amendment of soil with extracted HA and FA was discriminated by E-nose as indicated by rise in NAI. The individual sensor data analysis of S1 to S7 revealed that the five out of eight sensors of the sensory array of E-nose instrument exhibited high sensitivity. This suggests that if the sensitive sensors are exclusively used in the E-nose instrument it could be a good screening tool for the indicative measurement of soil fertility. The knowledge of presence of HA and FA in concentrations will facilitate even augmenting them in soils where these fertile factors are scantily present. Out of the eight sensors in the array of E-nose, five sensors consistently exhibited high response. The study suggests a possibility of improvisation of the E-nose equipment by assembling only maximally responding sensors.

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