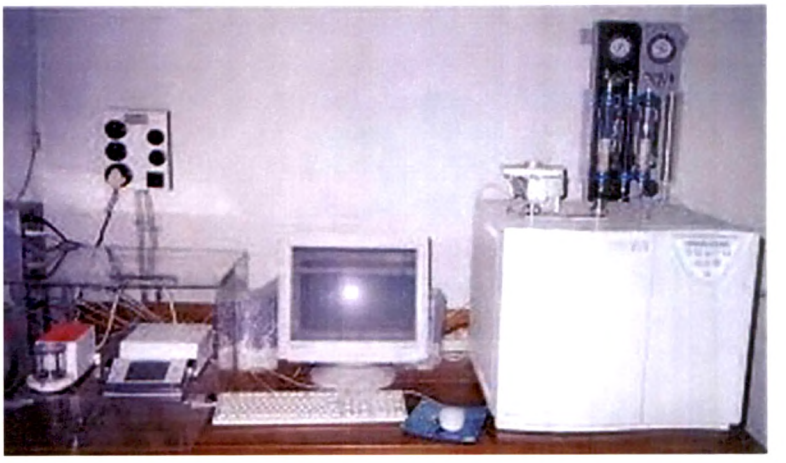
CHAPTER-5

CHEMICAL COMPOSITION

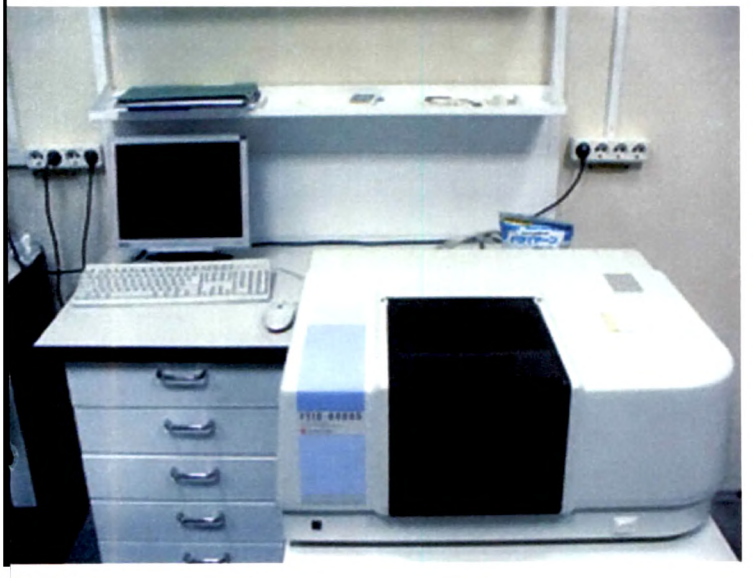
UV SPECTROPHOTOMETER



CHNS ANALYSER

SEM- EDS

AAS



FTIR

ICP-AES

5.0 Chemical composition of soils

5.1 FTIR characterization

FTIR spectroscopy offers a number of important advantages over other methods. It is a rapid, non-destructive method, requires minimal pre-treatment of samples (only air–drying and sieving), it is highly accurate and free of chemical reagents and harmful waste production. Fourier Transform Infrared (FTIR) spectroscopy is a commonly used technique capable of distinguishing the principal chemical classes in soil organic matter, such as carbohydrates, lignins, cellulose, fats and / or lipids and proteinaceous compounds, through the vibrational characteristics of their structural chemical bonds.

Fourier Transformed Infrared spectroscopy (FTIR) of the soils was performed on FTIR 8400 S Shimadzu model using KBr disc at Choksi Laboratory, Makarpura, Vadodara, and their spectra were interpreted for characteristic peaks. Fig.nos.21, 22, 23, & 24 show spectra of Kerala Black, Kerala Brown, Dwarka, Vadodara respectively and Table no. 19 shows peaks and intensities attributed to chemical bonds of all four soils.

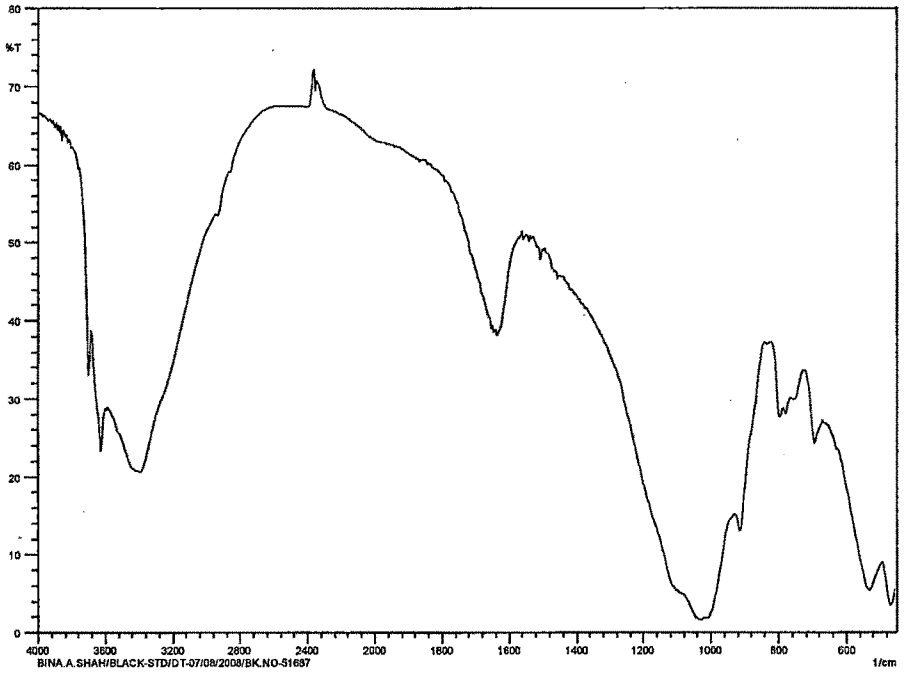


Fig.no: 21 FTIR Spectra of Kerala Black

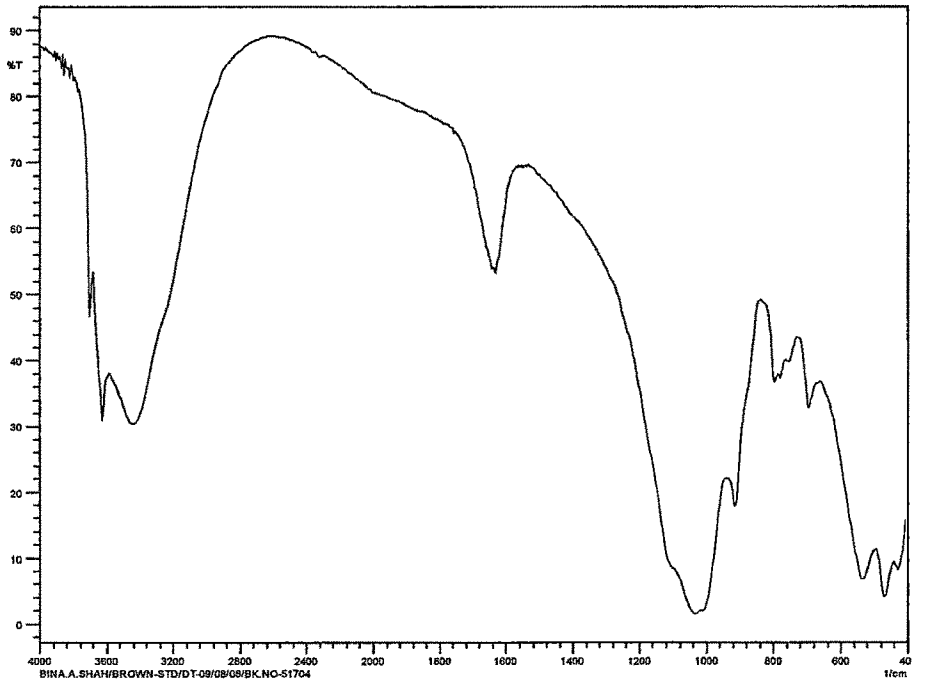


Fig. no. 22 FTIR spectra of Brown

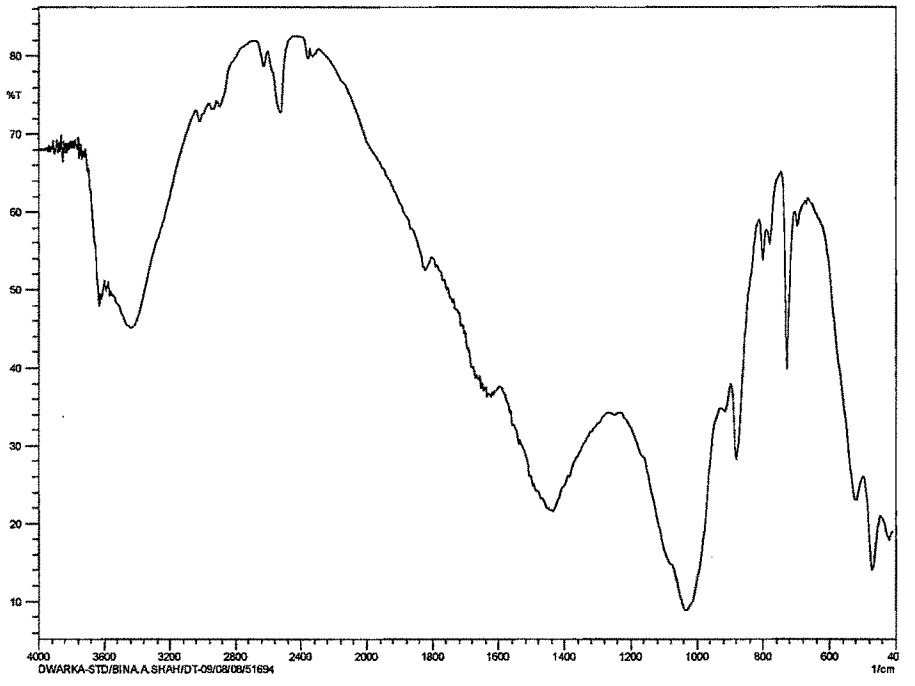


Fig no 23 FTIR spectra of Dwarka

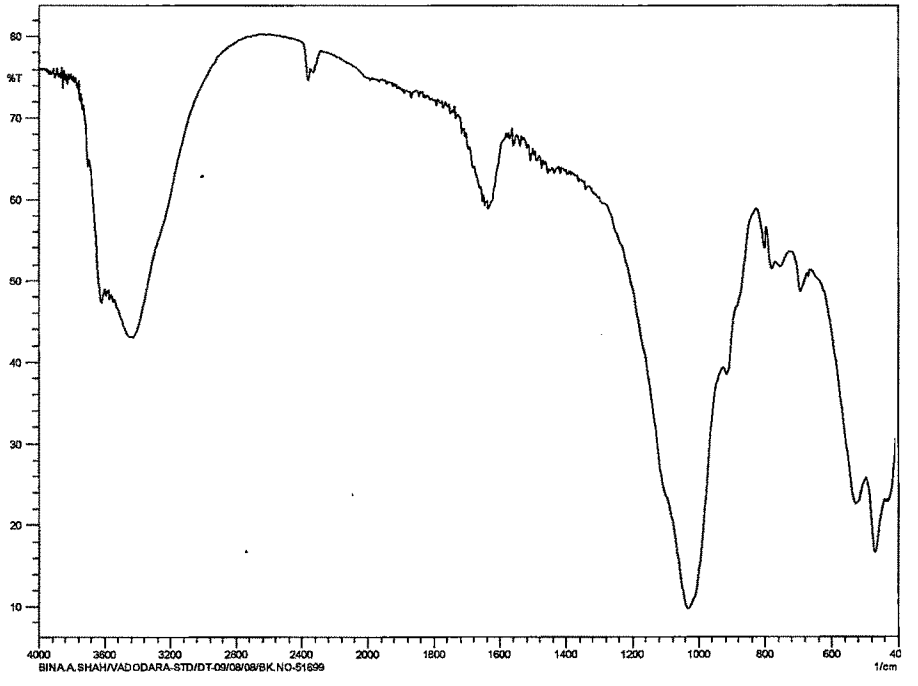


Fig. no. 24 FTIR spectra of Vadodara

Table no: 20 Comparison of FTIR data of all four soils

(ref: An introduction to practical infra-red spectroscopy A.D.Cross, R.Alan Jones. 1969 Third edition)

Sr. no.	Range in cm-1	Kerala Black		Kerala Brown		Dwarka		Vadodara		Assignment
		Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	
1	200-500	430.14	6.624	430.14	8.166	420.5	17.936	420.5	22.93	C-I stretching,,
		468.72	3.549	468.72	4.126	470.65	14.35	468.72	16.667	"
	600-500	532.7	5.413	534.3	6.677	518.87	22.999	526.58	22.613	"
	400-550	430.14	6.624	430.14	8.166	420.5	17.936	420.5	22.93	
		468.72	3.549	468.72	4.126	470.65	14.35	468.72	16.667	Iron oxide
		532.7	5.413	534.3	6.677	518.87	22.999	526.58	22.613	
2	690-550									C-Br axial
	750-700	694.4	24.34	694.4	32.877	694.4	58.193	694.4	48.755	C-Br equatorial
	710-695	"	"	"	"	"	"	"	"	NO_2 deformation
	760-745	"	"	"	"	"	"	"	"	NO_2 deformation
3	730-650					727.19	39.728			C-Cl monochlorinated primary compounds
	780-750	754.19	29.895	754.19	39.902			754.19	51.821	C-Cl equatorial ,
	800-700	779.27	28.101	779.27	37.436	779.27	55.828	777.34	51.538	C-Cl polychlorinated compounds
	800-700	796.63	27.587	796.63	36.785					IO_3 -1
	790-770									all nitraamines
	790-770									P-C stretching P-CH_3
	790-720	779.27	28.101	779.27	37.436	779.27	55.828	777.34	51.538	usually 2 bands Furans C-H in& out of plane defor.

Sr. no.	Range in cm-1	Kerala Black		Kerala Brown		Dwarka		Vadodara		Assignment
		754.19	29.895	754.19	39.902	727.19	39.728	754.19	51.821	
4	810-790	796.63	27.587	796.63	36.785					BrO_3^-
5	860-800	833.28	37.005			800	53.853	802.4	54.044	C-H out of plane deformation & Benzene ring substitution
						852.56	45.009			1:2:3:4 substitution
6	920-800					879.57	28.267			C-O stretching
	850-750	833.28	37.005			800	53.853			R-O-N=O trans & cis formNO nitrites NO vibrations
	1680-1610	1633.76	38.176	1631.8	53.263	852.56	45.009	1633.76	58.803	
						879.57	28.267			
						1608.69	37.014	1633.76	58.803	
7	920-890	912.36	13.038	912.36	17.798	914.29	34.397	914.2	38.5	" " MnO_4^- ions
	850-840									
	930-920	"	"	"	"	"	"	"	"	pyrolles N-H, C-H in, out of plane defor.
	970-950	"	"	"	"	"	"	"	"	tert.aliphatic N-oxides N-O stretching vib.
	998-914	"	"	"	"	"	"	"	"	alkyl nitro compounds
8	1660-1620	1633.76	38.176	1631.8	53.263			1633.76	58.803	$S_2O_3^{-2}$
	1130-1080	1031.95	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	SO_4^{-2}
9	1100-900	1031.95	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	all silicates
		912.36	13.038	912.36	17.798	914.29	34.397	914.2	38.5	
	955-890	912.36	13.038	912.36	17.798	914.29	34.397	914.2	38.5	All OH vib carboxylic acids
10	1040-1030	1031.95	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	aliphatic primary amines

Sr. no.	Range in cm-1	Kerala Black		Kerala Brown		Dwarka		Vadodara		Assignment
	1090-1070	"	"	"	"	"	"	"	"	C-N stretchiing vib
	1045-1030	"	"	"	"	"	"	"	"	Pyridines only
	1090-1025	"	"	"	"	"	"	"	"	N-H & C-H in& out of plane deformation 1:2:5 tri substitution
		"	"	"	"	"	"	"	"	aromatic bromo compds.C-X stretching vibration
	1090-1020	"	"	"	"	"	"	"	"	SI-O-Si & Si-O-C
	1050-970	"	"	"	"	"	"	"	"	all P-O alkyls asym.P-O-C str.
	1070-1030	"	"	"	"	"	"	"	"	sat.or unsat. Sulphoxides S=O stretching vib.
	1055-1030	1031.95	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	Thiophens C-H in & out of plane defor. 2-substitution
	940-905	912.36	13.038	912.36	17.798	914.29	34.397	914.2	38.5	
	865-840	833.28	37.005			852.56	45.009			
	1035-1015	1031.95	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	Pyrroles N-H, C-H in& out of plane defor.1 subst.
	1040-1020	"	"	"	"	"	"	"	"	Pyrroles N-H, C-H in& out of plane defor 2 subst.
	1040-1030	"	"	"	"	"	"	"	"	Pyrroles N-H, C-H in& out of plane defor 1:2:5 tri subst.
10	1045-1030	"	"	"	"	"	"	"	"	3 mono subst. Pyridines only
	920-890	912.36	13.038	912.36	17.798	914.29	34.397	914.2	38.5	3 mono subst. Pyridines only
	820-770	779.27	28.101	779.27	37.436	779.27	55.828	777.34	51.538	pyridines & 1- oxides
	730-690					727.19	39.728			3 mono subst. Pyridines only
		694.4	24.34	694.4	32.877	694.4	58.193	694.4	48.755	
	1040-1030									4mono subst pyridine 1-oxides only

Sr. no.	Range in cm-1	Kerala Black		Kerala Brown		Dwarka		Vadodara		Assignment
	850-790									"
	815-770									2:6 disubst ,pyridine C-H in & out of plane pyridine
	1100-950	1031.950	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	PO4-2,HPO4-1,H2PO4-
	1295-1265					1271.13	34.185			2 monosubstitution pyridines only
	1055-1040	1031.95	1.558	1032	1.5	1031.95	8.913	1031.95	9.75	pyridines & 1- oxides
	780-740	779.27	28.101	779.27	37.436	779.27	55.828	777.34	51.538	pyridines only
11	1255-1245					1249.91	33.981			$C(CH_3)_3$ skeletal vibs. Out of plane
12	1410-1310					1384.94	25.925			tertiary alcohols, phenols
	1260-1180					1359.86	28.433			
						1249.91	33.981			
13	1480-1400					1435.09	21.528			C-H str. Pyrimidines
	1300-1150					1249.91	33.981			Carbonates C-O stretching vib
14	1320-1210					1271.13	34.185			Carboxylate ion CO2 of carboxylic acids
	1610-1550					1359.86	28.433			
	1420-1300					1384.94	25.925			
14	1450-1410					1435.09	21.528			CO_3^{-2} ,carbonate
	880-800	833.28	37.005			800	53.853	802.4	54.044	
						852.56	45.009			
						879.57	28.267			

Sr. no.	Range in cm-1	Kerala Black		Kerala Brown		Dwarka		Vadodara		Assignment
	1440-1395					1435.09	21.528			Combination band of C-O str&OH def of carboxylic acid
15	1520-1470			1506.46	47.908					C=Cstretching vib aromatic homocyclic compds
	1465-1430			1456.3	45.373					
15	1655-1610					1608.69	37.014			ortho COC_6H_4OH or (NH_2)
	1650-1620	1633.76	38.176	1631.8	53.263			1633.76	58.803	amido acids NH
	1680-1650	"	"	"	"			"	"	Intramolecular H bonded acids of carboxylic acids
	1790-1720					1766.85	51.158			C=O stretching vibrations amide -1 band
	1825-1815					1822.79	52.44			C=Ostretching vibrations acyclic anhydrides
	1755-1745									C=O of COOH or COOOR
16	2450-2270	2399.53	67.405	2318.5	86.023	2353.23	79.69	2312.73	76.655	P-H stretching
	2310-2350	"	"	"	"	"	"	2359.02	74.609	Humic acids
	2700-2560	2451.61	67.42			2522.98	72.804			P-OH, OH stretching
	2600-2400	2470.9	67.43							free N-D
		2505.62	67.493							
		2578.91	67.486							
17	2880-2650	2617.49	67.313			2625.21	78.698	2831.31	78.878	CHO C-H stretching and defor. Vib., 2 bands may appear
	975-780	2854.74	59.028			2735.15	81.377			
18	2990-2980							2933.83	73.286	C-H stretching Cyclobutanes
	2925-2875	2931.9	53.575					2895.25	73.463	

Sr. no.	Range in cm-1	Kerala Black		Kerala Brown		Dwarka		Vadodara		Assignment
19	3040-3010					3016.77	71.619			$CR_1R_2=CHR_3$
	850-790									
20	3100-2600	2617.49	67.313							Amino acids containing an NH2 group
		2854.74	59.028							NH3+ stretching
		2931.9	53.575							NH3+ def. Amino-acid I band
	1665-1585	1633.76	38.176	1631.8	53.263	1608.69	37.014	1633.76	58.803	
	1550-1485			1506.46	47.908					NH3+ def. amino-acid II band
20	3550-3330	3417.98	20.767	3419.9	30.653	3419.9	45.245	3419.9	43.088	primary amines N-H stretching vib asymmetrical
	3400-3200	3394.83	20.609	3441.1	30.361	3429.55	45.161	3441.12	42.998	NH_2-(C)n-$CO_2^-M^+$
	3450-3250	3387.11	20.707							N-H stretching
	3390-3260									
	3500-3400									N-H stretching free bonded pyrroles aromatic heterocyclic
										5 membered ring
	3335-3030									NH_4^+
	3440-3420									N-H stretching secondary amides free NH
21	3670-3580	3620.51	23.24	3601.2	37.12			3618.58	47.201	free OH O-H stretching vib. alcohols
		3697.66	33.013	3622.4	31.027			3626.29	47.157	
				3697.7	46.815			3699.59	64.024	

Results

Carbon content

The **total carbon content** was associated with the main polysaccharide envelopes at 3300 and 1030 cm^{-1} , lignin like compounds 1513,1450,1371,1265, and 835 cm $^{-1}$ and aliphatic structures at 2920 and 2850 cm-1 (fats waxes and lipids) (Waiser T.H. 2007). Kerala Black was found to be very rich in aliphatic carbon compounds showing many peaks in the regioin--2000 to 3000cm^{-1}.

The absorption of soil at 1030cm-1 indicates that Carbon is in increasing content in the order Brown > Black >Dwarka>Vadodara. These results were in correlation with , C content obtained by chemical oxidation. (Reb ekka RE 2008, Urselmans TT 2006, Cheng-Wen Chang 2000, Sorensen LK 2005, Wetterlind J 2008).

Nitrogen content

Total nitrogen content was associated with 3550-3420, 1655-1610, 1720 cm^{-1} absorption bands (Artz RRE 2008, Siebielec G 2004). All four muds had a strong absorption band in N-H stretching frequencies i.e. RNH_2, R_2NH-----3400-3500 (two) (3417 cm-1) and 1031 cm-1 ,a C-N stretching frequency, indicating presence of aliphatic amines.

Minerals

Diagnostic peaks of kaolinite at 3700 and 467cm-1 were found in all soils except that in Dwarka, but there was strong absorption at 467cm-1 in Dwarka soil indicating partial presence of kaolinite. Due to overlapping of several bands in this region, may be 3700cm-1 peak must not have been detected in Dwarka. Mineral interference also manifests itself in the1030cm-1 band which is a characteristic of polysaccharide (Farmer 1974), which was strongly present in all the four soils.

Bacteria

Rinnan and Rinnan (2007) observed that the regression coefficients of the microbiological variables were rather similar to those of organic matter, concluding

that NIR (Near Infrared Spectroscopy) detected a combination of soil constituents containing organic functional groups, which are related to the studied microbiological variables like PLFA (Phospholipid fatty acid,)). Spectral ranges used for calibrations of soil organic carbon was 1732-1914, 2092-2630 cm-1 and for bacteria was 1195-1913,2092-2274,2452 -2632cm-1 (Zornoza R 2008). **Absence of absorption peaks in bacterial ranges and the organic carbon associated with it , indicated absence of bacteria which was confirmed by its microbiological testing described in section 5.9.**

Iron oxide

Iron oxide content of soils has been predicted from different spectral regions of the IR, based on characteristic absorption features at 550-650,750-950cm^{-1}(Ben-Dor and Banin 1995)and 1406 and 2449cm-1 (Ben –Dor 2006**). There was an increase in depth of absorption from 400 to 550cm-1 and in the broad feature at 900 cm-1 indicating that Vadodara soil was much richer in iron oxide content than other soils.** (Summers D 2009) .This was confirmed by colour and SEM-EDS data of the soil.

Carbonate

The carbonate absorption features at 1800 ,2350,and 2360cm-1 are used to predict calcite in soils (Ben-Dor and Banin, 1990). A clear band around 1450 cm-1 is observed, which is associated with the v3 carbonate bending and increases with total carbonate content. **Presence of these absorption bands was seen only in Dwarka soil and that prediction was confirmed by evolution of CO_2 gas on addition of concentrated H_2SO_4 and potassium dichromate for estimation of organic carbon described in section 5.4 of the text.** This was the reason why Total carbon(organic and inorganic) was observed highest in Dwarka by CHNS and SEM-EDS data.

Humic acid

Absorption of humic acids is found in 2310-2350 cm-1 and around 1700-2150 cm-1(Chang CW 2002, Cozzolino D 2003). All the soils were rich in humic acids but

looking at the intensities, Brown soil seemed to be the richest. Determining humic acid in our soils in soil: water extract, supported this data. (Section 5.8).

Spectral bands indicative of 'carboxylates' which include contributions from vibrations of aromatic and aliphatic carboxylates (R COO-), and /or aromatic C=C structures (1650-1600 and 1426 cm^{-1}) were also present in our soils and increase in absorption intensity of this band illustrated progressive free acid release with increasing humification .The transmission intensity at 1633.76cm-1 in Black was 38.176, in Brown was 53.263 and in Vadodara was 58.803 which indicated that Black and Brown had more humification than other soils and this data was confirmed by Humic acid content as measured in section 5.8 of the text.

Apart from the organic structures discussed above, all four soils also possessed chemical structures like pyridines, pyrroles, aldehydes, amides, phosphorus, silicates, bromo compounds and manganese. (Pyridines-779cm^{-1},pyrroles-3500-3400cm^{-1},aldehydes-2880-2650cm^{-1}, amides-1790-1720cm^{-1},phosphorus-2450-2270cm^{-1},silicates-1031cm^{-1}, bromo compounds-694,796, 1031cm^{-1},manganese-912cm^{-1}). Presence of chlorine , carbonates, phosphorus, silicates, and manganese was also confirmed by AAS , ICP-AES, and SEM-EDS data.

5.2 Chemical composition by SEM-EDS

Soil is made up of organic and inorganic matter having innumerable constituents but here we have concentrated only on elemental composition and organic carbon content. These parameters were determined by different methods.

Method

Determination of elements of soil was done by Scanning Electron Microscope (SEM) attached with LEO 435 VP SEM with Oxford ISIS 300-EDS (Energy Dispersive System)equipment , at Metallurgical Dept. ,Faculty of Technology &Engineering, M.S.University of Baroda, Vadodara. Soil samples were placed on carbon studs and put in the equipment for scanning.

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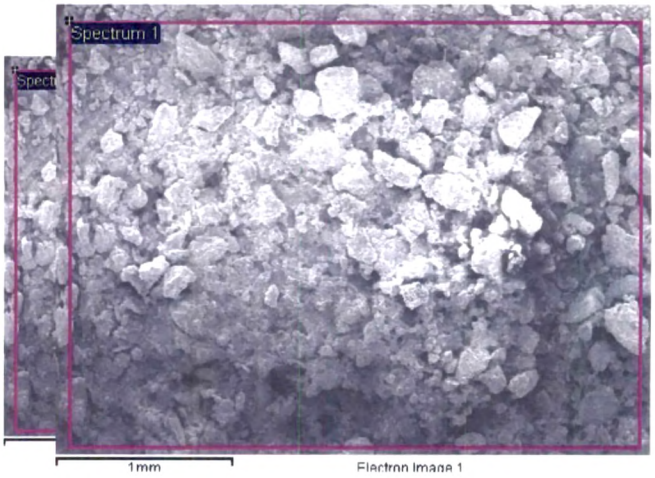
Spectrum processing :
No peaks omitted

Processing option : All elements analyzed (Normalised)
Number of iterations = 4

Standard :
C CaCO3 1-Jun-1999 12:00 AM
O SiO2 1-Jun-1999 12:00 AM
Na Albite 1-Jun-1999 12:00 AM
Mg MgO 1-Jun-1999 12:00 AM
Al Al2O3 1-Jun-1999 12:00 AM
Si SiO2 1-Jun-1999 12:00 AM
S FeS2 1-Jun-1999 12:00 AM
K MAD-10 Feldspar 1-Jun-1999 12:00 AM
Ca Wollastonite 1-Jun-1999 12:00 AM
Ti Ti 1-Jun-1999 12:00 AM
Fe Fe 1-Jun-1999 12:00 AM

Element	Weight%	Atomic%
C K	3.78	6.20
O K	55.31	68.15
Na K	0.51	0.44
Mg K	1.40	1.14
Al K	8.24	6.02
Si K	17.27	12.12
S K	3.28	2.01
K K	1.32	0.67
Ca K	0.61	0.30
Ti K	0.55	0.22
Fe K	7.72	2.72
Totals	100.00	

Comment: BLACK 1



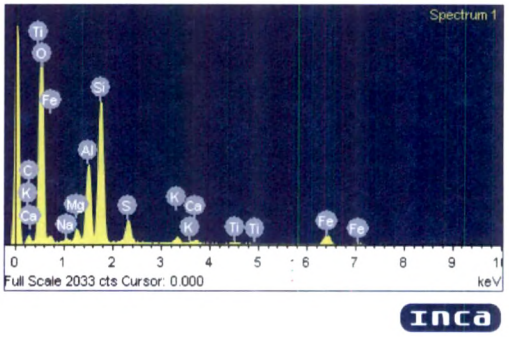


Fig.no.25 SEM-EDS graph of Kerala Black

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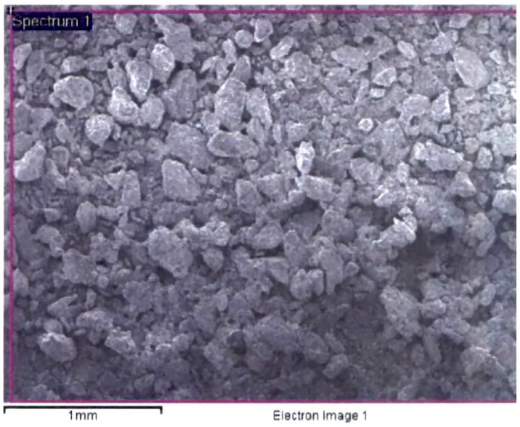
Spectrum processing
No peaks omitted

Processing option All elements analyzed (Normalised)
Number of iterations = 4

Standard
C CaCO3 1-Jun-1999 12 00 AM
O SiO2 1-Jun-1999 12 00 AM
Na Albite 1-Jun-1999 12 00 AM
Mg MgO 1-Jun-1999 12 00 AM
Al Al2O3 1-Jun-1999 12 00 AM
Si SiO2 1-Jun-1999 12 00 AM
Cl KCl 1-Jun-1999 12 00 AM
K MAD-10 Feldspar 1-Jun-1999 12 00 AM
Ti Ti 1-Jun-1999 12 00 AM
Fe Fe 1-Jun-1999 12 00 AM

Element	Weight%	Atomic%
C K	4 89	8 06
O K	53 16	65 77
Na K	0 75	0 65
Mg K	1 03	0 84
Al K	8 89	6 52
Si K	19 23	13 55
Cl K	0 47	0 26
K K	1 38	0 70
Ti K	0 70	0 29
Fe K	9 51	3 37
Totals	100 00	

Comment: BROWN 1



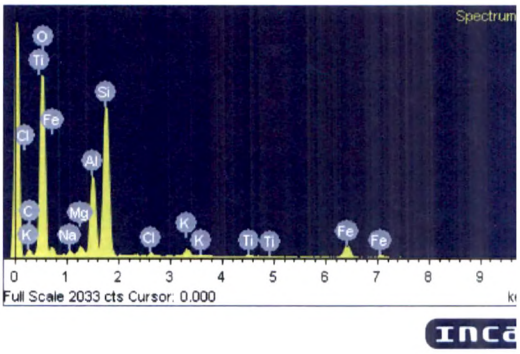


Fig.no.26 SEM-EDS graph of Kerala Brown.

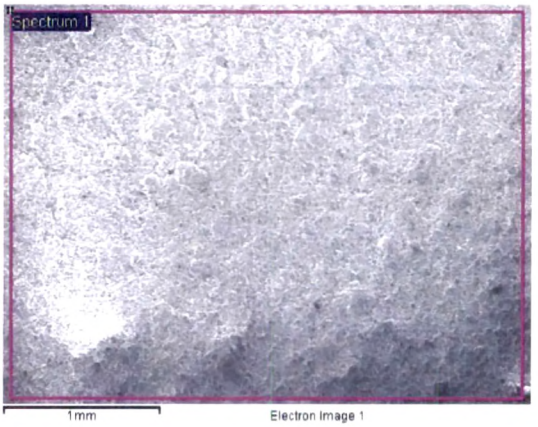
BEENA 12/18/2007 2:07:43 PM

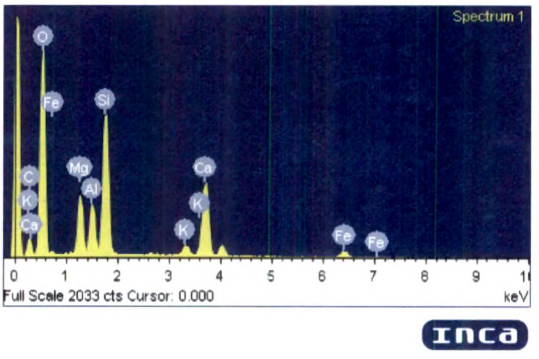
Spectrum processing :
Peak possibly omitted : 4.530 keV

Processing option : All elements analyzed (Normalised)
Number of iterations = 4

Standard :
C CaCO3 1-Jun-1999 12:00 AM
O SiO2 1-Jun-1999 12:00 AM
Mg MgO 1-Jun-1999 12:00 AM
Al Al2O3 1-Jun-1999 12:00 AM
Si SiO2 1-Jun-1999 12:00 AM
K MAD-10 Feldspar 1-Jun-1999 12:00 AM
Ca Wollastonite 1-Jun-1999 12:00 AM
Fe Fe 1-Jun-1999 12:00 AM

Element	Weight%	Atomic%
C K	5.66	9.13
O K	55.69	67.46
Mg K	5.29	4.22
Al K	4.08	2.93
Si K	12.43	8.58
K K	1.31	0.65
Ca K	12.05	5.83
Fe K	3.49	1.21
Totals	100.00	





Comment: DWARKA 1

Fig.no. 27 SEM-EDS graph of Dwarka

BEENA

12/18/2007 2:00:00 PM

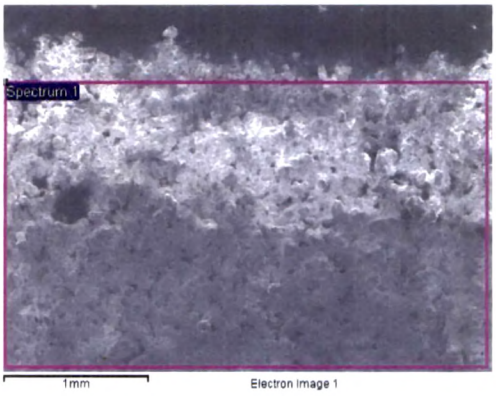
Spectrum processing
No peaks omitted

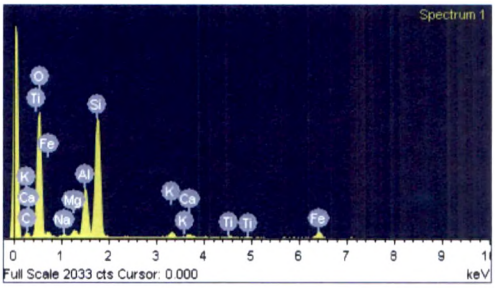
Processing option All elements analyzed (Normalised)
Number of iterations = 3

Standard
C CaCO3 1-Jun-1999 12 00 AM
O SiO2 1-Jun-1999 12 00 AM
Na Albite 1-Jun-1999 12 00 AM
Mg MgO 1-Jun-1999 12 00 AM
Al Al2O3 1-Jun-1999 12 00 AM
Si SiO2 1-Jun-1999 12 00 AM
K MAD-10 Feldspar 1-Jun-1999 12 00 AM
Ca Wollastonite 1-Jun-1999 12 00 AM
Ti Ti 1-Jun-1999 12 00 AM
Fe Fe 1-Jun-1999 12 00 AM

Element	Weight%	Atomic%
C K	2 17	3 61
O K	55 03	68 75
Na K	0 68	0 59
Mg K	1 02	0 84
Al K	7 92	5 87
Si K	22 72	16 17
K K	1 63	0 83
Ca K	0 93	0 46
Ti K	0 79	0 33
Fe K	7 12	2 55
Totals	100 00	

Comment VADODARA 1





inca

Fig.no.28 SEM-EDS graph of Vadodar

Result & discussion

The results are shown in fig. nos. 25, 26, 27, 28 and summarized in table. no. 20 and fig.no.29.

Table no.: 20 Composition of soil by SEM-EDS

Sr. No.	Elements	Black %	Brown %	Dwarka %	Vadodara %
01	C	3.78	4.89	5.66	2.17
02	O	55.31	53.16	55.69	55.03
03	Na	0.51	0.75	_	0.68
04	Mg	1.40	1.03	5.29	1.02
05	Al	8.24	8.89	4.08	5.87
06	Si	17.27	19.23	12.43	22.72
07	Cl	_	0.47	_	_
08	S	3.28	_	_	_
09	K	1.32	1.38	1.31	1.63
10	Ca	0.61	_	12.05	0.93
11	Ti	0.55	0.70	_	0.79
12	Fe	7.72	9.51	3.49	7.12

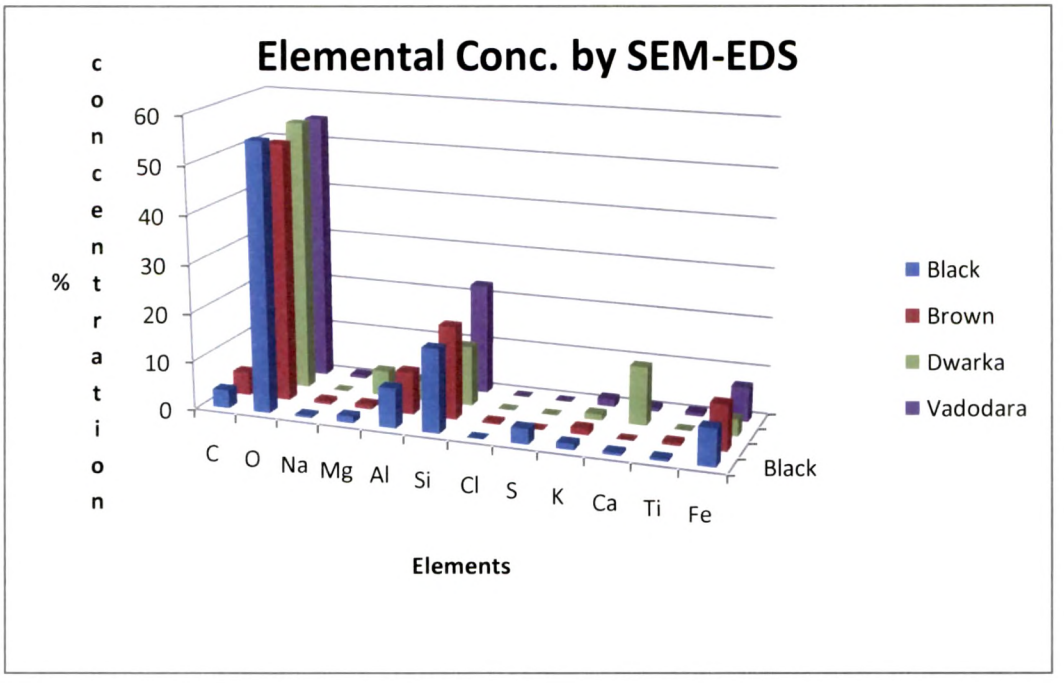


Fig.no:29 Concentration of elements as measured by SEM-EDS

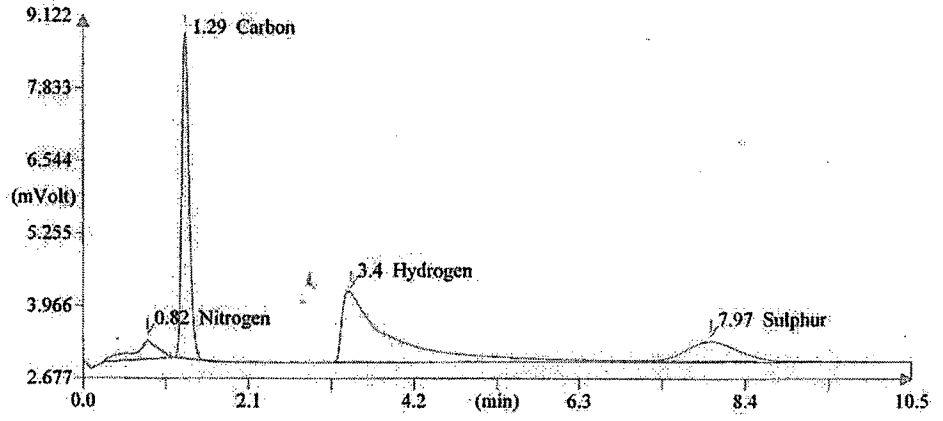
It could be observed from the table no. 20 that Dwarka had the highest amount of carbon (5.66%) and Vadodara had the least (2.17%). Carbon content of soil includes many compounds which are medicinally important , as already discussed in Introduction chapter. So carbon content of muds can be an important parameter for grading them with regards to their therapeutic activity. Moreover Magnesium and Calcium also play an important role in psoriasis, eczema and acne (section 6) and it was observed that Magnesium and calcium was highest in Dwarka as measured by this method. Presence of high concentration of Calcium and carbon in Dwarka confirmed presence of calcite which was also predicted from observing its colour. Moreover least concentration of iron in Dwarka (3.49%) was once again confirmed by its colour. Sulphur was detected only in Kerala Black and this was confirmed by its typical sulphurous smell.

5.3 CHNS analysis

CHNS analysis was conducted at SAIF (Sophisticated Analytical Instrument Facility) , IIT Bombay, on Thermo Finnigan CHNS analyser(Model EA 1112).The details of operating conditions and the graphs of the tests are given in fig. nos. 30(Black), 31 (Brown), 32 (Dwarka) and 33(Vadodara).

Title: SAIF-IIT, Powai, Mumbai

Operator ID:	KJD
Company name:	ThermoFinnigan
Method filename:	E:\PEN DRIVE DATA\Thermo _Finnigan\chn-2009\28-04-2009-CHNS.mth
Method name:	NCHS
Analysed:	04/28/2009 15:50
Printed:	05-11-2009 12:26
Elemental Analyser method:	
Sampler method:	
Sample ID:	28-04-2009-017-BLACK-STD-CHN-12 (# 17)
Analysis type:	UnkNown
Chromatogram filename:	28-04-2009-017-BLACK-STD-CHN-12.dat
Calibration method:	Least Squares to Linear fit
Sample weight:	2.608
Protein factor:	6.25



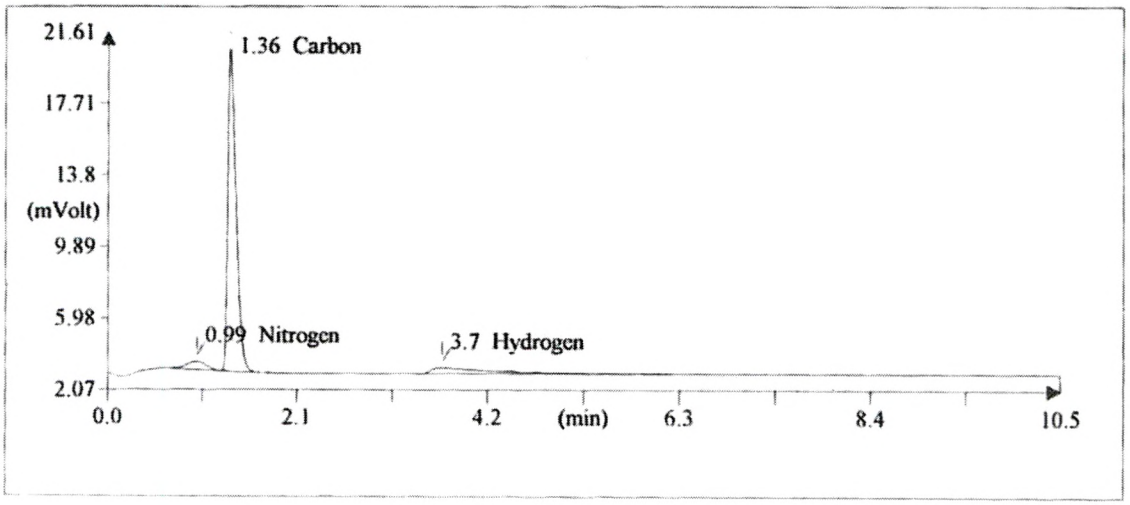
Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Component
1	0.817	72681	0.924	Nitrogen
2	1.292	374454	2.761	Carbon
3	3.400	560311	1.554	Hydrogen
4	7.967	159461	3.751	Sulphur
		1166907	8.989	

Fig.no.30 CHN analysis chart of Black soil

SMT. NANSA MEHTA LIBRARY

Title: SAIF-IIT, Powai, Mumbai

Operator ID:	KJD
Company name:	ThermoFinnigan
Method filename:	C:\Eager 300 for EA1112\data\Sys_data_example\chn-2009\10-06-2009-CHNS.mth
Method name:	NCHS
Analysed:	06/10/2009 15:08
Printed:	06-24-2009 12:11
Elemental Analyser method:	
Sampler method:	
Sample ID:	10-06-2009-007-DWARKA-STD-CHN-26- (# 7)
Analysis type:	UnkNown
Chromatogram filename:	10-06-2009-007-DWARKA-STD-CHN-26-.dat
Calibration method:	Least Squares to Linear fit
Sample weight:	2.671
Protein factor:	6.25



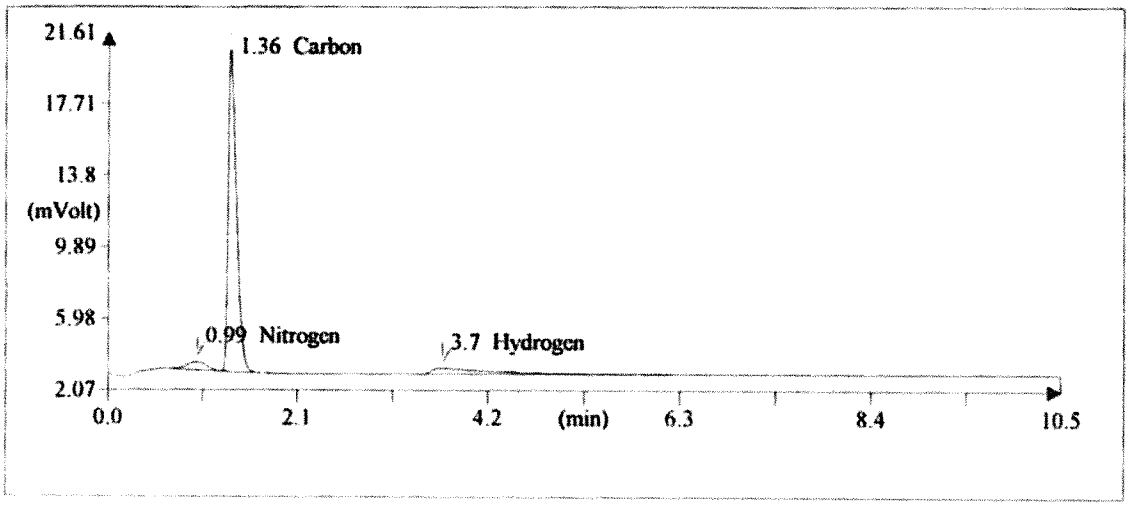
Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Comp
1	0.992	70375	0.366	Nitr
2	1.358	1096034	8.898	Carb
3	3.700	187256	0.571	Hydr
		1353665	9.836	

Fig.no.32 CHN analysis chart of Dwarka soil.

C.L. MANSA MEHTA LIBRARY

Title: SAIF-IIT, Powai, Mumbai

Operator ID:	KJD
Company name:	ThermoFinnigan
Method filename:	C:\Eager 300 for EA1112\data\Sys_data_example\chn-2009\10-06-2009-CHNS.mth
Method name:	NCHS
Analysed:	06/10/2009 15:08
Printed:	06-24-2009 12:11
Elemental Analyser method:	
Sampler method:	
Sample ID:	10-06-2009-007-DWARKA-STD-CHN-26- (# 7)
Analysis type:	UnkNown
Chromatogram filename:	10-06-2009-007-DWARKA-STD-CHN-26-.dat
Calibration method:	Least Squares to Linear fit
Sample weight:	2.671
Protein factor:	6.25

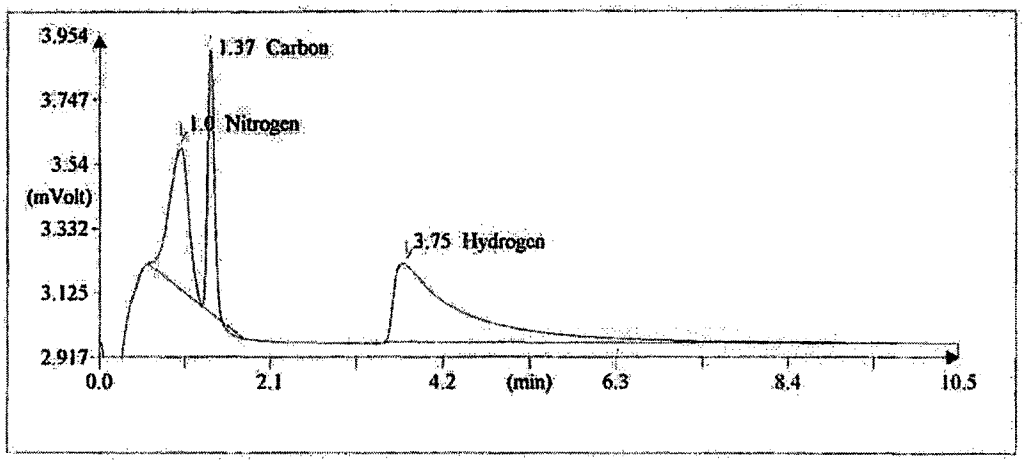


Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Comp
1	0.992	70375	0.366	Nitr
2	1.358	1096034	8.898	Carb
3	3.700	187256	0.571	Hydr
		1353665	9.836	

Fig.no.32 CHN analysis chart of Dwarka soil.

Title: SAIF-IIT, Powai, Mumbai

Operator ID:	KJD
Company name:	ThermoFinnigan
Method filename:	C:\Eager 300 for EA1112\data\Sys_data_example\chn-2009\10-06-2009-CHNS.mth
Method name:	NCHS
Analysed:	06/10/2009 16:47
Printed:	06-24-2009 12:15
Elemental Analyser method:	
Sampler method:	
Sample ID:	10-06-2009-016-VADODARA-STD-CHN-26- (# 16)
Analysis type:	UnkNown
Chromatogram filename:	10-06-2009-016-VADODARA-STD-CHN-26-.dat
Calibration method:	Least Squares to Linear fit
Sample weight:	2.12
Protein factor:	6.25



Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Compon
1	1.000	76028	0.585	Nitrog
2	1.367	45995	0.289	Carbon
3	3.750	145543	0.574	Hydrog
		267566	1.448	

Fig.no. 33 CHN analysis chart of Vadodara soil.

Result

Table no: 21 Carbon , Hydrogen , Nitrogen content of soils:

Sr.No.	Element %	Black	Brown	Dwarka	Vadodara
01	Carbon	2.761	0.717	8.898	0.289
02	Hydrogen	1.554	1.161	0.571	0.574
03	Nitrogen	0.924	0.466	0.366	0.585
04	Sulphur	3.751	-	-	-

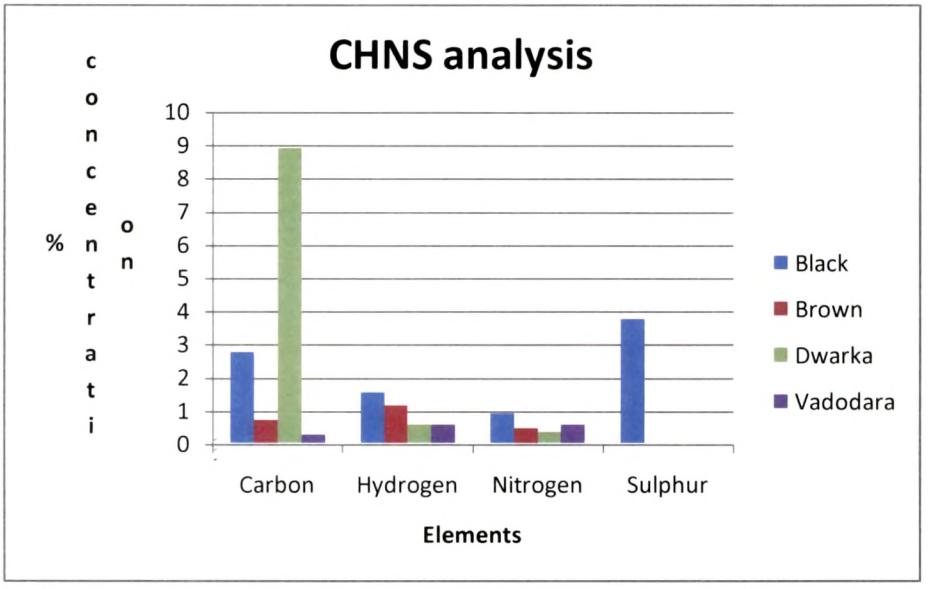


Fig.no.34 Composition of soil by CHNS analyzer

Data shown in table no. 21 and fig. no.34 indicated that Dwarka contained the highest amount of Carbon while Vadodara had the least. Hydrogen and Nitrogen content was highest in Black and Sulphur was present only in Black. Presence of Sulphur could be detected by its typical sulphurous smell (mild) coming out of Black soil.

5.4 Organic Carbon content (Chemical digestion method)

Various methods are available for the determination of organic carbon through dry combustion and wet digestion. The dry combustion method gives absolute values and useful for very accurate estimation of organic and total carbon. For routine work and easily oxidizable carbon determination, most widely acceptable methods are modified Walkley-Black method (Walkley-Black 1934) and colorimetric method (Datta NP 1962).

Determination of Organic Carbon (Datta et al 1962)

Principle

The oxidation of soil organic matter is carried out by dichromate –sulphuric acid mixture and the intensity of the green colour of the chromium sulphate formed is measured to give directly the amount of carbon oxidized.

Reagents

1. 1N (ARgrade) potassium dichromate (49.04 g/L)

2. Concentrated sulphuric acid (sp.gr. 1.84)

3. Sucrose (AR grade), anhydrous

Method

200 mg of soil (passed through sieve size 44#particles i.e. 300um) was accurately weighed and transferred to a 250 ml clean and dried Volumetric flask. 10 ml of 1N $K_2Cr_2O_7$ freshly prepared was added and shaken a little followed by 20 ml. of Concentrated sulphuric acid , shaken again and kept for 30 minutes on glass sheet. (so that laminated table top does not get damaged by the heat evolution).Then the contents were centrifuged to a clear state. The green chromium sulphate colour of the supernatant layer was read in the UV-1601 spectrophotometer Shimadzu at λ_{max} of 660nm adjusting the blank solution without soil. Note; On addition of conc.

Sulphuric acid to Dwarka sample, lots of effervescence was observed which indicated the presence of carbonates.

Reaction

$K_2Cr_2O_7 + 4\ H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3O$

C (organic carbon) $+ 2O \rightarrow CO_2$

Preparation of standard calibration curve

Accurately weighed samples (4, 8, 12, 16, 20mg) of anhydrous sucrose (AR) were transferred to clean and dry volumetric flasks.10 ml of 1N $K_2Cr_2O_7$ and 20 ml of concentrated $H_2SO_4$was added to each flasks and shaken and kept for 30 min. The clear solutions were examined under UV-1601 spectrophotometer (Shimadzu) at λ_{max} of 660nm using blank solution without sucrose. The absorbance is tabulated in table no.22.

Table no.22 Calibration of sucrose

Sr.no.	Conc. of sucrose in mg/30ml	Conc.sucrose ug/ml	Quantity of carbon (mg)	absorbance
01	0	0	0	0
02	4	0.133	1.68	0.09±0.002
03	6	0.200	2.52	0.131±0.008
04	8	0.266	3.36	0.18±0.001
05	10	0.333	4.2	0.23±0.003
06	12	0.400	5.04	0.28±0.004
07	14	0.466	5.88	0.33±0.005
08	16	0.533	6.72	0.37±0.002
09	20	0.666	8.4	0.47±0.002

N.B. here carbon content = sucrose x 0.42 because the carbon content of sucrose is 42%.

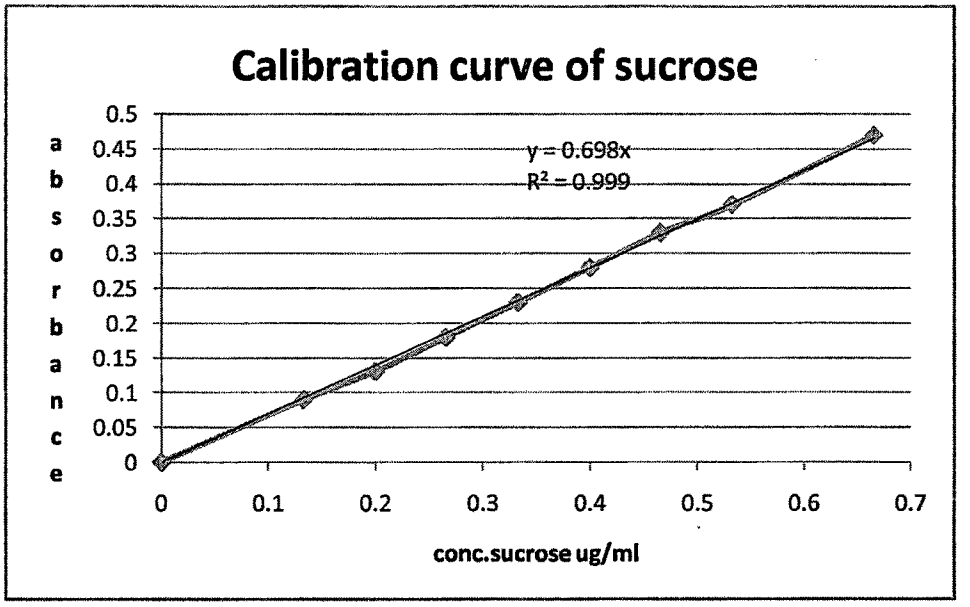


Fig.no:35 Calibration curve of sucrose

Organic carbon content of soils (Datta NP 1962)

Method

Accurately weighed 200mg of each soil sample(passed through #44 sieve) was taken in a 100ml stoppered volumetric flask and to it ,10ml of $1NK_2Cr_2O_7$ and 20 ml of conc. sulphuric acid was added. It was shaken slightly and kept for 30min. It was then centrifuged and the clear supernatant was examined under UV 1601 spectrophotometer (Shimadzu) at 660 nm.. Carbon thus measured was easily oxidisable organic carbon in soils and is tabulated in table no.23.

Table no.: 23 Organic Carbon content of soil: (passed through #44 sieve)

Soil	Kerala Black	Kerala Brown	Dwarka	Vadodara
% organic Carbon content	0.03±0.04	0.127±0.027	0.005±0.059	0.029±0.005

Carbon content in soil was measured by three methods: (1) SEM-EDS method (2)CHN analyzer {carbon combustion} (3) Organic carbon –chemical oxidation method.

Table no; 24 Comparison of Carbon content measured by 3 methods

Sr.No.	Soil	SEM-EDS % C	CHN analyzer % C	Chemical oxidation % C
01	Black	3.78	2.761	0.032±0.04
02	Brown	4.89	0.717	0.127±0.027
03	Dwarka	5.66	8.898	0.005 ±0.059
04	Vadodara	2.17	0.289	0.029±0.005

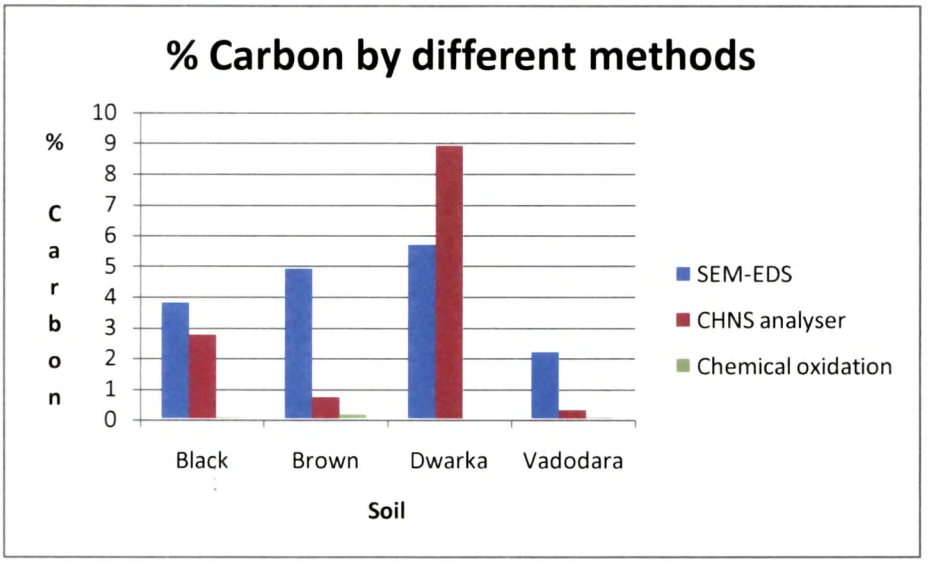


Fig. no. 36 % Carbon by different methods

Discussion:

CHN\S\O analyzer

The CHNS(O) Analyzer finds utility in determining the percentages of Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen of organic compounds, based on the

principle of "Dumas method" which involves the complete and instantaneous oxidation of the sample by "flash combustion". The combustion products are separated by a chromatographic column and detected by the thermal conductivity detector (T.C.D.), which gives an output signal proportional to the concentration of the individual components of the mixture. This method finds greatest utility in finding out percentages of C, H, N, S, (O) in organic compounds which are generally combustible at 1800° C. There are different techniques for the determination of CHN \ CHNS \O. It brings a new level of precision, accuracy, speed of analysis and ease of operation. The built in chromatographic column converts the compound and elutes it in the form of NO_2,CO_2, SO_2,H_2O which are then detected with the help of Thermal Conductivity Detector(www.iitb/SAIF.com).

Energy-Dispersive X-ray Spectroscopy (EDS)

When an element is bombarded with a particle beam, in this case, an electron beam, the specimen will release some of the absorbed energy as x-rays. Much of the time, the energy is the result of changes in the speed of an electron, which is random; however, when this interaction removes an electron from a specimen's atom, frequently an electron from an outer shell (or orbital) occupies the vacancy. When an outer electron occupies a vacancy, it must lose a specific amount of energy to occupy the closer shell. This amount is readily predicted by the Laws of Quantum Mechanics and usually much of the energy is emitted in the form of X-rays. Two methods are used to determine the x-rays that are produced: (1) energy-dispersive analysis separates and detects x-rays of specific *energy* and displays them as histograms, whereas (2) wavelength-dispersive analysis uses the reflection of x-rays off of a crystal at a characteristic angle to detect x-rays of specific *wavelength*. (www.charfac.umn.edu/instruments/eds_sem_primer.pdf)

Chemical oxidation:

The oxidation of soil organic matter is carried out by dichromate –sulphuric acid mixture and the intensity of the green colour of the chromium sulphate formed is measured to give directly the amount of carbon oxidized.

CHN analyser can measure total amount of dry combustible elements (in form of CO_2, NO_2, H2O SO_2) and SEM-EDS measures excitation energy while colorimetric method measures easily oxidisable organic carbon.

Due to variation in the principles of measuring carbon content by different equipments and methods, we got different quantities of carbon but the pattern remained the same i.e.highest was in Dwarka and least in Vadodara. Since the chemical method determines only the **easily oxidisable organic carbon** it was observed that Black had the highest **organic carbon** while Vadodara had the least. This was also confirmed by other physical observations seen in 5.1 and 4.1 section of this text (IR data , and Colour of soil). Since Dwarka was rich in carbonates which is inorganic, and easily combustible (ref: IR data, colour), CHN analyzer and SEM-EDS method showed highest amount while chemical digestion method measured chromium sulphate produced as a result of oxidation of **organic carbon**, the carbonate moeity evolved as effervescence and could not be detected by UV hence, there was vast difference of carbon content measurement in Dwarka as measured by colorimetric method and CHN analyser and SEM-EDS method.

5.5 Organic carbon content of soil particulate fractions

Powdered soil which was passed through 40# size sieve was further classified into other fractions by sieve shaker (Jayant Brand Rotap Sieve Shaker), using sieves nos. 44, 60, 85, 120, 150, 170, 200, 500.(ASTM). Time period of shaking was 20 min. Carbon content of these fractions was determined by chemical digestion method mentioned in section 5.4 of the text and results are shown in table no. 25 and fig.no.37.

Table No:25 Carbon content of soil according to particle size

Avg. particle size in um	Black	Brown	Dwarka	Vadodara
	%C	%C	%C	% C
>302.5	0.0275±0.02	0.108±0.04	0.0017±0.05	0.029±0.01
215	0.029±0.06	0.112±0.05	0.0019±0.03	0.031±0.009
150	0.030±0.1	0.115±0.03	0.0021±0.003	0.032±0.01
112.5	0.030±0.08	0.126±0.04	0.0026±0.008	0.033±0.02
97.5	0.035±0.04	0.132±0.03	0.0033±0.02	0.034±0.005
82.5	0.038±0.03	0.136±0.01	0.0039±0.01	0.0339±0.04
50	0.039±0.04	0.138±0.04	0.0041±0.01	0.0343±0.03
<25	0.0404±0.08	0.140±0.06	0.0052±0.01	0.0355±0.006
Passed through 44# sieve i.e unclassified whole powder	0.032±0.04	0.127±0.027	0.005±0.059	0.029±0.005
% rise in C in <25um than C in 44# passed whole powder	26.52	10.55	5.11	22.5

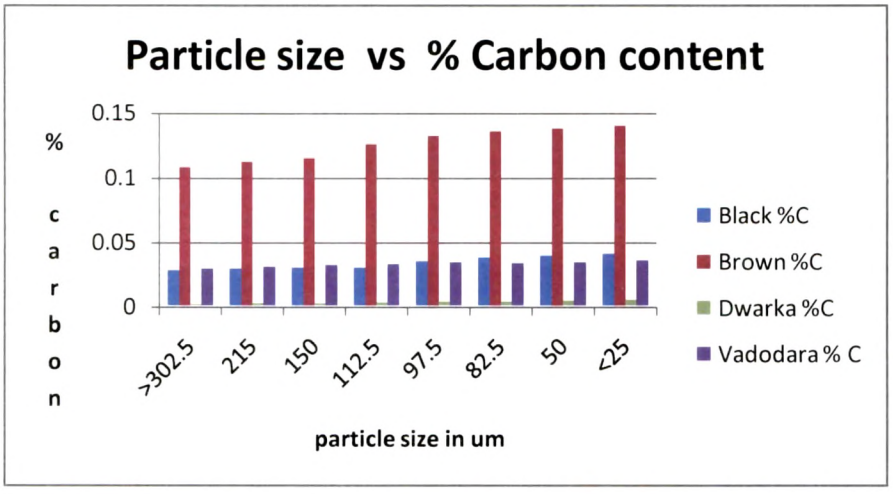


Fig. No.37 Average particle size vs % carbon content.

Results

It could be observed that as particle size decreased, there was slight increase in %organic carbon content of the soil fractions but the last fraction i.e. <25 um showed approximately 5% increase in carbon content in Dwarka ,22% in Vadodara, 10% in Brown and 26% rise in Black, than carbon content in unclassified powder.

Discussion

A study was conducted to measure carbon content of different particle sizes of soils by IR methods and they also observed that carbon and nitrogen content increased as particle size decreased (Cozzolino D 2006). In a study by Zimmermann M et al(2007) on different soils for organic carbon content in various particle size fractions, they repeated that about 29% of organic carbon in bulk soil was contained in the sand + aggregate fraction, and 51% in the silt +clay fraction. Thus results of our study also showed that organic carbon content of the soils varied with their particle size fraction.

5.6 Chemical Composition by AAS (Atomic Absorption Spectrometry)

Method

5gms of soil sample was weighed, leached in distilled water, filtered through ashless pulp, made upto 100ml and analysed on AAS(Atomic absorption spectrometer model Solaar S2). This test was conducted at Jewel Metallochem Lab. Pvt. Ltd., Mumbai(www.jewelmetallochem.com) and the results are tabulated in table no.26.

Results

Table No 26 Chemical composition of soils by AAS

Sr. no.	Elements	Kerala Black	Kerala Brown	Dwarka	Vadodara
01	Sulphur	0.008%	< 2 ppm	< 2 ppm	< 2 ppm
02	Chloride	0.020%	0.50%	0.030%	0.01%
03	Chromium	< 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm
04	Potassium	0.023%	0.009%	0.011%	0.009%
05	Sodium	0.008%	0.006%	0.023%	0.011%
06	Aluminium	0.011%	0.004%	0.001%	0.10%
07	Iron	0.18%	<0.40 ppm	< 0.4 ppm	0.026%
08	Calcium	0.035%	0.004%	0.016%	<0.50
09	Magnesium	0.22%	0.004%	0.007%	<0.40 ppm
10	Manganese	0.009%	0.001%	<0.7 ppm	<0.70 ppm

11	Phosphorus	0.022%	0.007%	0.004%	0.007%
12	Cadmium	< 0.50 ppm	<0.5 ppm	< 0.5 ppm	<0.5 ppm
13	Cobalt	< 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm
14	Copper	< 0.50 ppm	<0.5 ppm	< 0.5 ppm	< 0.5 ppm
15	Lead	< 0.5 ppm	<0.50 ppm	< 0.5 ppm	< 0.5 ppm
16	Zinc	< 0.5 ppm	< 0.5 ppm	< 0.5 ppm	< 0.5 ppm
17	Nickel	0.001%	0.001%	< 4 ppm	0.002%

Result & Discussion

Presence of trace elements like Cobalt, Chromium, Cadmium, Nickel, Copper, Lead, Zinc were very low (<5 ppm) indicating that possibility of toxicity due to them was negligible. (US FDA 2005, IS 6608) .

Compared to other three soils Kerala Black was rich in water soluble calcium, magnesium, and potassium while Dwarka was rich in water soluble sodium. As water soluble Chloride, Manganese, Iron, Aluminium have little influence on psoriasis, eczema and acne they were not monitored for further studies like invitro diffusion and clinical . In analogous to reports of SEM-EDS, CHNS analyses, Black showed concentration of sulphur as 0.008%, while the other soils showed concentration less than 2 ppm.

Low acidity of Black soil may be attributed to high concentration of calcium compared to other soils because sodium ions are held less strongly by the micelle calcium ions and are therefore more easily hydrolysed than calcium ions and hence the pH of sodium saturated soils (Dwarka and Vadodara) was higher than that of calcium saturated soils.

The data shown by SEM-EDS method showed the **total** concentration of elements while AAS showed concentration of elements **soluble in water.** Hence the concentration of elements as measured by AAS were comparatively less than that by SEM-EDS method. Nevertheless, both of these data were important because former was of interest to study adsorption, while the latter to study absorption of constituents into the skin.

5.7 Chemical Composition by Inductive coupling method: (ICP-AES) (Inductively Coupled Argon Plasma Atomic Emission spectrometry)

Method

200 mg of soil was accurately weighed, 20 ml of distilled water was added to it and intermittently shaken and kept aside for 2 hrs. It was then centrifuged at 800 rpm for 10 min. and the supernatant was taken for analysis by ICP-AES. This test was conducted at Metallurgical Services , Mumbai (www.metallurgicalservices.co.in).

Instrument used: iCAP 6300 model of Thermo Electron Corporation, U.S.A. with Radial plasma. High energy echelle cross-dispersion Optical system with focal length 383mm and spectral band pass of 7pm at 200nm. The Optical wavelength range covered is 167-818nm.

Detector: RACID86 charge Injection Devices .High performances solid state CID camera systems with 291,600 individually addressable detector elements in a 540x540 array.

Sample introduction; through a three-channel peristaltic pump with adjustable speed. Demountable plasma Torch with 1.5mm center tube and glass cyclonic type spray chamber and concentric glass nebulizer.

The samples were analysed for five , dermatologically important elements (P, Mg, Na, K, Ca) only and the results are shown in table no.27. and fig no. 38.

Table No: 27 Composition of soil elements as measured by ICP-AES

Sr.No.	Elements	Kerala Black ug/ml	Kerala Brown ug/ml	Dwarka ug/ml	Vadodara ug/ml
01	Phosphorus	0.26	0.163	0.332	0.501
02	Magnesium	45.61	10.37	5.94	2.79
03	Sodium	38.64	93.5	36.73	33.92
04	Potassium	3.16	10.5	5.71	2.16
05	Calcium	27.88	4.81	10.59	6.94

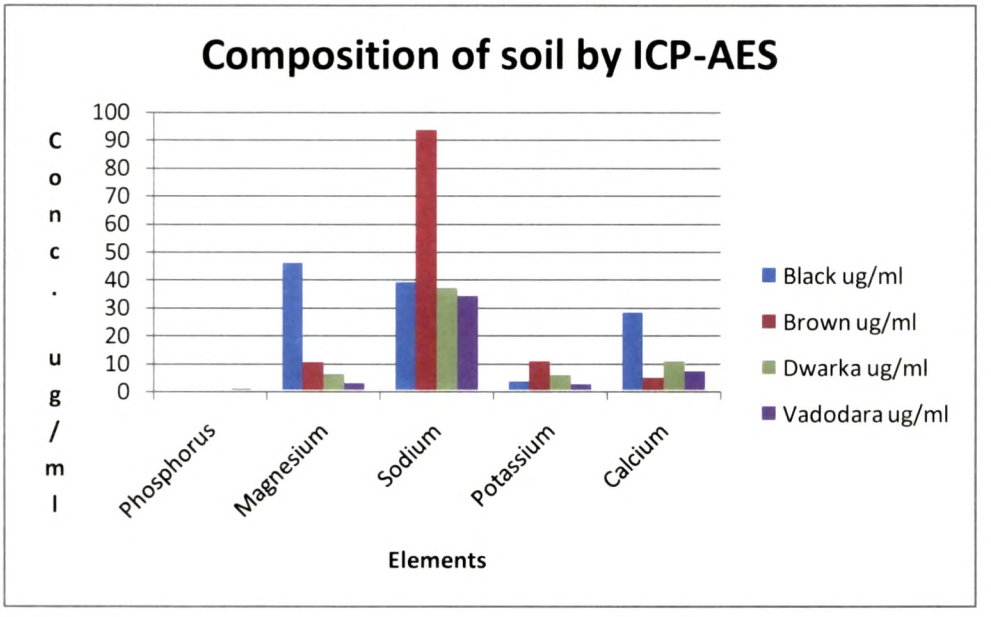


Fig. no: 38 Composition of soil by ICP-AES

Discussion

Kerala Black soil was found very rich in water soluble magnesium and calcium while Brown was rich in sodium and Vadodara in phosphorus. Calcium and Magnesium as determined by AAS also showed the same pattern i.e. highest in Kerala Black. Just as stated earlier, these elements play important role in skin pathology in disease like psoriasis and eczema. This data is in accordance with the electrical conductivity of soil which is a measure of dissolved salts.

5.8 Humic acid content of soil

Substances including fresh and incompletely decomposed organic residues from plants and animals make up "soil organic matter" and Humus. Humus consists of substances like humic acids , fulvic acids, humins, hymatomelanic acid and products of advanced decomposition of organic residues and products resynthesized by micro-organisms (protein like substances, carbohydrates, waxes, fats, tannins, lignins etc.) (Kononova MM 1966).

Soil humus refers to the amorphous organic materials remaining in soils after extraction of the water soluble fraction and exclusion of particulate organic materials. Humus consists of a mixture of humic substances and nonhumic biomolecules (Sumner ME 2000). Their chemical composition is complex and is still under investigation, although resorcinol, vanillic acid, ferullic acid and benzoic acid represent recurrent components (Sato.T 1987).

Humification of organic residues entering the soil depends upon their chemical composition and upon conditions in the soil influencing the activity of the micro-organisms.

On reaching the soil, organic residues of plant and animal origin undergo diverse changes brought about by various factors. Some of these changes can undoubtedly take place without the participation of micro-organims and animals: they can be classed as follows:

1.Destructive changes due to the physical action of natural factors (the effect of atmospheric precipitation, wind) and to the action of man (soil Cultivation).

2. Changes in the chemical nature of organic residues under the direct action of water, light, air and reaction of the medium, e.g.the oxidation of fats and resins in light and oxidation of aromatic compounds at alkaline pH.

It cannot be assumed that, because these (organic) compounds occur in small amounts in the soil, they are of lesser interest, as many of their functions (action as stimulants and inhibitors of plant growth, as antibiotics and as vitamins) are manifested only when they are applied in small amounts.(Kononova MM 1966).

Only a small part of the humus substances in the soil are in a free state: a larger part occurs with various forms of linkage to the mineral part of the soil. This linkage must be destroyed if humus substances are to be converted into a soluble state. The character of the link between humus substances and clay minerals remains obscure. Authors like Khan and Gapon (Khan DV 1946, Khan DV 1950, Khan DV 1959, Gapon 1937) consider that the complexes between humus substances and clays are probably formed by bridging through the exchangeable cations Ca, Mg and Al.

Humic acids are usually regarded as polymers of aromatic compounds, and there is little doubt that aromatic structures are incorporated in the complex. They have been demonstrated by spectroscopy (Wagner GH 1965) by the detection of phenolic groups in functional-group analyses, by the release of simple aromatic compounds in various degradation procedures, and by the fact that artificial humic acids with many of the properties of natural humic acids can be prepared in the oxidative polymerization of simple phenols (Flaig W 1960).

Absorption in the UV region Is attributable to the presence of multiple bonds and to unshared electron pairs in organic molecules. Light absorption of humic compounds appears to increase with increases in (1) the degree of condensation of the aromatic rings that they contain (Kononova MM 1966), (2) the ratio of carbon in aromatic nuclei to carbon in aliphatic or alicyclic side chains (Kasatochkin VI 1964. (3) total carbon content (Kleist H 1966) and (4) molecular weight (Kleist H 1966). These

linkages or groups confer color on organic substances and are called chromophores. Groups which by themselves do not confer color but which increase the color of chromophores are referred to as auxochromes. Typical chromohores known to occur in humic compounds are C=C and C=O: auxochromes that are likely to be present are C-OH, C-NH_2 and others.

UV spectra of most humic compounds are featureless, with the optical density decreasing as the wavelength increases. Occasionally an indication of a maximum can be discerned in the 210-260-um region (Sato O 1967, Schnitzer M 1968). It is noteworthy that UV spectra of humic compounds of diverse origins are very similar in spite of differences in elementary composition, sedimentation characteristics, and other properties (Ziechmann W. 1964)..

The optical properties and, in particular, the optical density of humus substances depend on their chemical structure. It has been suggested that the optical density of organic substances is directly proportional to their conjugated double bond content (Cherkesov AI 1957). Correspondingly, the optical density of alkaline solutions of humic acids with equal carbon content characterizes the ratio of the carbon in the aromatic net to the carbon in the side radicals. These properties were first determined, during the study of humic acids isolated from peat, coal and various soils, by Oden S (1919) and later by Aleshin SN (1950) .

At the present time sufficient data are available to give evidence of differences in the nature of the humic acids of different soils. Differences in the nature of the humic acids were also clearly revealed by the determination of optical properties: this method was first used by Oden S. (1919) and subsequently by Fromel W (1937) .

For humic acids of mineral coal (Kukharenko TA 1953) and for soil humic acids(Kononova MM 1950) a direct relationship between the light absorption of solutions of humates and the degree of condensation of the aromatic ring was reported.

Many scientists have worked hard to characterize humic acid. They have found that humic acid is a high molecular weight compound having mol .wt. of about 1350 (Oden S 1919). It has now been established that humic acids have a complex

structure: at least two main components-compounds of phenolic or quinoid nature and nitrogen containing compounds (amino-acids, peptides) participate in the formation of their molecule (Kononova MM 1966).

Preparation of calibration curve of Humic acid

Method

The composition of humic acid slightly varies from soil to soil based on its demographic origin and so to find out humic acid content of each soil, we prepared calibration curve of marketed humic acid as standard.

Pure Humic acid (90%) was supplied by HiMedia Laboratories Pvt Ltd., Mumbai. Solutions of 5, 10, 20, 30, 40, 50 ug/ml were prepared in distilled water. Maximum absorption wavelength was observed to be 224nm. This coincided with the λ_{max} of humic acid isolated from our soil. The absorbance data of which is given in table no.28 and fig.no. 39.

Table no: 28 Calibration data of Pure humic acid.

Sr.no.	Concentration ug/ml	Absorbance at λ 224nm
1	0	0
2	5	0.1442 ±0.034
3	10	0.249 ±0.012
4	20	0.4718±0.017
5	30	0.6893±0.015
6	40	0.9229±0.019
	50	1.1351±0.019

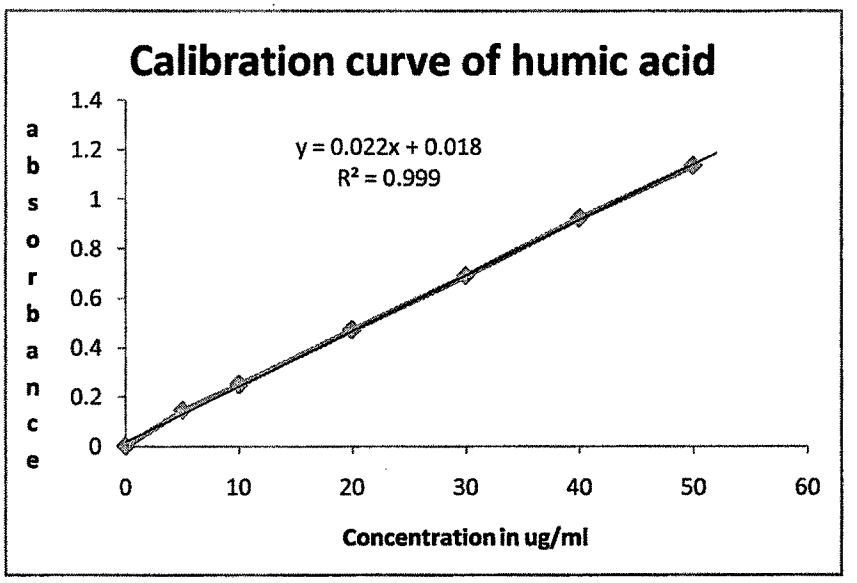


Fig.no. 39 Calibration curve of humic acid

Isolation of Humic acid from soil samples:

200 mg of each soil 120# passed sieve was taken in a 100 ml volumetric flask and to it 20ml of 0.5 M NaOH was added, shaken on a mechanical shaker for 1 hr. and kept overnight. Next day it was centrifuged and the liquid decanted into another beaker. To this, conc. HCl was added to achieve pH of 1-1.5 . Humic acid was precipitated at the bottom of the beaker. The upper liquid was decanted and the precipitate (humic acid) was air dried and weighed. It was then dissolved in 20 ml of distilled water and the samples observed under UV1601 spectrophotometer (Shimadzu make). The maximum absorption wavelength found was 224nm.

Table No: 29 Concentration of humic acid in soil

Sr.No.	Soil	% conc. w/w of soil
01	Kerala Black	0.182±0.014
02	Kerala Brown	0.122±0.022
03	Dwarka	0.086±0.0006
04	Vadodara	0.076±0.008

Result & Discussion

It was observed that Kerala Black contained highest amount of humic acid i.e. 0.182% (w/w of soil) as given in table no. 29. This was also confirmed by its black colour which indicates richness of humus and least bulk density .

Confirmation of presence of humic acid by soil's FTIR spectra

Results from infrared spectroscopy have shown the aromatic nature of soil and peat humic acids (Kumada K 1958 , Goulden JDS 1959).

Major carbon content of the soil is from humic acid. Humic acid contains 56% of C and 35% oxygen and about 4% of Nitrogen (McLaren DA 1971) which is exhibited in COO- ,C=O,OH groups. **Presence of these functional groups were found in soil IR spectra indicating presesnce of humic acid in the soil samples.**

Presence of 1633cm-1 band indicated absorption of intermolecular H bonded acids of carboxylic acid , 2310-2350 cm-1 band indicated humic acid. 3417 cm-1 of N-H stretching, 2880-2650 cm-1 band of CHO-CH stretching , 1520-1430 cm-1 aromatic C=C stretching, 1470-1435cm-1 band of aliphatic stretching C-CH3 -CH. , 3670-3580 cm-1 stretching vibrations of O-H alcohols, are all indications of humic acid.

5.9 Microbial Examination

Method (Zeev Maor 2006)

5 gms of each soil was taken in a sterile container and shaken with 10 ml of Sterile distilled water on a test tube shaker(vortex shaker) for 5 minutes. This suspension was kept aside for 120 mins .for contact time. One loopful of the supernatant was inoculated on Blood Agar, MacConkey's Agar and Nutrient Agar plates in triplicate . The plates were incubated at 30° C for 7 days and observed at 48hrs and 7 days.

Table No: 30 Microbial growth in soils **after 48 hrs** of incubation at 30° C

Sr. No.	Mud	Observation Nutrient agar	MacConkey'Agar	Blood Agar
01	Kerala Black	No growth	No growth	No growth
02	Kerala Brown	No growth	No growth	No growth
03	Dwarka	No growth	No growth	No growth
04	Vadodara	No growth	No growth	No growth

Table No: 31 Microbial growth after 7 days of incubation at 30° C

Sr. No.	Mud	Observation Nutrient agar	MacConkey'Agar	Sheep Blood Agar
01	Kerala Black	+	+	++
02	Kerala Brown	+	+	++
03	Dwarka	+	+	++
04	Vadodara	+	+	++

+ means little growth of colony forming units (CFUs)

++ means more growth of CFUs

Results

No growth of any colony was observed in any of the plates incubated for 48 hrs. as stated in table no.30. But at the end of 7 days of incubation, plates of nutrient agar and MacConkey's agar showed presence of few hundred colony forming units but sheep blood agar showed rich growth of colonies as indicated in table no.31. Endospore forming bacteria were most commonly encountered, Bacillus type morphologies were found.

Discussion

Discrepancy between the large colony counts obtained on sheep blood agar(SBA) and low counts on nutrient agar and MacConkey 's agar may be attributed to the presence of a dormant microbial population that does not form colonies even on rich, nonselective media, but can be activated by sheep blood. As most of the colonies that developed on SBA were endospore forming bacilli, the dormancy could be related to a low efficiency of germination of bacterial endospores on the other media, rather than to the impaired multiplication capacity of vegetative forms (Henis Y 1987). This indicated that, preservative would be necessary to be added, if the formulations prepared from mud included water.

5.10 Antimicrobial Activity

Method (Zeev Maor 2006)

Antimicrobial activity of mud was tested on Muller Hinton agar(supplied by Hi-Media Chemicals) plates on which suspensions of test organisms (0.1ml of *E.Coli. S.aureus, C.albicans* in Sterile distilled water at OD_{550} of 0.01) were evenly spread. Discs of 0.1ml of mud were prepared by filling sterile plastic 1ml syringe with mud

suspension (1:2 soil :water ,contact time 2hrs) and raising the piston to press out 0.1ml portion on the precut sterile disc. These discs were placed in sterile area. Three hours after inoculation with the test organism up to 4 discs were placed on the surface of the M.H Agar plates and incubated for 36 hrs at 37^0C and *C.albicans* at 28^0C. The appearance of inhibition zones around the discs was recorded. The tests were performed in triplicate. The strains of test organism used were as follows:

Escherichia Coli : ATCC (American Type Culture Collection) 8739 on MHA (Muller Hinton agar)

Staphylococcus aureus: ATCC 6538 on MHA (Muller Hinton agar)

Candida albicans: :ATCC 10231 on SDA(Sabourd Dextrose agar)

(Test organisms were gifted by Food and Drug Laboratory ,Govt.of India .Vadodara)

Table No: 32 Zones of inhibitions of different muds in mm

Sr.No.	Mud	E.Coli	S.aureus	C.albicans
01	Kerala Black	13.0±0.2	12.0±0.5	6.5±0.3
02	Kerala Brown	13.5±0.3	12.5±0.4	7.0±0.2
04	Dwarka	11.5±0.4	13.0±0.1	10.0±0.4
03	Vadodara	14.0±0.1	15.0±0.2	9.5±0.3

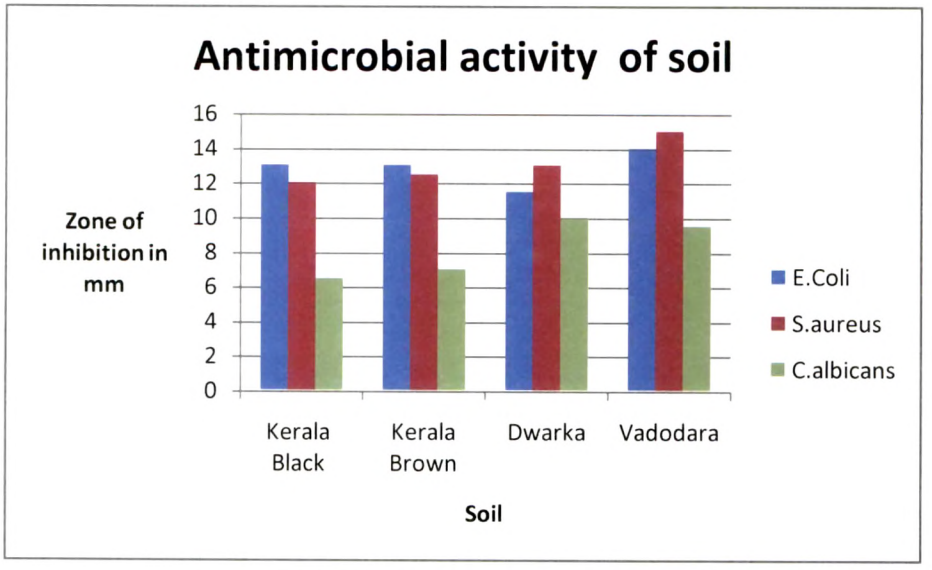
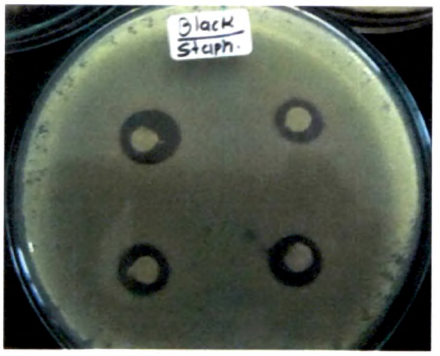
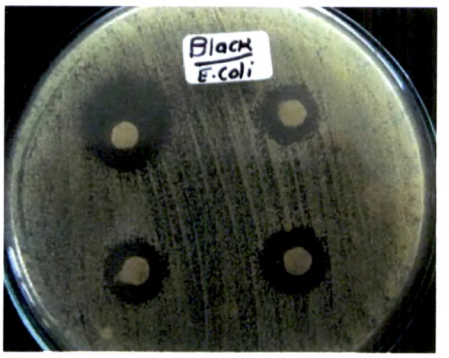


Fig.no. 40 Antimicrobial activity of soil

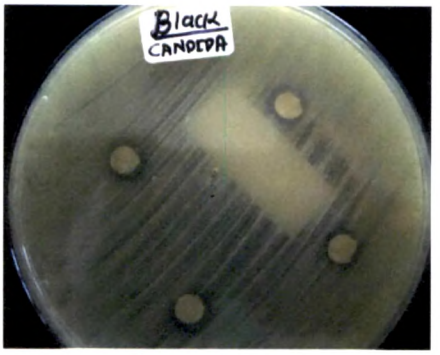
Fig . No: 41 **Zone of inhibition (ZOI)**



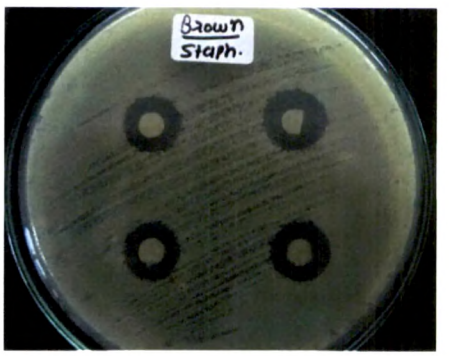
(a) ZOI of Kerala Black on *S.aureus*



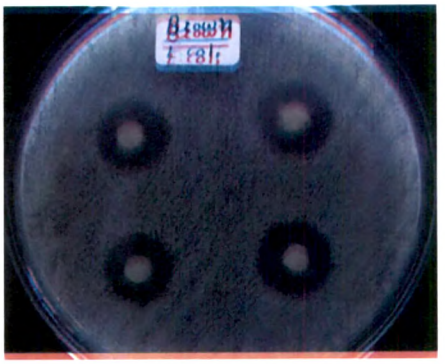
(b) ZOI of Kerala Black on *E.Coli*



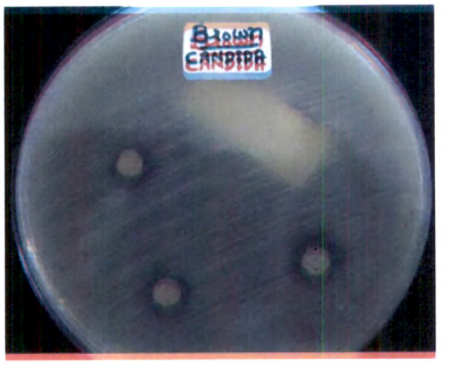
(c) ZOI of Kerala Black on *C.albicans*



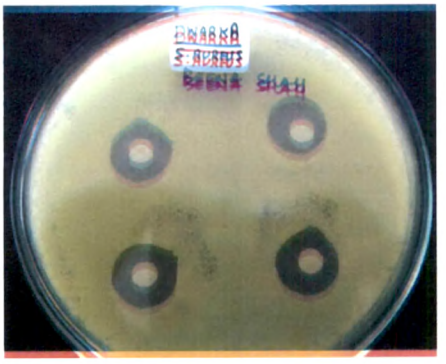
(d) ZOI of Kerala Brown on *S.Aureus*



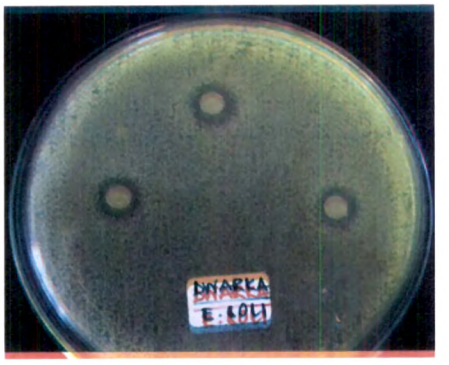
(e) ZOI of Kerala Brown on *E.coli*



(f) ZOI of Kerala Brown on *C.albicans*

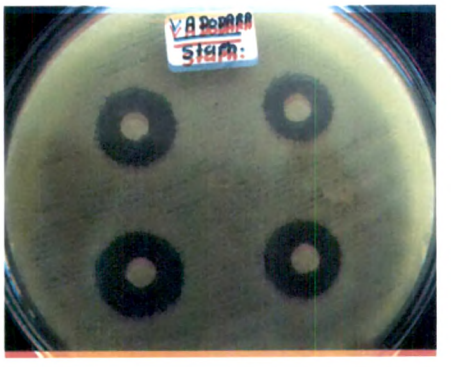


(g) ZOI of Dwarka on *S.aureus*



(h) ZOI of Dwarka on *E.Coli*

(i) ZOI of Dwarka on *C.albicans*



(j) ZOI of Vadodara on *S.aureus*

(k) ZOI of Vadodara on *E.coli*

(l) ZOI of Vadodara on *C.albicans*

Results

Zones of inhibition of all four muds for E.coli and *S.aureus* were in the range of 12mm to 15mm while on Candida albicans it was in the range of 6mm to 10mm as shown in table no.32, fig.no.32, 40 and 41. It was observed that Vadodara showed best anti microbial activity against *E.coli* and *S.aureus* while Dwarka showed good activity against fungi *Candida albicans*.

Discussion

The appearance of clear inhibition Zones around discs of mud placed on a lawn of different test organisms indicated its high bactericidal activity against gram-negative *E.Coli* and gram positive *S. aureus* while it was a little bit less effective against fungi *Candida albicans.* Many studies have been conducted to elucidate the antimicrobial activity of mud (Zeev Maor 2006, Ren A 1985, Sass E 1977). Antimicrobiocidal activity of muds may be attributed to the high salt concentration and in particular presence of sulphur in Kerala Black mud .

The zones of inhibitions obtained by antimicrobial activity of erythromycin, ampicillin and flluconazole on *S.aureus, E.coli,* and *candida albicans* respectively were 30mm, 15mm, and 12mm respectively as studied by Vineeta Singh et al (2009). From the reports of this study we can conclude that our soil samples' antimicrobial activity was comparable to that of ampicillin and fluconazole.

Together with vitamins and growth promoting substances, antibiotics are produced in the soil by micro-organisms. This group includes streptomycin , globisporin, penicillin, aureomycin, terrramycin and others: the observed activity of these substances depends on the soil characteristics (Krasil'Nikov NA 1958), for instance the nature of humus (Bekker EE 1959). Antibiotics can be taken up by plants, as has been demonstrated by Krasil'nikov (1958) and Winter and Willecke(1951) .The substantial size of antibiotic molecules (mol.wt. 300-500 or higher) does not prevent their penetration into the plant in an unchanged form(Krasil,nikov 1958, Scheffer F 1956). Though no such studies are conducted on human cells, but since antibiotics are absorbed through human skin is a fact we can assume that antibiotics may be absorbed through human skin from soil also or otherwise it may show its activity on surface skin , in psoriasis, eczema and acne.

Probably antibacterial activity may be the reason why no growth was seen on nutrient agar, MacKonkey's agar and sheep blood agar after 48 hrs of incubation. But the growth after 7 days of incubation may be attributed to the presence of spore forming bacteria in the soil.

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