CHARACTERIZATION OF HUMIC ACID FROM THE COMPOSTED WASTES FROM AGROBASED INDUSTRIES: SUGAR INDUSTRIES AND PAPER MILL

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Ms. JAYA TIWARI

SCHOOL OF ENVIRONMENTAL SCIENCES
JAWAHARLAL NEHRU UNIVERSITY
NEW DELHI-110067 (INDIA)
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जवाहरलाल नेहरू विश्वविद्यालय
Jawaharlal Nehru University
SCHOOL OF ENVIRONMENTAL SCIENCES
New Delhi- 110 067, INDIA

CERTIFICATE

This is to certify that the research work embodied in this thesis entitled **"Characterization of Humic Acid from the Composted Wastes from Agro based Industries: Sugar Industries and Paper Mills."** has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. This work is original and has not been submitted in part or full for any other degree or diploma to any university or institute.

Jayatiwari
Jaya Tiwari
(Candidate)

16/7/08
Prof K.G. Saxena
(Dean)

Prof. AL. Ramanathan
(Supervisor)

Gram : JAYENU TEL : 26741557, 26742575, 26742676
SES : 26741538 (Direct) 26704302. 4303. 4304 Fax : 26741502

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LATE BABA AND DAADI

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Chapter 1

INTRODUCTION

1.1 OVERVIEW

India is largely an agricultural country. Agricultural activities produce essential food items for our consumption along with various by products like fodder etc. Hence most of the leading industries are based on agricultural products like sugar industry, distilleries, paper mill, dairies, edible oils and vanaspati (Table1.1)

Out of the total pollution contributed by the industrial sub-sectors, 40% to 45% of the total pollutants can be traced to the processing of industrial chemicals and Food products and agro-based industries together contribute 65% to 70% of the total industrial waste-water in terms of organic load (Manivannan, 2005). For hundreds of years, sugar has been one of the most important components of the human diet universally used. Sugar is produced in 121 countries and approximately 70% is obtained from sugar cane, a very tall grass with big stems which is largely grown in tropical countries (Contreras,et al., 2009).

India is the second largest sugar producing country in the world and it discharges about 5,45 and 7.5 million tonnes annually of pressmud or bagasse and molasses respectively, as wastes (Satisha and Devrajan, 2007). Sugar industry belongs to the most important processing industries in India.. Pressmud generates intense heat (65°C), foul odour and takes long time for natural decomposition. The crop residues bagasse and trash are very poor in N and need to be mixed with other, N-rich, organic wastes in order to provide nutrients and an inoculum of microorganisms (Elvira et al., 1996).

Pressmud or filter cake, a waste by-product from sugar factories, is a soft, spongy, amorphous and dark brown to brownish material which contains sugar, fiber, coagulated colloids, including cane wax, albuminoids, inorganic salts and soil particles. By virtue of the chemical composition and high content of organic carbon, the usefulness of pressmud as a valuable organic manure has been reported by several workers.(Kapur, 1995; Tiwari et al., 1998; Ramaswamy, 1999)

Distillery waste forms one of the major causes of water pollution in India. About 88% of the raw materials used by the distillery industry end as waste. In the current level of production technology used by the Indian distilleries, approximately 13 to 15 litres of waste water generated in the production of litre of alcohol. Besides carrying appreciable organic load, spent wash (waste water) is colored and highly acidic having odd smell and it also contains appreciable amount of dissolved organic and inorganic matter all of which serious environmental problems. (Murgaragavan, 2002)

Around 40.72 million/m^3 spent wash is generated annually from distilleries in India, considerable amount of plant nutrients are available in distillery effluent like: N = 1660 to 4200 mg/L, P = 225 to 3038 mg/L, K = 9600 mg/L, Cl = 7238 to 42096 mg/L, Ca = 2050 to 7000 mg/L, Mg = 1715 to 2100 mg/ L, SO_4 = 240 to 425 mg/L. It also contains plant growth promoters like gibberellic acid (GA) and indol acetic acid (IAA) nearly 3246 mg/L with good fertilizers value. (Murgaragavan, 2002)

Approximately 400 pulp and paper mills, are there in India in which the raw materials used may be wooden pulp or bagasse from sugar industry, generate significant quantities of solid and liquid wastes, carry significant quantities of fibre, fines, filler and other wet end additives that contribute to total suspended solids (TSS), Chemical oxygen demand (COD), and biochemical oxygen demand (BOD) exists in India (Yadav, 2006).

Concern for disposal of paper mill sludge in landfills has increased over the last 10 years. Landfilling and incineration of by-products have become unfavorable means of disposal. Alternatives for disposing of paper mill sludge currently are being sought. The high organic matter content and low trace metal and organic pollutants in pulp and paper mill sludge suggest that these residuals may provide a valuable resource for soil amendments. As supplies for traditional organic amendments including peat moss, composts, and farm manures are diminishing near urban areas, opportunities for beneficial use of paper mill sludge on land have increased (Bellamy et al 1995)

S. No.	Industry	Specific Water Consumption (Cubic meters)	Waste Water generation, (Cubic Meter)	Pollution load (in terms of Kg of BOD)
1.	Dairy(Integrated) (per kilo litre of milk)	8.7	6.0	11.0
2.	Edible Oils & Vanaspati (per tonne oil)	3.0	2.0	7.5
3.	Fermentation			
	(i) Brewery (per Kilo litre of beer)	11.5	9.5	24.0
	(ii) Distillery (per kilo litre of alcohol)	130.0	90.0	600.0
	(iii) Maltry (per tonne of grain)	8.5	3.5	2.0
4.	Pulp & Paper (per tonne of Paper)	300.0	250.0	375.0
5.	Stratch (Maize Products) (per tonne of maize)	8.0	5.5	44.0
6.	Sugar (Per tonne of cane crushed)	2.0	0.4	0.5

Table 1.1: Water Consumption, Wastewater Generation and Organic Pollution Load in Agro-based Industry (Source: CPCB, 2000)

1.2 PROBLEMS ASSOCIATED WITH AGRO-BASED INDUSTRIAL WASTES

For the present work problems of three industries have been taken into consideration viz: Paper and Pulp mill, Distilleries and Sugar Mill

Although the wastes of these industries are biodegradable but still some major problems are associated with the disposal of these untreated organic wastes in water body. Which create following critical problems:-

- **a)** Increase the biological oxygen demand (BOD) and chemical oxygen demand (COD) of the water body hence decrease in dissolved oxygen.
- **b)** Causes bad odour and colouring of the surface water makes them unfit for human consumption.
- **c)** Disinfectant by products like Tri Halo Methane (THMs) is formed when disinfectant (chlorine or bromine) reacts with natural organic matter, which is carcinogenic in nature.
- **d)** Dumping of organic wastes in landfill sites without pre-treatment contaminates the ground water.
- **e)** High levels of suspended solids can also cause problems of both water opacity and blanketing of river or lake beds which results in anaerobic decomposition releasing hydrogen sulphide into the aquatic ecosystem. (www.epa.gov/history/topics/dioxins/01.htm)
- **f)** Fiber is discharged from pulp mills, smothering habitat and consuming oxygen that is essential to fish and other organisms.
- **g)** Chemical contaminants in the effluent of pulp and paper mills are known to cause diseases (including cancer and reproductive failure) in fish and in people who eat large quantities of contaminated fish.
- **h)** Sulphur emissions contribute to acid rain, and all the problems associated with it.

Thus in view of above mentioned serious problems associated with the agro based industries the recycling and utilization of their wastes and by products through development of an economically viable, socially acceptable and environmentally sensible technology is urgently needed. The large-scale accumulation of these wastes will pose disposal and pollution problems. The necessity to preserve natural resources and the optimisation of use of non-renewable energy has encouraged the recycling and recovery of organic waste as an alternative to dumping and incineration (ADEME, 1994).

The organic waste recycled in agriculture, residual sludge generated by wastewater treatment is a source of organic matter rich in both phosphorus and nitrogen. It can contribute to the rehabilitation of degraded soils by its fertilising and other soil-improving qualities. Nevertheless, direct agricultural use of organic waste is limited by the presence of pathogens, by fermentation of any unstable organic matter and by the organic and inorganic pollutants it contains (Lavoie and Marchand, 1997; Dudka and Muller, 1999) To overcome the risks incurred by the direct use of this waste in agriculture, treatment is required to minimise and eliminate the undesirable effects and to optimise the efficiency of the materials once applied to the soil.

Composting is the science of converting organic matter to useful products by the action of various organisms. Decomposition as a process occurs in nature at various levels. To attain the goal of having quality end products, various modifications have been applied to this natural process with a careful monitoring of the process. Composting is associated with the reclamation, recycling, treatment, and disposal of wastes. Reclamation and recycling are means of saving and reusing natural resources. Disposal has become a less desirable option because of environmental concerns.

It has been proved that composting is the best pre-treatment option to overcome these problems (Ouatmane et al., 2000; Amir and Hafidi, 2001), composting is carried out at high temperatures around 50–70 ^{0}C to destroy all the pathogens. Numerous insitu bacteria degrade the readily available organic components or transform them into stable humic components (Garcia et al., 1992; Diaz-Burgos et al., 1994; Amir et al., 2004).

The composting process mainly involves a battery of actions carried out by the interplay of various organisms that form a web of life. Composting is generally defined as the biological oxidative decomposition of organic constituents in wastes of almost any nature under controlled conditions (Sharma *et al.* 1997).

In this process, the organic substances are reduced from large volumes of rapidly decomposable materials to small volumes that continue to decompose slowly. The process brings the ratio of carbon to other elements into a balance, thus providing nutrients to plants in the absorbable state. The various components are necessary for the process to progress smoothly and to obtain good quality compost as the end product. Under controlled conditions, natural decomposition progresses faster and yields a quality product.

The rate of composting, like the rate of plant or animal growth, can be affected by many factors. The key factors are nutrient balance (C: N ratio), moisture content, temperature, and aeration. In addition, the organic substance selected for composting should be free of any toxic compounds such as detergents, surfactants, phenolic, and pharmaceutics, which will pose health risks either directly or through their metabolic, degraded products. It is therefore very important to minimize such materials in the input source.

Humic substances (HS) which are described as refractory, dark colored, heterogeneous organic compounds represent quantitatively and qualitatively the most important components not only of soil organic matter but also of non-living organic matter in all environmental compartments (Stevenson., 1994; Senesi and loffredo, 1999). The HS formation, which is termed as humification consists of complex decomposition and resynthesis processes. Humification has also been defined as the prolonged stabilization of organic substances against biodegradation (Knabner, 2002).

Humic substances (HS), of which humic acids (HAs) and fulvic acids are the major fractions, are formed naturally in soil and environment through the decay and resynthesis of organic residues Humic acids are considered as natural polyelectrical organic compounds of complex structure involving a proportion of more or less condensed aromatic ring with a large number of attached hydroxyl-, phenoxyl-, and carboxylic-reactive groups (Mishra et al., 2000)

The structural complexity humic acid creates opportunity for a broad range of chemical interactions. The polysaccharides provide hydrophilic surface where as phenol compounds are responsible for the hydrophobic surface makes HA as natural surfactant. Besides this humic acid immobilizes the various ecotoxicant including heavy metals, pesticides, polyaromatic hydrocarbons, azodyes etc. thus because of binding interaction sorptive interactions, interactions with the various physiological function of living organisms, the humic acid can be efficiently used to remediate the environmental pollutant.(Perminova and Hatfield., 2005).

The various beneficial properties of humic acid has encouraged several researchers and scientists to develop various methods and techniques to study humic acid like and fulvic acid like fractions of organic amendments. It includes elemental and acidic functional group analysis, gel-filtration chromatography, electrophoresis, iso-electrofocusing, pyrolysis, thermochemolysis and ultraviolet/visible, infrared, nuclear magnetic resonance (NMR), electron spin resonance (ESR) and fluorescence spectroscopies (Senesi et al., 1996; Chen, 2003).

Results of these studies have shown that, in general, HS-like fractions from organic wastes differ from native soil humic substances (HS) for their lower aromatic and carboxylic group content and higher aliphatic character and levels of N-containing and polysaccharide like structures (Senesi et al., 1996; Chen, 2003). During the maturation and stabilization of any organic amendment, organic matter (OM) mineralization and humification occur, and the HS-like components undergo concomitant changes.

In view of above reasons the present work attempted to study the elemental and structural changes involved in humification processes, which focuses on the use of organic wastes generated from agro based industries as soil amendment, in order to achieve that the organic wastes were composted for six months , humic acid was extracted from the composted wastes at the interval of two months then various elemental and functional group analysis, optical properties, gel electrophoresis techniques, FTIR, H^1 and C^{13} NMR were used for the characterization of humic acid and for the evaluation of the quality of organic waste during composting process.

The above objectives have been achieved by following analysis:

1) Extraction and quantification of humic acid from the composted wastes of agro based industries.
2) Elemental and UV-Visible spectroscopy characterization of Humic acid by estimating the percentage of Carbon, Nitrogen and Hydrogen and E_4/E_6 ratio.
3) Structural characterization to identify various aliphatic and aromatic functional groups in the humic acid extracted from composted organic wastes and soil by using FTIR, ^{1}H-NMR, ^{13}C- NMR analysis.

4) Molecular weight characterization of humic acid by Gel electrophoresis and Maldi- TOF-Mass Spectrometery.
5) To compare the elemental composition of humic acid from the wastes of sugar industry and paper mill industry at three stages of composting i.e. Two months, four months and six months.
6) To quantify and characterize the humic acid in soil collected from near by area of industry where these wastes are dumped.
7) To investigate the maturity and stability of compost of waste from agro-based industries by using various humification indices and by observing abundances of aromatic and aliphatic functional groups at different stages of humification.
8) To study the spatial variation in the nature of Humic acid extracted from the industrial wastes of diverse climatic zone.
9) Analysis of major cations and anions and dissolved organic carbon (DOC) in ground water samples and to study its impact of industrial wastes.

Literature Review

Chapter 2

LITERATURE REVIEW

2.1 OVERVIEW

Over three-fourth of fresh water draw by the domestic and industrial sector, return as domestic sewage and industrial effluents which inevitably end up in surface water bodies or in the groundwater, affecting water quality (Mukherjee and Nelliyat, 2006)

The improper and indiscriminate disposal methods of industrial effluents and sludge lead to the loss of nutrient resources and also economic loss (Yadav and Garg, 2009).In developing countries like India, industrial effluents often get mixed with domestic sewage and it is not collected or treated properly even in Metro cities (Minhas and Samara, 2004) When treatment is not adequate, application of domestic wastewater on land and disposal in surface water might cause various environmental problems, like groundwater contamination (bacteriological and chemical), soil degradation, and contamination of crops grown on polluted water (McCornick et al., 2004, 2003 and Scott et al., 2004).

The continuous disposal of industrial effluent on land, which has limited capacity to assimilate the pollution load, has led to ground water pollution, but economically and ecologically acceptable disposal of industrial sludge is becoming great challenge to industries as well as scientists due to the high cost of sludge stabilization reactors, dehydration systems and transportation of sludge to disposal sites.

Among various kinds of industries the agro-based industries produce huge quantities of liquid and solid wastes and their treatment results into the generation of large quantities of sludge.

Therefore Major portion of agro based industry is either dumped in landfill sites or disposed off in river without any pre-treatment, which further creates the organic load on the ground water and river water respectively (Norbu, 2002).

The disposal of wastes of agro based industry leads the environmental problems in the nearby areas of the industry. These wastes are an important source of pollution owing to its relatively high content of organic matter in the form of sugars, tannins, polyphenols, polyalcohol, pectin and lipids in addition to lignin, cellulose and pectin in these organic wastes (Hamadi, 1993).

2.2 IMPACT OF AGRO BASED INDUSTRIES ON THE GROUND WATER

Large number of research work on water pollution (ground and surface water) caused by effluents from sugarcane processing industry, distillery and pulp and paper mill and other industries are available often, which has been reviewed in this chapter.

In any urban area like National Capital Territory (NCT) Delhi, there are many possible sources of groundwater contamination, viz. unlined drains, sanitary landfill sites, septic tanks and cesspools, industrial and domestic effluents, ex-filtration from the sewage system and gasoline stations (Aryal et al. 2006; Kumar et al. 2006). Groundwater contamination largely depends on land-use pattern as quality of subsurface water typically reflects the land use and soil type present on the surface. Therefore, it is essential to distinguish between the contributions by natural weathering and anthropogenic inputs. Contamination can have adverse effect on human health and stock lives. Studies on nutrient chemistry in groundwater have been widely reported during recent decades in many parts of the world (Rajmohan and Elango 2005).

Irrigation, waste water infiltrations, leaching from unlined drains and landfill sites can increase the nutrients in groundwater. Nitrate (NO_3^-) contamination in drinking water is a significant public health concern. Many studies were reported on the adverse health effects of nitrate in groundwater and surface water like the threat of methemoglobinemia causing severe oxygen deprivation, especially in children, neurological problems, renal failure and increases in stomach cancer (Shuval and Gruener, 1977; O'Riordan and Bentham, 1993).

Remedial steps to control the pollution need mapping of contaminant in groundwater, tracing the source of contaminants and their pathways. The contamination can take place due to natural reasons like in the case of nitrate as it can release from nitrate rich soils or due to anthropogenic means like wastewater, leachates or excessive use of fertilizers. Seasonal changes also have key control on the speciation and amount of leachates regardless of the total nutrient concentration. Hence, the determination of the sources of NO_3^- in groundwater is an important first step in the process of improving groundwater quality.

Similarly, excess salinity in groundwater can arise due to one or more of natural or anthropogenic reasons. Excess salinity not only affects human health, live-stocks and agri-products but it can not simply be consumed due to its saline taste. The increase in groundwater salinity may be caused by a single process or a combination of different processes including anthropogenic related activities like recycling of water through irrigation return flow, increase in salinity due to water logging and evaporative concentration of salts or due to influx of natural saline water, such as sea water intrusion near coast, or due to geogenic inputs (Banner et al., 1989; Vengosh et al., 1999; Sharma et al., 2004; Kumar et al., 2007).

The groundwater salinity can also be of climatic origin. The evaporation of surface water in the shallow basins of arid and semiarid zones like Delhi concentrates its salt content. The translocation of these salts down the soil profile with the infiltrating water adds and increases the salt content of its shallow aquifer. Thus in this paper, an effort has been made with the objective to distinguish between the contributions of natural weathering and anthropogenic inputs in nitrate and salinity problem of the area, to reveal the spatial distribution and predict the seasonal changes in various nutrients

(Yuce, 2007) studied the spatial distribution of groundwater pollution in the Porsuk river basin, Turkey. It was found that the ground water in alluvium aquifer of the Eskisehir plain is polluted by waste water transported through Porsuk river basin , agricultural - industrial activities, river – ground water interactions and unsanitary landfill.

The liquid residues during the industrial phase of the production of alcohol are: liquor, sugar cane washing water, water from the condensers and from the cleaning of the equipment, apart from other residual water. This extract is extremely polluting as it contains approximately 5% organic material and fertilizers such as potassium, phosphorus and nitrogen. The amount of water used in this process is large, generating a high level of liquid residues called as spent wash (Borrero et al., 2003).

Spent wash from distillery disposal into the aquatic ecosystem is hazardous and has high pollution potential. High COD, BOD, total nitrogen and total phosphate content of the effluent may result in eutrophication of natural water bodies (Fitz Gibbon et al., 1998).

According to Savant et al., 2006 pulp and paper manufacturing is one of the oldest and largest industries in India with an installed capacity of about three million metric tones per annum finished product. Since on an average about 300m^3 water is consumed per ton of paper produced, the industry generates a huge volume of highly colored and toxic effluents. About 500 different chlorinated organic compounds have been identified in paper mill effluent the high chemical diversity of these pollutants causes a variety of clastogenic, carcinogenic, endocrinic and mutagenic effects on fishes and other aquatic communities in recipient water bodies (Ali and Sreekrishnan, 2001; Karrasch et al., 2006)

(Chikatur et al, 2003) studied the impact of west coast paper mills (WCPM), Dandeli on Kali River. They repoted that WCPM releases its effluent from the papermaking process into the Halamaddi Nalla, which flows through the WCPM and merges with the Kali River half a kilometer from the mill. A December 1999 ISRO study commissioned by the mill to asses the possibility of ground water contamination found that several kilometers stretch of Kali River was affected and ground water was contaminated in village situated along the river.

At Sjasstoj, Russia, a wide area of land is contaminated due to effluents from pulp and paper mill situated on the southern shore of lake Ladoga and eutrophication of lake ladoga has also increased with pollution of the lake water. The concentration of dissolved organic carbon was found as high as 44 mg/l and adsorbable halogens as 130 μg/l in ground water samples collected from nearby areas of paper industry (Schoenheinz et al., 2002)

The examination of soil and water pollution levels in the Abu drainage area (Meerut, Uttar Pradesh) indicated that indiscriminant discharge of effluents from a wide range of industries (including sugar mills) resulted in serious effect on the local flora and fauna and water was found to be unsuitable for human consumption, domestic use and irrigation purposes (Singh et al., 2000).

At Sonai (Maharashtra), effluents from the Mula sugar factory, released into a stream flowing through the area, had infiltrated the underlying aquifer. Relative to the discharge point, the resulting plume of polluted groundwater extended only a few meters upstream, but more than 400 m on either side of the waterway downstream. This resulted in a zone of polluted ground water extending for more than 3.5 Km^2. It was recommended that the base of sugar factory lagoons and the stream used for effluent discharge should be water proofed for the protection of ground water which was the only source available locally for drinking and agricultural purposes (Pawar et al., 1998)

Singh et al., 1996 examined the effects of industrial effluents on ground water quality in urban industrial units including a sugar factory and distillery, in Sardarnagar (Gorakhpur, Uttarpradesh) and found that both hand pump and boreholes yielded poor water quality. In another study evidence of adverse impacts was observed on water quality of the river Ramganga (between Moradabad and Bareily), arising from discharges of sewage waste, and effluents generating from sugar, rubber and paper industries (Singh et al., 1998).

Sugar mill and distillery effluents resulted in deterioration of many aspects of water quality in the river Gelabil (Assam) for 8 km downstream of the discharge point. During periods when the factory was closed, pollution level of the river was negligible. (Baruah et al., 1993)

We found that the effluents of sugar mills, distilleries and paper mills are rich in organic and inorganic nutrients (N,P,K) thus in order to avoid surface and ground water pollution the effluents can be reused in composting of organic solid waste of various agro based industries and an useful compound i.e. humic acid can be extracted from composts. Definition, properties and formation of humic substances has been discussed in details in the following sections of this chapter.

2.3 HUMIC SUBSTANCES

Humic substances (HS) are natural products, which constitute most important refractory organic carbon in the geosphere. They are present ubiquitously in soil, sediment and aquatic environment. They are composed of carbon, hydrogen, oxygen, nitrogen and sulphur. These elements are always present in HS regardless of their origin country or continent (Rocha et al, 1999; Davis et al., 2001; Mc Donnell et al., 2001)

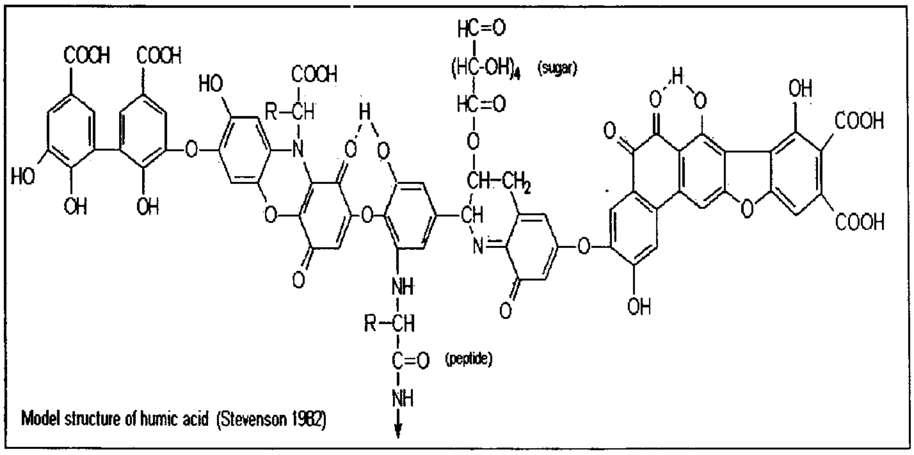
They are yellow to black in appearance, acidic, polydisperse (having different composition and molecular weights), polyelectrolyte, paramagnetic, with extremely complex structure and unique biological and physiochemical features, and finally hit a diagenic state where it is difficult to change them further. Both their dark color and paramagnetism most likely result from the electronic structure of their core: aromatic subunits, and extended (electron) system in the semi-quinone moieties and electron donor-acceptor nature (Senesi et al., 1991).

Humic substances are known to be of high molecular mass polyhydroxycarboxylates which contain poly-aromatic and aliphatic subunits. The degree of ionization of these macromolecules is governed by the amount of ionized phenolic and carboxylic groups, which is the function of pH of the solution (Arno, 2002). Their most common classification is based on the solubility at different pH values such as humic acid (HA),insoluble at pH < 2 (under acidic condition); fulvic acid (FA), soluble at all pH values and Humin (HU) insoluble at all pH values (Davis et al., 2001; Karen et al., 2005)

HA is one of the most important forms of HS. HA plays an important role in various fields such as agriculture, industry, environment and biomedicine. On the otherside they can raise aquatic environment pollution, induce cytotoxity for many mammalian cells.

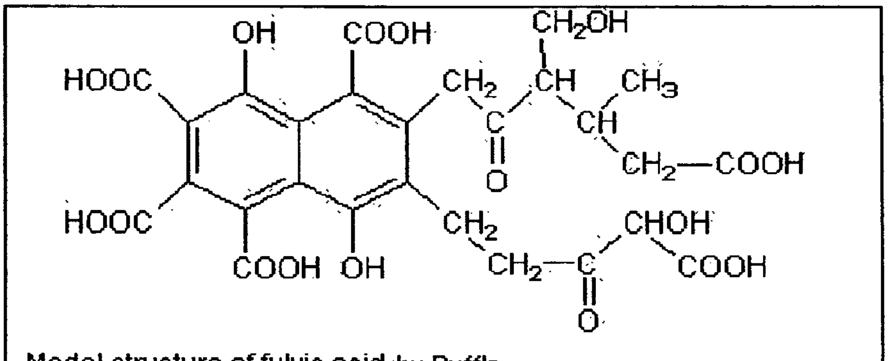
Thurman, 1985 defined humic acid as those precipitate in acid and those in solution are Fulvic acid. Generally the Fulvic acid acids are more soluble in water because it contains more carboxylic and hydroxyl functional groups and is lower in molecular weight, ranges between 800-2000 dalton. Humic acid is larger than 2000 dalton and is often colloidal in size. Humic substances are heterogeneous high molecular weight organic materials which are ubiquitous in terrestrial and aquatic environments. They are resistant to microbial degradation and are not considered to be dynamically involved in microbial metabolism, especially in anoxic habitats. Humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups.

The hypothetical structure for humic acid, shown in figure, contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings.



Model structure of humic acid (Stevenson 1982)

The hypothetical model structure of fulvic acid (Buffle's model) contains both aromatic and aliphatic structures, both extensively substituted with oxygen - containing functional groups.



Model structure of fulvic acid by Buffle

Elemental composition of humic substances and several plant materials (by Kononova, 1966)

Substances	% dry ash-free basis			
	C	H	O	N
Fulvic acids	44 - 49	3,5 - 5,0	44 - 49	2,0 - 4,0
Humic acids	52 - 62	3,0 - 5,5	30 - 33	3,5 - 5,0
Proteins	50 - 55	6,5 - 7,3	19 - 24	15,0 - 19,0
Lignin	62 - 69	5,0 - 6,5	26 - 33	-

2.4 THE HUMIC ACID / FULVIC ACID RATIO

The percentage of the humus which occurs in the various humic fractions varies considerably from one soil type to another. The humus of forest soils is characterized by a high content of fulvic acids while the humus of peat and grassland soils is high in humic acids. (Figure)The humic acid / fulvic acid ratio usually, but not always, decreases with increasing depth.

Humic acid / fulvic acid ratios of some surface soils (by Kononova, 1966)

Soil	Humic acid/ Fulvic acid ratio	Soil	Humic acid/ Fulvic acid ratio
Chernozem ordinary	2.0 - 2.5	Gray forest	1.0
Chernozem deep	1.7	Sod podzolic	0.8
Chestnut dark	1.5 - 1.7	Tundra	0.3

HA are involved in numerous reactions with a wide range of substances, including metals, organic and inorganic pollutants in soils and water.(Yates et al., 1997; Riggle and von Wandruska, 2002; Zhou et al., 2005).

Novel understanding of the nature of HA indicate that they are complex associations of small polydispersed mixtures of heterogenous polyelectrolyte, and behave as a weak polyelectrolyte acid that can be investigated by analytical techniques. Most of the acidity in the HA is due to the carboxylic, phenolic-OH and /or enolic groups which dissociate to develop negative charges in a wide pH range. The degree at which these groups are ionized or protonated depends at the environmental pH values.

Their abundance and distribution have a significant influence in the interaction between HA and a wide range of substances (Machesky, 1993; Ritchie and Perdue, 2003; Plaza et al., 2006. One of the most important properties of HA is its large buffer capacity in a wide pH range, which arises essentially from the dissociation of acidic functional group which they are particularly rich (Ceppi et al., 1999; Campitelli et al, 2003)

The addition of organic amendments (compost or vermicompost) could contribute to the soil buffer capacity, and /or exert a marked control on the physiochemical behaviour, through their HA content; this issue together with their charge development and acid –base properties has been studied extensively in the recent years by (Avena et al., 1999; Campitelli et al., 2003; Gondar et al., 2005). Therefore, a better knowledge of the chemical and physiochemical properties of HA will contribute to understand the mechanisms that control an optimum supply of nutrients for crops, as well as many soil physical and chemical characteristics are affected by the organic amendment application. (Chrstl and Kretzschmar, 2001; Garcia, et al., 2004).

Chaturvedi et al, 2006 studied the sorption kinetics and leachability of heavy metals from the contaminated soil amended with natural sorbent humus soil in combination with synthetic hydroxyapatite have been investigated for immobilization and clearly demonstrated that humus soil along with hydroxyapatite could potentially be a cost effective stabilization agent for treating heavy metal contaminated soil

Misra and Pandey, 2005 studied on immobilization of heavy Metals in contaminated soil using Nonhumus–Humus soil and hydroxyapatite. The findings suggest that amending the nonhumus soil with humus soil and hydroxyapatite can immobilize toxic metals more effectively and also reduce their bioavailability.

Conte et al., 2004, used humic acids as natural surfactants in the washings of highly contaminated soils where as (Yamada and Ozaki, 1998) worked on the behavior of humic substances as precursors of Trihalomethanes in environmental water.

Lovely, 1996 showed that some microorganism found in soils and sediments are able to use humic substances as electron acceptors for the anaerobic oxidation of organic compounds and hydrogen. This electron transport yields energy to support growth.

2.5 FORMATION OF HUMIC ACID

It has been known that HS are a form of environmental organic matters that are formed during the physical, chemical and microbiological transformation process (humification) of dead tissue of animal and plant. It is made-up of discrete, well-defined molecules but is a class of substances that are produced and reside in terrestrial (soil organic matter (SOM)) and aquatic natural organic matter (NOM) forming a major component of both the soil and aquatic carbon pools (Rocha et al., 1999; Davis et al., 2001). Approximately 80% of the total carbon in terrestrial media and 60% of the carbon dissolved in aquatic media are made up of HS (Eladia et al., 2005)

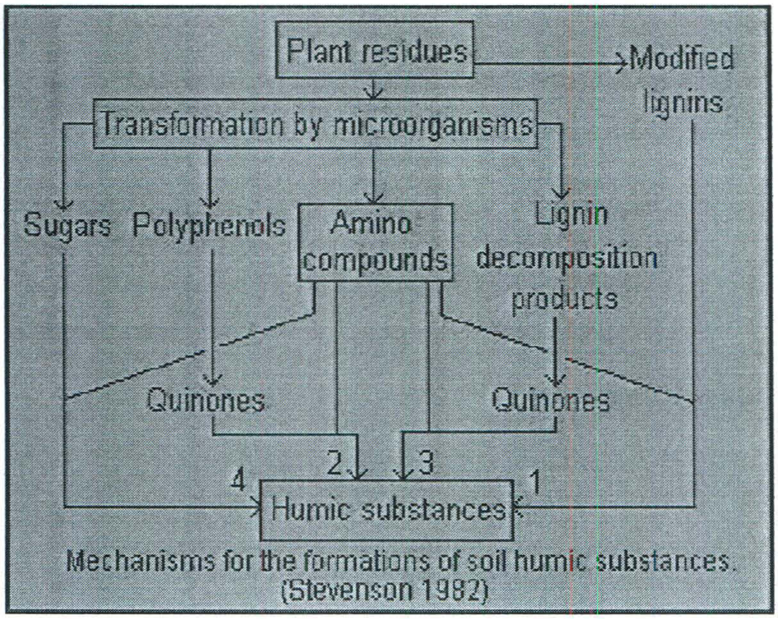
Some theories have been used to explain the origin of HS such as sugar- amine condensation theory and the lignin theory or the polyphenols theory. Currently, most investigators suppose that HS have origin in lignin (Oglesby et al., 1967; Eladia et al., 2005). Polyphenols come mostly from lignin during its biodegradation and probably play a key role in the formation process. Various techniques are being used to characterize HS.

2.6 THEORIES OF HUMIC ACID FORMATION

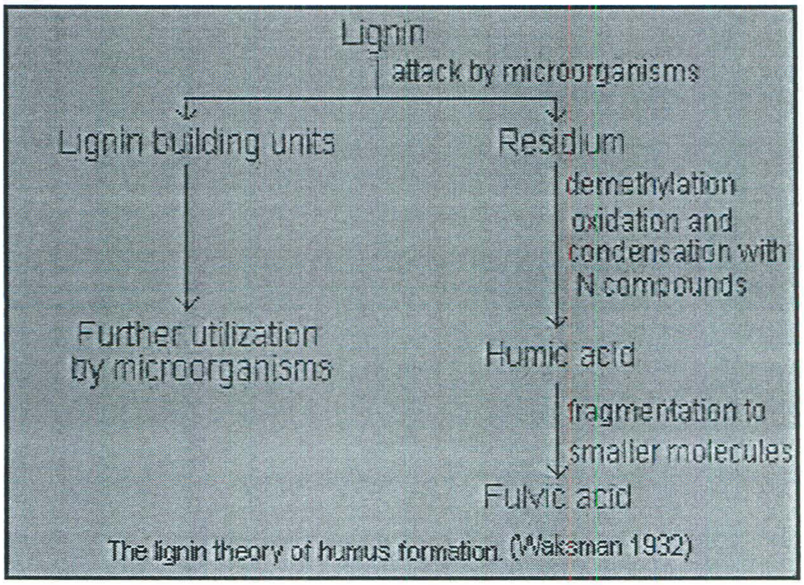
(www.ar.wroc.pl/weber/powstaw2.htm)

The formation of humic substances is one of the least understood aspects of humus chemistry and one of the most intriguing. Studies on this subject are of long-standing and continued research can be justified on theoretical and practical grounds. several pathways exist for the formation of humic substances during the decay of plant and animal remains in soil, the main ones being shown in the picture: The classical theory, popularized by Waksman,1932, is that humic substances represent modified lignins (pathway 1) but the majority of present-day investigators favor a mechanism involving quinones (pathway 2 and 3). In practice all four pathways must be considered as likely mechanisms for the synthesis of humic and fulvic acids in nature, including sugar-amine condensation (pathway 4). These four pathways may operate in all soils, but not to the same extent or in the same order of importance.

A lignin pathway may predominate in poorly drained soils and wet sediments (swamps, etc.) whereas synthesis from polyphenols may be of considerable importance in certain forest soils. The frequent and sharp fluctuations in temperature, moisture and irradiation in terrestrial surface soils under a harsh continental climate may favor humus synthesis by sugar-amine condensation.



Mechanisms for the formations of soil humic substances. (Stevenson 1982)



The lignin theory of humus formation. (Waksman 1932)

2.7 CHARACTERIZATION OF HUMIC SUBSTANCES

Recycling of organic wastes from industrial and domestic sources by means of composting has arisen in recent years as an alternative to classical soil amendment (Gonzalez-Vila et al, 1999; Ait Baddi etal., 2004a, b; Amir et al., 2004). These organic residues have to be pretreated in order to stabilize them and to reduce their initial toxicity. Non-composted residues or immature composts applied to agricultural soils degrade rapidly, may cause phytotoxicity due to the presence of simple organic acids, and decreases in oxygen and nitrogen concentrations, among other harmful effects on soil properties (Mustin, 1987; Jimenez and Gracia, 1992), the content in potential organic i.e polycyclic aromatic hydrocarbons and inorganic (heavy metals) pollutants of composts must also be controlled, as these contaminants may migrate to groundwater or accumulate in plants (Oleszczuk and Baran , 2005; Alvarenga et al., 2007)

Therefore in order to study more in depth these possible structural associated with humic acid formed during humification process of organic waste is an important point to consider when assessing the maturity and properties of the final product. (Fuentes et al., 2007)

The most common techniques to obtain structural and compositional information on humic substances which have been discussed here are as follows, Elemental Analysis, UV-Visible,, Infra-Red Spectroscopy, ^{1}H NMR and ^{13}C NMR Spectrometry, MALDI-TOF-MS and Gel Electrophoresis methods (Belzile et al., 1997).

2.8 ELEMENTAL COMPOSITION AND E_4/E_6 RATIO

Elemental composition, mainly hydrogen, carbon, oxygen and nitrogen contents of humic substances, can establish the nature and origin of these compounds (McDonnell et al., 2001) This data is normally used in terms of atomic ratios (H/C, O/C and C/N), which give information on different types of humic substances, structural changes of humates and structural formula in soils, composts and sediments. Various studies have been taken up for establishing elemental composition of humic and fulvic acids from different sources like marine and costal sediments (Sensi, 1994).

Sen and Chandra, 2007 during evaluation of the organic matter transformation in vermicomposting of sugar industry wastes have observed that the drift of C/N as a function of time was an important index used for the assessment of the efficiency of the vermicomposting process and compost maturity. The decline observed for the C/N ratio from an initial of 21.92 in the ptbc to a final value of 10.20 in the 60 day vermicompost indicated higher organic matter decomposition and the attainment of a suitable degree of organic matter stabilization.

Bustamante et al., 2008 studied the carbon and nitrogen transformation in the evaluation of compost stability. They evaluated the quality of the compost obtained from solid wastes of winery and distillery industry by means of co-composting with animal manures. They monitored a number of parameters through out the composting process such as p, electrical conductivity, organic matter, water soluble carbon , water soluble polyphenols, different forms of nitrogen(organic nitrogen, ammonium and nitrate) and humification indices (humification ratio, humification index, percentage of humic aid like C, polymerization ratio and cation exchange capacity),as well as the germination index. It was also observed that organic matter losses followed first order kinetics equation and mixture with the lowest C/N ratio showed the highest initial ammonium contents, probably due to the higher and more labile N content of manure. The increase in the cation exchange capacity revealed the organic matter humification during composting, where as other humification parameters, such as the humification ratio and the humification index, did not show the expected parameters.

Campitelli and Ceppi, 2008 reported a comparison, from chemical and physicochemical point of view, between the HA extracted from stabilized organic matter obtained through composting and vermicomposting and native soil HA by various analytical techniques, elemental and functional group composition, FTIR, and potentiometric titration. According to the results obtained, the HA derived from the less evolved organic materials (compost and vermicompost) showed different humification levels (higher in composting than in vermicomposting process), higher aliphatic nature, higher nitrogen compounds content, lower oxidation degree, lower charge development, differences in the acidity strength of the acidic functional groups and differences in the heterogeneity than those extracted from evolved material (soil).

Sellami et al., 2008 assessed the maturity of composted olive mill wastes using UV spectra and humification parameters. In their study the humification process was evidenced by quantifying the humic substances and the generally accepted humification indices; (1) the ratio of humic acid (HA) carbon to fulvic acid (FA) carbon (C_{ha}/C_{fa}), (2) the ratio of water soluble carbon (C_w) to total organic nitrogen (C_w / N_{org}), (3) and the ratio of humic acid carbon to total organic carbon C_{ha}/C_{org} and by determining the absorbance ratios; E_2/E_4, E_2/E_6, E_4/E_6 the results showed that the time required to reach maturity was dependent on the chemical properties of the initial raw materials used. They confirmed maturation by an increase in nitrogen in HA, in C_{ha}/C_{fa} and a concentration of C_w, which included a high proportion of easily bio-degradable organic compounds such as sugars, amino-acids and peptides, decreased during composting process.

Campitelli et al, 2006 have compared chemical and physico-chemical characteristics of humic acids extracted from three different sources i.e. compost, soil and amended soils and found H/C ratio to be higher for composted soils, indicating high degree of maturation.

According to Agnelli et al., 2000, the H/C ratio is a measure of aromaticity; highly aromatic macromolecules have H/C<1. The O/C ratios are the index for the abundance of oxygen containing functional groups while C/N ratio indicates biological activity. They have found that the C/N ratio decreases from fine earth to rock fragments. The lower C/N ratios of rock fragments suggest a higher degree of N incorporation in the humic structure, indicative of consistent biological activity.

Wilson et al., 1999 also found that N/C ratio increases with increase in molecular weights, suggesting that N-containing compounds are concentrated in the higher molecular fractions and are less degradable. Riffaldi et al., 1993 attributed the high nitrogen value to the incomplete hydrolysis of proteinaceous constituents. According to Piccolo et al. (1992), high values of nitrogen and sulphur are indicative of high contents of non-humified biomolecules (polysaccharides and polypeptide) in the materials

2.9 SPECTRAL STUDIES

Recent advances in molecular spectroscopy have helped to derive valuable information on structural aspects of Humic substances isolated from diverse sources. UV-Visible, Fluorescence, IR, NMR and ESR spectra have been widely used for the said purpose. UV-Visible spectra of humic acids are featureless. However, it had been a general observation that different humic acid fractions have distinguishable colour differences. (Swift et al., 1970) had reported that the optical extinction coefficients of lower molecular weight humic acid fractions are greater than their counterparts. A review by (Shnitzer and Khan 1972) on the UV-Visible spectral studies of humic substances indicate that the light absorption of humic substances appears to enhance with an increase in: the degree of condensation of the aromatic rings they contain, the ratio of carbon in aromatic nuclei to carbon in aliphatic or alicyclic side chains, the total carbon content and the molecular weight.

Although the visible absorption spectra does not provide much insight into chemical structure of humic acid molecules, the ratio of optical densities of humic acid and fulvic acid solution at 465 and 665 nm is often used for characterization of humic substances. This ratio often referred to as E_4/ E_6 is independent of concentration of humic materials, but varies for humic acid extracted from different sources (Kononova,1996), a low ratio is indicative of relatively high degree of condensation of aromatic humic substances (Agnelli et al., 2000 and Chen et al., 1977)

UV spectroscopy was used as a tool for monitoring humification and proposing an index of the maturity of compost. A new index was obtained by UV spectroscopy using the deconvolution of an unknown spectra of compost to maturity indices usually used, such as total extraction of humic substances (rate of extraction), IP (Index of Polymerization) or the humic and fulvic acid ratio.UV spectroscopy makes it possible to approach the global chemical structure of molecules as a whole, in particular their aromatic structure.

2.10 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

IR spectra of Humic substances show bands at the following frequencies: 3400 cm^{-1} (hydrogen bonded OH), 2900 cm^{-1} (aliphatic C-H stretch), 1720 cm^{-1} C=O of COOH, C=O stretch of Ketonic C=O), 1630 cm^{-1} (aromatic C=C, hydrogen bonded C-O of carbonyl or quinine, COO^{-1}), 1450 cm^{-1} (aliphatic C-H), 1400 cm^{-1} (COO^{-1},aliphatic C-H), 1200 cm^{-1} (C-O stretch of OH^{-} deformation of COOH) and 1050 cm^{-1} (Si-O of silicate impurities) (Senesi and Sipos, 1985, Shin et al., 1999). The bands are broad, most likely because of extensive overlapping of individual absorptions. Although IR spectra of humic materials tell little about the chemical structure of humic nuclei, they provide worthwhile information on the distribution of functional groups. The IR spectra reflects the preponderance of oxygen – containing functional groups, that is –COOH, -OH and >C=O in humic materials. (Aiken etal., 1985, Stevenson, 1994, Baglieri et al., 2006, Campitelli et al., 2006, Agnelli et al., 2000)

2.11 NUCLEAR MAGNETIC RESONANCE (NMR)

Nuclear magnetic resonance (NMR) spectroscopy has been widely used for the study of humic substances (Hatcher et al., 2001), and although specific compound identification is not possible with this technique, information can be provided on the distribution of various functional group present. Peaks in the NMR spectrum can usually be assigned to specific functional groups in complex materials such as humic substances. Despite this, NMR techniques have been valuable in advancing the understanding of humic substances, for example, H^1 and C^{13} NMR has been used to examine the aromaticity /aliphatic nature of HS, leading to the discovery that they are primarily aliphatic and not predominantly aromatic in nature, as once thought (Malcom, 1990). NMR studies have also found that humic acids are composed of partially degraded plant constituent's components, (Wershaw et al., 1990), that fulvic acid is a separate chemical entity to humic acid and that perhaps phenolic carbons are minor rather than major components of HS.

2.12 PROTON NUCLEAR MAGNETIC RESONANCE (H^1-NMR)

H^1-NMR spectroscopy has become a standard method in many chemistry labs. A wide range of structural questions can be answered by its application, since there are clear cut relationships between the data content in H^1-NMR spectra and the arrangement of protons in the molecules under investigation.

Proton NMR spectroscopy is used for estimating the relative abundance of various humic substance functional groups. Spectra of humic and fulvic acids dissolved in alkaline or neutral functional groups. Spectra of humic and fulvic acids dissolved in alkaline or neutral solution provide information that is useful for characterizing their aromatic and aliphatic components. H^1-NMR spectra of humic substances dissolved in $CDCl_3$,CCl_4, deutrated water (D_2O), deutrated dimethyl sulfoxide (DMSO-d_6) and aqueous solutions of NaOD showed resonance signals at δ=1.0-1.7 ppm (aliphatic protons), δ= 1.7-2.5 ppm (protons on carbons adjacent functional groups such as carbonyls, aromatic and double bonds),δ=4.0-4.4 (probably indicating some lactone structure) and δ=7.3-7.9 (aromatic protons). Some of the authors have divided these proton resonances into the following categories: 0.5-2 ppm(methyl and methylene protons), 2-5 ppm (methyl and methylene protons linked to hetero atoms) and 5-10 ppm (aromatic protons) (Gracia et al ., 1994) Francioso et al., 2003 and Baglieri et al., 2006 have allocated the region of the spectrum 0.5-3 ppm to the h forms linked to aliphatic groups, the region 3-4.2 ppm to the H forms linked to aromatic molecules. Signals of <0.5 ppm are attributed to organic Si components such the compounds (Si-R and Si-O-R) in accordance with (Kovac et al., 2002)

2.13 CARBON-13 NUCLEAR MAGNETIC RESONANCE (C^{13}-NMR)

C^{13}-NMR spectra have several advantages over conventional H^1-NMR. It provides direct information on the carbon skeleton of the organic substances in addition, the larger chemical shifts recorded by this technique allow signals to be resolved for individual carbon atoms with only slightly different intrinsic magnetic environment.

Researches have categorized C^{13} NMR spectra into different regions depending on their aliphatic and aromatic characters as: 0-105 ppm (Aliphatic-C), 105-165 ppm (Aromatic-C) and 165-85 ppm (C in COOH groups) (Agnelli et al., 2000 and Schnitzer, 1991); while others have divided NMR spectra into five regions according to chemical shifts as 0-45 ppm (Alkyl-C), 45-110 ppm (O-alkyl C), 110-160 ppm (Aromatic-C) , 160-190 ppm (Carboxylic C), 190-220 (Carbonyl C). Aromatic carbon content for bulk samples and various fractions (<1, 1-3,-5, 5-10, 10-30, 30-100, 100-300 KDa) vary from 27.0 to 39.8%. The C^{13} NMR spectra also suggests that H- and C- substituted aryl carbons may account for more than 80% and that the O-substituted aryl carbon is less than 20% of the total aryl carbons (Li et al., 2004)

Studies conducted by Schnitzer (1986, 1991) to characterize soil humic and fulvic acids revealed that; (1) humic acid contains more of carbon, hydrogen, nitrogen and sulphur, and less of oxygen than does the fulvic acid; (2) Phenolic-OH, -COOH, total acidity, alcohol – OH and Ketonic >C=O group contents of fulvic acid are appreciably higher than those of the humic acid (however, the later contains more quinonoid >C=O groups per unit weight; (3) about 90% of oxygen in humic acid can be accounted for by the oxygen carrying functional groups, but in the fulvic cid nearly all the oxygen re held in similar functional group; and (4) E_4/E_6 ratio is considerably higher than that of humic acid implying that the fulvic acids have lower particle size and /or molecular weight distribution as compared to the humic acids.

2.14 MATRIX ASSISTED LASER DESORPTION/ IONIZATION TIME OF FLIGHTMASS SPECTROMETRY (MALDI-TOF-MS)

Determination of the HA structure is a complex problem. Recently a number of various soft ionization spectrometric techniques like Electrospray Ionization (ESI) (Stenson et al, 2002, 2003) or the matrix assisted laser desorption/ionization time of flight mass spectrometery (MALDI-TOF-MS) and lately laser desorption/ionization time of flghtmass spectrometry (LDI-TOF-MS) are being applied more and more to the characterization of humic substance (Gajdosova et al., 2000, 2001, 2003; Kujawnskai et al., 2002; Pena-Mendez, 2005, 2007).

Application of MALDI-TOF-MS or LDI-TOF-MS to characterize HA to give complex mass spectra is preferred over Py-GC-MS because soft ionization technique is less destructive (Havel et al., 2001). In the case of LDI-TOF-MS, there is a danger of segmentation which needs to be avoided, while MALDI-TOF-MS also has limited use, not only due to fragmentation of HA, but also because of the formation of aggregates or clusters. Formation of cluster in MALDI has been reported by Pacheco et al., 2003, therefore, for both techniques careful optimization of the parameters affecting measurement is necessary.

Pena-Mendez et al., 2005 have taken mass spectra of Humic acid (HA) from different sampling sites (Antartica, Brazil, Czech republic, Mexico and USA) and origin (Plant, soil, peat, and coal derived were obtained by laser desorption/ionization time of flight mass spectrometry (LDI-TOF-MS). They found that reproducible mass spectra of HA samples were obtained, the majority of peaks are observed in m/z region 100-1000 Da.Very similar structural groups (patterns) of the peaks are present in the m/z range 717-918 Da for HA samples of quite different origins, countries or continents. After applying principal components and cluster analysis, they demonstrated that the most of the Antartica HA samples show distinguishable differences when compared with humic acids from other continents and of different origin. In another conducted by Gajdosova et., 2003, they have also found the similar peak pattern between m/z 800-950 for all humic acid samples.

2.15 GEL ELECTROPHORESIS

There is no end to the techniques used to characterize humic substances but gel electrophoresis is another tool to separate humic substances according to their molecular weight. There are different view points regarding the sizes and shapes of humic substances in the scientific community. One suggested that humic substances are macromolecular and assume random coil conformations in solution; a second proposed that humic substances re molecular associations of relatively small molecules held together by weak interaction forces; third considered that HS remain as micelle or "pseudomiceller" structures in solution.

Trubetskoj et al., (1997) have fractionated soil by size-exclusion chromatography (SEC) on sephadex G-75 using water, Tris-HCl or 7M urea as eluents, or by ultrafilteration in the presence of 7M urea or water. These fractionated HAs were classified as 100K (normal retention size.100,000); 30K (1000000-30,000); 10K (30,000-10,000) and 5K (10,000-5). Several pools from each chromatography and all ultra filtration (UF) retentates were analysed by polyacrylamide gel electrophoresis (PAGE).results have shown that page in the presence of denaturing agents can be successfully used for checking the purity of HA fractions obtained by both SEC and UG

2.16 PREVIOUS WORK ON THE CHARACTERIZATION OF HUMIC ACID

Francioso, et al., 2007, investigated the structural features of the humic like fraction (HLF) from olive pulp (OP), its effluents originated from the fermentation processes for hydrogen (EH_2) and methane production (ECH_4) and humic acid (HA) from soil amended with each of these materials. A considerable structural C NMR spectrometry and degradation methods modification emerged between the HLF, in particular from the (ECH_4) effluent, which was characterized by a high content of polyphenolic and polypeptidic substances. The structural interpretation performed by 2D NMR indicated the presence of aliphatic and aromatic protons while the sugar-like content and $O\text{-}CH_3$ groups decreased with respect to the soil control HA.

Structural change involved in humification processes of organic materials with diverse origin was studied by Fuentes et al., 2007 using ^{13}C CPMAS NMR, ^{1}H NMR, Fourier transform infrared spectroscopy (FTIR) and high pressure size exclusion chromatography (HPSEC). The results obtained indicated that humification seems to be associated with an increase in the aromatic character of the systems, with an increase in the aromatic character of the systems, with the presence of phenol groups as principal substituents and a reduction in oxygen containing functional groups, principally carboxylic groups as well as the development of molecular fractions with larger size.

Amir. et., al, 2006, they characterized Humic acid extracted from sewage sludge during composting by thermochemolysis – gas chromatography – mass spectrometry. Ceppi et., al, others, 2005 characterized humic acids extracted from different organic compost and soil from Argentina by capillary electrophoresis and mass spectrometry and observed the differences and similarities between soils HAs and compost HAs. Amir, 2003, has characterized estimated molecular weight of Humic acids extracted from sewage sludge during composting and of their sephadex ^{R} gel fractions by Fourier transform infrared, UV-spectroscopy, and E_4/ E_6 ratio . They found that fraction of high molecular weight excluded from gel largely prevails. This fraction consists of polycondensed lignin- like structure bearing peripheral lipidic and peptidic chain. The fraction retained on gel is characterized by a low and medium molecular weight and is composed low- condensed lignin constituents with high acidic functional group content, they also observed during composting the increase in aromaticity and polycondensation degree of Humic acids, and decrease in aliphatic peptidic and lipidic compounds.

Tanaka, 2001, studied the structure of Humic acid dissolving in aqueous solution by ATR–FTIR spectroscopy and isolated two types of fractions of humic acid (Aldrich chemical), one is dominated by aliphatic structure and another is dominated by aromatic structure and the functional groups of both the fractions were compared with the transmission Fourier transform infrared spectroscopy with KBr pellet (KBr spectra) and with diffuse reflectance (DR) spectra and found that bands of the ATR spectra for both fractions are in well agreement with those of (KBr and DR spectra). This shows that the functional groups of the humic acid dissolving in aqueous solution could be characterized by using the ATR-FTIR spectroscopy.

Pandey et al., (1999) studied that free radicals play an important role in the binding of organochlorine pesticides and heavy metals with humic acid by forming a soluble complexes. They found the generation of free radicals in case of interaction of humic acid with ∞–HCH (Hexachlorocyclohexane), Cr, Cu and a hyperfine splitting pattern was noticed only in case of Mn. But such phenomena does not occur with Ca, Cd, Pb, As, Ni, Co, Zn and Fe.

Li et al., 2004, fractionated humic acid into eight fractions by ultra filtration apparatus with membranes having seven molecular cut-off and of the eight HA fractions using elemental analysis, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), Fourier transform infrared spectroscopy (FTIR), Ultra-violet- visible (UV-Vis) and solid state ^{13} C-nuclear magnetic resonance spectroscopy. The UV-VIS spectroscopy an PY-GC-MS analyses indicate that the fractions with lower molecular weights have more heterogeneous functional groups, greater O/C atomic ratios and higher contents of oxygen an lignin derived aromatic structural units. Conversely, the HA fractions with higher molecular weights have lower contents of oxygen and aromatic structural units that correspond to greater H/C and lower

O/C atomic ratios, which suggests that Has formed under the same biogeochemical conditions may consist of macromolecule with a range of chemical, structural and molecular properties.

Baddi et al., 2003, studied the evolution of the humic acids generated during composting of olive mill wastes by elemental analysis and spectroscopic methods (Fourier Transform Infra Red: FTIR and Nuclear magnetic Resonance; C13 NMR), in order to determine the stability and maturity of the final compost. During Composting, the humic acids presented a decrease of the peptidic and aliphatic structures and an increase of the aromatic structures. This confirms the stability of the final product after 12 months of composting. It results from this study that the quality of the humic substances generated could be a useful indicator to evaluate the maturity of the final products and too assess its agronomic value.

Mansuy et al., 2001, performed humic acid characterization in highly polluted river sediments by infrared spectroscopy and Py-GC-MS in identifying the different organic contributions to HA and in differentiating compound having a natural origin (terrestrial inputs, such as lignin derived compounds) from anthropogenic compounds (sterols and to a lesser extent fatty acids). The former belong to the structure of humic acids while the latter are mainly free lipids trapped in the HA structure that cannot be fully recovered by solvent extraction.

The above literature review indicated that lot of work has been carried out on humification and characterization of humic acid from various kinds of organic waste like Olive mill waste, municipal solid waste etc, all over the world. In India as well several works have been carried out on composting of municipal solid waste, pressmud and paper sludge. Some studies have also been carried out on the metal binding capacity of humic acid, but none have carried out the detailed studies on the elemental and functional groups characterization of humic acid extracted from the composted pressmud and paper sludge using distillery effluents. Hence, the present work has attempted to characterize the sludge and its components like Humic acid and to get an insight into the impact of these agro industrial waste dumping on the ground and surface water quality particularly in two climatic zones of India Maharashtra (Tropical) and Uttar Pradesh (Subtropical).

Study Area

Chapter 3

STUDY AREA

3.1 OVERVIEW

The major sugarcane producing states in the tropical areas of India includes Maharashtra, Andhra Pradesh (AP), Tamilnadu (TN), and Gujarat where the yield is around 70 to 100 thousand tons per ha and Subtropical regions include Uttar Pradesh, Bihar, Punjab and Haryana where the yield is around 40 to 70 thousand tons per ha. This region faces very extreme of weather. The high productivity of sugarcane renders these regions of India most suitable to establish maximum units of sugar mills, molasses based distilleries and paper mills. Sugar, distillery and paper industries are generally occur in vicinity of each other because in most of the cases, the discarded materials from one segment may be a by-product and can be used as raw material for other segments, For example, the molasses and the bagasse are discards from a sugar mill; but, they are the raw materials in distilleries and pulp & paper mills respectively.

Thus the agro-based industries of two different climatic zones (Tropical and Subtropical) have been chosen as the study area for the present work. The two different climatic zones have been selected for the comparatives studies of various parameters. Samples includes effluents, ground water, solid waste and soil were collected from the sugar mills, paper mills, distilleries and near by areas of the aforesaid industries of Lucknow, Faizabad and Meerut in UttarPradesh (subtropical) and Aurangabad and Nasik in Maharashtra (Tropical).

3.1 LUCKNOW (UTTAR PRADESH)

3.1.1 LOCATION

Lucknow district is located in the central part of the state of Uttar Pradesh, lying between latitudes $26^{0}30'$ to $27^{0}10'$ north and $80^{0}32'30''$ to $81^{0}\ 12'$ east longitude extending over an area 2560 sq km.,with a total population of 27, 62,01 persons (census of India 1991).

3.1.2 RIVER

The district of Lucknow forms part of great Indo-Gangetic plain and is situated in between the Ganga and Ghaghra rivers. Though, the district is almost a plain area, but the levels of northern partis comparatively higher than the southern part. The Gomti River is flowing across the district from northwest to southeast direction.

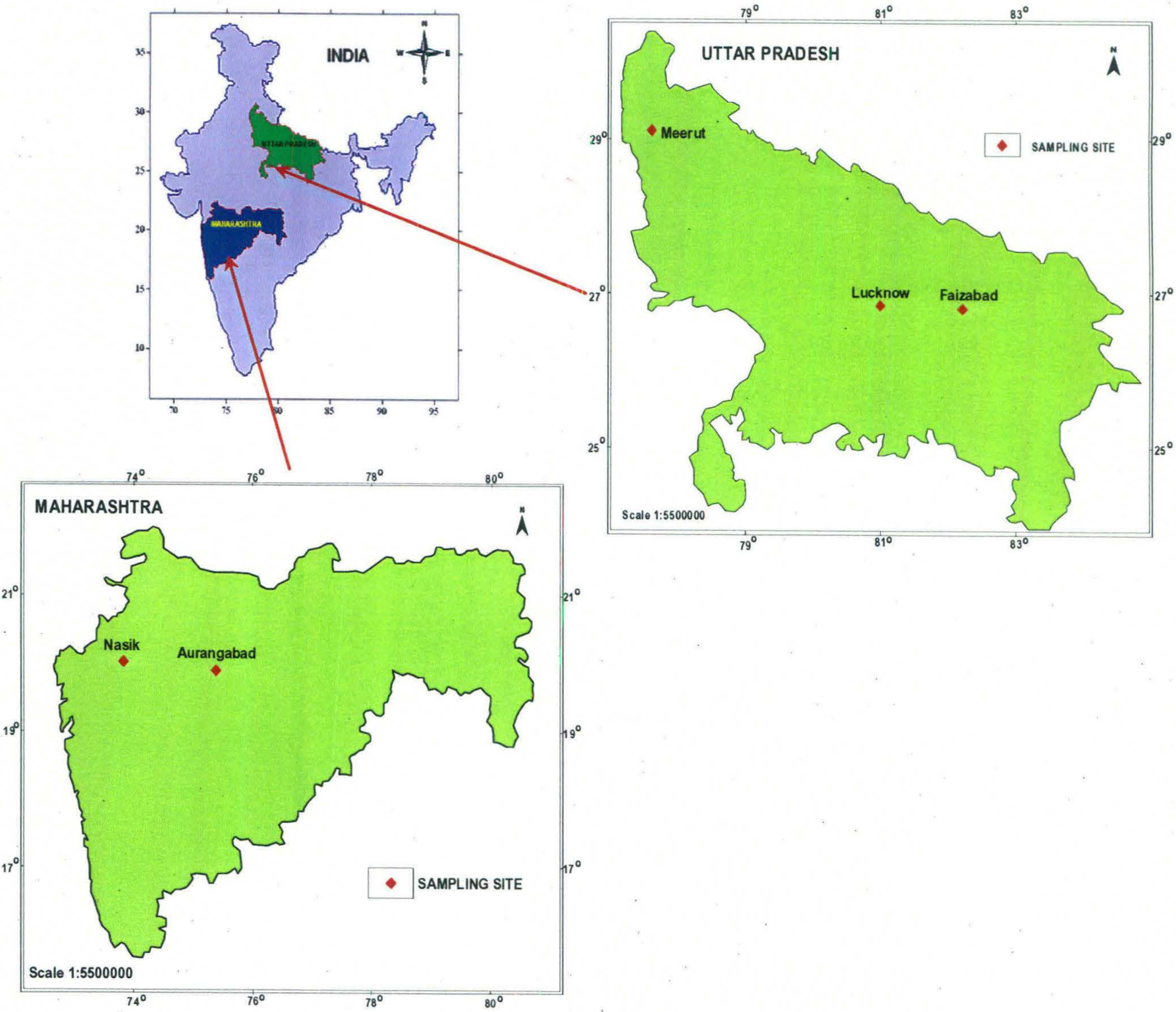


Figure 3.1 Map showing location of Study Area

3.1.3 CLIMATE AND RAINFALL

The maximum temperature of the district is 43.6^{0}Celcius while the minimum temperature is 5^{0}C. The average rainfall is 963 mm.

3.1.4 GEOLOGY

Geologically, the district is part of Indo Gangetic plain, so the rock type is alluvium in character.

3.1.5 SOIL TYPE

The soil associations of the different parts of the district are of two types i.e. (a) Aridisol (saline and saline alkai soil (b) Alfisol (older alluvial soil).

3.1.6 AGRICULTURAL ACTIVITIES

Net sown area of the district is 60.6 percent followed by forest 4.5 percent, salinity and uncultivable land 9.4 percent and other fallow land 13.4 percent and 1.3 percent grassland. From the agricutural point of view, wheat cultivation (44.0%) occupies predominant position followed by paddy (30.2%), pulses (12.0%), sugarcane (4.0%), oilseeds and other crops (10.7%).Traditionally, Lucknow has been a *mandi* town for mangoes, melons, and grains grown in the surrounding areas. The district economy is mainly based on agriculture followed by small-cum-cottage industries. According to 1991 census, the urban and rural population was 17, 31,224 persons and 10, 31,577 persons respectively. Out of the total workers, 11.0 percent are engaged in agricultural activities. Sugarcane-growing plantations and sugar industries are also in close proximity. This attracted Mohan Meakins Brewery to set up a unit based on molasses in the city. Meakins was incorporated in 1855 and is Asia's first commercial brewery.

3.1.7 INDUSTRIES

Four medium scale industries such as (Aeronautical limited, Eveready flush light company, India Cooper paper mill company limited etc), 7,686 small scale industries (i.e. cotton textile, rubber products, paper and board mills, distilleries, dairies and milk product etc.) are located mainly at Lucknow and nearby areas of Lucknow.

(Source: Singh et al., 2008)

3.2 FAIZABAD (UTTAR PRADESH)

3.2.1 LOCATION

Faizabad district which literary means the city of beneficence lie between 26^{0}24'30'' to 26^{0}52'30'' north latitude and 81^{0}42'30''to 82^{0}28'30'' east longitude occupying an area of 2764 sq kms.

3.2.2 RIVER

River Ghagra (Saryu) is the main river of the district makes its northern boundary.

3.2.3 CLIMATE AND RAINFALL

The Faizabad experiences a subtropical climate which usually having more or less extreme type i.e. a very hot summers and chilly winters. The maximum and minimum temperature varies from 43.7^{0}C to 1.2^{0}C in summers and winters respectively. The average annual rainfall of the district is 1400 mm.

3.2.4 GEOLOGY

Geologically the district lies in the region which is formed of Pleistocene to sub-recent alluvial deposits of the rivers of indo-Gangetic system where older rock surface have completely buried under the thick recent alluvium. The district has no exploitable minerals except Kankar (calcareous matter).

3.2.5 AGRICULTURAL ACTIVITIES

Agriculture is the main occupation of the people of the district as more than 80% of the total working population is engaged in agricultural activities. Paddy is the principal crop of the district followed by wheat, gram, sugarcane, peas, arahar and potato.

3.2.6 INDUSTRIES

Sugar and cement Industries are the major industrial units of the districts as these dominate the larger employment of the people in the district. Small and medium scale industries are also not much less in the district. They are iron and aluminum, textile and agro based manufacturing.

Source: (Gazette Of Uttar Pradesh, 1991)

3.3 MEERUT

3.3.1 LOCATION

Meerut is situated in the central part of the Ganges-Yamuna Doab, falling between latitudes 29 ^{0} 00'N to 29 ^{0}20'N and longitudes 77 ^{0} 30'E to 78 ^{0}00' East in Meerut district of Uttar Pradesh, and covering on area approximately 2060 km^2.

3.3.2 RIVER

Holy River Ganga flows along the eastern boundry of Meerut city North-South direction.

3.3.3 CLIMATE AND RAINFALL

Climatically, the area belongs to dry sub-humid to moist-humid category with hot summers and cold winters. The temperature varies from 3^0 C to 4^0 C in January to 43^0 C to 45^0 C in May or June. The normal annual rainfall varies from 1050 mm in the north to 650 mm in the south. Around 90% of annual rainfall occurs in monsoon season (June to October).

3.3.4 SOIL

Two soil pedons (an Aquic and an Udic Hapulstalf) represents the wheat – sugar cane belt of indo-gangetic alluvium plain of Meerut district.

3.3.5 INDUSTRIES

Meerut City is famous as an industrial city. It is rich in agriculture area with such pockets of land that do not fit in for crop purpose. It is thus ideal for industry. Existing and prospective industry: Textile, Transformer, Sugar, Distillery, Chemical, Engineering, pulp and paper mill.

Source: (Gazzette Of Uttar Pradesh, 1991)

3.4 AURANGABAD (MAHARASHTRA)

3.4.1 LOCATION

The district of Aurangabad and is situated between the parallels of 19° 17' 30" and 20° 40' 10" north latitude, and between the meridians of 74° 39' 30' and 76° 40' east longitude, covering an area of about 6,986 square miles. Its greatest length from east to west is 130 miles, and its greatest breadth from north to south 95 miles.

3.4.2 RIVER

Aurangabad District is located mainly in Godavari Basin and its some part towards North West of Tapi River Basin. This District's general down level is towards South and East and North West part comes in Purna–Godavari River basin. The other sub rivers of Godavari are Purna, Shivna, Kham, Dudhna, Galhati and Girja rivers.

3.4.3 CLIMATE

In Aurangabad rainy season starts from the month of June to September- and October to February- Winter Season and March to May Summer Season. The Average rain fall of Aurangabad District is 734 mm and the minimum temperature is 5.6^0C maximum temperature is 45.9^0C.

3.4.4 GEOLOGY

The district is covered by Deccan Trap basaltic flows of Upper Cretaceous to Lower Eocene age. These flows are further classified as vesicular/zeolitic basalt, greenish colored amygdaloidal basalt, jointed / fractured basalt and hard massive basalt.

3.4.5 SOILS

According to the Revenue Survey Department, the soils are roughly divided into three classes i.e regar, masab, and garab; or utam, madhiam, and kanist. The first class soils are of a fine uniform texture, and vary in color from deep black to dark brown; the second class of uniform but coarser texture, and of a reddish color; and the third class of coarse gravelly or loose friable texture, varying from light brown to grey.

3.4.6 INDUSTRIES

Aurangabad has many large and small scale industries, beside many automobile and pharmaceutical companies it several agro-based industries like Shaw Wallace distillery near chikalthana industrial belt and Vishwa paper mill on Paithan road.

(Source: Gazzette of Maharashtra, 1991)

3.5 NASIK (MAHARASHTRA)

3.5.1 LOCATION

Nasik district, lying between 19° 35' and 20° 52' North latitude and 73° 16' and 74° 56' East longitude, with an area of 15,582'0 km.2 (6,015 sq. miles) has a population of 23,69,221 with 20 towns and 1,628 inhabited villages and 4 uninhabited villages as per the census of 1971.

3.5.2 GEOLOGY

The Great Trap region of the Deccan covers the whole district. It is entirely of volcanic formation. The volcanic portion consists of compact, stratified basalts, and an earthy trap. The basalts are the most conspicuous geological feature. To the west they lie in flat-topped ranges, separated by valleys, trending from west to east. In some flows the- basalt is columnar and then it weathers into the fantastic shapes. The formation at the base of the traps is chiefly amygdaloidal, containing quartz in vertical veins, crystals and zeolitic minerals, especially apophyllite weathering into a grey soil. The absence of laterite, which caps the summits of the hills to the south, is a curious feature in the geology of the area.

3.5.3 SOILS

As regards the soil, the valleys are filled with disintegrated basalt of various shades from grey to black, washed down by rain. It is of argillaceous nature. This soil is not favorable to the growth of large trees but it is very fertile for cereals and pulses. The black soil contains high alumina and carbonates of calcium and magnesium with variable amounts of potash, low nitrogen and phosphorus. The red soil is less common and is suitable for cultivation under a heavy and consistent rainfall.

3.5.4 CLIMATE AND RAINFALL

The climate of this district is characterized, by dryness except in the south-west monsoon season. The year may be divided into four seasons, the cold season from December to February followed by the hot season from March to May and the south-west monsoon season from June to September followed by the post-monsoon season during October and November.

The rainfall in the district is under the influence of south-west monsoon. However, its distribution all over the district is, in a considerable degree, affected by the relief of the district. There is an uneven distribution of the rainfall. On the one hand, the extreme west of the district receives maximum rainfall of 148 inches, on the other; it dwindles down to 20 inches in the central and eastern sectors of the district.

3.5.5 AGRICULTURAL ACTIVITIES

The economy of Nasik district is primarily agrarian in character. Agriculture is still the main-stay of approximately 74 per cent of the total population of the district. In this chapter an attempt is made to describe the various aspects of agriculture in the district. As the topography of the district affects the agricultural industry the district is situated partly in the basin of the Tapi River and partly in that of the Godavari.

3.5.6 INDUSTRIES

Nasik is an ideal centre for the establishment of an industrial estate as it has all the facilities required for such a project. Nasik district has total 7 agro-based industries which includes Sugar Factories, Paper mills and distilleries

(Source: Gazette of Maharashtra, 1991)

Material & Methods

Chapter 4

METHODS AND MATERIALS

4.1 COLLECTION OF WATER SAMPLES AND RAW MATERIALS FOR COMPOSTING AND CHARACTERIZATION OF HUMIC ACID

Fresh press-mud and paper sludge were collected from sugar mill and paper mills of Uttar Pradesh and Maharashtra respectively. Soil sample was also collected from the area in vicinity of distillery of Aurangabad for background study. The waste waters from the above mentioned industries were collected to study there physico-chemical properties. The groundwater of Lucknow, Nasik and Aurangabad were collected to study the impact of wastes from these agro based industries. The water samples were analyzed for the major cations and anions, dissolved organic carbon and other physico-chemical parameters.

4.2 PHYSICO-CHEMICAL PARAMETERS OF PRESSMUD, PAPER SLUDGE, SOIL AND WASTE WATER

pH, EC, Temperature and moisture content, Total nitrogen and Total organic carbon were calculated by following standard methods:

4.2.1 pH and EC

The pressmuds , paper sludge and soil of desired locations were air dried, crushed and passed through a 2mm sieves before the analysis, then the organic wastes of agro based industries and soil were analyzed for the pH, EC at a rate 1:5 (W:V) for organic wastes and 1:2.5 for soil by their respective electrodes.

4.2.2 Moisture content

The moisture content of the soil/ samples at any time more or less depends on its water holding capacity and environmental condition with time.10g of fresh solid wastes and soils were kept in hot-air oven at 105^0C in clean-dry petri-dishes separately. After the time interval of 24, 48 and 72 hrs they were weighed. The moisture content is normally expressed in percentage on weight basis (g of water/100 g oven dry soil) (Hesse, 1971)

% of moisture on dry wt basis = WmX100/Ws
Where, Wm= Wi-Ws
Wi= Initial weight of soil.
Ws= Dry weight of soil

4.2.3 TOTAL ORGANIC CARBON ORGANIC CARBON (WALKLEY- BLACK WET COMBUSTION METHOD)

500 mg of organic waste and soil sample were weighed in a 500ml Erlenmeyer flask.10 ml 1N $K_2Cr_2O_7$ solution was added with a pipette and swirled to mix, then 20ml of H_2SO_4 from a dispense was added carefully and mixed it gently. Since the reaction created enormous amounts of heat, hence solution was allowed to cool to room temperature (20-30 minutes). After which 20ml distilled water was added. Swirled again to mix . 5 drops of ferroin indicator was added and titrated the excess (unused part) of chromic acid with ferrous sulfate or ferrous ammonium sulfate to clear blue- brown end point (T ml) same procedure was followed with a blank (Bml)

Calculation:

$\% \ C_{org} = (B-T) \times N \times 3 \times 1.4 \times (100/\text{mg oven dry soil})$

$\% \text{ Organic matter} = \%C_{org} \times 100/58$

4.2.4 DIGESTION METHOD FOR TOTAL NITROGEN

Digestion Method for total nitrogen analysis: Samples were digested by using digestion method of Anderson (1993). 0.2 g of finely grounded sample was taken in digestion tube and 4.4 ml of digestion mixture (prepare by dissolving 0.42g Selenium powder and 14g lithium sulphate in 350 ml 30%(v/v) H_2O_2 and mixed. In this mixture 420 ml H_2SO_4 was added carefully while cooling in ice bath) was added in each tube. This was digested at 90^0C till solution becomes colorless. The solution was added in each tube stirred the final volume was made up to 100 ml by adding distilled water. The clear solution was used for analysis.

4.2.4.1 TOTAL NITROGEN

Nitrogen was determined by Kjeldahl Method by using steam distillation. Free ammonia was liberated from the solution by steam distillation in the presence of excess alkali. The distillate is collected in a receiver containing excess boric acid indicator and nitrogen is determined by acid titration.

Distillation process was done by transferring 10ml aliquot to reactor chamber and adding 5ml alkali mixture (prepared by dissolving 500g NaOH and 25g sodium thiosulphate in water and final volume was made up to 1000ml with distill water) and starting distillation immediately. About 25ml of distillate was collected in 5 ml boric acid –indicator solution (prepared by 0.3 g bromocresol green and 0.165 g methyl red were dissolved in 500ml of 95% ethanol to make the indicator mixture. 20g of boric acid was dissolved in 700ml distilled water and cooled and 200ml of 90%ethanol was added. 20 ml of mixed indicator was added to it and mixed well by shaking. 0.05N NaOH was added to it till 1ml of indicator with 1ml distilled water changes color from pink to pale green (okalibo,1993) the solution was then made up to 100 ml).The distillate was titrated against N/140 HCl to a grey point using micro burette.

Kjeldahl Nitrogen was calculated using the following equation:

$$\%N = \frac{(a-b)*N \text{ of HCl}*1.4}{V*S}$$

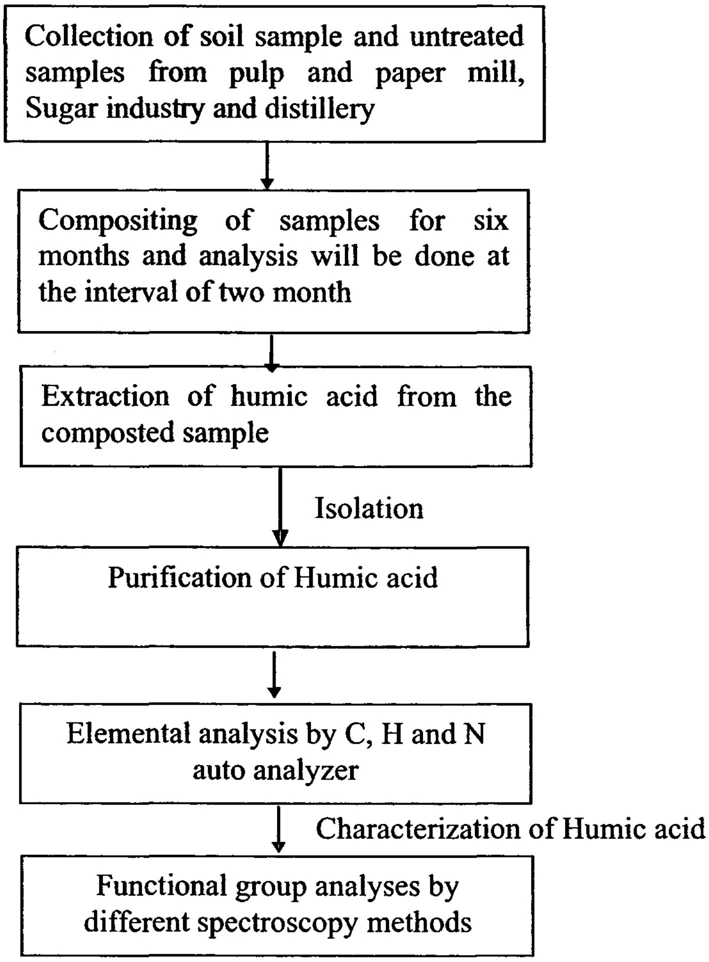


Fig 4.1 Flow chart showing different steps involved in Experimental Setup

4.3 METHOD OF COMPOSTING

Fresh press-mud a by-product from sugar mill and paper sludge was used as a base [main] material for composting. 3 Kg of each pressmud, paper sludge and soil from Aurangabad, Lucknow and Faizabad were kept in a concrete cemented pots and left for a week to dry up the samples then spent wash and microbial innoculum uniformly added at weekly intervals upto six months i.e. 180 days. The ratio of pressmud and spent was 1:3 (Satisha and Devrajan, 2007). The same ratio was maintained in case of paper sludge and soil samples. Spent wash (15lts) and microbial innoculum (3g) was added in each sample of pressmud, paper sludge and soil and mixed thoroughly by mechanical means using scooper and aeration was done by turning the compost mass over every fortnight. The microbiological activity was monitored and assessed by measuring the temperature of the heap (fig 4.1)

4.4 EXTRACTION OF HUMIC SUBSTANCES FROM THE COMPOSTED WASTES AND SOIL

20 g of composted samples were equilibrated to a pH value 1 to 2 with 1 N HCl at room temperature. Solution volumes were adjusted to 100 ml with MQ water. The suspensions were kept on shaker for 1 hr and then the supernatant were separated from the residue by centrifugation at 8000 rpm for 30 min followed by decantation. Supernatant were saved for the isolation of fulvic acid. Composted samples were neutralized with 1M NaOH was added. The suspensions were kept for intermittent shaking for 24 hr. the alkaline suspension was allowed to settle overnight and supernatant was collected by centrifugation and decantation. Supernatant was acidified with 6M HCl with constant stirring to pH 1 and then the suspension was allowed to stand for 12 to 16 hours to separate the humic acid (precipitate).Humic acid fraction was redissolved by adding a minimum volume of 0.1M KOH under N_2. Solid KCl was added to attain a concentration of 0.3 M $[K^+]$ and then centrifuged at high speed to remove the suspended solids. The humic acid was reprecipitated by adding 6M HCl, with constant stirring to adjust pH at 1 and the suspension was allowed to stand again for 24hr.The supernatant was centrifuged and discarded. The humic acid precipitate was suspended in 0.1M HCl / 0.3M HF solution in a plastic container and kept overnight for shaking at room temperature. Suspension was centrifuged and the HCl/HF treatment was repeated so that the ash content is below 1% humic acid was the freeze dried and kept at 4^0C (fig 4.2).

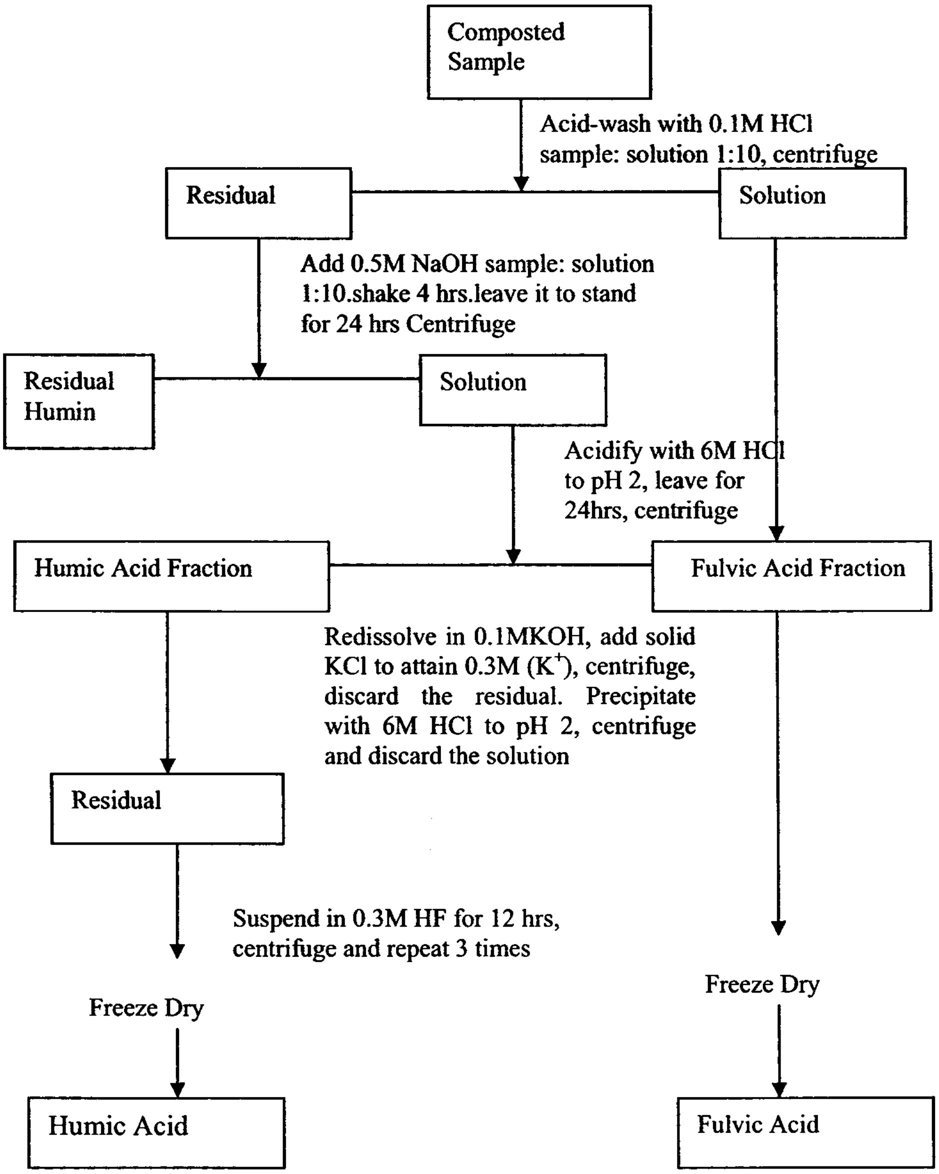


Fig 4.2 Showing different steps involved in the Extraction of Humic acid from the organic waste and soil

(Source:Ussiri and Johnson;2003)

4.5 CHARACTERIZATION OF HUMIC SUBSTANCES ELEMENTAL ANALYSIS

The mass percentage of carbon, hydrogen and nitrogen in each humic acid sample were analysed by EURO EA elemental analyzer. Humic acids composed of carbon, hydrogen, nitrogen and oxygen as major elements, along with sulphur and halogens present in practically negligible quantities.

4.6 UV-VIS SECTROPHOTOMETRIC MEASUREMENTS

All UV-VIS spectrophotometric absorbance measurements were performed by recording UV-Vis spectra within 800-200 nm range against blanks consisting of 0.1M NaOH for HA and FA determination. A rise in pH leads to an increased absorbance because of the increased electron density of chromopohres. To compare all the spectra of HA and FA, ph of all the samples were adjusted to 7. Since the maxima of the spectra can vary for different sediment samples, the UV-VIS spectrum for each sample was reorded rather than measurement at single wavelength. The E_2/E_3 (absorbance at 250m divided by absorbance at 365 nm) and $E_4/.E_6$ ratios (the absorbance at 465 nm divided by the absorbance at 665 nm) were calculated. (Chen et al., 1977; Malcom, 1989; Lassen et al., 1994)

4.7 INFRARED SPECTROMETRY

The pellets were preheated to 65^0C for 2 hr to eliminate the moisture interference,1mg of the predried sample was kept at 45^0C for about 18hr, mixed with 300mg of similarly pre dried sample KBr and grounded for 15 sec. A control pellet is also formed in the same manner, following which both the pellets are placed in vaccum oven at 100^0C for 2 hr. the pellet containing HA is scanned in double beam mode against the KBr blank (Stevenson and Goh, 1974). The spectra were recorded using Perkin Elmer RXI FTIR spectrophotometer.

This analysis was performed at SAIF, CDRI, Lucknow and Department of chemical engineering, BHU.

4.8 PROTON NMR SPECTROMETRY

Proton NMR spectra of 10 mg/ml solution of Humic acid in D_2O solvent were recorded in bruker DRX-300 (300 MHz FT NMR) spectrophotometer. The signal for D_2O was used as reference and set to 4.8 ppm chemical shift. The proton NMR was performed at Zydus Cadila, Ahamedabad.

4.9 C^{13} NMR SPECTROSCOPY

Liquid-state C^{13} NMR spectra recorded in 0.5M NaOD (50mg in 0.5 ml) on a Bruker 250 MHz instrument. Spectral width was set to 17 KHz and Pulse width 3.5µs (45^{0}C). The spectra were referenced to external TMS (tri-methyl silane) (Thomsen et al, 2002). Liquid-state C^{13} NMR spectra recorded at DRDO, New Delhi and Zydus Cadila, Ahmedabad.

4.10 GEL ELETROPHORESIS

4.10.1 SDS PAGE

SDS PAGE is a well-established technique for the separation of proteins. Polyacrylamide gels can also be used for the separation of humic acids on the basis of their molecular weight this parameter was performed at SES, JNU

4.10.2 METHOD

Purified humic acid samples were separated on 185 tris-cl SDS-Page. Page was run in vertical electrophoresis unit (Amarsham Pharmacia 600V), 20cm x 20 cm. glass gel pates were assembled according to manufacturer's instruction and acrylamide solution was poured into the gaps between the glass plates leaing sufficient space for stacking gel. 18% resolving gel was casted mixing 18% (v/v) acrylamide: bisacrylamide (29:1), m375mM Tris-cl pH 8.8 nd 0.1% SDS v/v. 0.1% APS and 6-7µl TEMED was used for the polymerization of the gel.After the polymerization was completed overlay was poured off and top was washed with deionised water. Stack gel was prepared mixing 5% (v/v) acrylamide: bisacrylamide (29:1), 125mM Tris-cl pH 6.8, 0.1% SDS. 0.1 % APS and 4-5µl of TEMED was used for the polymerization of the gel also.Stack gel was poured directly onto the surface of resolving gel, and immediately clean Teflon comb was inserted into the stacking gel solution between the glass plates. Comb was removed after the polymerization of the stack gel; wells were properly washed with deionised water and gel was mounted in the electrophoesis apparatus. (Cavani et al., 2003)

4.10.3 SAMPLE PREPARATION

Purified humic acid samples were dissolved in sample buffer (50 mM Tris-cl pH 6.8, 2%SDS (v/v), 10% glycerol (v/v). approximately 1mg humic acid was dissolved in 50µl sample buffer 20µl of each sample was loaded into the individual wells in predetermined order. Pre-stained protein weight molecular marker was run along with humic acid samples. Unused well were filled with IX sample buffer. Top and bottom reservoirs were filled wit tris-glycine electrophoresis buffer (25mM Tris-base, 250mM glycerine 0.1% SDS. Electrophoresis apparatus was attached to electric power supply (Amarsham Phrmacia). Gel was run at 25mA for 3hrs at constant volts. Gel was run until marker dye reached at the bottom of resolving gel. The glass plates were removed from the electrophoresis apparatus; gel was placed onto a transparent sheet, orientation was marked and it was scanned. The significant portion of humic acid is colored and can be visualized on the gel without staining.

4.11 MATRIX-ASSISTED LASER DESORPTION / IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF-MS)

This technique is characterized by a very high sensitivity in the range of subpicomoles and works with a mass accuracy and precision of 0.1–0.01% (Kaufmann 1995). During the MALDI process the 1mg of humic acid dissolved in 1 ml NaOH is mixed with an excess of a "matrix'' compound on a plate. A laser is then fired at the mixture and the energy from the laser is passed from the matrix to the analyte. This process causes ions of the analyte to be produced in the gas phase which are accelerated through a time-of-flight (TOF) tube in which they travel at a speed related to their mass and are detected by a mass analyzer to generate the mass spectrum. Generally mass spectra were measured in linear positive mode. MALDI-TOF was used in the present study primarily as it allows the detection of compounds with high and low masses with improved sensitivity. This analysis was done School of life sciences, JNU, New Delhi.

4.12 SAMPLE PREPARATION FOR MASS SPECTROMETRY ANALYSIS

All HA samples were dissolved in 0.036 *M* NaOH solution (1 mg in 1 ml 0.036 M NaOH). In order to perform MS analysis, 1 ml of such solution was dropped to a sample plate, dried in an air stream at room temperature and inserted into the vacuum chamber of the 24 instrument. After deep vacuum was reached (10 Pa) the mass spectra were measured. Always the resulting spectra were accumulated from at least 100 shots. (Gajdosova, et al., 2003)

4.13 WATER ANALYSIS

4.13.1 pH

pH was measured in unfiltered samples of groundwater surface water and waste water. The pH was measured by "CYBERSCAN 510". The glass electrode was conditioned (4M KCl as filling solution) and calibrated with buffer solution of pH 4.7 and 9.2. The temperature knob was set in accordance with the temperature of the water samples. The samples were stirred continuously in order to maintain homogeneity before noting down the pH.

4.13.2 EC (ELECTRICAL CONDUCTIVITY)

Conductivity was measured in micro-Siemens (µS) using "Systronics Conductivity Meter (P602)". The instrument was calibrated and set for 0.01M KCl solution (1413µS at 25^0C).

4.13.3 BICARBONATE (HCO_3^-)

The bicarbonate content was determined following the potentiometric titration method (APHA, 1995). Bicarbonate standards ranging from 100-1000 mg/l was prepared from $NaHCO_3$. 50 ml sample and a series of bicarbonate standards were titrated against 0.02N HCl. The end point was noted at pH 4.5. A standard graph was plotted between bicarbonate standards and volume of acid consume. The readings of sample were noted down from this graph.

4.13.4 ANIONS (CHLORIDE, FLUORIDE, BROMIDE, PHOSPHATE, NITRATE, SULPHATE IONS)

These anion were analyzed by using 'Dionex' Ion chromatograph with Ion Pac AS12A analytical column and Ion Pac AG12A guard column .The eluent used was 2.7mM sodium carbonate/0.3 mM sodium bicarbonate, the eluent flow rate maintained was 1.5-2.0 ml/min, expected background conductivity was14-16 µS for sample volume 10µl and maximum operating pressure was 3,500 psi.

4.13.5 CATIONS (SODIUM, POTASSIUM, CALCIUM AND AMMONIUM IONS)

Cations were analysed by using "Dionex" chromatograph with Ion Pac CS12A analytical and CG12A guard column, sample volume was 10µL, eluent used was 22mM H_2SO_4, the eluent flow rate was 1.0 ml/min at room temperature expected background conductivity was < 2-3µS, the operating pressure was below 4,000 psi

4.13.6 DISSOLVED ORGANIC CARBON

The 100 ppm stock solution was prepared from potassium per thalate ($C_8H_5O_4$) salt. Then standards ranges between 5 to 25 mg/l were prepared by serial dilution of stock solution. 10 ml of samples and standards were added to 50ml Erlenmeyer flask; 0.4 ml of buffer solution of pH 2 was added. Stirred it at a moderate speed for 10 min persulphate powder pillow was added to sample and to reagent blank vial.0.3 ml deionized water was added to reagent blank vial and 0.3 ml of prepared sample was added to the sample vial. Mixed it properly then blue indicator ampules was inserted in each vials. The vial assemblies were capped tightly and placed in the COD reactor for 2 hrs at 103 -105^0C. The vial assemblies were removed carefully from the reactor and placed them in a test tube rack. The vials were allowed to cool for one hour for accurate results. The optical density of these vials was taken at 430 nm (Hach process).

4.14 MULTIVARIATE ANALYSIS

4.14.1 CORRELATION MATRIX

Correlation matrix was constructed among the various composting parameters to assess the maturity and stability of the composts and between the various water quality parameters for the groundwater.

4.14.2 FACTOR ANALYSIS

Factor analysis for groundwater samples were computed using Statistical package SPSS-10.3". The extraction method for the factor analysis was "Principal Component Analysis" and "Varimax Rotation" was used for deriving factors.

4.14.3 CLUSTER ANALYSIS

Cluster analysis is another data reduction method which was used to classify entities with similar properties for cluster analysis single linkage method was used. In this method the distance between the clusters was determined by the distance of the two closest objects (nearest neighbour) in the different clusters.

4.16 GEOCHEMICAL PLOT

4.16.1 PIPER DIAGRAM

Piper diagrams were drawn by using "Aqua" software to classify groundwater samples into various types. Piper diagram has been widely used to study similarities and differences in the composition of waters and to classify them into certain chemical types. The water types demonstrated by the piper's diagram show the essential chemical character of different constituents in percentage reacting values, expressed in milligram equivalent.

4.16.2 SODIUM ADSORPTION RATIO (SAR)

The Sodium absorption ratio (SAR) is an important parameter for determination of suitability of irrigation water. It is an index of sodium/alkali hazard. This index quantifies the proportion of sodium (Na^+) to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in a sample. The sodium adsorption ratio (SAR) values for each water sample were calculated by using following equation (Richard 1954).

Result and Discussion

CHAPTER 5(I)

CARBON AND NITROGEN TRANSFORMATION DURING COMPOSTING OF AGRO INDUSTRIAL WASTE AND THEIR MATURITY ASSESSMENT BY HUMIFICATION PARAMETERS

Composting of organic waste is a well established method to obtain their chemical stabilization, biological maturation and sanitization before application to agricultural land. It consists of a managed biological transformation of organic matter operated by aerobic microorganisms which leads to an extended mineralization to carbon dioxide, ammonia and water, so that the end-product is relatively stable, reduced in quantity as compared to the initial amount of waste, and free from disturbing odors, pathogens and seeds. Basically, composting is an accelerated version of the processes involved through the provision of the most favorable conditions for microbial activity (Bernal et al., 1998)

Stability and maturity are two important factors for the successful use of organic wastes in agriculture with limited risks for the environment. Generally, the maturity is associated with plant growth potential or phytotoxicity (Lannotti et al., 1993), where as stability is often related with the compost's microbial activity. Both maturity and stability usually go hand in hand, since phytotoxic compounds are produced by the microorganism in unstable composts (Zucconi et al., 1985).

Nutrient balance is very much dependent on the type of organic matter being composted. Carbon provides the preliminary energy source and nitrogen quantity determines the microbial population growth. Hence, maintaining the correct C: N ratio is important to obtain good quality mature compost (Pace et al. 1995). At the same time, the most dangerous effect of immature compost utilization is an immobilization of nitrogen, polysaccharides and nutrient in the soil by micro organisms (Bernal et al, 1998).during bioconversion of the materials, concentration of carbon will be reduced while that of nitrogen will be increased, resulting in the reduction of C: N ratio at the end of the composting process.

Various parameters that have been used to assess the quality and maturity of composts include the organic matter degradation, C/N and C/H ratio, Humification Index, E_4/E_6 ratio and humus content of the finished compost (Garcia et al., 1992; Huang et al., 2001) and spectroscopic measurements including UV–VIS, FTIR and NMR (Hsu and Lo, 1999; Smidt ., 2001) which have been discussed in the next chapter.

Results of physico-chemical properties of waste water from distillery and paper mill have shown in table 5.1.1 and 5.1.2 pH, EC, Temperature and percentage of organic matter (OM) of composting materials and individual elemental analysis, HI, C/N, C/H and Cha of humic acid from the composted material at various stages have been shown in table 5.1.3 and 5.1.4 .

5.1.1 PHYSICO-CHEMICAL PARAMETERS OF WASTE WATER OF DISTILLERY AND PAPER MILL

The table 5.1.2 shows the waste water of distillery (spent wash) and paper mill were acidic with pH ranges between (3.94 - 6) and loaded with various organic and inorganic salts, resulting in high EC (10098-17245 µS/cm) since the input is plant originated, the spent wash also contains considerable amounts of plant nutrients and organic matter. Potassium ranges from 391-5849 mg/l. Calcium, Magnesium, Sulphate and Chloride were also present in appreciable amounts. 742.43 mg/l of Cl^- was detected in the waste water of paper mill of Aurangabad which is due to excessive use of chlorine as bleaching agent for the whitening of paper. Since these waste waters are rich in nutrients (K, Mg, Ca etc) hence, they can be used as a source of plant nutrients as well as soil amendments. The high concentration of Calcium (453-1045 mg/l) in waste water indicated their potentiality for reclaiming the sodic soils similar to that of gypsum.

5.1.2 PHYSICO-CHEMICAL PARAMETERS OF COMPOSTING MATERIALS (PRESSMUD, PAPER SLUDGE AND SOIL)

pH and EC

Values given in Table 5.1.3 shows that the initial and final pH of all the composting materials were within the acceptable range i.e. 6-8, suggesting its suitability for composting (Rinke, 1992, Miller, 1993). Organic matter degradation produce an increase in EC values in all the composting materials which result in the production of inorganic compounds and the increased relative concentration of other ions due to the mass loss of the materials (Paredes et al., 2001). In the later phases of composting the EC value of paper sludge Faizabad (PSF) and Soil Aurangabad (Soil A) showed a decrease in EC, which could be explained by the volatilization of ammonia and the precipitation of mineral salts, as observed earlier by the (Huang et al., 2004)

TEMPERATURE EVOLUTION OF THE COMPOST

Temperature is one of the main parameters used to monitor the composting process, since its evolution is depends on many of the biological reactions that take place, as well as it is also associated with its capacity of the process to reduce the pathogen contents (Stentiford, 1996).

All the composting mixture showed a typical composting temperature (>45^{0}C) during the first two weeks of composting and maintaining the thermophilic phase for approximately 78, 80, 110 and 125 days for pressmud Faizabad (PMF), pressmud Aurangabad (PMA), paper sludge Faizabad (PSF) and soil of Aurangabad (Soil A) respectively. Fig1 shows the PSF and soil A reached the thermophilic values faster and had a longer thermophilic phase than PMF and PMA. This could be due to the different character of the organic matter (OM) contained in the raw materials used , but no significant increase in temperature was observed after the homogeneity of the OM degradation conditions.

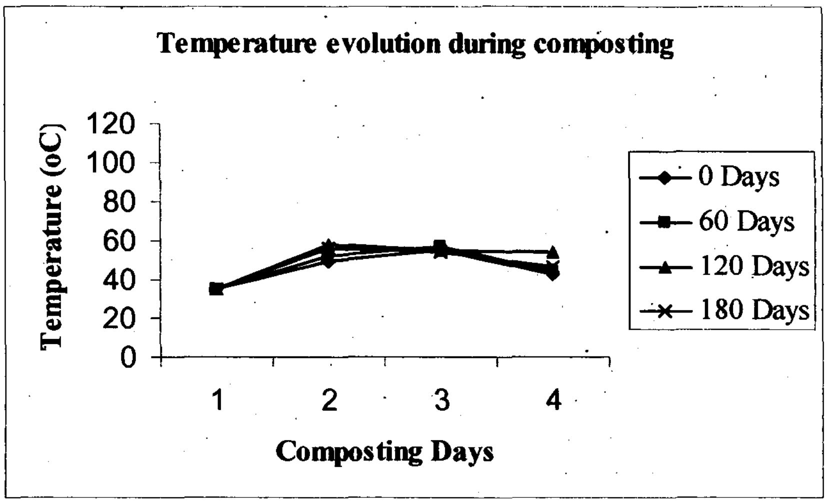


Fig 5.1.1 Showing temperature evolution during composting

ORGANIC MATTER DEGRADATION

The initial organic matter concentration of PMF and PMA was 89.78% and 85.47 % respectively, which is higher than that of the PSF (69.7 %) and Soil A (37.85%) Table 5.1.3. The final value of PMA, PMF, PSF and Soil A were 69.66, 70.5, 59.34 and 28.7 % respectively. This shows that organic matter of composted wastes decreases, where as the humic acid like fraction (Cha) increased in all the composting mixtures in the composting process. The decreasing concentration of organic matter indicates the degradation of organic materials during the composting process and the increased humic acid like fractions demonstrates the greater degree of humification of organic matters.

5.1.3 ELEMENTAL ANALYSIS

CARBON AND HYDROGEN RATIO (C/H)

The table (5.1.4) shows the decreasing pattern of carbon (C %) and hydrogen (H %) of humic acid in all the cases during composting. This reduction could be attributed to the loss in total dry mass due to losses of C as CO_2 and H as water vapour (H_2O) (Hamoda et al, 1998).The C/H ratio shows the degree of maturity of the humic substances and can also be considered as a source indicator of organic matter. In the present work C/H ratio shows an increasing trend in case of pressmud of Faizabad but no trend was observed throughout the composting process of humic acid in other samples thus it cannot be related to the degree of maturity in general.

NITROGEN EVOLUTION DURING COMPOSTING

Usually the nitrogen concentration increases during composting of organic waste but in the present study percentage of nitrogen (N %) in paper sludge increased from 0.80% to 6.55% during composting only, probably as a consequence of a concentration effect due to the weight reduction of the compost as observed by other authors (Predes et al., 2000; ferrer et al., 2001). Decreasing trend was observed in humic acid PMF (4.95% to 3.86%), PMA (5.41 % to 4.49%) and Soil A (5.75% to 4.87%), which could be due to their low C/N ratio at initial stage or may be consumed by the microbes present in the materials of organic wastes in building up their colony or lack of oxygenation.

CARBON AND NITROGEN (C/N) RATIO

The initial C / N ratio obtained in this study ranged from 8.28 lowest for the Soil A and 65 highest for the PSF, the final values of C/N ratio i.e. 9.22, 9.21, 6.33 and 6.61 for the humic acid extracted from PMF, PMA, PSF, SoilA respectively, suggests that all composts had reached an acceptable degree of maturation, since they were < 20, Golueke, (1981). However, in HA of PMF, PMA and soil A, the initial C/N ratio was already low i.e. < 15 thus this maturity parameter cannot be used for these composts, but its possible evolution i.e. deceasing trend of C/N during composting was given a good indication of the organic matter (OM) degradation process. However using a single parameter as a maturity indicator is insufficient and crossbreeding several parameters are usually required to assay the maturity of compost and is directly related to the humification process which can be measured by the HI= Cha/Ct*100, and E_4/E_6 (Amir et al., 2003; Rivero et al., 2004)

5.1.4 E_4/E_6 RATIO (OPTICAL PROPERTY)

The ratio of optical density or extinctions at 465 and 665 nm expressed as E_4/E_6., has been used by many investigators for the characterization of humic compounds (Konova, 1966; Campbell et al., 1967; Rashid and Vilks, 1977b). With increased humification, condensation and molecular weight, the E_4/E_6 values decrease. The ratio therefore can be used as an index of the degree of humification or condensation of humic molecules.

The E_4/E_6 ratio is related to the molecular weight and the oxygen content of the humic substances (Chen et al, 1977). The E_4/E_6 ratio is extensively used in soil science to indicate the humification degree (decomposition of organic matter); the progressive humification is indicated by a decreasing E_4/E_6 quotient. The scattering of monochromatic light in a dilute solution of macromolecules or colloidal particles is closely related to its weight, size and interaction of the particles in solution. Thus $E4/E_6$ data suggest the aggregation level, alongwith higher values indicating a more aggregated humic macromolecule (Pertusatti and Prado, 2007).

The initial values of E_4/E_6 ratio for HA extracted from PMF, PMA, PSF and Soil A as reported in table (5.1.4) were 9.59, 14.34, 7.8 and 7.85 respectively and their final values 3.69, 3.82, 9.54 and 3.08 respectively indicating a decreasing pattern in most of the Has. This is close to already reported values by the (Campitelli., 2008) which decreases with the humification process.

5.1.5 HUMIFICATION INDEX (HI)

The ratio between humic acid fraction (C_{ha}) and total carbon (C_t) in natural system such as compost, sludge and soil gives us humification index (HI). The greater the index is, the greater part of the organic substance has been transformed into humic acid (Ciavatta et al, 1993). The humic acid fraction, humification index and total carbon of organic waste and soil have been shown in the Table 4. In the present study the C_{ha} and HI of all the composting materials showing an increasing trend, indicating that a large proportion of organic waste is converted into humic acid.

5.1.6 CORRELATIONS BETWEEN MATURITY AND STABILITY

Using the each value of samples in their different stages of composting, correlations is found between the maturity and stability parameters. It is clear from the correlation matrix shown in table 5.1.5 that the humic acid like fractions (Cha) has good positive correlation with the humification index (HI) due to their close interdependence. This demonstrates that the rate of humification increased with more humic acid like formation was formed.

The percentage of organic matter (% OM) is positively correlated with the % of Carbon, % Hydrogen present in humic acid and the E_4/E_6 ratio which indicates that during composting process there was loss of organic matter, As a result Carbon, Hydrogen concentration and E_4/E_6 ratio significantly decreased. However the % OM was negatively correlated with the humification Index (HI) which showed the loss of organic matter with increase in rate of humification. Similarly the carbon percentage (% C) was strongly but negatively correlated with the HI. As the C/N ratio is one of the most suitable parameter to describe compost's degree of maturity, but it had not shown correlation with any other composting parameter. Thus it can be concluded that inspite of the prevailing good correlation between the various humification indices (i.e. HI, Cha, OM%, E_4/E_6) the compost of organic waste had not reached to its complete maturity, which could be due to excessive mineralization of organic matter with decrease of Nitrogen concentration in most of the composting materials except in the case of paper sludge.

Location	**pH**	**Moisture**	**Organic Carbon %**	**Total Nitrogen %**	**C/N**	**Cha (g/Kg)**	**HI**
PMF	7.3	55	39.5	1.5	26.33	10	2.63
PMA	7.45	50	38	1.27	29.92	10.5	2.66
PSF	7.8	59.8	28	1.12	27.45	16.25	6.16
SOIL A	6.98	52.45	13.34	0.98	13.61	6	44.98

Table: 5.1.1 Physico-chemical properties of raw materials used for composting

PMF : Pressmud of Faizabad
PMA : Pressmud of Aurangabad
PSF : Paper Sludge Faizabad
SOIL A : Soil Aurangabad

Samples	pH	EC	TDS	Cl	NO_3	SO_4	Na	K	Mg	Ca	DOC
Distillery LKO	4.5	17040	9530	532.4	30.03	9708.45	246.4	5600.3	1170.67	958	3930
Distillery Aurangabad	5	16895	8678	478.95	58.5	8756.8	305.9	5450.6	1775.98	1045.64	4150.95
DistilleryFaizabad	4.5	17245	9583	558.07	45.24	7650	680.7	5760.08	1559	1004.07	4085.63
Distillery Meerut	5.5	15460	8245	675	55.69	6980.9	526.62	5849.7	1680.6	899.75	4254.5
Paper mill Faizabad	5.65	12598	6583	345.6	40.08	5770.52	1085.97	768.82	458.9	453.2	850
Paper mill Auragabad	3.94	16140	9900	742.43	33.65	6205.88	955.66	391.23	148.52	687.92	298.07
Paper mill Nasik	4.45	10098	5770	268.79	38.16	7234.53	2117.7	905.4	206.95	592.57	740
Paper mill Meerut	6	15930	10,630	350.27	42.44	7856..75	1989	432.55	303.45	783.4	7180
Mean	4.9425	15175.8	8614.88	493.939	42.9738	7472.44	988.494	3144.84	913.009	803.069	3186.14
Min	3.94	10098	5770	268.79	30.03	5770.52	246.4	391.23	148.52	453.2	298.07
Max	6	17245	10630	742.43	58.5	9708.45	2117.7	5849.7	1775.98	1045.64	7180

Table5.1.2 Showing physico-chemical parameters of distillery spent wash and paper mill waste water(units)

Time Period	pH	EC (dS/m)	Temperature (^{0} C)	Organic Matter (OM) %
PMF				
T0	7.45	1.89	35.00	89.78
T2	7.15	2.04	50.00	83.69
T4	7.10	2.28	55.00	77.80
T6	7.00	2.45	42.50	69.66
PMA				
T0	7.3	1.8	35	85.47
T2	7.1	2.12	52.5	81.9
T4	7.05	2.3	56.8	78.23
T6	6.87	2.55	44.5	70.54
PSF				
T0	7.4	2.1	35	69.7
T2	7.1	3.02	58	65.43
T4	7.06	3.67	55	61.67
T6	6.7	2.9	45.5	59.34
Soil A				
T0	7.2	1.45	35	37.85
T2	7.08	2.12	56.5	33.57
T4	7.2	2.3	54	31.89
T6	6.98	2.25	46.7	28.7

Table 5.1.3 Evolution of principal parameters of composting material during humification process

Time Period	**%C**	**%H**	**%N**	**C/N**	**C/H**	**E4/E6**	**Cha**	**HI**
PMF								
T0	49.57	5.2	4.95	10.01	9.53	9.59	10	2.53
T2	46.96	5.07	4.56	10.30	9.26	9.76	30.5	7.72
T4	41.53	3.85	3.93	10.57	10.79	8.8	42.58	10.78
T6	35.69	2.84	3.86	9.25	12.57	3.69	48.62	12.31
PMA								
T0	50.52	4.38	5.41	9.34	11.53	14.34	10.5	2.76
T2	48.66	5.11	4.75	10.24	9.52	9.82	31.45	8.28
T4	47.47	4.98	4.88	9.73	9.53	7.06	41.78	10.99
T6	41	4.37	4.49	9.13	9.38	3.82	49.87	13.12
PSF								
T0	52.6	5.1	0.8	65.75	10.31	7.8	7.25	2.59
T2	47.5	5.05	5.6	8.48	9.41	8.96	18.25	6.52
T4	45.08	4.63	5.29	8.52	9.74	9.27	24.25	8.66
T6	44.5	4.14	6.55	6.79	10.75	9.54	29.75	10.63
Soil A								
T0	47.57	4.14	5.75	8.27	11.49	7.85	6	4.51
T2	40.65	4.02	5.68	7.16	10.11	5.62	10.7	8.05
T4	37.63	3.32	5.99	6.28	11.33	4.7	31.95	24.02
T6	32.19	3.14	4.87	6.61	10.25	3.08	47.5	35.71

Table 5.1.4 Characteristics of the organic matter of the wastes mixtures at different stages of humification process

	pH	**EC**	**Temp**	**OM%**	**C**	**H**	**N**	**C/N**	**C/H**	**E4/E6**	**Cha**	**HI**
pH	1.00											
EC	**-0.68**	1.00										
Temp	-0.41	0.40	1.00									
OM%	0.25	-0.12	-0.17	1.00								
C	**0.52**	-0.23	-0.30	**0.60**	1.00							
H	0.37	-0.09	-0.01	**0.61**	**0.88**	1.00						
N	-0.39	0.27	0.31	-0.33	-0.21	-0.18	1.00					
C/N	0.45	-0.16	-0.39	0.16	0.43	0.32	**-0.86**	1.00				
C/H	0.01	-0.16	-0.38	-0.28	-0.33	**-0.73**	0.03	-0.03	1.00			
E4/E6	0.36	-0.07	-0.19	**0.59**	**0.78**	0.60	0.11	0.05	-0.12	1.00		
Cha	**-0.64**	0.31	0.39	0.02	**-0.67**	-0.46	-0.02	-0.34	0.01	**-0.55**	1.00	
HI	-0.42	0.24	0.30	**-0.60**	**-0.84**	**-0.67**	0.18	-0.30	0.11	**-0.68**	**0.65**	1.00

Table 5.1.5 Correlation matrix between Chemical Characteristics and Maturity Indices

Chapter 5 (II)

CHARACTERIZATION OF HUMIC ACID FROM COMPOSTED WASTES OF PAPER MILL SUGAR MILLS AND TREATED SOIL

Humic acids are heterogeneous macromolecules naturally occurring in soils, sediments and organic wastes. They are among the major organic constituents actively participating in the global C cycle and controlling the fate of organic and inorganic pollutants in surface aquatic environments (Stevenson, 1994; Christl et al., 2001).

It has been shown that their chemical composition and structural properties are major factors governing their chemical and biological reactivity in the environment. For instance, the aliphatic portion of humic acids normally absorbs more hydrophobic pollutants than aromatics and polar components do (Kopinke et al., 2001; Salloum et al., 2002).

Many researchers are therefore interested in elucidation of the structure of humic acids. Some recent studies have proposed a supra molecular humic structure associated with weak interaction between many small molecules, to interpret the chemical properties of humic acids (Piccolo et al., 1996, 2002). Several physical and chemical methods are commonly used for characterizing structural and molecular properties of humic acids. These include infrared spectroscopy (IR), ^{1}H and/or ^{13}C nuclear magnetic resonance spectroscopy (1H & ^{13}C-NMR ;), .fluorescence spectroscopy, electron spin resonance spectroscopy (ESR), as well as chemical and thermal degradation techniques (Aiken et al., 1985; Stevenson, 1994).

Because of the compositional complexity, spectroscopic methods alone can only provide bulk information on molecular structures of humic acids. In comparison, chemical and/or thermal degradation techniques yield a large number of structural components resulting in more detailed molecular information on the various compositional features of humic macromolecules (Christman et al., 1989; Griffith and Schnitzer, 1989; Stevenson, 1994; Chefetz et al., 2002(a); Francioso et al., 2002; Li et al., 2004)

5.2.1FTIR ANALYSIS

Wave length	**Attribution**
3300 -3500 cm^{-1}	O-H vibrations of the hydroxyl groups of phenols, alcohols and carboxyl functions and N-H vibrations from amide ad amines.
2925 cm^{-1}	Symmetric CH stretching in-CH_3 and $-CH_2-$ of aliphatic chains
2840 cm^{-1}	Asymmetric CH stretching in $-CH_3$ and $-CH_2-$ of aliphatic chains
1725-1710 cm^{-1}	C=O stretching in carboxylic acids and /or in carbonyls, Ketones and aldehydes.
1654-1640 cm^{-1}	C=O stretching in quinones and /or in ketonic acids and primary amides
1540-1510 cm^{-1}	Aromatic C=C stretching and /or N-H deformation and C=N stretching in 2^0 amides
1460-1440 cm^{-1}	Aliphatic C-H deformation of structures such as fatty acids and waxes occurring in composts
1400-1380 cm^{-1}	O-H deformation, C=O stretching of phenols, anti- symmetric, COO- stretching and aliphatic C-H deformation.
1260-1200 cm^{-1}	C-OH stretching of aromatic groups and C-O-C stretching of aryl ethers and phenols.
1170 cm^{-1}	Alcohol function vibrations.
1080-1030 cm^{-1}	C-O-C stretching of carbohydrates
800-817 cm^{-1}	C-H deformation of substituted aromatic groups.

Table 5.2.1 Showing the attribution of various wavelengths in FTIR analysis.

(Source: Baddi et al., 2003)

Infrared bands were interpretated according to (Stevenson, 1982; Senesi and Plasza., 2007 and Fukushima., 2009). The FTIR spectra of each HA were observed. In each spectrum the major absorption bands were assigned as follows :- at approximately 3410 cm^{-1}, which corresponds to O-H stretching of hydroxyl groups of alcohols, phenols and organic acids as well as N-H groups. Two bands at 2850-2980 cm^{-1} are caused by symmetric and asymmetric stretching vibrations of C-H and CH_2 and CH_3 groups. The band (a shoulder in some cases) at 1715 cm^{-1} is attributed to the C=O stretching vibration of COOH, ketones, aldehydes and esters. The band around at 1640-1660 cm^{-1} may be related to aromatic c=c stretching; peak or shoulder at approximately 1510-1550 cm^{-1}' N-H deformation of aromatic amine or amide (amide=II band); peak at 1380-1480 cm^{-1} was attributed O-H deformation and C-H deformation of CH_2 and CH_3 groups, a weak peaks at 1220-1280 cm^{-1} was produced by amides or ethers and a broad band between 1120 and 980 cm^{-1} with a sharp peak centered near 1045 cm^{-1} was related to C-O stretching of polysaccharides or polysaccharide-like substances, as well as silicate impurities. Bands at 1126 and 1045 cm^{-1} are also characteristic of aromatic C-H in plain deformation for syringyl and guaiacyl alcohols, two structural component of lignin (Stevenson, 1994; Pretsch et al., 1998; Sun and Tomkinson, 2002; Amir et al., 2004; Ait Baddi et al., 2004a,b).

In general, transformations that occurred during the different composting processes were reflected by a decrease in the bands at 2925-2850 cm^{-1} (except for humic acid from pressmud of Aurangabad) and at 1040 cm^{-1}, and an increase in those at 1715, 1640, 1460 and 1400 cm^{-1}. The rise in these bands suggests an increase in carbonyl groups (COOH, Ketones, aldehydes, esters) as well as aromatic, phenolic and quinone structures. The changes observed by FTIR indicated the decrease, during composting, in aliphatic and polysaccharide structures and the increase in more oxidized and, probably, polycondensed aromatic components.

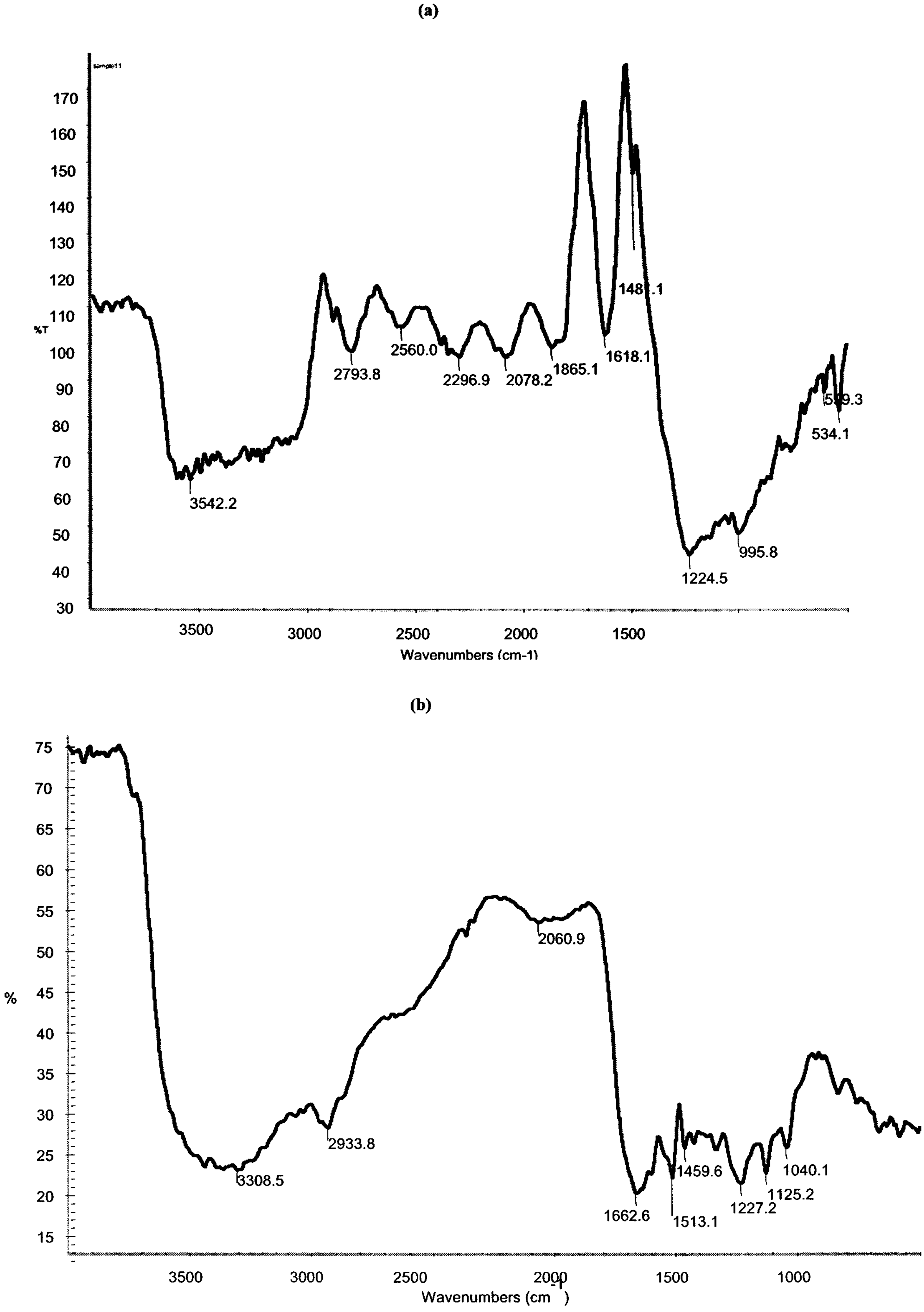


Fig 5.2.1 FTIR spectra of Humic acid extracted from pressmud of Aurangabad Intial and Final stage

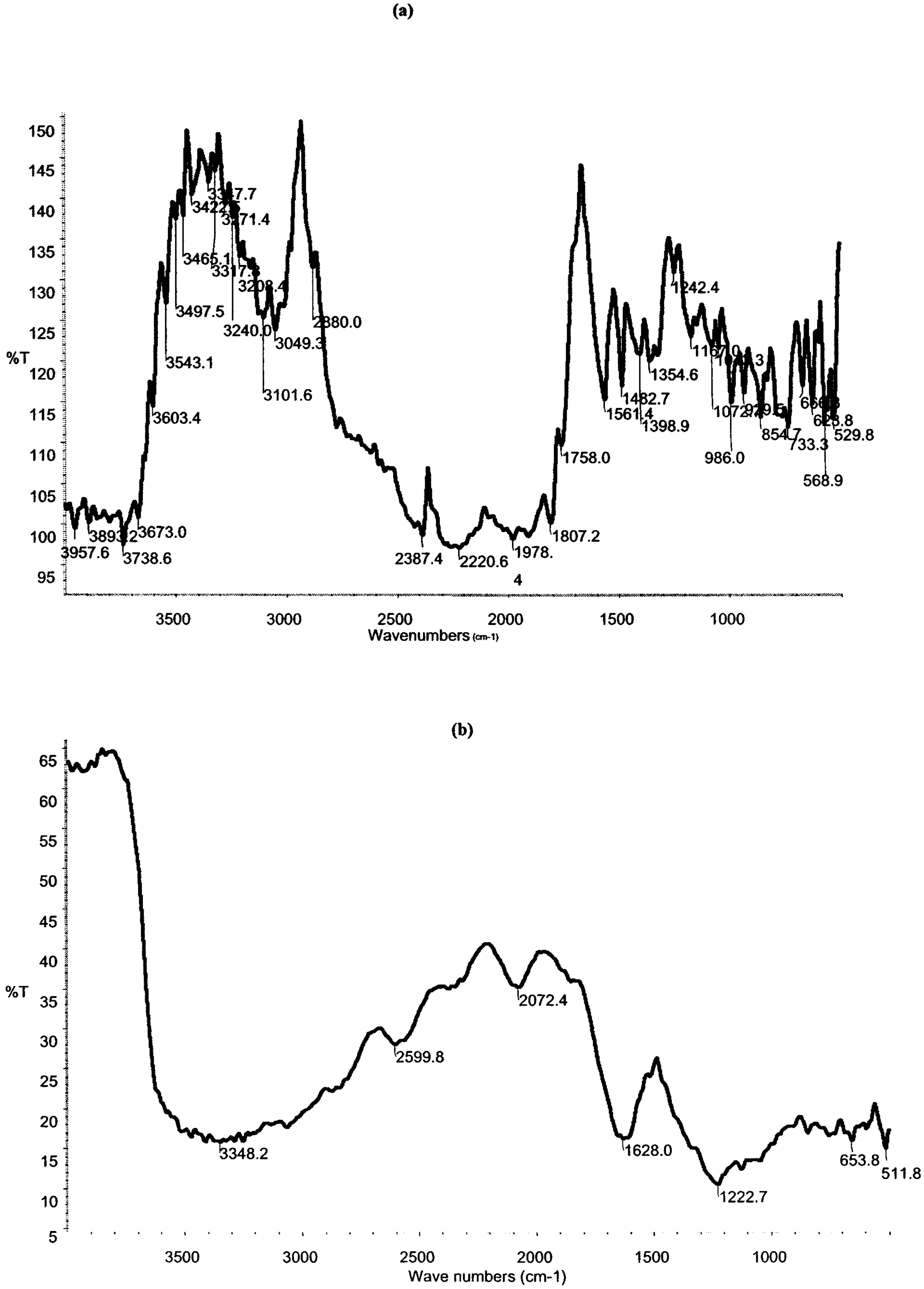


Fig 5.2.2 FTIR spectra of Humic acid extracted from pressmud of Faizabad Intial and Final stage

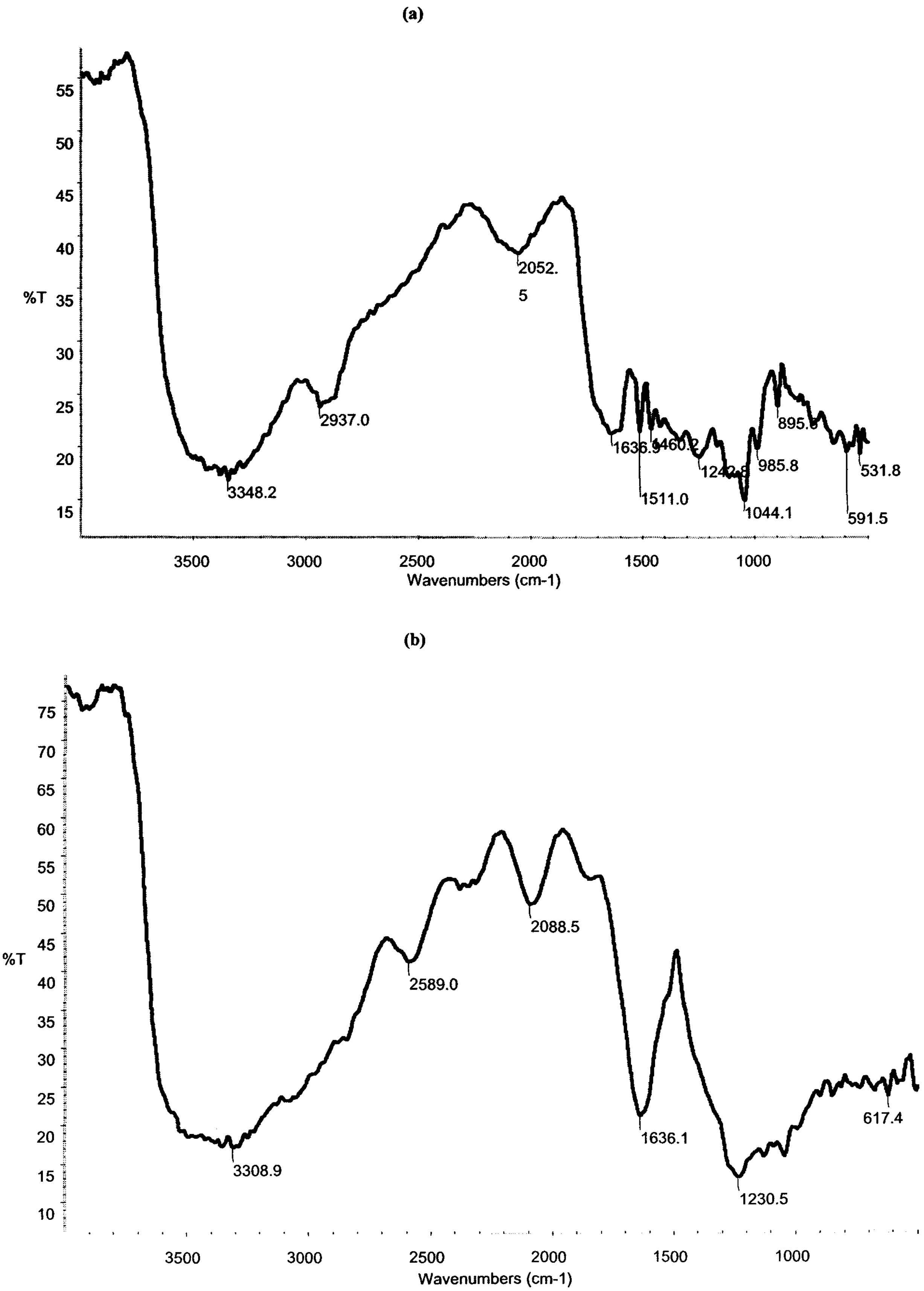


Fig 5.2.3 FTIR spectra of Humic acid extracted from paper sludge of Faizabad Intial and Final stage

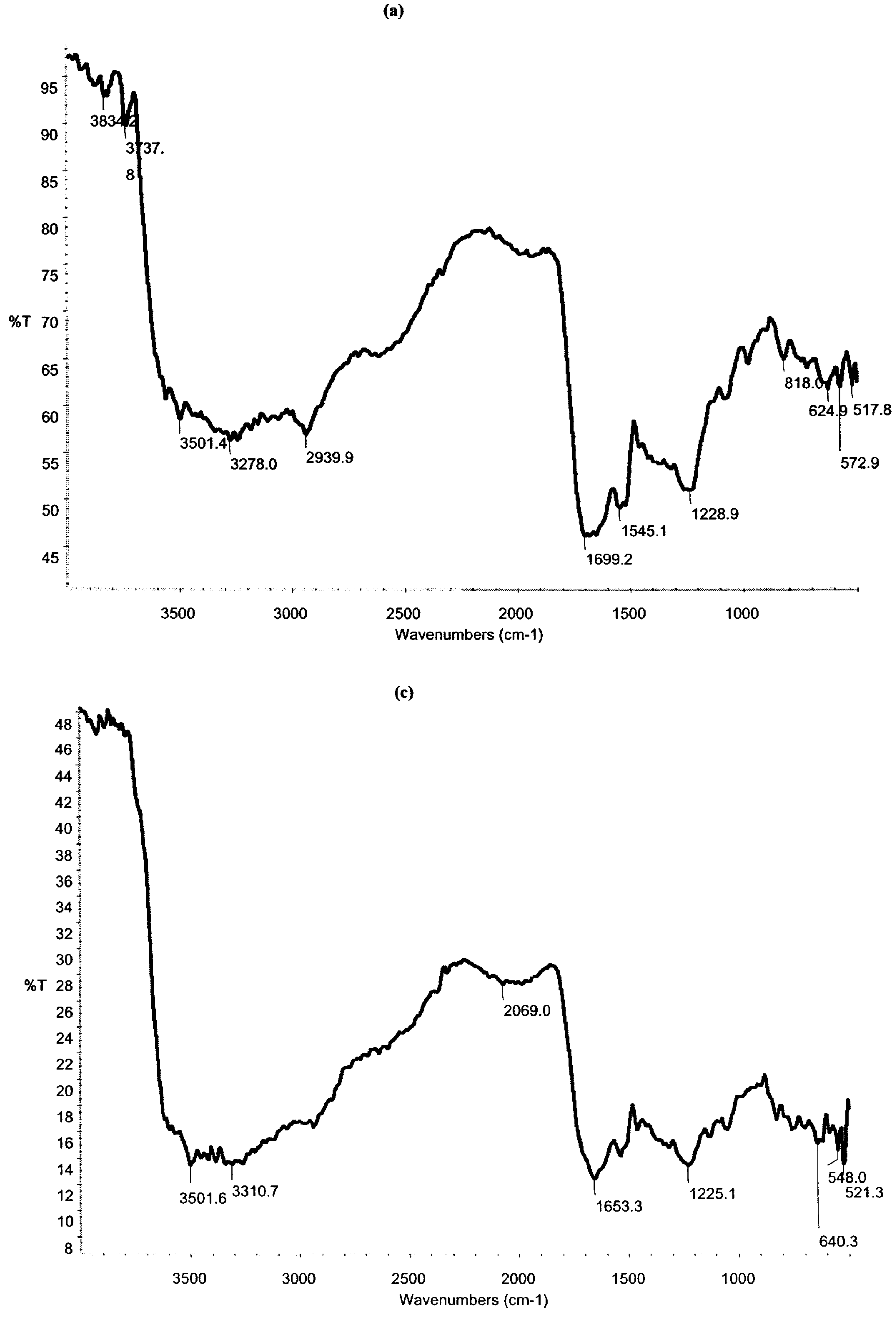


Fig 5.2.4 FTIR spectra of Humic acid extracted from soil of Aurangabad Intial and Final stage

5.2.2 ^{1} H NMR SPECTROSCOPY

Proton NMR spectroscopy is used for estimating the relative abundance of various humic substance functional groups. Spectra of humic acids dissolved in alkaline or neutral solution provide information that is useful for characterizing their aromatic and aliphatic components. General assignments of the proton NMR peaks are given in table 5.2.2.

Table 5.2.2 Major Proton resonance of humic materials

δ(relative to TMS as 0) ppm	**Assignment**
13.0	Carboxylic acid protons
10.0	Hydroxyl protons
6.0-7.5	Aromatic protons
4.0-5.5	Lactone protons
3.7	Methoxyl protons
2.6	Aliphaticprotons attached to C-α to benzene ring
1.3	Aliphatic protons β to benzene ring
0.9	Aliphatic protons γ to benzene ring

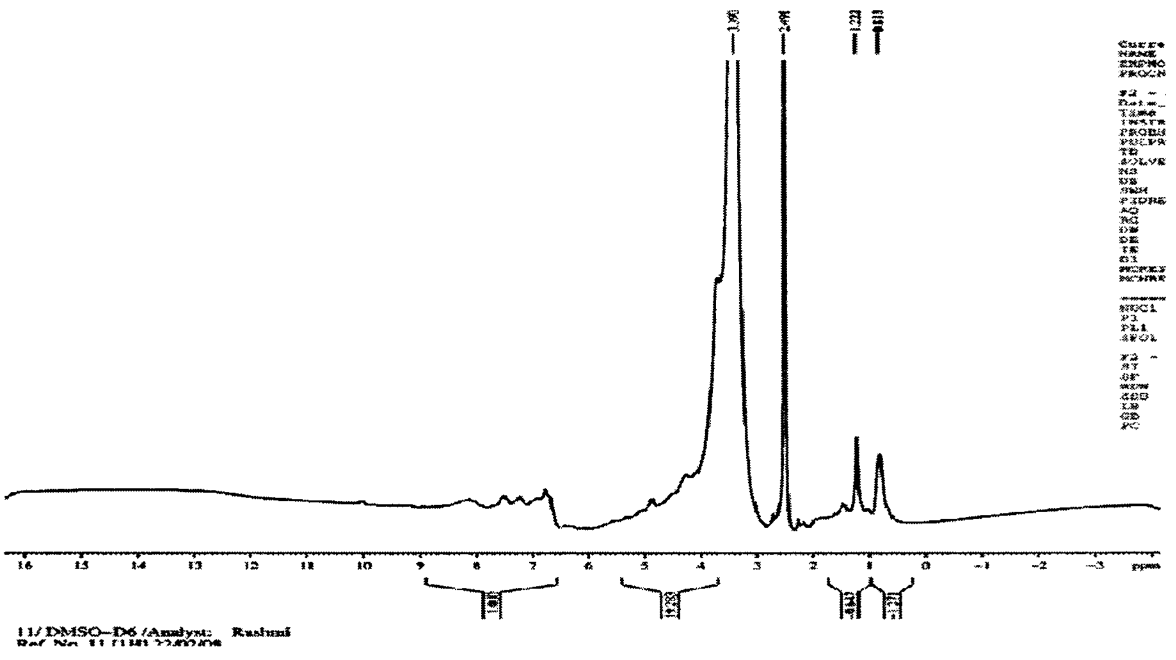
(Source: Aiken et al., 1985)

In the present study the ^{1}H-NMR spectra were divided into four chemical shift ranges and their assignments and interpretations (δ ppm; relative to tetramethylsilane) are given according to (Peuravaori and Pihlaja, 1998). The four main chemical shift ranges representing different types of hydrogen atoms were briefly: HA1 (cα. 0.2± 1.48 ppm; methyl and methylene protons of carbons directly bonded to other carbons); HA2 (cα. 1.48± 2.92 ppm; methylene and methine protons attached to aromatic rings, or carboxyl and carbonyl groups); HR±O (ca. 2.92±4.21 ppm; consists chiefly of protons attached to carbon atoms bound to oxygen); HAr (ca. 5.94±8.14 ppm; protons predominated by aromatic and not by olefinic carbons.

In the region of resonance of aliphatic protons, peaks around 0.815 are present in almost all the ^1H NMR spectra of humic acid extracted from different source which were assigned to protons of terminal methyl groups. Where as signal between 1.2 and 1.8 are attributed to methylene and methine protons. The peaks appearing in the range of 2.0-2.8 ppm can be assigned to protons adjacent to functional groups with electronegative atoms (carboxyl, amide, carbonyl or ester groups). Resonance for proteins also appears between 1.5 and 2.8 ppm.The signals covering the 3.2-4.4 ppm range include contributions fro\m H on C_α to an oxygen or nitrogen atom (Chen et al 2000, Kovac et al., 2004)

Protons attached to amino methine and /or methylene groups bonded to amide functional groups show resonance at 3.1-3.35 ppm (Montoneri et al., 2003), although having more intense signals. More intense peaks were observed between 3.7-3.9 ppm which were attributed to CHOH and CH_2OH groups. In the case of composts these functional groups may indicate the presence of methoxyphenylpropyl repeating units which typically occur in lignin, and /or the presence of polysaccharides moieties (Adani et al.,2006) The pressmud of Aurangabad (PMA) and paper sludge (PSF) have shown strong peaks in this region which might be due to high lignin content and polysaccharides moieties as compared to PMF (Pressmud Faizabad) but with the progress of humification process the intensity of peaks in this region have increased in all the samples except in soil which might be due to excessive degradation of lignin or polysaccharides.

The protons in aromatic region of all the spectra have shown significant increase with the period of humification process, which shows the stabilization and condensation process involved during humification process.



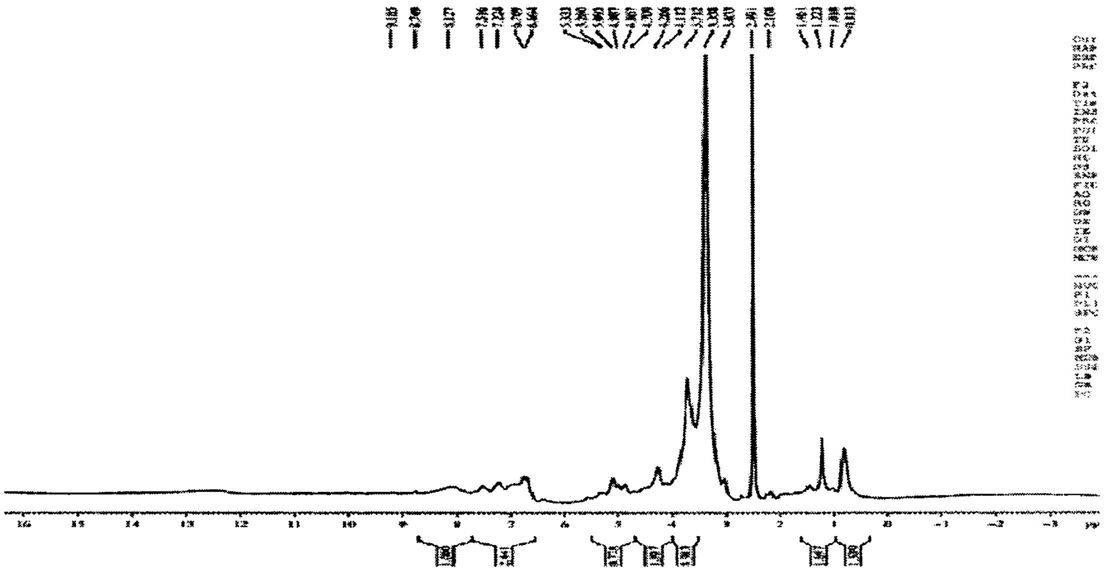


Fig5.2.5 showing proton NMR of humic acid from pressmud Aurangabad during controlled and last phase of composting

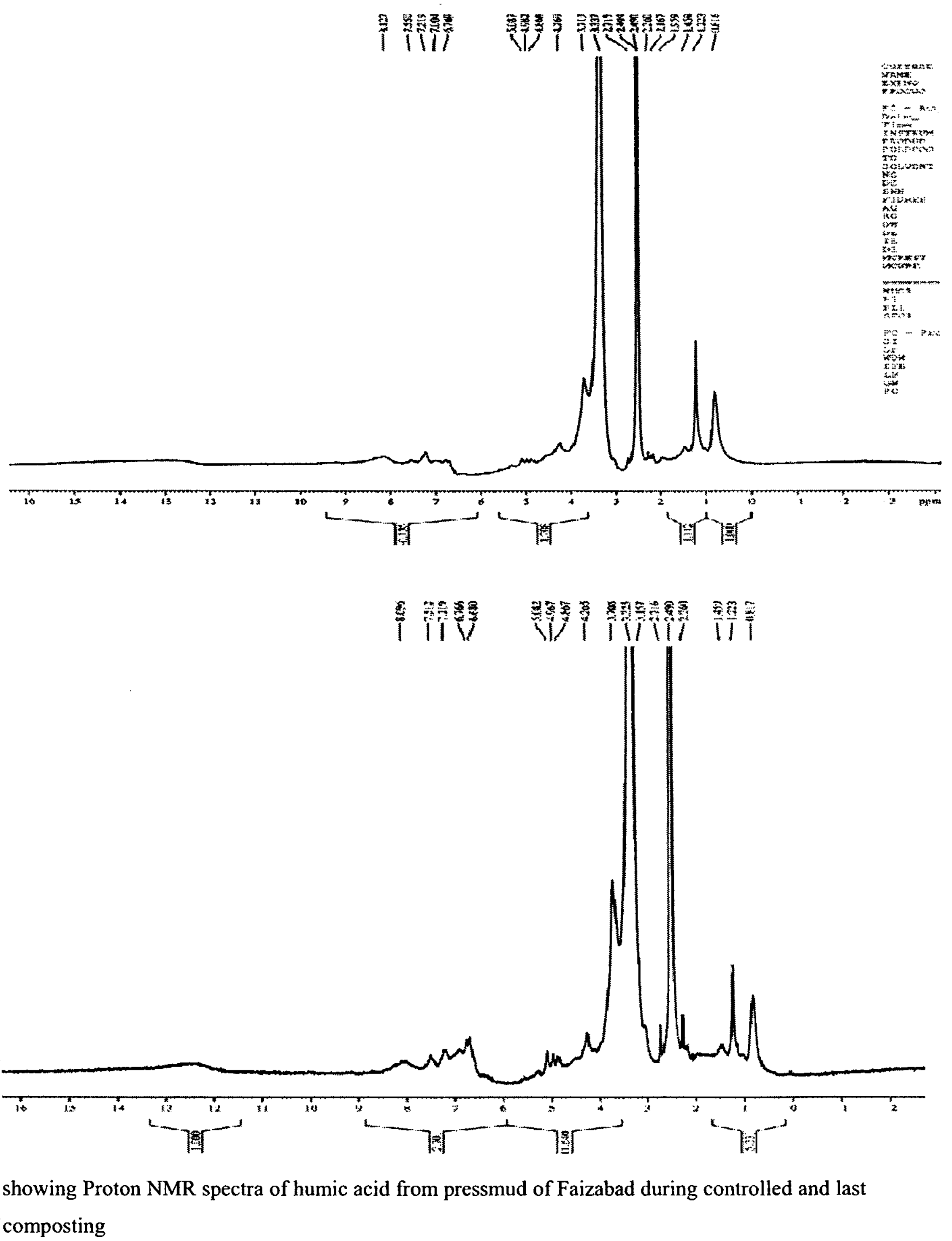
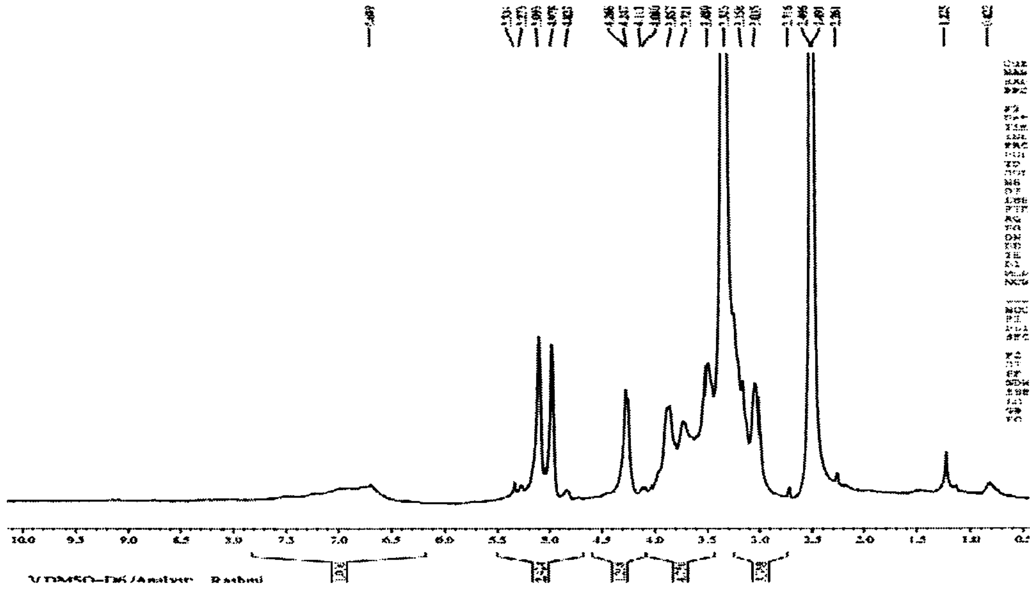


Fig5.2.6 showing Proton NMR spectra of humic acid from pressmud of Faizabad during controlled and last phase of composting



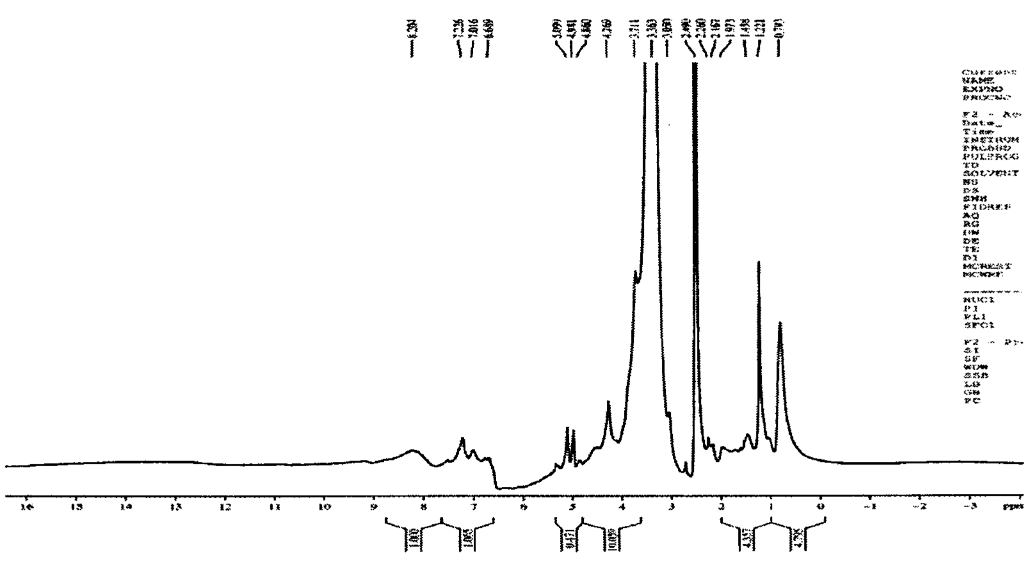


Fig5.2.7 showing Proton NMR spectra of humic acid from papersludge of Faizabad during controlled and last phase of composting

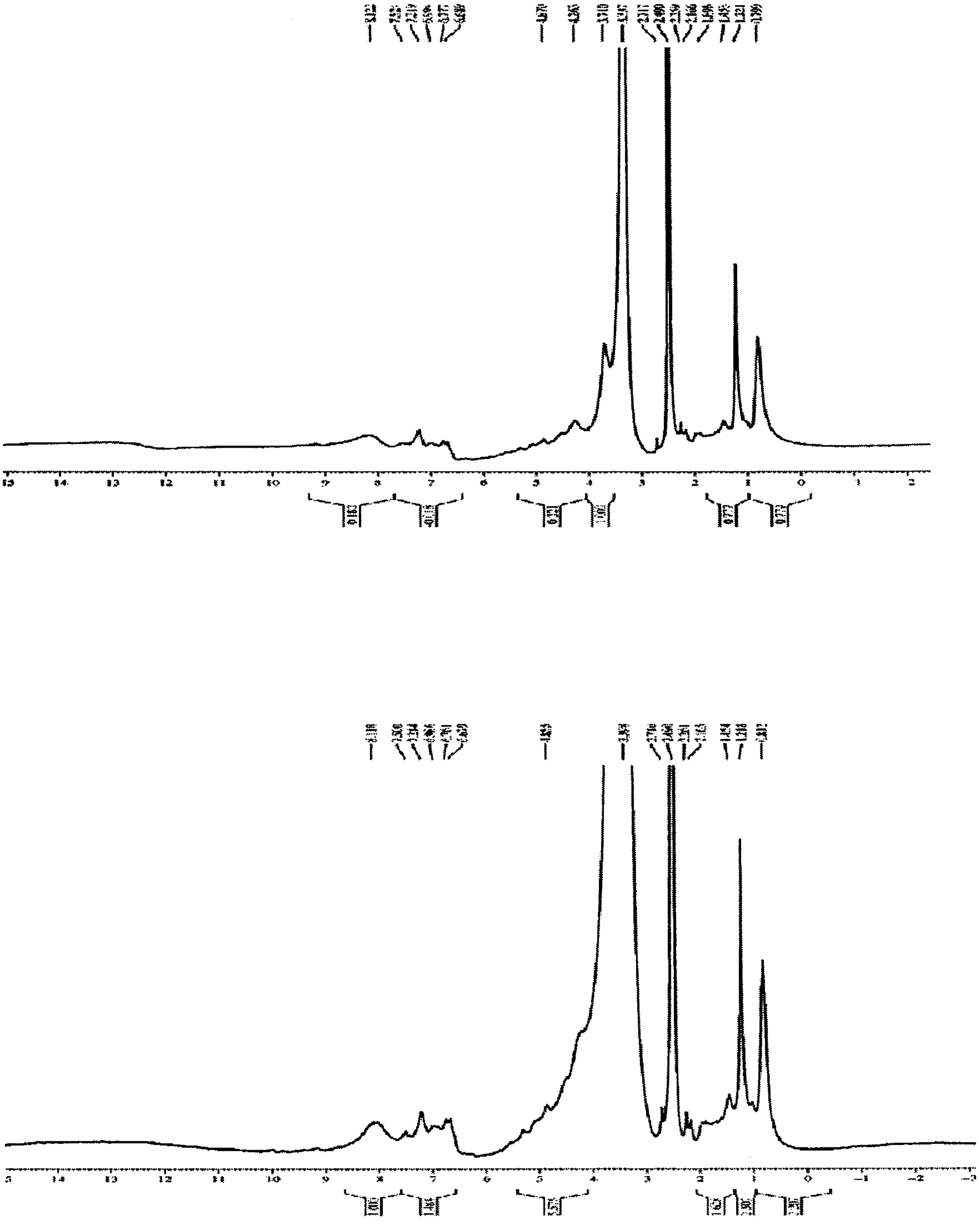


Fig5.2.8 showing: Proton NMR spectra of humic acid from soil of Aurangabad during controlled and last phase of composting

5.2.3 ^{13}C-NMR SPECTROSCOPY

Although ^{1}H NMR spectroscopy has been used for humic substance functional group characterization, analysis by ^{13}C NMR is generally preferred when detailed structure has to be elucidated. Because of the greater chemical shift dispersion of ^{13}C NMR spectroscopy, spectral overlap is reduced and minor structural changes can be detected more readily. Furthermore, ^{13}C NMR directly detects the carbon nuclei of the humic substance than the adjacent proton, allowing observation of ketones and carboxylate functional groups. These features make ^{13}C NMR especially useful for measuring the relative abundance of aliphatic, carbohydrate, aromatic and carboxyl carbons in humic samples. General assignments of the ^{13}C NMR peaks are given in table 5.2.3

Chemical shift δ (ppm)	**Attributed chemical groups**
0 - 50	Paraffinic carbon C in alkyl chain
50-110	Aliphatic carbon substituted by oxygen and nitrogen
110-130	Olefinic carbons; unsubstituted aromatic carbon
130-145	Carbon-substituted aromatic carbons
145-160	Oxygen or nitrogen substituted aromatic carbons
160-200	Carboxylic carbon, esters or amide

Table 5.2.3 Showing major ^{13}C NMR resonance
(Source: Baddi et al., 2003)

The ^{13}C NMR spectra of the humic acids from the various stages of composted wastes are shown in fig.5.2.9 to 5.2.16 and discussed as follows:

In the aliphatic carbon region, the signal at 16.5 ppm is characteristic of a terminal methyl group. The signals at 20 to 32 ppm are attributed to methylene groups in long-chain fatty acids (Preston and Schnitzer, 1984) while the signal at 37 ppm is originated from (-CH2) n in long alkyl chains. Ouatmane, 2000, attributed the occurrence of the latter peak to humic acids containing branched or long-chain aliphatic structures. In the present case of humification of various organic waste, the aliphatic structures were much more abundant in the first two months of composting.

The resonances observed between 40 and 105 ppm are from carbohydrates with oxygen-containing substituents (quinines, ketones, aldehydes and alcohols) and amino acid chains containing glycine (δ 42.6 ppm), arginine (δ 41.5 ppm) and lysine (δ 40.0 ppm) . However, the signals at 66, 70 to 80, 89 and 105 ppm are due to the presence of the - δ D glucopyranose unit of cellulose. In this region, the signal at 55 ppm is due to aromatic methoxy groups (-O-CH3). This peak was particularly intense during the first month of composting. In the region of aromatic carbons, the spectral range covered by the resonances indicated the variety of the substituents on the aromatic rings. Signals between 115 and 130 ppm are from strongly protonated aromatic structures.

Resonances between 130 and 147 ppm suggest carbon substitution of aromatic rings. Thus, the signals at 118 to 130 ppm are due to non-substituted aromatic carbons while the peak at 150 ppm is attributed to phenols and other aromatic rings substituted with carbon, oxygen or nitrogen. Similarly, the signals at 150–160 ppm are attributed to phenols and also to nitrobenzene and aromatic ethers. Carboxyl, amides and esters give an intense signal at 175 ppm. The strength of this peak reached a maximum at the 6th month of composting which could be related to an increase in the levels of nitrogen in the humic acids from the composted wastes and soil.

The spectra of the humic acids from composted wastes are very similar to those from manure, presenting a similar signal at 56 ppm corresponding to a methoxy carbon, low proportions of aromatic carbon and high levels of carboxyl and alkyl C-O groups. This suggests the presence of carbohydrates incorporated in the tannins or lignins.

The ^{13}C-NMR spectra were divided into eight chemical shift ranges and their assignments and interpretations (δ ppm; relative to tetramethylsilane) are given as follows. The main eight chemical shift assignments representing different kinds of carbon atoms were discussed briefly: CA (ca. 0±50 ppm; aliphatic carbons); CB (ca. 50±60 ppm; mainly methoxyl carbons (±OCH3)); CC (ca. 60±90 ppm; C±O resonances arising from, e.g., carbohydrate-type compounds); CD (ca. 90± 110 ppm; e.g. anomeric carbons of polysaccharides); CE (ca. 110±140 ppm; unsubstituted and alkyl-substituted aromatic carbons); CF (ca. 140±160 ppm; phenols, aromatic ethers or amines); CG (ca. 160± 190 ppm; represents largely resonances due to carboxyl carbons) and CH (ca. 190±220 ppm; carbonyl carbons of aldehydes and ketones).

It is clear from the ^{13} C-NMR spectra obtained in the present study that both the aliphatic as well as aromatic carbons are increasing during composting process, which reflects that during composting, the unstable organic compounds such as aliphatic materials are transformed through intense microbial activities into more stable humic compounds with more oxidized, olefinic or aromatic structures that could include more polycondensed rings as observed earlier by Amir et al., 2004.

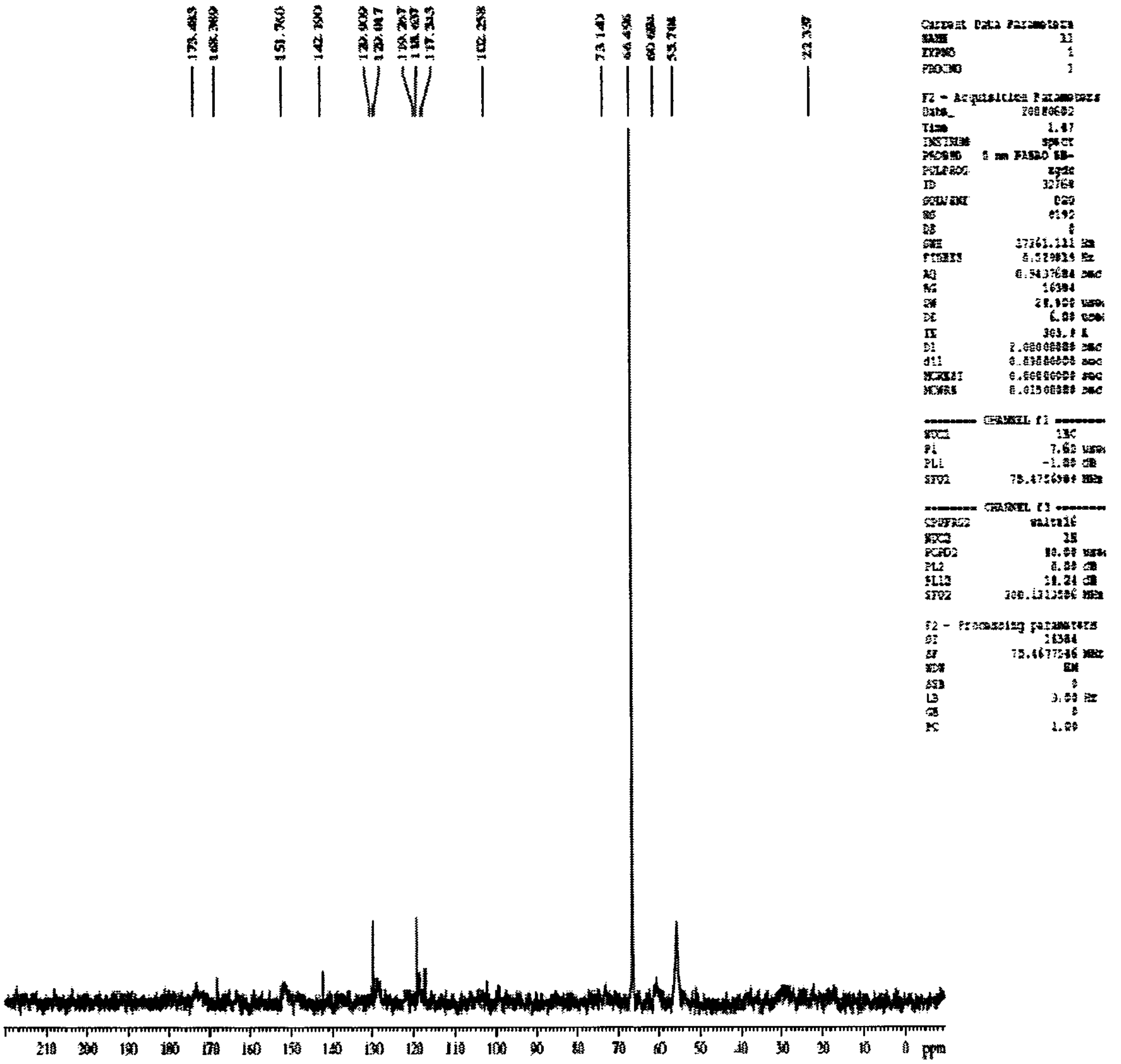


Fig 5.2.9 C^{13} NMR spectra of humic acid extracted from pressmud of Aurangabad at initial stage

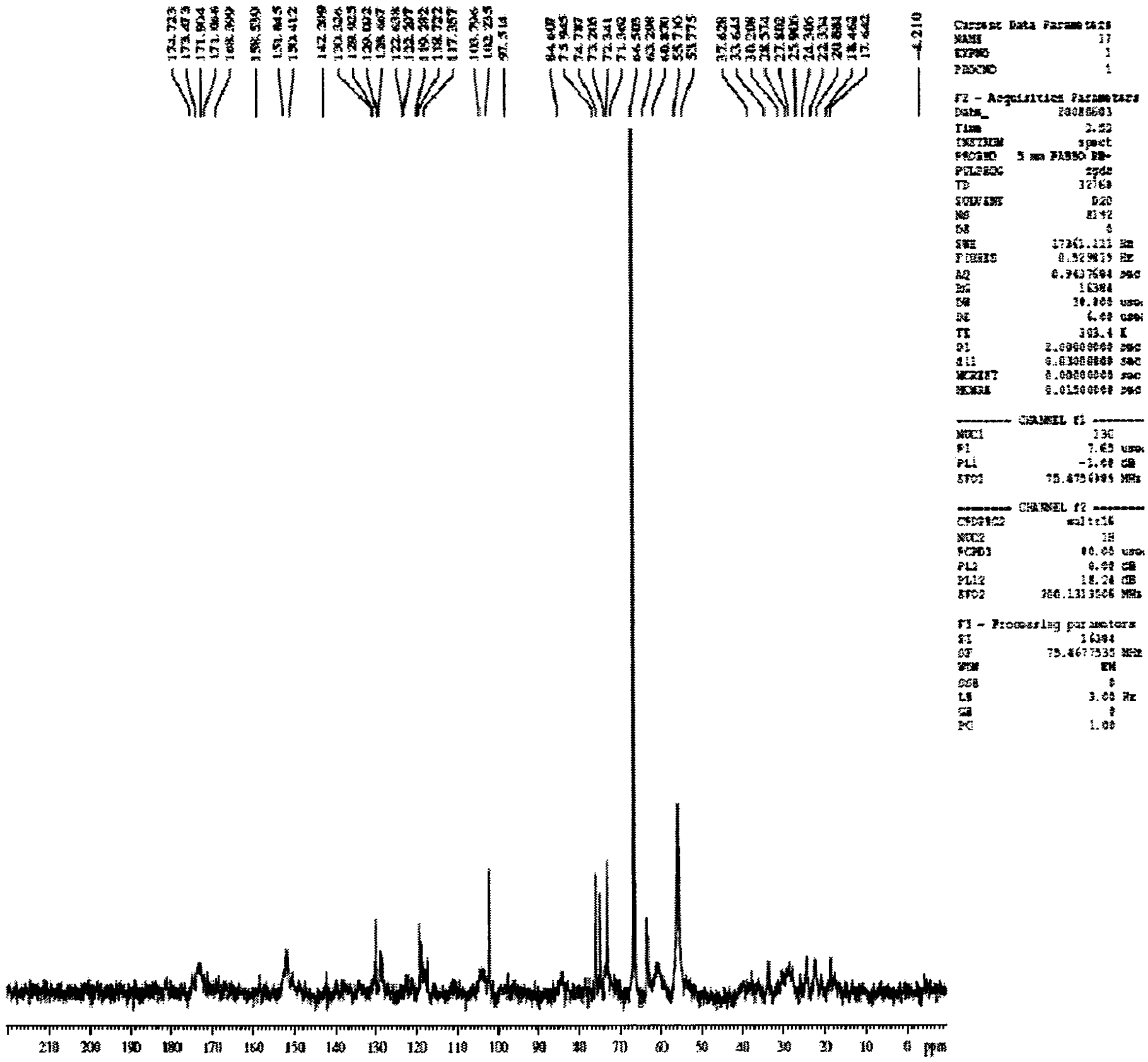


Fig 5.2.10 C^{13} NMR spectra of humic acid extracted from pressmud of Aurangabad at final stage

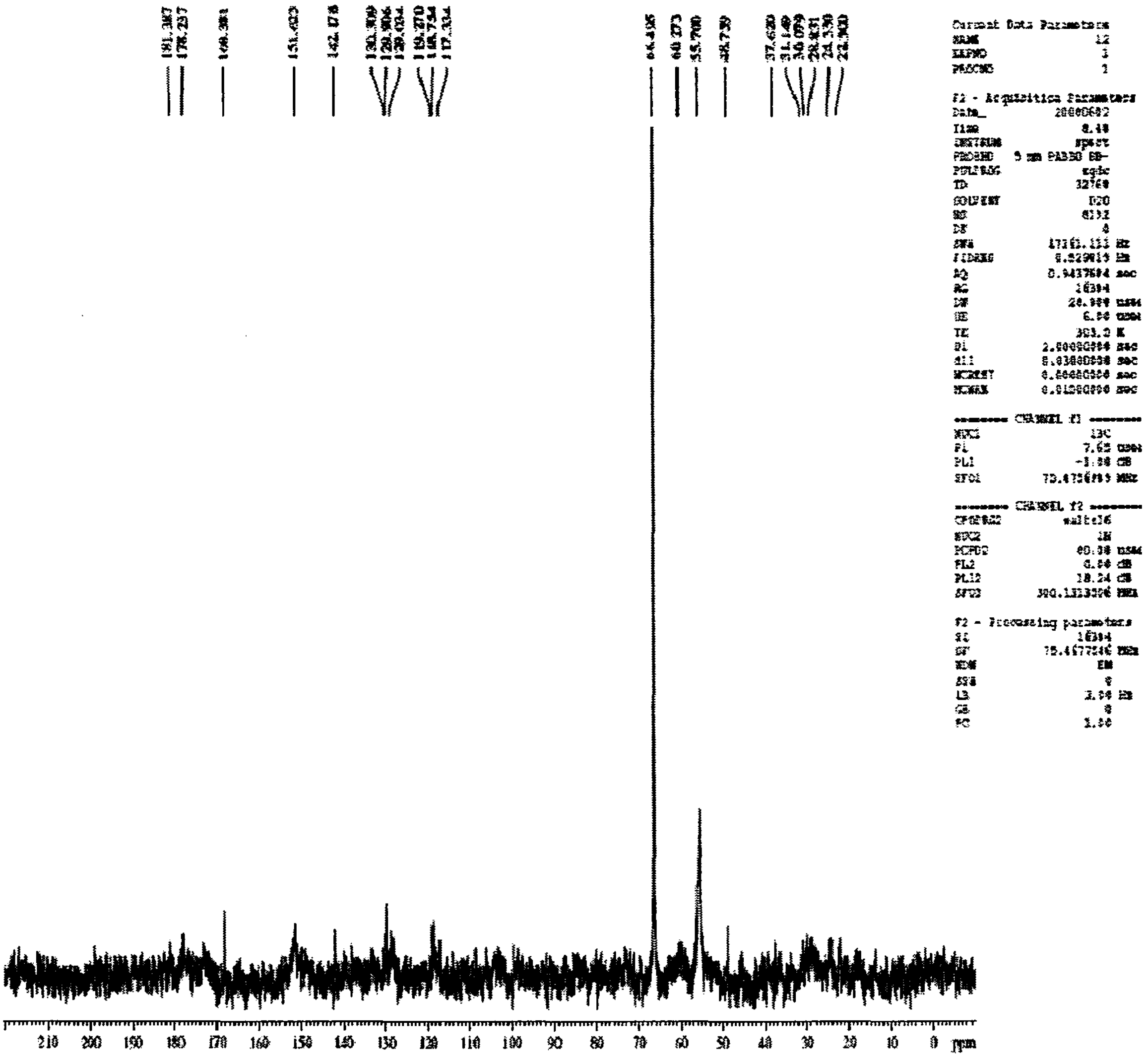


Fig 5.2.11 C^{13} NMR spectra of humic acid extracted from pressmud of Faziabad at initial stage

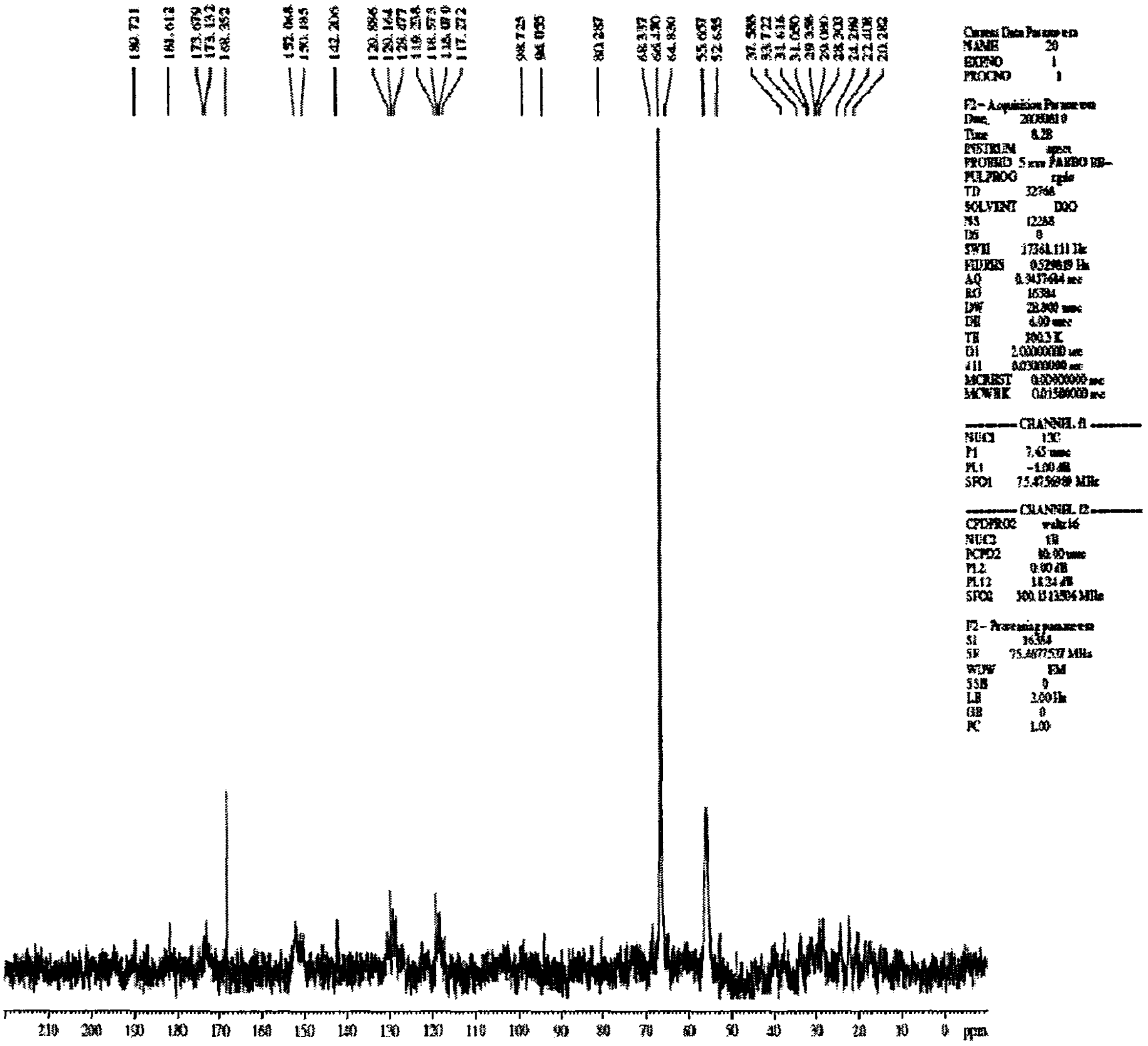


Fig 5.2.12 C^{13} NMR spectra of humic acid extracted from pressmud of Faziabad at Final stage

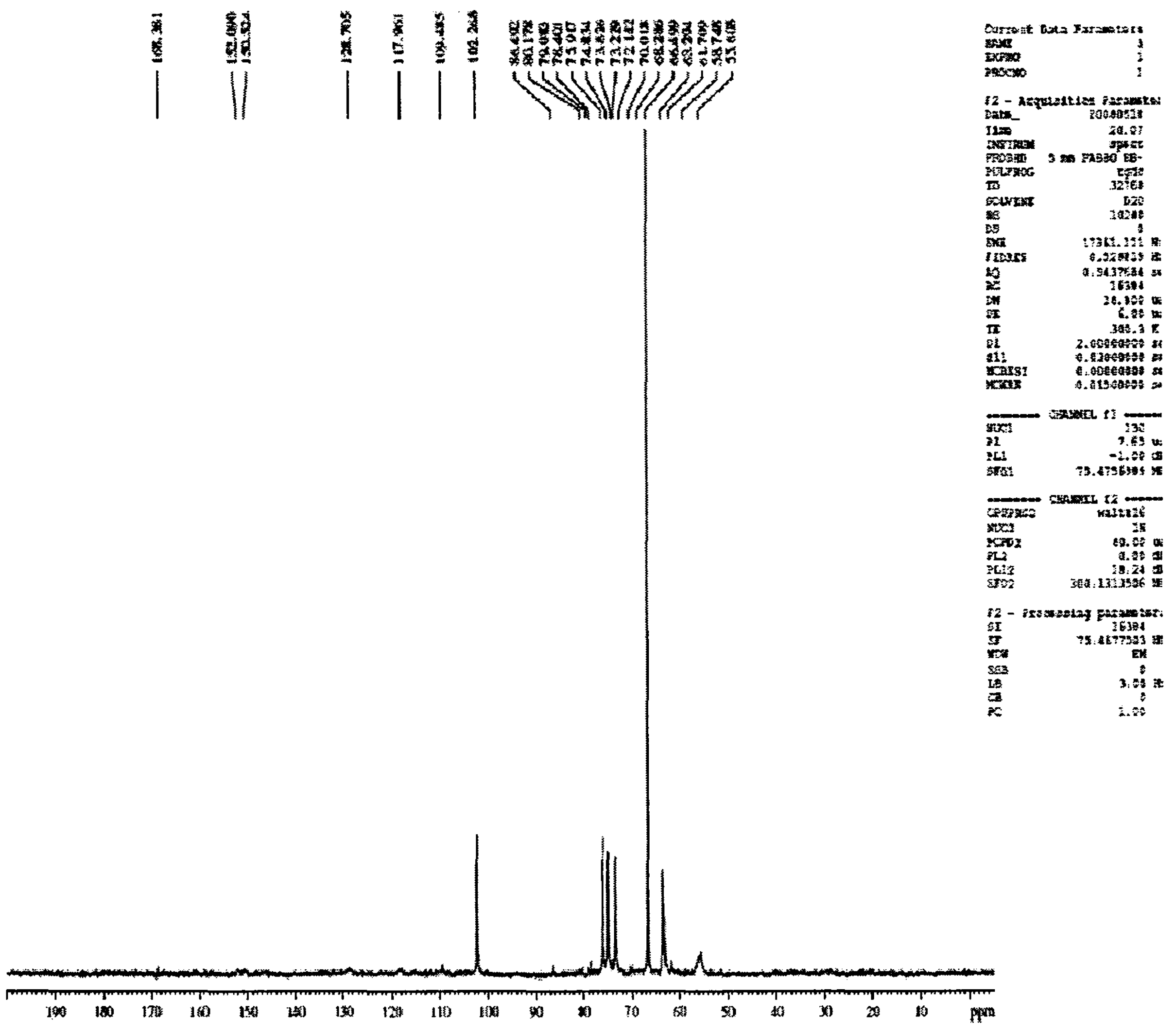


Fig 5.2.13 C^{13} NMR spectra of humic acid extracted from paper sludge of Faziabad at initial stage

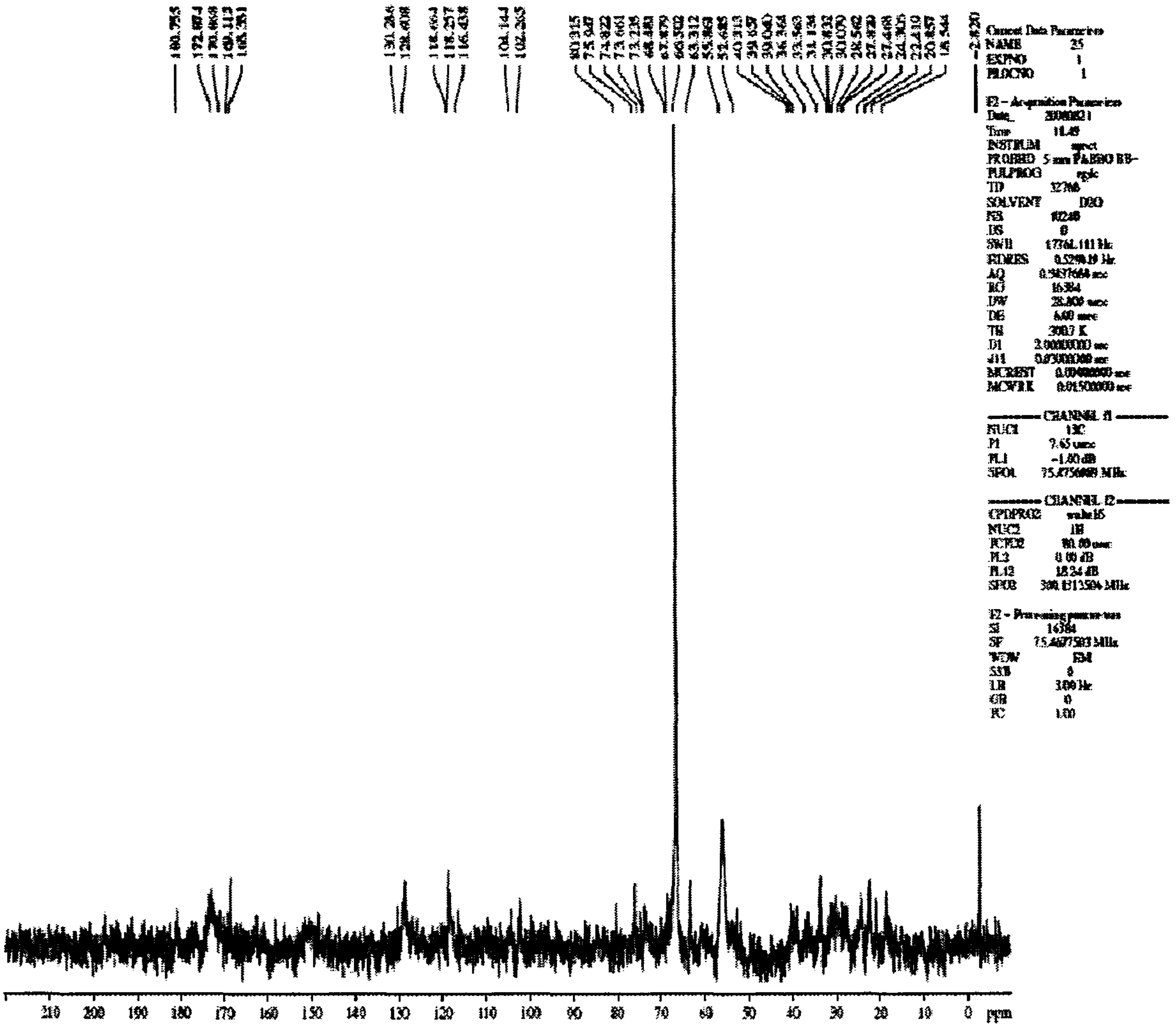


Fig 5.2.14 C^{13} NMR spectra of humic acid extracted from paper sludge of Faziabad at final stage

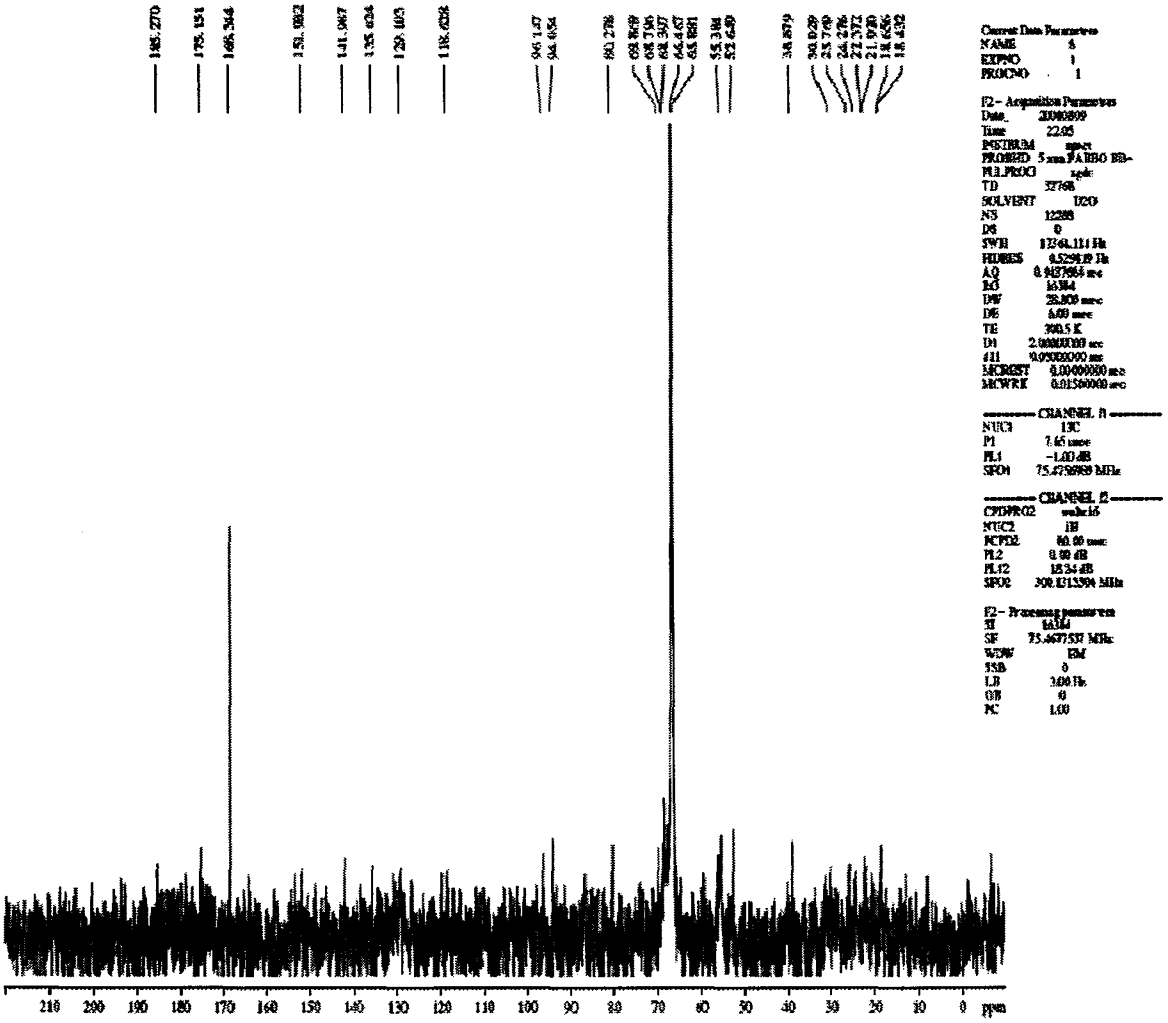


Fig 5.2.15 C^{13} NMR spectra of humic acid extracted from soil of Aurangabad at initial stage

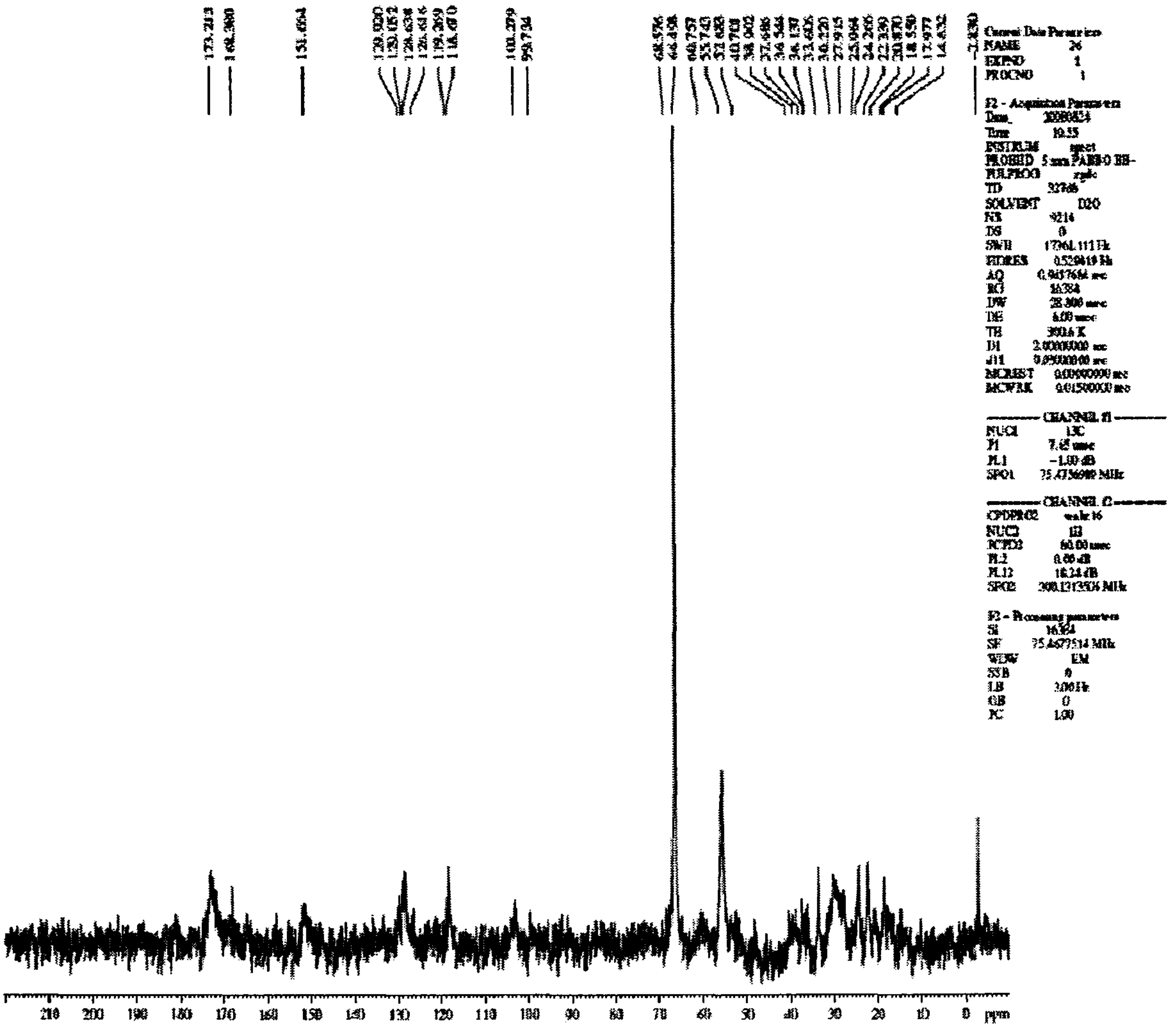


Fig 5.2.16 C^{13} NMR spectra of humic acid extracted from soil of Aurangabad at final stage

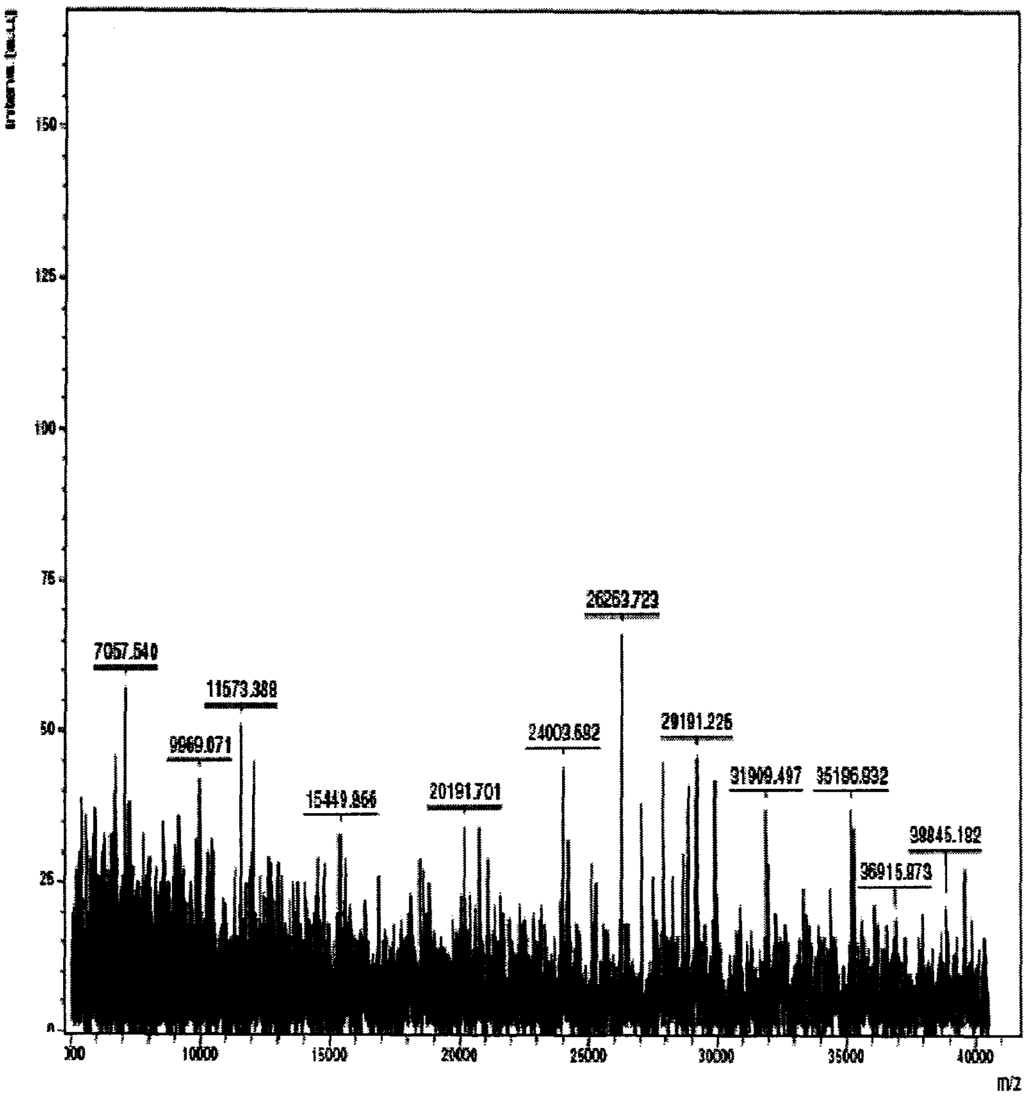
5.2.4 MOLECULAR WEIGHT CHARACTERIZATION OF HUMIC ACID EXTRACTED FROM COMPOSTED PRESSMUD AND PAPER SLUDGE

Though humic acids are being studied for many years now, but their sizes and shapes are still a matter of debate among scientists. Two different view points were suggested: first, humic substances are macromolecular and assume random coil conformations in the solution: second, HS are molecular associations of relatively small molecules held together by weak interaction forces.

In the present study matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and gel electrophoresis were used to determine the molecular weight distribution of the humic acid extracted from the composted wastes of sugar mill and paper mill and compared with the result of gel electrophoresis.

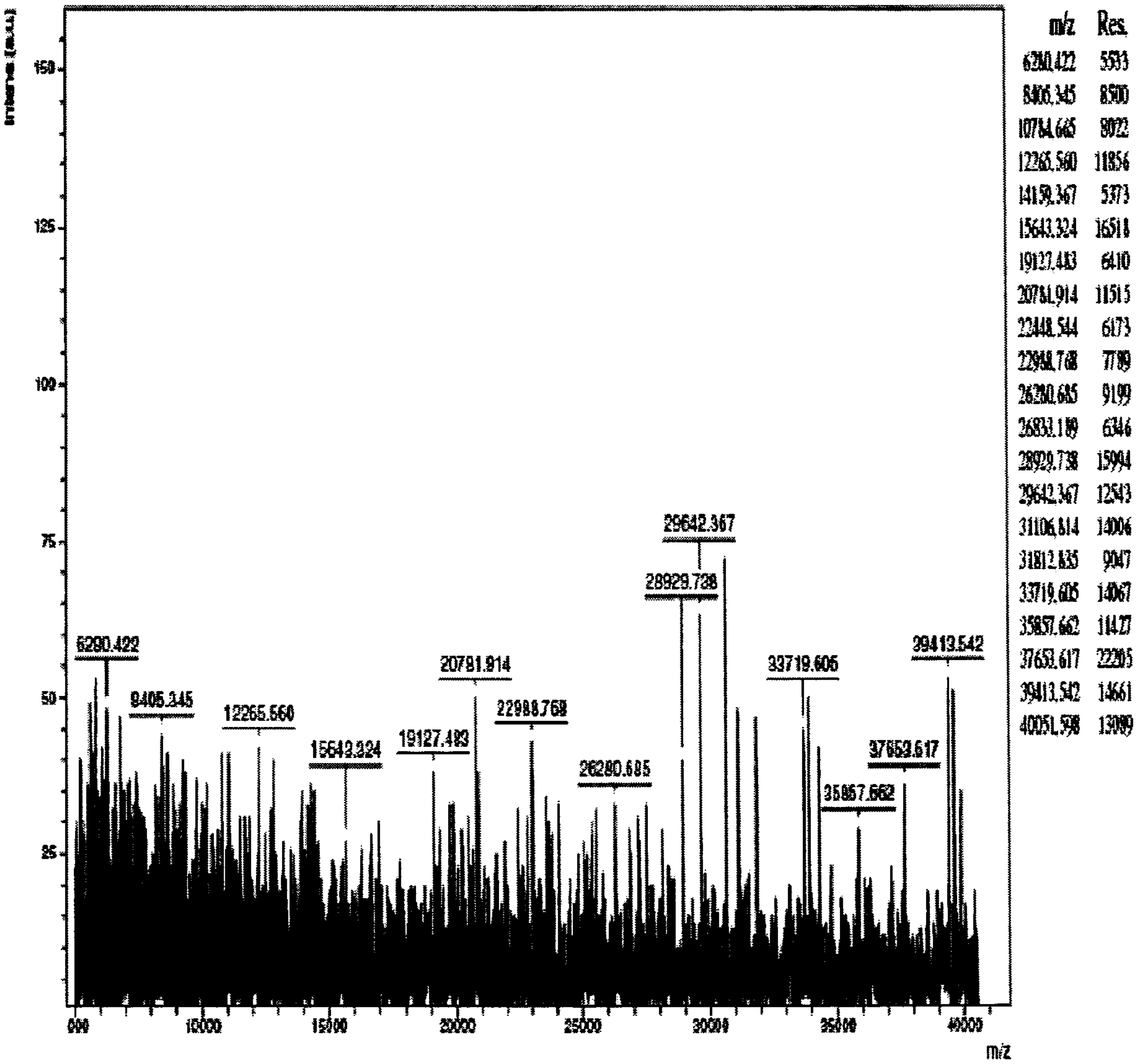
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) is a new "soft" technique used to ionise compounds from low mass up to approximately 350,000 Da (Kaufmann, 1995) It is especially valuable in the detection and characterization of bio-macromolecules present in mixtures, such as peptides, proteins, glycoconjugates, oligonucleotides, and synthetic polymers (Gross and Strupat 1998)

It was found that humic acids are the mixture of same and /or similar compounds and the results of MALDI-TOF-MS were supported by the conventional method of Polyacrylamide Gel Electrophoresis (PAGE) for determining the molecular size distribution of the humic substances.



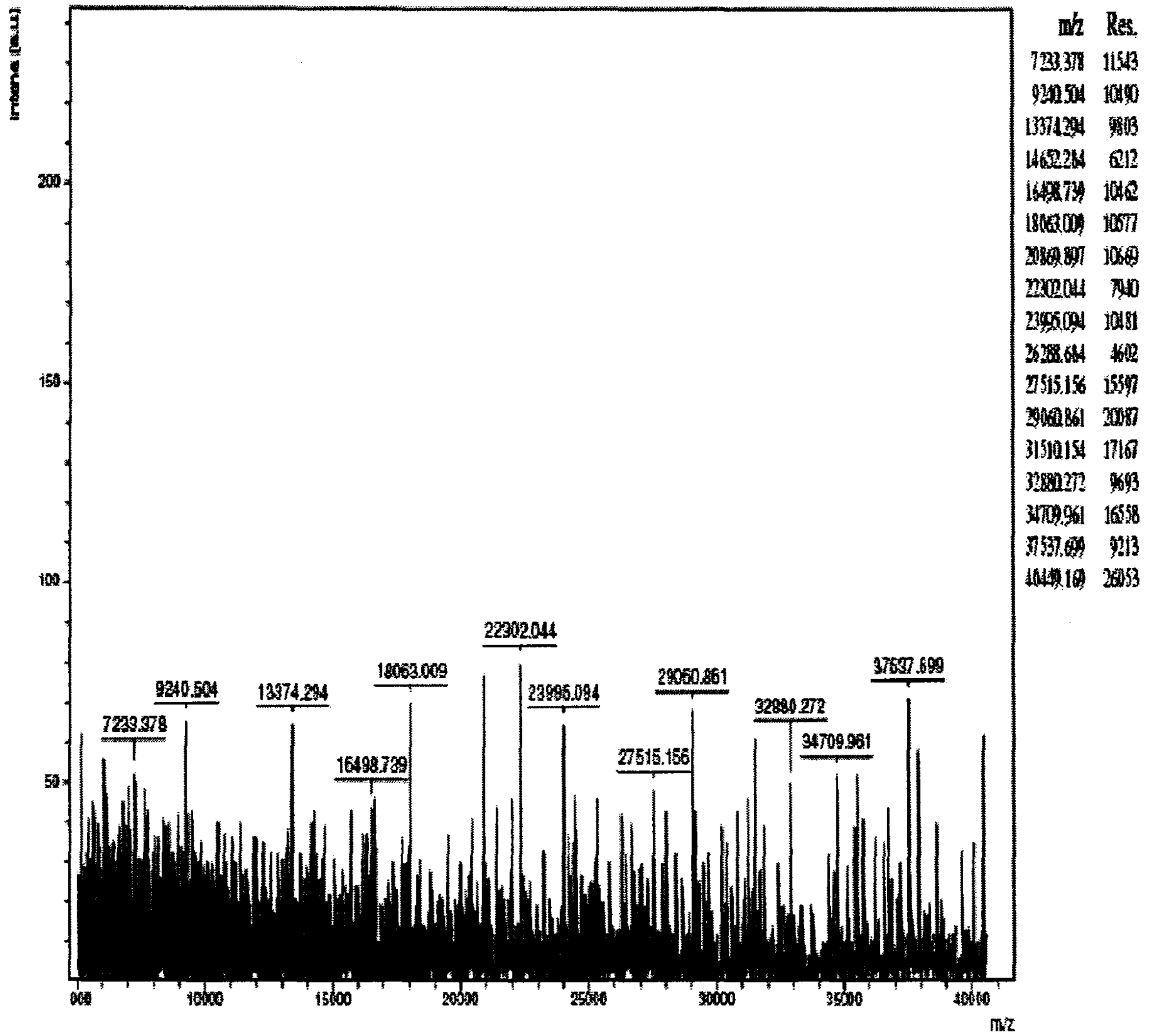
m/z	Res.
5379.459	8180
7057.540	7778
8553.832	7130
9171.990	9195
9969.071	10196
11573.388	8551
12059.848	7522
13037.543	14838
14555.561	14158
15449.866	10422
17458.503	8013
18080.375	2928
18456.588	16277
20191.701	6974
23330.944	7223
24003.682	16697
26263.723	20774
27898.119	5245
28873.179	18065
29191.225	16965
31909.497	14909
33345.017	15959
35186.832	10055
36915.873	13129
38845.182	1215
39866.279	17117

Fig 5.2.17 Maldi-TOF mass spectra of humic acid extracted from Pressmud of Aurangabad



m/z	Res.
6280.422	5533
8406.345	8500
10784.665	8022
12265.560	11856
14159.367	5373
15643.324	16518
19127.483	6410
20781.914	11515
22448.544	6173
22988.768	7789
26280.685	9199
26833.189	6346
28929.738	15994
29642.367	12543
31106.814	14006
31812.835	9047
33719.605	14067
35857.662	11427
37653.617	22305
39413.542	14661
40051.598	13089

Fig 5.2.18 Maldi-TOF mass spectra of humic acid extracted from Pressmud of Faizabad.



m/z	Res.
7233.378	11543
9240.504	10490
13374.294	9803
14652.264	6212
16498.739	10462
18063.009	10577
20869.897	10669
22302.044	7940
23995.094	10481
26288.684	4602
27515.156	15597
29060.861	20087
31510.154	17167
32880.272	9693
34709.961	16558
37537.699	9213
40449.169	26053

Fig 5.2.19 Maldi-TOF mass spectra of humic acid extracted from Paper sludge of Faizabad.

Gel Electrophoresis

One of the oldest techniques for approximate measurement of biological compounds is Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE). Here, addition of SDS to the samples is done to make their charge to mass ratio equal, which allows the separation of the molecules purely on their molecular weights. In the present study, we have tried to determine the molecular weight of humic acid by separating them on SDS-PAGE , Protein molecular weight markers ranging from 97.4 KDa to 14.3 KDa was used as standard to compare the size of humic acids. We observed three bands of different sizes as well as of different intensities (Fig) . One prominent band in the range 14.3 – 20.1 as per protein molecular marker was observed. Another distingusing band below 14.3 was observed. These results support the theory that smaller molecule of humic acid aggregate to form the higher molecular weight of humic acid.

PAGE analysis

Electrophoresis of humic acid samples, isolated from the compost of different organic waste is shown in Fig 5.2.28 and Fig. 5.2.29. All Humic acids formed four naturally colored fractions, named A, B, C and D with different electrophoretic mobility (EMs): A – start zone, that did not move into the gel; B – narrow zone in the mid part of the gel; C and D – two zones in the bottom of the gel, combined into fractions C + D due to relatively close electrophoretic behavior. The PMA, PMF, PSF and SOIL A showed the same numbers and EM of individual bands of humic fractions. Similar results were obtained by Trubetskaya et al., 2001

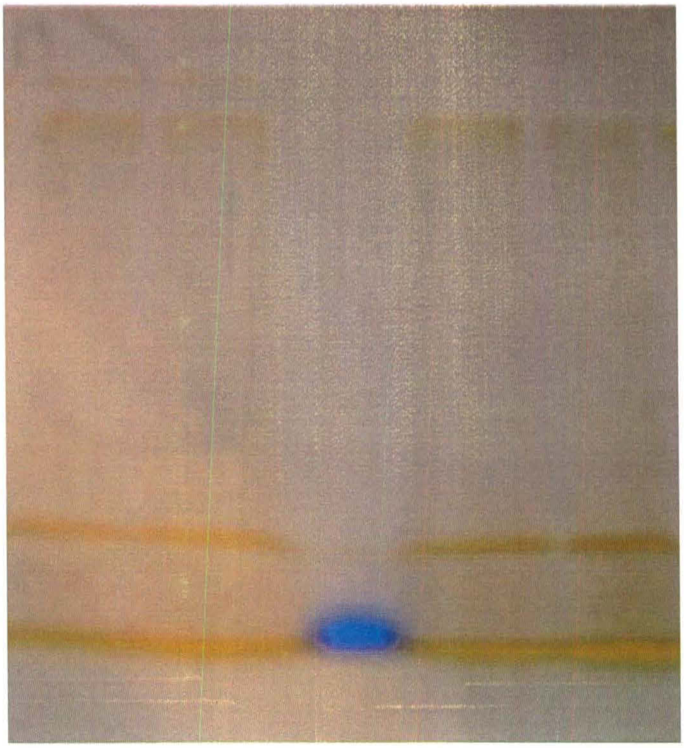


Fig 5.2.20 Electrophoresis of 0.25mg of humic acid from compost of pressmud (1&2), paper sludge (3) and soil (4) of Aurangabad and Faizabad in 10% polyacrlamide gel in presence of denaturing agents. The A, B and C+D are discrete natural coloured zones of Humic acid

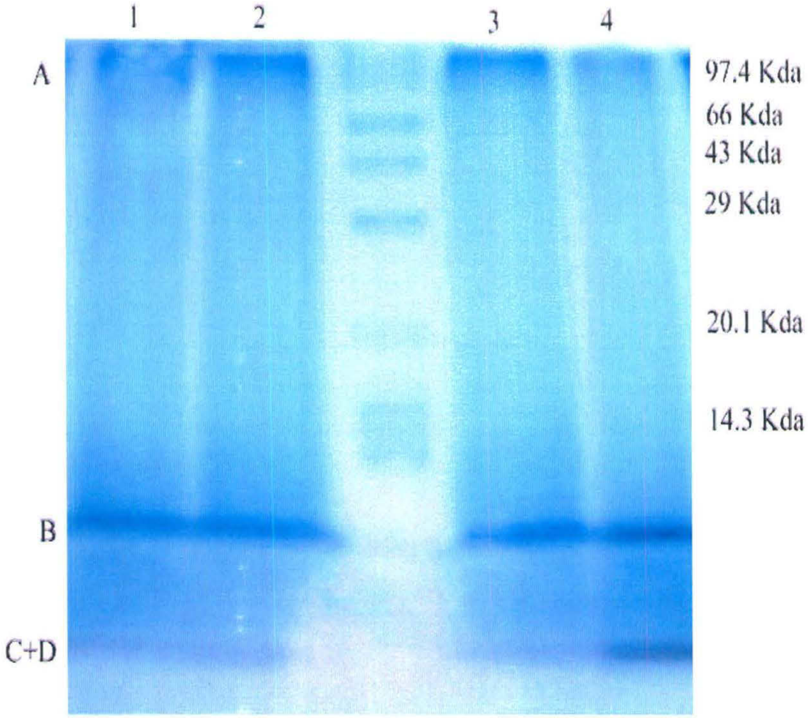


Fig 5.2.21 Fig 5.2.20 Electrophoresis of 0.25mg of humic acid from compost of pressmud (1&2), paper sludge (3) and soil (4) of Aurangabad and Faizabad in 10% polyacrlamide gel in presence of denaturing agents. The A, B and C+D are discrete zones (after staining) of Humic acid

Chapter 5 (III)

IMPACT OF AGRO BASED INDUSTRIES ON THE GROUNDWATER QUALITY OF ADJOINING AREAS OF INDUSTRIES

Untreated or partially treated wastewater contains a wide variety of inorganic, organic, and biological contaminants. Even treated wastewater, commonly referred to as effluent, may contain elevated concentrations of various chemicals including chloride, nitrate, hydrocarbons, and metals. Untreated and partially treated wastewater may be stored in ponds and lagoons or be discharged on the land or into an open water system. Treated wastewater may be used as irrigation water for agricultural purposes or as artificial recharge to augment groundwater supplies. Leaking treatment facilities, the storage of wastewater in ponds and lagoons, and the discharge of untreated or partially treated wastewater on the land or into an open water course carries the risk that infiltration of contaminants will take place and groundwater will be contaminated. (Singh, et.al., 1996)

The hydrogeochemical processes help to get an insight into the contributions of rock-water interaction and Industrial influence on groundwater quality. These geochemical processes are responsible for the seasonal and spatial variations in groundwater chemistry (Matthess, 1982; Kumar et al., 2006).Groundwater chemically evolves by interacting with aquifer minerals or internal mixing among different groundwater along flow-paths in the subsurface (Domenico, 1972; Wallick and Toth, 1976; Toth, 1984). Schuh et al. (1997) indicated that increase in solute concentrations in the groundwater were caused by spatially variable recharge, governed by microtopographic controls. Further, the weathering of primary and secondary minerals is also contributing cations and silica in the system (Freeze and Cherry 1979; Jacks 1973; Bartarya 1993).

This chapter focuses on to study the impact of industrial waste on the quality of ground water collected from the adjoining areas of the agro-based industries in Lucknow, Aurangabad and Nasik. Multivariate analysis, such as factor analysis and cluster analysis was used simply as a numerical method of discovering variables that are more important than other data for representing parameter variation or demonstrating hydrochemical processes. This technique helps to simplify and organize data set in order to make useful generalizations and insight.

5.3.1 BRIEF INTRODUCTION ABOUT AURANGABAD, NASIK AND LUCKNOW

The districts Aurangabad lies between 19^0 to 20^0 N Latitude and 74^0 to 76^0 E longitude in the Deccan Plateau Zone. The Godavari is the main river flowing west to east and Purna, Shivana, Khelna, Kham, Dhudhana, Giraja etc., are the sub rivers flowing in the district. The soils are mostly formed from igneous rocks and are black, medium black, shallow and calcarious types having different depths and profiles, agriculture is dependent on Monsoon rainfall. (Gazette of Aurangabad, 1991)

Nashik, a major industrial town situated at Latitude$19\ ^0$ - 33' and 20^0 - 53' North and Longitude $73\ ^0$ – 16' and 75^0 –6' East in Northern Maharashtra, is located at a height of 565 meters above mean sea level at a distance of 180 Km from Mumbai (Bombay), The main rivers flowing in the district are Godavari, Kashyapi, Darna, Girna, Kadwa and Nasardi (Nandini),. There are two industrial eastates namely Satpur having 1600 Acres and 750 no. of units and Ambad having 1400 Acres area with 850 nos. of units. (MPCB, 2000)

The Lucknow district comes under the Ganga alluvial plain which is basically formed by the Himalayan derived sediments and serves as transient zone between the Himalaya (source) and the Bay of Bengal (sink). The plain experiences a strong geochemical weathering leading to fractionation and elemental partitioning under sub-tropical climatic conditions controlled by heavy monsoon rainfall with long periods of drought and high ambient temperatures. It plays an important role in geochemical dynamics of this region as compared to the Himalayan area. The chemical weathering of the Ganga Alluvial Plain sediments is more intense than in the Himalaya. In the Ganga Alluvial Plain, the loss of Na, Ca and Sr during weathering is noticeable. Weathering products of the Ganga

Alluvial Plain are the results of incomplete alteration of deposited alluvial sequences under humid sub-tropical climate. (Singh,et al., 2008)

The Gomti,River is a tributary of the Ganges River. The Gomti originates near Madho Tada, Pilibhit, India. It extends 900 km (560 miles) through Uttar Pradesh and meets the Ganges River near Saidpur after 240 km the Gomti enters Lucknow, through which it meanders for about 12 km. At the entrance point water is lifted from the river for the city's water supply. The major sources of pollution in the Gomti are:

- Industrial waste and effluent from sugar factories and distillaries.
- Domestic waste water and sewage from habitations.

The river collects large amounts of human and industrial pollutants as it flows through the highly populous areas of Uttar Pradesh. The river flows in the great alluvial plain, which is of Pleistocene-Holocene origin, and redistributes the primary weathered sediments of the Gangetic alluvial plain derived from the Himalaya. Lucknow is a capital city in the Gangetic plain, with an urban population of more than 1.6million and some industrial units. The urban effluents, including industrial and municipal waste, flow into the freshwater river through small open drainages. The river sediments are made up of fine sand, silt, and clay. The fine silt and clay (<20 micrometer fraction) content in the sediment samples ranges from 6 to 40% with an average of 20%. The sediment is dominated by Quartz, followed by feldspar and mica.
(http://www.gisdevelopment.net/application/environment/water/mi08_238.htm

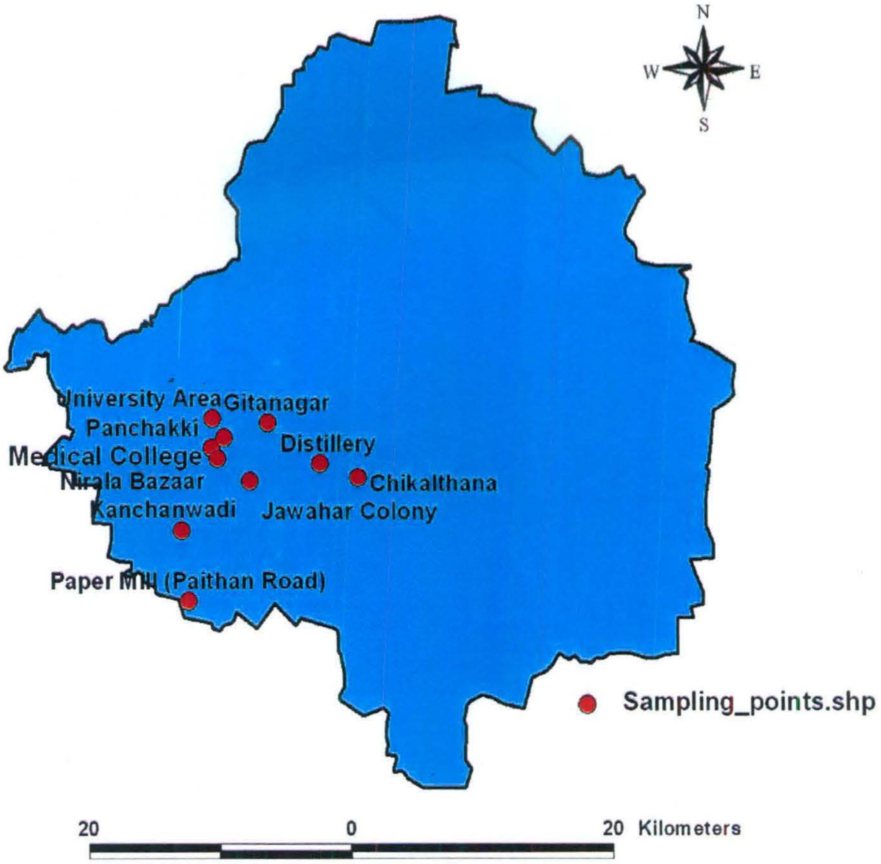


Fig 5.1.1 Map showing ground water sampling locations in Aurangabad district of Maharashtra

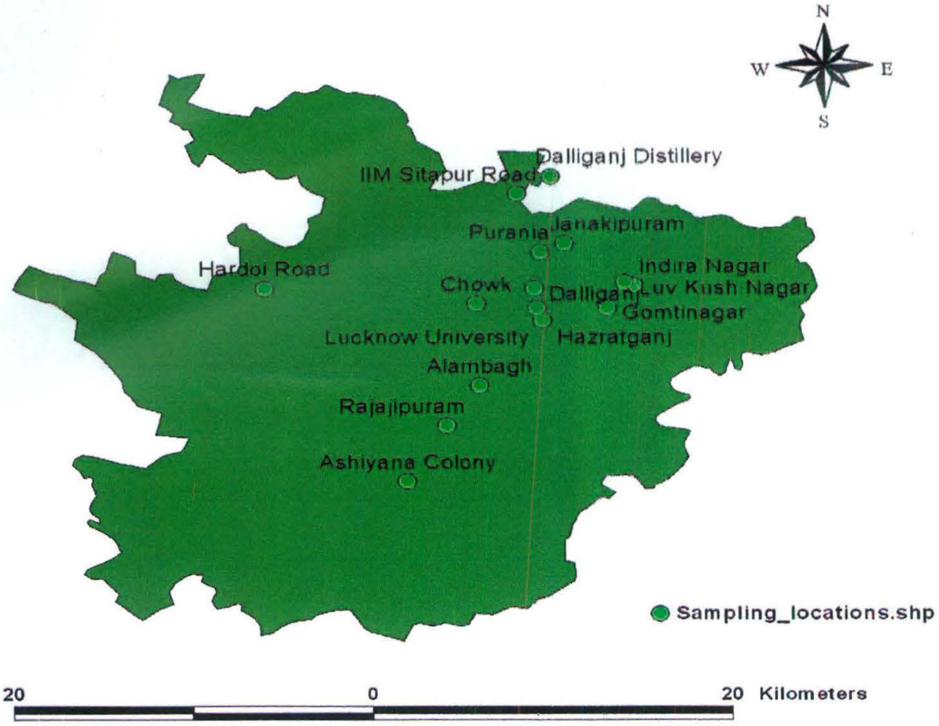


Figure 5.3.1 Map showing ground water sampling locations in Lucknow district of Uttar Pradesh.

The principal constituents of ionic species and their distribution in natural waters vary greatly depending on the geographical formations, soil type and human activities. mportant ionic species (Cation & Anion) in all natural waters that influence water quality and represent the principal chemical constituents are listed below:

Cation	**Anions**
Calcium (Ca^{2+})	Bicarbonate (HCO_3^-)
Magnesium (Mg^{2+})	Carbonate (CO_3^{-2})
Sodium (Na^+)	Chloride (Cl^-)
Potassium (K^+)	Sulfate (SO_4^{2-})
Iron (Fe^{2+})	Nitrate (NO_3^-)
Manganese (Mn^{2+})	Phosphate (PO_4^{3-})

Source: (Jain,et.al, 2007)

Table 5.3.1 major cations and anions in natural water

Elements/radicals	**Guideline values (mg/l)**
Chloride	250
Sulfate	500
Nitrate	50
Fluoride	1.5
Iron	2
Manganese	0.5
Copper	2
Nickel	0.02
Lead	0.01
Zinc	3
Arsenic	0.01
Cadmium	0.003
Chromium	0.05
Mercury	0.001

Table 5.3.2: Drinking water quality standards as recommended by WHO, 1996

S.No	Parameter	Desirable limit (mg/l)	Permissible limit (mg/l)
1	pH	6.5 – 8.5	No relaxation
2	Iron(Fe)	0.3	1.0
3	Chloride(Cl)	250	1000
4	Fluoride(F)	1.0	1.5
5	Calcium(Ca)	75	200
6	Magnesium(Mg)	30	100
7	Copper (Cu)	0.05	1.5
8	Mangnese(Mn)	0.1	0.3
9	Sulphate(SO_4)	200	400
10	Nitrate(NO_3)	45	100
11	Cadmiun(Cd)	0.01	No relaxation
12	Zinc(Zn)	5.0	15
13	Alkalanity	200	600
14	Dissolve solid	500	2000

Table 5.3.3: Drinking water Characteristics (ISI:10500:1991) (BIS, 1991)

Ground water samples	pH	EC	TDS	HCO_3	F	Cl	NO_3	SO_4	Na
Vishva Papermill Aurangabad	6.9	1895	1345	245	1.21	560.74	75.66	160.56	336.9
Kanchanwadi Aurangabad	7.4	708	340	278	1.03	187.94	46.86	60.75	146.2
Medical collage Aurangabad	7.2	753	448	275	2.74	30.89	18.75	233.25	112.7
Industrial area Aurangabad	7.6	1297	928	355	0.44	331.2	15.4	113.34	73.72
Niralabazar Aurangabad	7.6	734	380	365	0.87	150	37	154.8	145.7
Chikalthana Aurangabad	7	1015	689	230	0.32	198	48	356.89	159.6
Jyotinagar Aurangabad	7.3	755	462	245	1.03	185	28.9	135.76	125
jubleepark Aurangabad	7.4	767	430	235	0.52	153.14	36.58	146.08	115.6
Rajabazar Aurangabad	7.3	799	495	265	1.03	85.04	30.78	98.9	104.8
Panchakki Aurangabad	7.3	823	565	275	2.45	118	6.67	195.5	125.6
Paper mill Nasik	6.7	879	575	260	3.56	175.34	6.48	70.98	110.4
Distillery Nasik	7	1255	823	295	1.39	135.9	42.09	176.66	155.5
Daliganj Lucknow	7	865	534	280	0.3	5.84	6.79	12.68	10.82
Distillery Lucknow	6.9	1095	711	270	1.03	38.07	58.9	127.95	99.45
Indiranagar Lucknow	6.9	687	409	265	1.04	22.18	29.97	43.5	1.4
Luv kushnagar Lucknow	6.8	650	334	260	1.39	18.64	13.85	19.44	1.96
Jankipuram Lucknow	7.4	1060	650	295	0.63	36.8	12.35	21.82	5.24
Hazaratganj Lucknow	7.3	986	539	280	0.57	25.96	15.45	10.67	30.8
Gomtinagar Lucknow	7.2	975	634	255	0.94	40.12	25.4	18.51	15.39
Puarania Lucknow	7.2	897	589	265	0.56	28.07	43.5	130.91	50.52
Alambagh Lucknow	7.4	785	368	285	0.12	32.09	20.02	51.73	65.5
Rajajipuram Lucknow	7.4	840	452	280	0.72	38.9	8.56	18.69	30.7
Chowk Lucknow	7.1	610	348	210	0.7	20.02	2.6	50.66	26.98
Hardoi road Lucknow	7.2	630	356	265	0.4	8	0	65.75	20.12
IIM Sitapur road Lucknow	7.5	680	388	279	0.16	33	0	36.29	32.69
University Lucknow	7.3	870	525	275	0.2	12.69	10.03	30.5	20.32
Ashiana Colony Lucknow	7.3	793	378	280	0.14	13.5	7.03	49.56	30.75
Mean	7.2	892	544	273	0.94	99.45	23.99	96	79.79
Minimum	6.7	610	334	210	0.12	5.84	0	10.67	1.4
Maximum	7.6	1895	1345	365	3.56	560.74	75.66	356.89	336.9

Continued……

Ground water samples	K	Mg	Ca	DOC	TZ+	TZ-	% error	SAR	Water Type
Vishva Papermill Aurangabad	1.96	95.6	145.6	3.5	29.94	24.44	10.12	5.31	Na-Cl
Kanchanwadi Aurangabad	1.44	50.15	79.09	3.2	14.53	11.93	9.83	3.15	Na-Cl
Medical collage Aurangabad	3.44	25.68	91.2	2.1	11.69	10.68	4.49	2.68	Na-SO_4
Industrial area Aurangabad	1.61	56.57	197.57	1.9	17.84	17.78	0.16	1.19	Ca-Cl
Niralabazar Aurangabad	2.55	40.7	118.5	1.5	15.72	14.08	5.5	2.93	Na-HCO_3
Chikalthana Aurangabad	4.98	26.46	207	1.8	19.62	17.57	5.45	2.77	Ca-SO4
Jyotinagar Aurangabad	1.09	42.5	118.79	1.3	14.95	12.58	8.61	2.5	Ca-Cl
jubleepark Aurangabad	0.97	38.95	120.04	1.5	14.3	11.83	9.46	2.34	Ca-Cl
Rajabazar Aurangabad	1.09	18.58	94.59	1.6	10.86	8.85	10.18	2.57	Ca-HCO_3
Panchakki Aurangabad	3.2	24.28	115.35	2.2	13.33	12.16	4.62	2.77	Ca-HCO_3
Paper mill Nasik	0.09	19.91	90.5	2.4	10.99	10.97	0.07	2.73	Na-Cl
Distillery Nasik	0.14	29.8	130.46	1.4	15.77	13.1	9.23	3.19	Na-HCO_3
Daliganj Lucknow	3.5	17.84	45.78	0.9	4.34	5.15	-8.6	0.34	Ca-HCO_3
Distillery Lucknow	1.89	13.78	112.9	0.6	11.17	9.17	9.82	2.35	Ca-HCO_3
Indiranagar Lucknow	5.34	29.05	102.75	0.5	7.76	6.41	9.48	0.03	Ca-HCO_3
Luv kushnagar Lucknow	2.97	21.03	90.59	0.56	6.44	5.49	8	0.05	Ca-HCO_3
Jankipuram Lucknow	3.8	16.87	80.96	1.6	5.78	6.56	-6.33	0.14	Ca-HCO_3
Hazaratganj Lucknow	1.03	12.45	45.64	1.5	4.69	5.66	-9.42	1.04	Ca-HCO_3
Gomtinagar Lucknow	1.98	16.71	60.9	10.5	5.16	6.16	-8.82	0.45	Ca-HCO_3
Puarania Lucknow	1.89	14.52	70.65	1.5	6.99	8.59	-10.31	1.43	Ca-HCO_3
Alambagh Lucknow	1.98	10.1	61.69	1.25	5.82	6.98	-9.04	2.35	Na-HCO_3
Rajajipuram Lucknow	1.9	15.98	50.08	1.8	5.22	6.33	-9.64	0.96	Ca-HCO_3
Chowk Lucknow	2.4	12.9	74.87	1.65	4.55	5.14	-6.06	0.91	Ca-HCO_3
Hardoi road Lucknow	1.68	16.56	96.8	0.6	7.14	5.96	8.99	0.5	Ca-HCO_3
IIM Sitapur road Lucknow	1.23	18.35	66.78	0.6	6.32	6.27	0.42	0.91	Ca-HCO_3
University Lucknow	3.66	15.34	50.8	0.8	4.8	5.67	-8.38	0.64	Ca-HCO_3
Ashiana Colony Lucknow	3.08	20.52	62.35	0.9	6.24	6.12	0.97	0.86	Ca-HCO_3
Mean	2.26	26.71	93.79	1.84	10.44	9.69	1.44	1.74	
Minimum	0.09	10.1	45.64	0.5	4.34	5.14	-10.31	0.03	
Maximum	5.34	95.6	207	10.5	29.94	24.44	10.18	5.31	

Table 5.3.4 showing various physico- chemical parameters, % error, SAR and Water type. All parameters in mg/l except EC (μs/cm) and pH Month: September and October Year: 2007

	pH	EC	TDS	HCO$_3$	F	Cl	NO$_3$	SO$_4$	Na	K	Mg	Ca	DOC
pH	1												
EC	-0.20	1											
TDS	-0.25	0.98	1										
HCO$_3$	0.537	0.11	0.06	1									
F	-0.40	0.03	0.10	-0.08	1								
Cl	-0.04	0.74	0.75	0.06	0.17	1							
NO$_3$	-0.20	0.57	**0.55**	-0.09	0.00	0.58	1						
SO$_4$	-0.06	0.26	0.33	-0.09	0.26	0.44	0.50	1					
Na	-0.09	0.61	0.61	-0.05	0.31	0.84	0.73	0.66	1				
K	-0.14	-0.13	-0.10	-0.05	-0.16	-0.22	-0.06	0.14	-0.24	1			
Mg	0.02	0.62	0.62	0.11	0.09	0.92	0.58	0.33	0.77	-0.11	1		
Ca	-0.07	0.46	0.54	0.13	0.13	0.67	0.48	0.75	0.58	0.07	0.58	1	
DOC	-0.00	0.24	0.26	-0.12	0.16	0.19	0.17	-0.03	0.12	-0.12	0.14	-0.034	1

Table 5.3.5 Correlation matrix of ground water samples collected from Aurangabad, Nasik and Lucknow

5.3.2 PHYSICO-CHEMICAL PARAMETERS

pH

The pH value of ground water collected from Lucknow and Aurangabad ranges from 6.75 to 7.65 which demonstrate slightly acidic or neutral. Ground water collected from the industrial premises shows slightly acidic in nature which may be due to excessive discharge of acidic waste water of paper mill and distillery.

EC and TDS

The EC values are useful tool to evaluate the purity of water. The EC value ranges from 610μS/cm to 1895μS/cm. Results indicate the higher values for ground water of Aurangabad as compared to Lucknow which is because of more industrial activities in Aurangabad. The TDS values ranges between 334 mg/l to 1345 mg/l .the maximum value is for the ground water collected from the paper mill of Aurangabad. The results show the values of EC and TDS are with in the permissible limits prescribed by WHO.

Calcium

It is derived mostly from rocks, and maximum concentrations come from lime stone, dolomite, gypsum, and gypsiferrous shale. Calcium is the second major constituent, after bicarbonate, present in most natural waters, with a concentration range between 10 and100 mg/l. Calcium is a primary constituent of water hardness and calcium level between 40 and 100 mg/l are generally considered as hard to very hard.

The calcium concentration in the ground water samples ranges from 41.69 mg/l to 207.00 mg/l. the minimum concentration found in the ground water collected from the residential areas and the maximum concentration of calcium reported in the sample taken from the Chikalthana industrial area of Aurangabad where Maharashtra distillery unit is present. As the waste water from distillery is rich in calcium thus it may be one of the reasons for the higher values of calcium ions.

Magnesium

Source of magnesium includes ferromagnesium minerals in igneous and metamorphic rocks and magnesium carbonate in limestone and dolomite. Magnesium salts are more soluble than calcium, but they are less abundant in geological formations. At high concentration in drinking water, magnesium salts may have laxative effects. They may also cause unpleasant taste at concentrations above 500 mg/l. For irrigation purposes, magnesium is a necessary plant nutrient as well as a necessary soil conditioner. Magnesium is associated with hardness of water, and is undesirable, in several industrial processes.

In the present analysis the magnesium values ranges between 10.10 mg/l to 95.60 mg/l which are well with in the limits., but again the higher values found in water samples collected from the adjoining areas of industries.

Sodium

The major source of sodium in natural waters is from weathering of feldspars, evaporates, and clay. Sodium salts are very soluble and remain in solution. Typical sodium concentrations in natural waters range between 5 and 50 mg/l. here in the present work the sodium concentration ranges between 1.40 mg/l to 336.90 mg/l. since the waste water of paper industries contains high amount of sodium, therefore the ground water of paper mill in Aurangabad and Nasik showed higher concentration of sodium.

Potassium

Potassium is less abundant than sodium in natural waters. Its concentration rarely exceeds 10 mg/l in natural waters. In highly cultivated areas, runoff may contribute to temporarily high concentrations as plants take up potassium and release it on decay. From the point of view of domestic water supply, potassium is of little importance and creates no adverse effects. The potassium concentration ranges between 0.09 mg/l to 5.34 mg/l in ground water collected from the Aurangabad and Lucknow, which were in the expected range.

Bicarbonate

Bicarbonate is the major constituent of natural water. It comes from the action of water containing carbon dioxide on limestone, marble, chalk, calcite, dolomite, and other minerals containing calcium and magnesium carbonate. The carbonate-bicarbonate system in natural water controls the pH and the natural buffer system. The typical concentration of bicarbonate in surface waters is less than 200 mg/l as HCO3. In groundwater, the bicarbonate concentration is significantly higher. The HCO_3^- concentration varied from 210-365 mg/l which renders the water alkaline in nature.

Chloride

Cl^- occurs naturally in some sedimentary bedrock layers, particularly shale. Cl^- is soluble in water and moves freely with water through soil and rocks. Cl^- is more persistent in nature than nitrate as it is not readily consumed by microorganisms. Cl^- is present in all natural waters, but mostly the concentration is low. Exception occur where the streams receive inflows of high chloride ground water or industrial waste water (Hem, 1991) High content of Cl^{--} may give a salty taste to groundwater and can corrode pipes, pumps and plumbing fixtures. People who are not accustomed to high chlorine in drinking water are subjected to laxative effects (WHO, 1996). Cl^-concentration in the study area varied from 5.84 to 560 mg/l. The higher value was found in ground water from paper mill of Aurangabad which could be due to use of chlorine as bleaching agent in paper mills.

Sulfate

SO_4^{2-} is a naturally occurring ion in almost all kinds of water bodies and is a major contributor to total hardness. SO_4^{2-} content more than 200 mg/l is objectionable for domestic purposes; beyond this limit, SO_4^{2-} causes gastro-intestinal irritation (WHO, 1996) Most of the water samples of all the cities (Aurangabad, Nasik and Lucknow) had SO_4^{2-} contents within permissible limits, ranging from 10.67 to 356.89 mg/l. higher values were observed in adjoining areas of industries which shows the impact of industrial activities.

Fluoride

Fluorite (Caf_2) is a common fluoride mineral. This mineral has a rather low solubility and occurs in both igneous and sedimentary rocks. In the present analysis The fluoride concentration varied between 0.12 mg/l to 3.56 mg/l. The fluoride concentration in ground water of Lucknow was well with in the limits prescribed by the BIS and WHO where as in some samples of Nasik and Aurangabad the F^- concentration was above the permissible limits. Since basaltic rocks are igneous rocks and according to Rankama and

Sahama , 1950 the rocks rich in alkali metals are higher in fluoride contents, thus could be one of the reason of high F^- concentration in Aurangabad and Nasik.

Dissolved Organic Carbon

The dissolved organic carbon (DOC) in ground water comes from either surface organic matter or from kerogen, the fossilized organic matter present in the geologic material of the auifer. In case of shallow ground water the DOC is from surface waters.Dissolved organic carbon (DOC) in ground water ranges from 0.2 mg/l to 15 mg/l with a median concentration of 0.7 mg/l. the majority of all ground water have concentrations of DOC below 2 mg/l. (Leenheer et al, 1974; Barcelone, 1984)

In the present study the dissolved organic carbon (DOC) ranges between 0.50 mg/l to 3.50 mg/l. The table demonstrates that dissolved organic matter is more in ground water samples of the Aurangabad and Nasik as compared to Lucknow because of more units of agro based industries in this belt.

5.3.3 COMPARISON BETWEEN PHYSICO-CHEMICAL PARAMETERS OF WASTE WATER AND GROUND WATER

The table 5.3.7 shows that waste water from agro based industries are acidic in nature where as the average value of pH of ground water is almost neutral. The electrical conductivity and total dissolved solids of industrial waste water is much higher than the ground water. The Dissolved organic carbon (DOC), major anions like NO_3, Cl, SO_4 and cations Ca, Mg, Na and K were found in higher concentration in waste water as compared to ground water. The observed higher concentration of most of the ions in comparsion to WHO guidelines shows the impact of industrial waste water on the quality of ground water.

Parameters	**Average values of waste waters**	**Average values of ground waters**
pH	4.9	7.2
EC (µS/cm)	15176	893
TDS (mg/l)	8614	544
Cl^- (mg/l)	493.94	99.45
NO_3 (mg/l)	42.97	23.99
SO_4 (mg/l)	7472.40	96
Na (mg/l)	988.49	79.79
K (mg/l)	3144.80	2.26
Mg(mg/l)	913	26.71
Ca (mg/l)	803	93.79
DOC (mg/l)	3186	1.84

Table 5.3.6 Shows a comparison between average values of wastewater and groundwater

5.3.4 MULTIVARIATE ANALYSIS

The data were prepared and processed in SPSS 11.0 and excel in window XP. Descriptive statistics, Factor analysis and cluster analysis were processed in SPSS. Factor analysis was used to understand the correlation structure of collected data and identified most important factors contributing the data structure. (Ashley and Lloyd, 1978)

For factor analysis Varimax rotation method was used and also used to find association between parameters so that the number of measured variable can be reduced. After obtaining correlation matrix and eigen values, factor loading were used to measure the correlation between variables and factors. These variables were then rotated by Varimax rotation technique to obtain new variability which is easy to interpret.

The table 5.3.6 represents the initial eigen value, percent of variance and cumulative percent of total variance and cumulative percent of total variance of ground water data. From the table it was inferred that the first four component together account for 75.845% of the total variance in which the first component accounts for 42.118%of the total variance. The second component explains 13.621 %, the third component 11.21 %and fourth component exhibit 8.89 % of the total variance. Guidelines have been developed for determining the number of factors to be used and ignored. For interpreting the data the method of Kaiser criterion was followed which retains only those factor having eigen values greater than 1. The first four factors having an eigen value greater than 1 has been used for further interpretation. The extracted components explain nearly 75.84% of the variability in the original variables. The number of eigen values can be estimated from a scree plot demonstrated in fig 5.3.1 as shown in this figure, the eigen value sharply decreased with in the first four components and then slowly stabilize for the remaining ones.

Factor 1: This factor has an Eigen value of 5.475 and accounts for 42.1% variance in the data. The variables are Na, Cl, TDS, EC, Ca, Mg and SO_4^{-2}.The first factor is characterized by very high loadings of Cl and Na and moderate to high loadings of TDS, Mg, EC, NO_3, Ca and SO_4. This factor reveals that the EC and TDS in the study area are mainly due to Na and CI which can be attributed to pollution load due to industrial activities.

Factor 2: This factor has an Eigen value of 1.771 and accounts for 13.6% variance in the data. The variables are pH and HCO_3., this factor can be attributed to weathering process.

Factor 3: This factor has an Eigen value of 1.457 and accounts for 11.2% variance in the data. The variables are K and SO_4.

Factor 4: This factor has an Eigen value of 1.157 and accounts for 8.9% variance in the data. The single variable is F. So, this factor can be attributed to natural geochemical process taking place in the area.

Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**
Cl	0.928			
Na	0.903			
TDS	0.845			
Mg	0.844			
EC	0.823			
NO_3	0.763			
Ca	0.752			
SO_4	0.625		0.586	
pH		0.854		
HCO_3		0.784		
K			0.603	
F				0.669
Eigen value	5.475	1.771	1.457	1.157
Percentage of variance	42.1	13.6	11.2	8.9
Cumulative percentage	42.1	55.7	67.0	75.9

Table5.3.7. Varimax Rotated component matrix, eigen value, percentage of total variance and cumulative percentage of groundwater

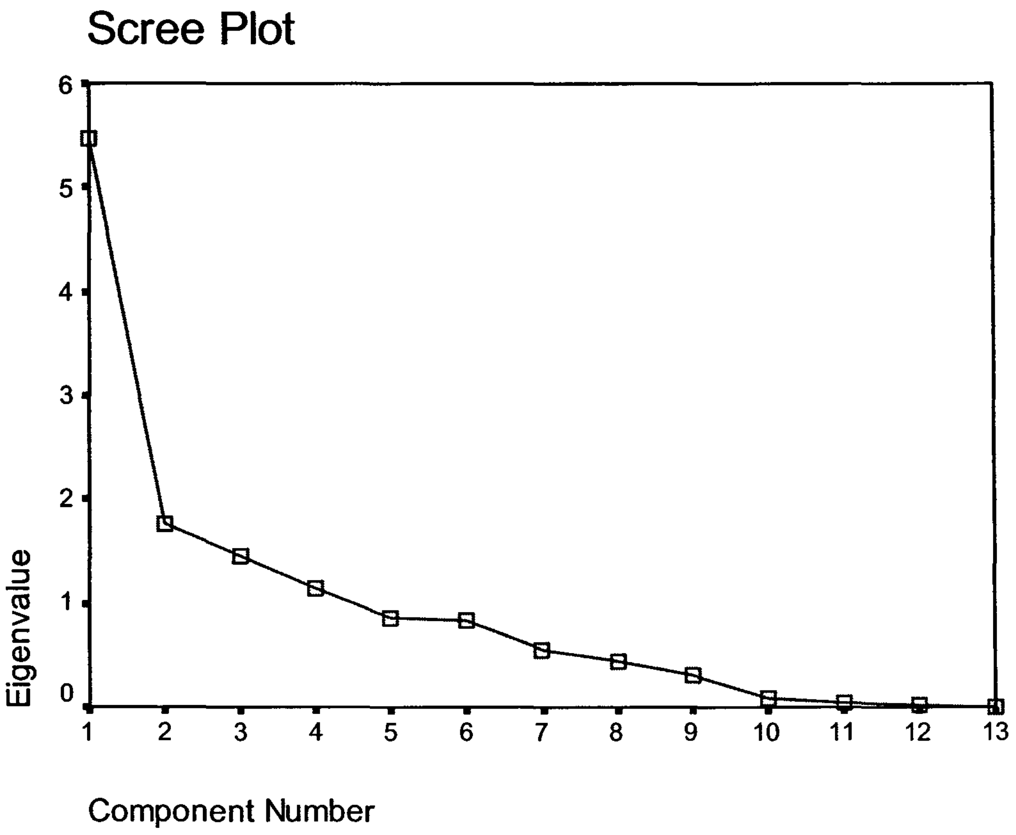


Fig 5.3.1 Scree plot of ground water

5.3.5 CLUSTER ANALYSIS

Cluster analysis is also another data reduction method that was used to classify entities with similar properties. Multivariate statistical method encompassing cluster analysis, factor analysis and principal component analysis have been successfully used in hydrochemistry for many years. Factor analysis was also used to find association between parameters so that the number of measured parameters can be reduced. Known associations were then used to predict unmeasured water quality parameters. The method divides a large number of homogenous groups on the basis of their correlation structure. For cluster analysis single linkage method was used. In this method the distance between the clusters was determined by the distance of the two closest objects (nearest neighbor) in the different clusters. (Pathak, et.al., 2008)

The dendrogram of various parameters produced two major groupings (Figure 5.3.2). The dendrogram shows that the association between EC and TDS parameters is more significant. The first cluster group shows close association between HCO_3^- and Mg^{++}. Samples belong to cluster 1 have high salinity, hardness, Ca, Mg, Na, K, Cl^-, DOC, SO_4^{--} and the figure shows that EC and TDS have grouped into cluster 2 and rest of the parameters form a separate cluster.

HIERARCHICALCLUSTER ANALYSIS

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

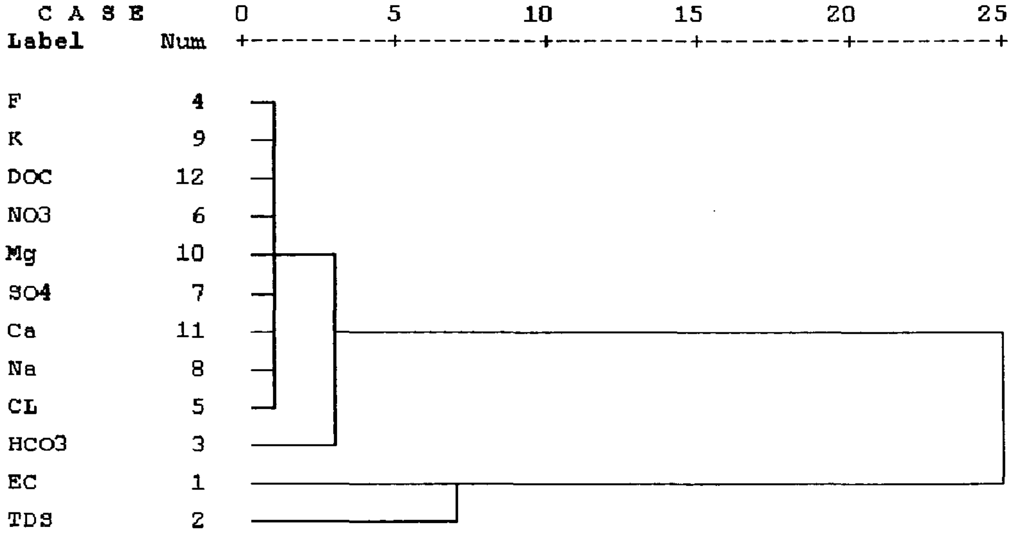


Figure 5.3.2 Dendrogram of 12 parameters of 27 cases

5.3.6 Chemical classification of ground water

Sodium Absorption Ratio

The Sodium Absorption Ratio (SAR) is an important parameter for determination of suitability of irrigation water. It is an index of sodium/alkali hazard. This index quantifies the proportion of sodium (Na^+) to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in a sample. The sodium adsorption ratio (SAR) values for each water sample were calculated by using following equation (Richard 1954).

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

where the concentrations are reported in meq/l.

Sodium adsorption ratio varies from 1.19 to 5.31 with an average of 2.82 in the groundwaters of Aurangabad whereas; it ranges from 2.73 to 3.19 with an average of 2.96 and 0.03 to 2.35 with mean value of 0.86 in Nasik and Lucknow groundwater samples respectively (Table .5.3.3).

Todd (1980) classified irrigation water with SAR values less than 10 as 'excellent' and the water is evaluated suitable for any crop. The suitability of the groundwater samples of the investigated areas for irrigation purpose was judged and classified based on Sodium Absorption Ratio. Based on the classification, the water samples of the study areas fall in excellent category for irrigation.

Piper Diagram

One method of comparing the results of chemical analyses of ground water is with a piper diagram (Piper, et.al., 1944)

The diagram (Figure 5.3.3) consists of two lower triangles that show the percentage distribution, on the milliequivalent basis, of the major cations (Mg^{++}, Ca^{++}, and Na^{+} plus K^{+}) and the major anions (Cl^{-}, $SO_4$2-and CO3 2-plus HCO3 -) and a diamond-shaped part above that summarizes the dominant cations and anions to indicate the final water type. This classification system shows the anion and cation facies in terms of major-ion percentages. The water types are designated according to the area in which they occur on the diagram segments. The cation distribution indicates that the most of samples of Lucknow, Aurangabad and Nasik in composition predominantly is Calcium type; where as some samples of Aurangabad is Na+ K type. In the anion triangle, for the ground water samples of Lucknow there is a tendency more towards carbonate/bicarbonate type water and samples of Aurangabad and Nasik ranges from Carbonte/ bicarbonate to mixed anion-type water.

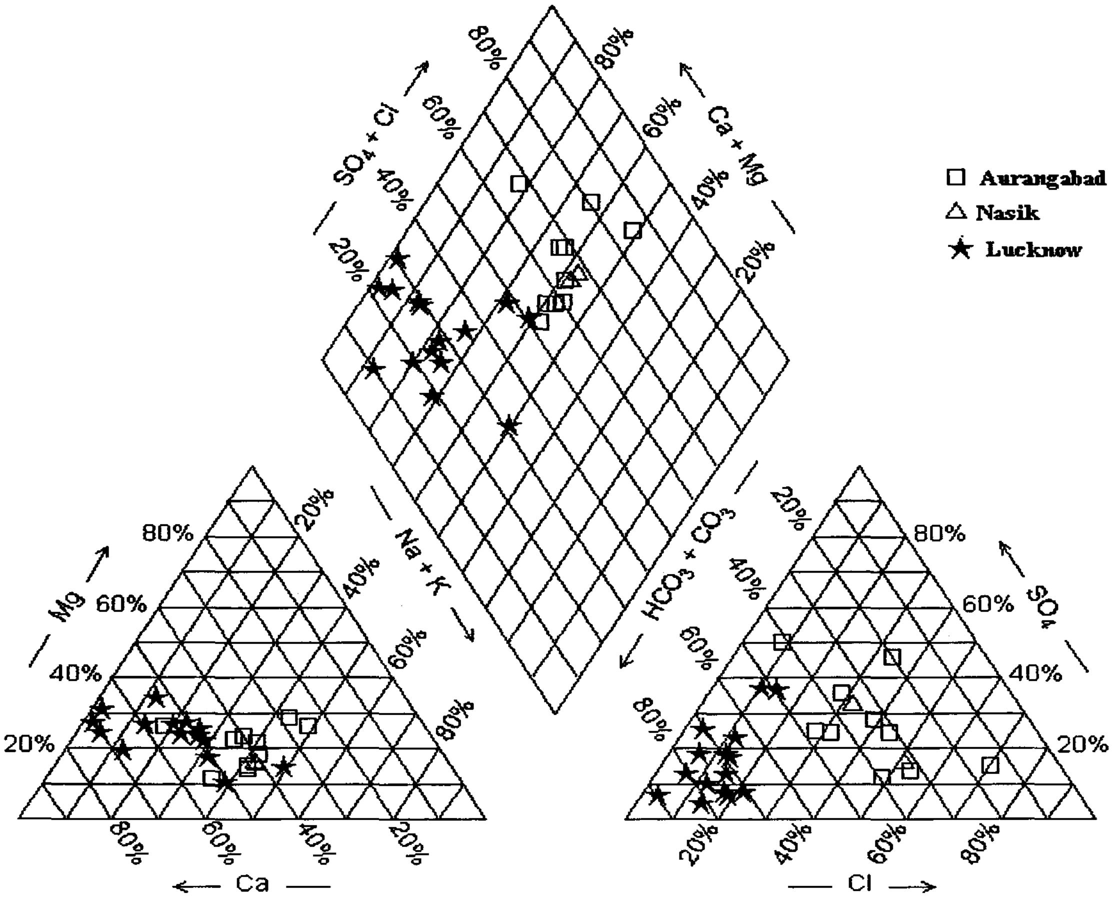


Fig 5.3.3 Piper diagram of ground water quality of Aurangabad, Luckonw and Nasik.

5.3.7 Conclusion

In Aurangabad and Nasik the host rock type is Basaltic which comprises mainly Quartz, Feldspar, Muscovite, Biotite, Olivine and Amphibole etc. the clay minerals (Biotite, Muscovite) are important because they provide very reactive surfaces and it releases Ca, Mg, SiO_2, HCO_3 in the water aquifer. Where as, in Lucknow, a alluvial plane which is made up of mainly sedimentary rocks comprises Calcite, Quartz, Clays, Dolomite etc thus here also the dominant anion is HCO_3^- ion and Ca and Mg are the dominant cation. This theory is supported by the results given in table 5.3.3 which shows that the dominant anion is HCO_3^- and cations are Calcium (Ca) and magnesium (Mg) in the gorund water of Aurangabad, Nasik and Lucknow.

Usually in ground water the Cl^- concentration is low but the table 5.3.3 demonstrates that samples collected from the locations adjoining to industrial site or with in the industrial site such as Chikalthana industrial area, paper mill, industrial area on the Jalna road, Aurangabad, paper mill and distillery in Nasik are showing excessive Cl^- concentration and Sodium Na^+ which is evidently shows the impact of paper mill and distillery on the ground water quality.

Summary and Conclusion

Chapter 6

SUMMARY AND CONCLUSION

The present study aimed at Characterizing humic acid which was extracted from the composted wastes of the Sugar industries (pressmud), papermill (Paper sludge)and spent wash treated soil of Aurangabad. In order to extract humic acid from the composted wastes, the raw organic waste was treated with distillery spent wash and microbial innoculum (mainly Pseudomonas striata, Bacillus polymyxa, Bacillus polymyxa and metarrhizium etc) and composting was done for six months. The various analyses were done with the extracted humic acid at the interval of six months. The characterization was done by elemental analysis to see the transformation of carbon, nitrogen and hydrogen during composting. Structural characterization was done by FTIR, Proton NMR and ^{13}C-NMR and molecular weight characterization was done by Maldi- Tof-MS and Gel electrophoresis.

Ground water chemistry studied by characterizing various parameters (pH, EC, TDS, major cations and major anions) to see the impact of industrial waste. Multivariate analysis was performed to study the Correlation between various parameters. The Sodium adsorption ratio (SAR) and piper diagram were used to define the water type and potentiality for irrigation purpose.

The salient features of the present study are discussed and concluded below.

- The extraction and quantification of humic acid from composted wastes revealed that the quantity of humic acid increased with the process of humification. It is also evident from the various humification indices.

- The elemental analysis demonstrated that the percentage of C and H of extracted humic acid decreased during composting or humification because of loss of carbon (C) as carbon dioxide (CO_2) and hydrogen (H) as water vapour (H_2O).

Except in paper sludge of Faizabad the nitrogen (N) percentage in humic acid decreased during the composting process which may be attributed to their low C/N ratio at the initial stage of humification of these organic waste or due to lack of oxygenation.

- Humic acid extracts from composted materials yielded high C/H ratio in pressmud of Faizabad and paper sludge of Faizabad, which showed the higher degree of maturity, but in other sample no regular pattern was observed in C/H ratio thus this study could not conclude about their degree of maturity by this parameter. The decreasing C/N ratio which had reached to less than 10 showed higher degree of maturity in all the samples especially in pressmud of paper sludge of Faizabad.

- The increasing C/H and decreasing C/N ratio during humification could be the result of the degradation of carbohydrates, polysaccharides or fatty aids, and their incorporation into the humic "core" of N containing groups through condensation of proteins and modified lignin, or sugar – amine condensation.

- The decreasing E_4/E_6 ratio in all the composts indicated a progressive degree of composting. It is also clearly indicated by the quantification of extracted humic acid (Cha) at various stage and humification index (HI). The higher Cha and HI indicated that a large proportion of different organic waste and spent wash treated soil was significantly converted into humic acid. Correlation matrix demonstrated strong correlation between HI and Cha. Good correlation was also observed among organic matter degradation, E_4/E_6 ratio, C and H percentage.

- The structural characterization by FTIR spectras revealed that transformation occurred during the different composting processes which is reflected by a decrease in the bands at 2925-2850 cm^{-1} which is attributed to aliphatic groups (except for humic acid from pressmud of Aurangabad) and at 1040 cm^{-1}, and an increase in those at 1715, 1640, 1460 and 1400 cm^{-1}. The rise in these bands suggests an increase in carbonyl groups (COOH, Ketones, aldehydes, esters) as well as aromatic, phenolic and quinone structures. The changes observed by FTIR indicated the decrease in aliphatic and polysaccharide structures and the increase in more oxidized and, probably, polycondensed aromatic components during composting.

- The pressmud of Aurangabad (PMA) and paper sludge have shown high lignin content and polysaccharides moieties as compared to PMF (Pressmud Faizabad) but with the progress of humification process the lignin content and polysacchaides have increased in all the samples except in soil which might be due to excessive degradation of lignin or polysaccharides.

- The proton (^{1}H) NMR studies demonstrated that the protons in aromatic region of all the spectra have shown significant increase with the period of humification process, which shows the stabilization and condensation process involved during humification process.

- It is clear from the ^{13}C-NMR spectra obtained in the present study that both the aliphatic as well as aromatic carbons are increasing during composting process, which reflects that during composting, the unstable organic compounds such as aliphatic materials are transformed through intense microbial activities into more stable humic compounds with more oxidized, olefinic or aromatic structures that could include more polycondensed rings.

- Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and gel electrophoresis were used to determine the molecular weight distribution of the humic acid extracted from the composted wastes of sugar mill and paper mill and compared with the result of gel electrophoresis.

- Electrophoresis of humic acid samples, isolated from the compost of different organic waste showed that all Humic acids formed four naturally colored fractions, named A, B, C and D with different electrophoretic mobility (EMs): A – start zone, that did not move into the gel; B – narrow zone in the mid part of the gel; C and D – two zones in the bottom of the gel, combined into fractions C + D due to relatively close electrophoretic behavior. The pressmud of Aurangabad(PMA), Pressmud of Faizabad (PMF), Paper sludge of Faizabad (PSF) and soil of Auranagabad (SOIL A) showed the same numbers and EM of individual bands of humic fractions.

- It was found that humic acids are the mixture of same and /or similar compounds and the results of MALDI-TOF-MS were supported by the conventional method of Polyacrylamide Gel Electrophoresis (PAGE) for determining the molecular size distribution of the humic substances.

- After characterizing humic acid from the wastes collected from industries of diverse climatic zones i.e. tropical (Aurangabad and Nasik) and subtropical (Lucknow and Faizabad) by various techniques, it was observed that no significant variation occur in nature of humic acid, thus no impact of spatial variation occurred on the character of humic acid.

- In Aurangabad and Nasik the host rock type is Basaltic which comprises mainly Quartz, Feldspar, Muscovite, Biotite, Olivine, Amphibole , clay minerals (Smectite group) , etc., are important because they provide very reactive surfaces and it releases Ca, Mg, SiO_2, HCO_3 in the aquifer system. Where as, in Lucknow, a alluvial plane which is made up of mainly sedimentary deposits comprises, Quartz, Clays, Calcite ,Dolomite etc and hence they have the dominant anion and cation as HCO_3^- and Ca and Mg .

- Ground water quality in Aurangabad has been deteriorated much as compared to Lucknow, this may be due to more industrial setups in Aurangabad. All the major cations and anions have higher values in ground water especially Chloride Cl^- and

Sodium Na^+ in ground water collected from the Vishwa paper mill of Aurangabad. This excessive concentration significantly revealed the industrial impact on ground water quality. It is also interesting to note that higher values of most of the parameters were found in the samples taken from the areas which are located in close vicinity to industries and lower values were observed in new settlements or residential areas away from industrial sites.

- The Factor analysis (principal component analysis) revealed that ground water quality of the study areas (Lucknow , Auarangabad and Nasik) is controlled by four factors such as natural weathering, secondary leaching, non-mixing and partial mixing of different types of water and industrial effluent impact.

- The hierarchial cluster analyses classify all the parameters in two main clusters. The dendrogram shows that the association between EC and TDS parameters is more significant. EC and TDS have grouped into cluster 2 and rest of the parameters form a separate cluster indicating two main sources that control water quality.

- The piper diagram of ground water demonstrated that the cation distribution for most of the samples of Lucknow, Aurangabad and Nasik in composition predominantly is Calcium type, where as some samples of Aurangabad is Na+ K type. In the anion triangle, for the ground water samples of Lucknow there is a tendency more towards carbonate/bicarbonate type water and samples of Aurangabad and Nasik ranges from Carbonate/ bicarbonate to mixed anion-type water.

- The suitability of the groundwater samples of the investigated areas for irrigation purpose was judged and classified based on Sodium Absorption Ratio. Based on this classification, the water samples of the study areas fall in excellent category for irrigation.

- This study found that, apart from natural weathering process that control the hydrogeochemistry of ground water, the waste water of agro based industries has significant impacts on the ground water quality. This is due to continuous disposal of industrial effluents on land, which has limited capacity to assimilate the pollution load, which ultimately led to groundwater pollution. This shows the clear impact of agro based industries and land use patterns on the quality of ground water.

References

REFERENCES

Adani, F.,Genevini,P., Tambone, F., Montoneri, E., 2006. Compost effect on soil humic acid: a NMR. study. Chemosphere 65, 1300-1307.

ADEME, 1994. La collecte et le traitement des de´ chets, Guide pratique pour les e´ lus de Midi—Pyre´ ne´ es. 8(4/5):183–193.

Agnelli, A., Celi, L., Degl'innocenti, a., corti., and ugolini, 2000. Chemical and spectroscopic characterization of the humic substances from sandstone-derived rock fragments. Soil Science, 165 (4): 314-327.

Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P., 1985. Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation and Characterization. John Wiley & Sons, New York.

Ait Baddi, G., Alburquerque, J.A., Gonza´ lvez, J., Cegarra, J., Hafidi, M., 2004a. Chemical and spectroscopic analyses of organic matter transformations during composting of olive mill wastes. International Biodeterioration and Biodegradation 54, 39–44.

Ait Baddi, G., Hafidi, M., Cegarra, J., Alburquerque, J.A., Gonza´ lvez, J., Gilard, V., Revel, J.-C., 2004b. Characterization of fulvic acids by elemental and spectroscopic (FTIR and 13C-NMR) analyses during composting of olive mill wastes plus straw. Bioresource Technology 93, 217–323.

Ait.Baddi, G., Hafidi, M., Gilard. V., Revel, J.C.,2003. Characterization of humic acids produced during composting of olive mill wastes: elemental and spectroscopic analyses (FTIR and 13C-NMR), Agronomoie, 661-666

Alvarenga et al., 2007 P. Alvarenga, P. Palma, A.P. Gonçalves, R.M. Fernandes, A.C. Cunha-Queda, E. Duarte and G. Vallini, Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land, *Environment International* 33 (2007), pp. 505–513

Amir S., Hafidi M., Bailly JR., Revel JC, 2006, "structural characterization of Humic acids, extracted from sewage sludge during composting by thermochemolysis – gas chromatography- mass spectrometry", process biochemistry, Vol 41,issue 2.

Amir, S., Hafidi, M., 2001. Valorisation de boues de stations d'e´ puration des eaux use´ es par un bioproce´ de´ ae´ robie ''compostage''.Annales de Chimie, Sciences des Mate´ riaux 26, 409–S414.

Amir, S., Hafidi, M., Merlina, G., Hamdi, H., Revel, J.C., 2004. Elemental analysis, FTIR, 13C-NMR of humic acids from sewage sludge composting. Agronomie 24, 13–18.

Anderson, J.M. and Ingram, J.S.I., 1993. Tropical soil biology and fertility: a Handbook of methods, 2nd Ed., CAB Nc: 221.

APHA, AWWA and WPCF, 1995. Standard methods for the examination of water and waste water 19th| edition Washington DC.TICLE IN PRESS

Arno K., Volker Romheld and Chen, Y, 2002, Cadmium binding by fractions of dissolved organic matter and humic substances from Municipal Solid Waste Compost, J.Environ.Qual., 31: 1885-1892.

Aryal RK, Murakami M, Furumai H, Nakajima F, Jinadasa HKPK (2006) Prolonged deposition of heavy metals in infiltration facilities and its possible threat to groundwater contamination. Water Sci Technol 54(6–7):205–212

Ashley RP, Lloyd JW (1978) An example of the use of factor analysis and cluster analysis in groundwater chemistry interpretation. J Hydrol 39:355-364

Avena,M.J., Koopal L.K. Van Riemsdijk,W.H., 1999, Proton binding to humic acids; electrostatic and intrinsic interaction ,J., colloid Interface Sci. 217, 37-48.

Baglieri, A., Ioppolo, A., Negre M. and Gennari, M., 2006. amethod fro isolating soil organic matter after the extraction of humic and fulvic acids.

Banff, Alta, June, 1984) Multivariate analysis in hydrogeochemistry: an example of the use factor and corre spondence analyses. Groundwater 27:27-34

Banner JL, Wasserburg GJ, Dobson PF, Carpenter AB, Moore CH (1989) Isotopic and trace element constraints on the origin and evolution of saline groundwaters from centural Missouri. Geochim Cosmochim Acta 53:383–398

Barcelona , M.J., 1984, TOC determination in ground water: Ground water, 22, 18-24.

Bartarya SK (1993) Hydrochemistry and rock weathering in a subtropical lesser Himalayan river basin in Kumaun, India. J Hydrol 146:149-174

Baruah, A.K., Sharma, R.N. and Baah, G.C. 1993, Impact of sugar mill and distillery effluents on water quality of river Gelabil, Assam, Indian Journal of Environmental Health, 35, 4, pp 288-293.

Bellamy, K.L., C. Chong, and R.A. Cline. 1995. Paper sludge utilization in agriculture and container nursery culture. J. Environ. Qual. 24:1074-1082.

Belzile, N., Joly, H.A. and Li, H., 1997. characterization of humic substances extracted from lake sediments. Canadian Journal of chemistry, 75: 14-27.

Bernal, M.P., Paredes,C., Sanchez, M.A., Cgarra, J., 1998. Maturity and stability parameters of composts prepared with a wide range of organic wastes . Biores. Technol. 63, 91-99

Borrero et al., 2003. M.A.V. Borrero, J.T.V. Pereira and E.E. Miranda, An environmental management method for sugar cane alcohol production in Brazil, *Biomass Bioenergy.* 25 (2003), pp. 287–299

Campbell, c.A., Paul, E.A., Rennie, D.A., Mccallum, J., (1967) Applicability of the carbon dating method of analysi to soil humus studies. Soil science 104, 217-224.

Campitelli, P and Ceppi, S., 2008. Effects of composting technologies on the chemical and phiscochemical properties of humic acids, Geoderma, 144, 325-333

Campitelli, P.A.,Velasco,M.I., Ceppi,S.B., 2006. Chemical and physicochemical characteristics of humic acids extracted from compost, soil and amended soil, talanta 69, 1234-1239

Cavani, L., Ciavatta, C., Trubetskaya, O.E., Reznikova, O.I., Afanas'eva, G.V. and trubtskoj, O.A., 2003. Capillary zone electrophoresis of soil humic acid fractions obtained by coupling size exclusion chromatography and polyacrylamide gel electrophoresis. J. of chromatography 983; 263-270

Ceppi S.B., Velasco M.I., Campitelli P.A., Mendez EMP & Havel .J., 2005 "Characterization of Humic acids originated from Argentinean Compost and soil by Capillary Electrophoresis and mass – spectrometry." , J..Chil.Chem.Soc. 50, no, 527-533

Chaturvedi P.K., Seth C.S. and Mishra V., 2006, Sorption kinetics and leachability of heavy metal from the contaminated soil amended with immobilizing agent (humus soil and hydroxyapatite), Chemosphere.

Chefetz, B., Salloum, M.J., Deshmukh, A.P., Hatcher, P.G., 2002a. Structural components of humic acids as determined by chemical modifications and carbon-13 NMR, pyrolysisand thermochemolysis-Gas chromatography/mass spectrometry. Soil Sci. Soc. Am. J. 66, 1159–1171.

Chen, J., Chiu, Ch., 2003. Characterization of soil organic matter in different particle-size fractions in humid subalpine soils by CP/MAS 13C NMR. Geoderma 117, 129–141.

Chen, Y., Katan, J., Gamliel, A., aviad, T., Snitzer, M., 2000. Invovement of soluble organic matter in increased plant growth in solarized soils. Biology and Ferility of soils32, 28-34

Chen,Y.,Senesi,N.,Schnitzer,M. 1977. Information provided on Humic substances by E_4/E_6 ratios. Soil Science Soc.Am.J, 41, 352-358.

Chikkatur, A., Rajmohan, P., Sastry,S., 2003 The impact of west coast paper mills, Dandeli, ESG note for Kali Bachao Andolan, workshop, 19th September.

Christl,l., Kretzschmar,R., 2001. Relating ion binding by fulvic and humic acids to chemical composition and molecular size.1.Proton binding .Environ.sci.Technology.35, 2505-2511

Christman, R.F., Norwood, D.L., Seo, Y., Frimmel, F.H., 1989. Oxidative degradation of humic substances from freshwater environments. In: Hayes, M.H.B., MacCarthy, P.,

Ciavatta, C., Govi, M., Pasotti, l., Sequi, P., 1993. Changes in organic matter during stabilization of compost from municipal solid wastes. Bioresource Technol. 43, 141-145.

Ciavatta,C.,Chirenje, T., Ma, L.Q., Martinez, g., 2004. Influence of compost on soil organic matter quality under tropical conditions, Geodema, 123, 355-361.

Contreras, A.M., Rosa, E., Perez.M, langenhour, H.V., Dewalf, J., 2008, Journal of cleaner production, 17, 772-779.

Davis, g., Ghabbour e.A. and C.steelink 2001. Humic acids: arvelous products o soil chemisty. J. Chem.. Educ., 78, 1609-1614.

Diaz-Burgos, M.A., Polo, A., Calcinai, M., Masciandaro, G., Ceccanti, B., 1994. Use of pyrolysis-gas chromatography to evaluate sludge humification. In: Senesi, N., Miano, T.M. (Eds.), Humic Substances in the Global Environment and Implications on Human Health. Elsevier, Amsterdam, pp. 1285–1289.

Dudka, S., Muller, W.P., 1999. Accumulation of potentially toxic elements in plants and their transfer to human food chain. Journal of Environmental Science and Health B 34, 681–708.

Elaida M.Pena-Mendez, Josef Havel and Jiri patocka 2005. Humic substances – compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine .J. Appl. Biomed, 3, 1-12

Elvira, C., Sampedro, L., Dominguez, J., Mato,S., 1996.Vermicomposting of wastewater sludge from paper pulp industry with nitrogen rich material.soil boil.Biochem.29, 759-762.

Ferrer, J., Paez, G., Mármol, Z., Ramones, E., Chandler, C., Marin, M., Ferrer, A., 2001. Agronomic use of biotechnologically processed grape wastes. Bioresource Technol. 76, 39–44.

FitzGibbon F, Singh D, McMullan G, Marchant R (1998). The effect of phenolic acids and molasses spent wash concentration on distillery wastewater remediation by fungi. Process Biochem. 33: 799-803.

Francioso, O., Ferrari, e., Saladine N., Montecchio, Gioacchini, P., Ciavatta C., 2007, TG-DTA, Drift and NMr characterization of humic like fractions from olive wastes and amended soil, Journal of Hazardoous Materials 149, 408-417.

Francioso, O., Sa´nchez-Corte´s, S., Casarini, D., Garcia-Ramos, J.V., Ciavatta, C., Gessa, C., 2002. Spectroscopic study of humic acids fractionated by means of tangential ultrafiltration. J. Mol. Struct. 609, 137–147.

Freeze RA, Cherry JA (1979) Groundwater. Prentice-Hall, Engle wood Cliffs, p 604

Fuentes,M., Baigorri, R., González-Gaitano, G. and José M.,Garcia,M., 2007,The complementary use of ^{1}H NMR, ^{13}C NMR, FTIR and size exclusion chromatography to investigate the principal structural changes associated with composting of organic materials with diverse origin,Organic geochemistry, 38,12,2012-2023

Fukushima, M., Yamamoto.K., Ootsuka, K., Komai, T., Aramaki, T., Horiya,S.,2009.Effect of the maturity of wood waste compost on the structural features of humic acds, Bioresource technology, 100, 791-797.

Gajdosova, D., Novotna,K., laska,k., Prosek, P., and Havel, J. 2003, Seperation and characterization of humic acids from antartica by capillary electrophoresis and matrix-assisted laser desorption ionization time of flight mass spectrometry inclusion complexes of humic acids with cyclodextrins, Journal of Chromatography. 1014, 117-127.

Gajdosova, D., Pokorna, L., and Havel, J., 2000, Humic substances versatile components of plant, soil and water. The Royal Society Cambridge, Cambridge.Davies,G., Ghabbou, E.A. (ed). Pp 133-149.

Gajdosova,D.,Pokorna,L., laska, K. Prosek, P., Havel,J. 2001. Humic substances structures, Models and functions, the Royal Society of Cambridge, Davies, G. and Ghabbour, E.. (ed) pp 121-131.

Garcia Gil, J.C., ceppi, S.B., Velasco, M.I., Polo,a., Senesi,N., 2004. Long term effects of amendment with municipal solid waste compost on the elemental and the acidic functional group composition and pH- buffer capacity of soil humic acids. Geoderma 121, 135-142

Garcia, C., Hernandez, T., Costa, F., 1992. Characterization of humic acids fromuncom posted and composted sewage sludge by degradative and non-degradative techniques. Bioresource Technology 41, 53–57.

Garcia,B.,Mogoll'on, J.L., Lopez,L.,Rojas,A., and Bifano,C. 1992. Humic and fulvic acid characterization in sediments from a contaminated tropical river. Chem.Geol., 118 (1-4), 271-287.

Gondal, D., lopez, R., fiol, S., Antelo, J.m., Arce, F., 2005. Chracterization and acid base properties of fulvic and humic acids isolated from two horizons of an ombrotrophic peat bog. Geoderma. 126, 367-374

González-Vila et al., 1999 F.J. González-Vila, G. Almendros and F. Madrid, Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil, *The Science of the Total Environment* 236 (1999), pp. 215

Griffith, S.M., Schnitzer, M., 1989. Oxidative degradation of soil humic substances. In: Hayes, M.H.B., MacCarthy, P., Malcolm, R.L., Swift, R.S. (Eds.), Humic Substances II. In Search of Structure. John Wiley & Sons, New York, pp. 69–98.

Gross J, Strupat K (1998) Matrix-assisted laser desorption /ionization mass spectrometry applied to biological macromolecules. Trends Anal Chem 17: 470–484.

Hamadi M., (1993) Valorisation et epuration des effluents des huilerus d'olive:l'utilite'de la microbiologie industrielle, olivae 46, 20-23

Hamoda, M. F., H.A. Abu Qdais and J. Newham. 1998. Evaluation of municipal solid waste composting kinetics. Resources, Conservation and Recycling 23: 209-223.

Havel, J.,Pena-Mendez, E.M., Luba, P., and Havlis, J. 2001. Recent development in humic acid characterization. Acido-basic and complexation properties, separation and reliable fingerprints by capillary electrophoresis and maldi-tof Mass Spectrometry. Ninth international meeting of the IHSS, Adelaide 1998.In understanding and managing organic health effects of nitrates in drinking water. Prog Water Technol

Hem,J.D, 1991,Study and interpretation of the chemical characteristics of natural water,U.S Geological survey water-supply paper 2254, Scientific Publishers, Jodhpur.

Hsu, J.M.., Lo, S, L., 1999, chemical and spectroscopic analyses of organic matter transformation duing composting of pig manure. Environmental Pollution 104, 189-196.

Huang, G.F., Wong, J.W.C., Wu, Q.T., Nagar, B.B., 2004. Effect of C/N on composting of pig manure with sawdust. Waste Manage. 24, 805-813.in: Piccolo A. (Ed.), Humic substances in terrestrial ecosystems,

Jacks G (1973) Chemistry of groundwater in a district in Southern India. J Hydrol 18:185-200

Jain,S.K., Agarwal, P.K and singh V.P.,2007, Hydrology and water resources of India , water science and technology library.

Jiménez and García, 1992 E.I. Jiménez and V.P. García, Determination of maturity indices for city refuse composts, *Agriculture Ecosystems and Environment* 38 (1992), pp. 331–343.

Kapur, M.L., 1995. Direct and residual value of sulphitation cane filter cake as a nitrogen source for crops.J.Indian Soc.Soil Sci. 43 (1), 63-65

Karen Heymann, Hamid mashayekhi and Baoshan Xing 2005. Spectroscopy analysis of sequentially extracted humic acid from compost. Spectroscopy letters 38, 293-302

Karrasch, B.; Parra, O.; Cid, H.; Mehrens, M.; Pacheco, P.; Urrutia, R.; Valdovinos, C.; Zaror, C., (2006). Effects of pulp and paper mill effluents on the microplankton and microbial self-purification capabilities of the Biobýo River, Chile. Sci.Total Environ., 359 (1-3), 194– 208.

Kaufmann R (1995) Matrix-assisted laser desorption ionization (MALDI) mass spectrometry: a novel analytical tool in molecular biology and biotechnology. J Biotechnol. 41: 155–175.

Kögel-Knabner I. 2002. The macromolecular organic composition in plant and microbial residues as input to soil. Soil Biol. Biochem. 34, 139-162.

Kononova, M.M., 1996 soil organic Matter.oxford;Pergamon press, 544.

Kopinke, F.D., Georgi, A., Mackenzie, K., 2001. Sorption of pyrene to dissolved humic substances and related model polymers. 1. Structure-property correlation. Environ. Sci.

Kovac, N., Faganeli, J., Bajt, O., Set, B., Orel, B., penna, n.,2004. chemical composition of macroaggregates in the northen Adriatic Sea. Organic geochemistry 35, 1095-1104.

Kujawinski, e.b., Freitas, m.A., Zang, X., Hatcher, P.G., green church, K.B and Jones, r.b.,2002. the application of electrispray ionization mass spectrometery (ESI MS) to the Structural characterization of natural organic matter. Org. Geochem., 33, 171-180.

Kumar M, Kumari K, Ramanathan AL, Saxena R (2007) A comparative evaluation of groundwater suitability for irrigation and drinking purposes in two agriculture dominated districts of Punjab, India. Environ Geology, Springer Pub http://dx.doi.org/ 10.1007/s00254-007-0672-3

Kumar M, Ramanathan AL, Rao MS, Kumar B (2006) Identification and evaluation of hydrogeochemical processes in the groundwater environment of Delhi. India Environ geology, Springer Pub 50:1025–1039

Lassen. P., Carlsen L., WarwickP., Randall A.and Zhao R.(1994). Radioactive labeling and characterization of humic mteials. Environ. Int., 20: 127-134.

Lavoie, J., Marchand, G., 1997. De´ termination des caracte´ ristiques a` conside´ rer d'un point de vue de sante´ et de se´ curite´ des travailleurs dans les centres de compostage des de´

chets domestiques. Etudes et Recherches : Rapport R-159. Institut de Recherche en Sante′ et en Se′ curite′ du Travail du Que′ bec, 37pp.

Lenheer, J.A., Malcolm, R.l., Mckinley, P.W., and Eccles, l.., 1974, Occurrence of dissolved organic carbon in selected ground water samples in the United states: U.S. Geological survey journal of research , 2, 361-369

Li, L., Zhao, Zh., Huang, W., Peng, P., Sheng, G., Fu, J., 2004. Characterization of humic acids fractionated by ultrafiltration. Org. Geochem. 35, 1025–1037.

Lovley D.R. Coates J.D., Blunt-Haris E.L., Phillips E.J.P. and Woodward J.C. (1996) Humic Substances As Electron Acceptors for Microbial Respiration. *Nature.* Vol 382, 1. pp 445-448

Malcolm,R.L., 1990,variations between humic substances isolated from soils, stream waters and ground waters as revealed by ^{13}C-NMR spectroscopy. In: MacCarthy, P., Clapp, C.E., Mlcolm, R.L. and P.R. (eds), Humic substances in soil nd crop sciences: Seleced Readings, American sci.of Agronomy Maison, pp 13-35.

Manivannan, R., 2005, “Recycling of Industrial Effluents” New India Publishing Agency, ISBN

Matthess G (1982) The properties of groundwater. Wiley, New York, p 498 Maya AL,

Loucks MD (1995) Solute and isotopic geochemistry and groundwater flow in the Central Wasatch Range, Utah. J Hydrol 172:31-59

McCornick, P. G., A. Hijazi and B. Sheikh (2004), “From Wastewater Reuse to Water Reclamation: Progression of Water Reuse Standards in Jordan”, in Scott, C., N. I. Faruqui and L. Raschid (eds.), “Wastewater Use in Irrigated Agriculture: Conronting the Livelihood and Environmental Realities”, Wallingford, UK; CAB International.

McCornick, P. G., S. R. Grattan and I. Abu-Eisheh (2003), "Water Quality Challenges to Irrigated Agriculture Productivity in the Jordan Valley", in Clemmens, A. J. and S. S. Anderson (eds), "Water for a Sustainable World – Limited Supplies and Expanding Demand", proceedings from the Second International Conference on Irrigation and Drainage, United States Commission on Irrigation and Drainage Conference, Phoenix, AZ, USA.

Mcdonnell, R., Holden, N.M., Ward, S.M., Collins, J.F., Parrell, E.P. and M.H.BHayes 2001. Characteristics of humic substances in the heartland and forested peat soils of the Wicklow Mountains. Biology and environment:Proceedings of the royal irish academy.

Miller, F.C., 1993. Composting as a process based on the control of ecologically selective factors. In: Meeting, F.B. (Ed.), Soil Microbial Ecology. Marcel Dekker, Inc., New York, pp. 515–544.

Minhas,P. S. and J. S. Samra (2004), "Wastewater Use in Peri-Urban Agriculture:Impacts and Opportunities", Central Soil Salinity Research Institute, Karnal, India.

Mishra V., Pandey A.K., and Pandy,S.D.,2000, stability constants of metal – humic acid complexes and its role in the environmental detoxification., Ecological and Environmental Safety 47,195-200.

Montoneri,E., Savarino, P., Adani, F., Genevini, P.l., Ricca, G., Zantti.F., Paoletti, S., 2003. polyalkylphenyl- sulphonic acid with acid groups of variable strength from compost. Waste Management 23, 523-535.

Mukherjee, Sacchidananda and Nelliyat, Prakash,Ground Water Pollution and Emerging Environmental Challenges of Industrial Effluent Irrigation: A Case Study of Mettupalayam Taluk, Tamilnadu (March 2006). Available at SSRN: http://ssrn.com/abstract=1021153

Murugaragavan,R., 2002. Distillery spentwash on crop production in dryland soils.M.Sc(Environmental Sciences) thesis, Tamil Nadu agricultural University, Coimbatore, India.

Mustin, 1987 Mustin, M., 1987. Le Compost. Gestion de la Matière Organique. Éditions François Dubusc, Paris.

Norbu.T Masters thesis on Pretreatment of Municipal Solid waste by windrow composting and vermicomposting, asian institute of technology, school of environmental resource and development, Thailand, August 2002.

O'Riordan T, Bentham G (1993) The politics of nitrate in theZ ˇ .UK. In: Burt TP, Heathwaite AL, Trudgill ST (eds) Nitrate—Processes, patterns and management. Wiley, New York, pp 403–416

Oglesby, R.T., Christman , R.F, and .H. driver , 1967. The biotransformation of ligin to humus fact and postulate. Adv. Appl. Microbiol., 9, 171-184.

Oleszczuk and Baran, 2005 P. Oleszczuk and S. Baran, Polycyclic aromatic hydrocarbons content in shoots and leaves of willow (*Salix viminalis*) cultivated on the sewage sludge-amended soil, *Water, Air and Soil Pollution* 168

Ouatmane, A., Dorazio, V., Hafidi, M., Revel, J.C., Senesi, N., 2000. Elemental and spectroscopic characterization of humic acids.

Pace, M.G., B.E. Miller and K.L. Farrel-Poe. 1995. The Composting Process October 1995. Extension, Utah State University. AG- WM 01

Pandey.A.K., Pandey.S.D., Misra.V and Viswanathan.P.N. (1999) Role of free radicals in the binding of organochlorine pesticides and heavy metals with humic acid. The *Sciences of The Total Environment.* Vol 231, issues 2-3, 1999 pp 125-133.

Paredes, C., Roig, A.,Bernal , M.P., Sanchez-Monedero, m.A., Cegarra,J.,2000, Evolution of organic matter and nitrogen during co-composting of olive mill waste water with solid organic wastes.Biol.Fert. Soils32, 222-227

Paredes,C., Bernal, M.P., Roig,A., Cegarra,J., 2001. Effects of olive mill waste water addition in composting of agroindustrial and urban wastes.biodegradation 12, 225-234.

Pathak, J.K., Alam, M., and Sharma, S., 2008, Interpretation of ground water quality uding multivariate statistical technique in Moradabad city, western Uttarpradesh state, India

Pawar, N. J.; Pondhe, G. M.; Patil, S. F. 1998. Groundwater pollution due to sugar-mill effluent, at Sonai, Maharashtra, India, *Environmental Geology* 34: 151–158.

Perminova I.V., Hatfield K., 2005, "Use of humic substances to remediate polluted environment from theory to practice, Springer, printed in the Netherlands, 3-36.

Peuravaori, J and Pihlaja, K., 1998, Multi-method characterization of lake humic matterisolated with two different sorbing solids, Analytica Chimica acta, 363, 235-247

Piccolo, A., Conte, P., Trivellone, E., van Lagen, B., Buurman, P., 2002. Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. characterization of size-separates by Pyr-GC-MS and 1H-NMR spectroscopy. Environ. Sci. Technol. 36, 76–84.

Piccolo, A., Nardi, S., Concheri, G., 1996. Micelle-like conformation of humic substances as revealed by size exclusion chromatography. Chemosphere 33, 595–602.

Piper, A.M., 1944, A graphic procedure in the geochemical interpretation of water analysis: American geophysical union transaction, 25, 914-923.

Preston, C.M., Schnitzer, M., 1984. Effects of chemical modifications and extractants on the carbon-13 NMR spectra of humic materials. Soil Science Society of America Journal 48, 305–311.

Pretsch, R., Clerc, T., Seibl, J., Simon, w., 1998. Tablas para la determination of the third german edition. Springer-verlag, Barcelona.

Rajmohan N, Elango L (2005) Nutrient chemistry of groundwater in an intensively irrigated region of Southern India. Environ Geol 47:820–830

Ramaswamy, P.P.,1999.Recycling of agriculturaland agro-industry waste for sustainable agricultural production. J. Indian Soc.soilSci.47 (4), 661-665.

Rankama Kalervo, and sahama, T.G., 1950, Geochemistry: Chicago, University of Chicago Press, 912.

Rashid, M.A., Vilks, G. 1977b environmental controls of methane production in holocenebasins in Eastern Canada. Organic chemistry 1, 53-59.

Report on environmental status of Nashik Region Maharashtra, Maharashtra pollution control board, 2000

Richard, L. A. (1954). Diagnosis and improvement of saline and alkali soils. Agricultural handbook 60 (pp. 160). Washington, DC: USDA.

Riffaldi, R. Levi-Minzi and A. Saviozzi., 1993, Humic fractions of organic wastes, *Agriculture, Ecosystems and Environment* 10. pp. 353–359

Riggle, J., Von Wandruska, R., 2002.conductometric characterization of dissolved humic materials. Talanta 57, 519-526

Rink, R., 1992. Composting methods. In: Rink, R. (Ed.), On-Farm Composting Handbook. Northeast Regional Agricultural Engineering Service, Cooperative Extension, Ithaca, NY, pp. 24–42.

Rivero, C., Chirenje, T., Ma, L.Q., Martinez, G., 2004. Influence of compost on soil organic matter quality under tropical conditions. Geoderma 123, 355–361.

Rocha, J.C., Sargentini, Jr.E., Toscano, I.A.S, Ros, A.H. and P.Burba, 1999. Multi – Method study on aquatic humic substances from the " Rio Negro"-amazonas state/ brazil. Emphasis on molecular size clssificatio on their metal content.J.Braz.Cem.soc. 10 (3) 169-175.

Salloum, M.J., Chefetz, B., Hatcher, P.G., 2002. Phenanthrene sorption by aliphatic-rich natural organic matter. Environ. Sci. Technol. 36, 1953–1958.

Satisha, G.C and Devarajan, L., 2007, Effect of amendments on windrow composting of sugar industry pressmud, Waste management, 27, 1083-1091.

Savant, D.V., Abdul- Rahman, R., Ranade, D.R., 2006. Anaerobic degradation of adsorbableorganic halides (AOX) from pulp and paperindustry waste water. Bioresource technology. 97, 1092-1104

Schoenheinz, D.,Grischek, T., Berenoy, V., Gutken, I., Shebesta, A., Hiscock, K.,Macheleidt, W and Nestler, W., 2002, ground water pollution at a pulp and paper mill at sjasstroj near lake ladoga, Russia, Geological society, London, Special publications, 193, 277-291.

Schuh WM, Klinekebiel DL, Gardner JC, Meyar RF (1997) Tracer and nitrate movements to groundwater in the Norruem Great Plains. J Environ Qual 26:1335-1347

Scott, C. A., N. I. Faruqui and L. Raschid-Sally (2004), "Wastewater Use in Irrigated Agriculture: Management Challenges in Developing Countries", in Chapter 1 in Scott, C.A., Faruqui, N.I., Raschid-Sally, L. (eds.), "Wastewater use in irrigated agriculture: Confroning the livelihood and environmental realities", Wallingford, UK; Colombo, Sri Lanka; Ottawa, Canada, CABI Publishing; IWMI; IDRC.

Sellami,F., Hachicha, S., Chotourou,M., Medhioub, K and ammar, e., 2008, Maturityassessment of compostd olive mill wastes using UV-spectra and humification parameters, Bioresource Technology, 99, 15, 6900-6907.

Sen, B and Chandra,T.S., 2007, Chemolytic and solid state spectroscopic evaluation of organic transformation during vermicomposting of sugar industry wastes., Biresource technology, 98, 680-1683.

Senesi N and Loffredo E 1999. The chemistry of soil organic matter. *In* Soil physical chemistry, Second Edition. Ed. D.L. Sparks, p. 239-370. CRC, Press LLC.

Senesi N., Miano,T.M., Provenzano, M.R. and Brunetti, G., 1991. Characterization , differentiation and classification of humic substances by Fluorescence spectroscopy. Soil sci., 152, 259-271.

Senesi, N., Miano, T.M., Brunetti, G., 1996. Humic-like substances in organic amendments and effects on native soil humic substances. In: Piccolo, A. (Ed.), Humic Substances in Terrestrial Ecosystems. Elsevier, Amsterdam, pp. 531–593 (Chapter 14).

Senesi, N., Plaza, C., Brunetti, G., Polo, A., 2007. A comparative survey of recent results on humic-like fractions in organic amendment and effects on native soil humic substances. Soil Biol. Biochem. 39, 1244–1262.

Senesi, N.and sipos, S. 1985. Molecular weight distribution, analytical and spectroscopic characterization of humic fractions sequentially isolated by organic solvents from a brown coal humic acid.Organic geochemistry, 8.157-162

Sharma B, Kumar M, Ramanathan AL (2004) Impact of Surface Water on the Groundwater Quality in and around Najafgarh Drain, Delhi. In: Ramesh R, Ramachandran S (eds) Freshwater management. Capital publishing Company, Delhi, pp 70–86.

Sharma, V.K., Canditelli, M., Fortana, F., and Cornacchia, 1997, Processing of urban and agro-industrial resides by aerobic composting: reiew Energy conversion and Management 38 (5) 453-478.

Shin, H.S., Monsallier, j.M. and choppin, G.R. 1999. spectroscopic and chemical characterization of molecular size fractionated humic acid. Talanta, 50, 641-647.

Shuval H, Gruener N (1977) Infant methemoglobinemia and otherhealth effects of nitrates in drinking water. Prog Water Technol

Singh, B.B., Srivastava, G.K and Gupta, R.P.1996.Effect of industrial effluents on ground water quality, at Sardarnagar, Gorakhpur (U.P) Journal of living world, 3,2,61-64

Singh, H.P., Mishra, J.P and Mahaver, L.R. 1998, observation on certain physico-chemical characteristics of water in relation to pollution of river Ramganga. Journal of Environmental biology, vol.19, No.4, 353-356.

Singh, I.B., Singh, S and Singh, M., 2008, Weathering in the Ganga Alluvial Plain: Geochemical signatures linking of the Himalayan source and the Bay of Bengal sink, Himalayan Journal of Sciences,vol.5(7)

Singh,R., 2000. Pollution in Abu Drainage – a preliminary report, Adv.plant Sci 13(1) 2000, 43-45.

Smidt, E., 2001. Eignung der FT-IR Spektroskopie zur Charakterisierung der organischen Substanz in Abf˙ allen. Doctoral Thesis, BOKU—University of Natural Resources and Applied Life Sciences, Vienna, Austria.

Stenson, A.C., Marshal, A.G. and Copper, W.T. 2003. Exact masses and chemical formulas of individual suwanee river fulvic acids from ultra high resolution ESI FT-ICR spectra, anl.chem., 75: 1275-1284.

Stenson, A.C., Marshall, A.G. and copper, W.T. (2002). Anal.cem, 75: 1275.

Stentiford, E.I., 1996. Composting control: principles and practice. In: de Bertoldi, M. (Ed.), The Science of Composting. Part I. Blakie Academic and Professional, Glasgow, UK, pp. 49–59.

Stevenson ,F.J. and Goh, 1974. Infrared spectr of humic acids elimination of interferencedue to hygroscopic moisture and structural changes accompanying heating with KBr. Soil Sci.11: 34-41

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions, second ed. John Wiley & Sons, New York.

Sun, R., Tomkinson, J., 2002. Comparative study of lignins isolated by alkali and ultra sound-assistd alkali extractions from wheat stra. Ultrasonics Sonochemistry 9, 85-93

Swift, R.C., Thornton, B.K. and Posner, A.M., 970. spectral Characterizatic of a humic acid fractionated with respect to molecular weight using an agar gel, soil sci., 110, 93.

Tanaka T, Nagao S, and Ogawa H, 2001, Attenuated total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy of functional groups of Humic acid dissolving in aqueous solution, Analytical Sciences, Vol 17. Publisher the Japan Society for analytical chemistry.

Thomson, M., Lssen, P, Dobel, S., Hansen, P.E., Crlsen, l. and Morgensen, B.b (2002). Characterization of humic materials of different origin: amultivariate approach for quantiying the latent properties of dissolved organic matter. Chemosphere, 9, 1327-1337.

Thurman, E.M. 1985. Organic geochemistry of natural waters. Martinus Nijhoff Dr W.Junk Publishers, dordrecht.

Tiwari, R.J., Bangar, K.S., Nema, G.K., Sharma, R.K., 1998. Long term effect of pressmud and nitrogenous fertilizers on sugarcane and sugar yield on a Typic Chromustert. J. Indian Soc. Soil Sci. 46 (2), 243–245.

Todd, D.K. (1980): Ground water Hydrology. – John Willey & sons publishers, New York

Toth J (1984) The role of regional gravity flow in the chemical and thermal evolution of groundwater. Proceedings of the first Canadian/ American conference on hydrogeology,

Trubetskoj,O.A., Trubetskaya, O.E., Afanas' eva, G.v., Reznikova,O.I. and saz-Jimenez, C.(1997). Polyacrylamide gel electrophoresis of soil humic acid fractionated by size exclusion chromatography and ultrafilteration. Journal of Chomatograhy, 767, 285-292.

Tubetskaya, O.E., Truetskoj, Ciavatta, C., 2001, evaluation of the transformation of organic matter to humic substances in compost by coupling sec-page, Bioresource Technology, 77, 51-56

Tuomela, M., Vikman, M., Hatakka, A., Ita¨ vaara, M., 2000. Biodegradation of lignin in a compost environment: a review. Bioresource Technology 72, 169–183.

Ussiri D.A.N and Johnson C.E., 2003, *Geoderma* 111 (2003) 123-149.

Vengosh A, Spivack AJ, Artzi Y, Ayalon A (1999) Geochemical and

Wallick EI, Toth J (1976) Methods of regional groundwater flow analysis with suggestions for the use of environmental isotope and hydrochemical data in groundwater hydrology, IAEA, Vienna, 37-64

Wershaw, L., Pinckney, D.J., Llaguno, e.c. and Vicente- beckett, v.1990. NMR characterization of humic acid fractions from different Philipine soils and sediments Analytica chimica acta, 232, 31-42

Wershaw, L.,Pinckney, D.J., llaguno, E.C. and Vicente- Beckett, V. 1990. NMR characterization of humic acid fractions from different Philippine soilsnd sediments Analytica Chimica acta, 232, 31-42.

Wilson, H.L.,Smith R.J. and Raulinson, c.1989. Particulate trace metal compounds and fluxes across the southeastern US continental shelf.Mar.chem., 27, 283-297.

Wilson, M.A. Ellis, AV., lee, G.S.H., Froster, P., Rose, H., Lu, x.Q. and young, B.R. (1999). Structure of molecular fraction of bayr humic substances: I. Low temperature products. Ind.Eng. Chem.., 38; 4663-4674.

www.ar.wroc.pl/weber/powstaw2.htm
www.ar.wroc.pl/weber/powstaw2.htm
www.censusindia.net/
www.cpcb.nic.in/sept 2000 ii.htm
www.cpcb.nic.in/sept97ii.htm
www.epa.gov/history/topics/dioxins/01.htm
www.epa.gov/history/topics/dioxins/01.htm
www.maharashtra.gov.in/english/gazetteer/index.html
www.regional.org.au/assi/supersoil2004/s7/poster/1891-mahimairajas.htm
www.regional.org.au/assi/supersoil2004/s7/poster/1891-mahimairajas.htm
(www.gisdevelopment.net/application/environment/water/mi08_238.htm

Yadav, A. and Garg, V.K., 2009. Feasibility of nutrient recovery from industrial sludge by vermicomposting technology. Journal of hazardous materials, 168 (1) 262-8

Yadav, S.K., 2006, Human healh implications due to water toxicity by pulp and paper mill, J. Hum. ecol., 20 (2): 91-96.

Yamada, E., Ozaki, T., and Kimura, M., 1998. determination and behavoiur of humic substances as precursors of trihalomethane in environmental water. Anal. Sci., 14, 327-332.

Yates, L.M.,Engebreston., Haakenson, T,J., Von wandruszka.R., 1997. Immobilization of aqueous pyrene by dissolved humic acid. Anal Chim Acta, 356, 295-300.

Yuce, G., 2007, Spatial distribution of ground water pollution in the Porsuk River Basin (PRB), Turkey, Int. J. Environ.Pollution, 30, 529-547.

Zhou,P., Yan, H., Gu, B., 2005. Competitive complexation of metal ions with humic substances. Chemosphere 58, 1327-1337.

Zucconi, F., Monaco, A., Forte, M., De Bertoldi, M., 1985.Phytotoxins during the stabilization of organic matter. In: Gasser, J.K.R. (Ed.), Composting of Agricultural and Other Wastes. Elsevier Applied Science Barking, Essex, UK, pp. 73–85.