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PHYSICO-CHEMICAL AND MICROBIOLOGICAL STUDIES OF INDUSTRIAL WASTES GENERATED IN WAZIRPUR AREA OF DELHI

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MASTER OF PHILOSOPHY

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CERTIFICATE

This is to certify that the research work embodied in this dissertation entitled "***Physico-chemical and Microbiological studies of Wastes Generated in Wazirpur Area of Delhi***" has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi for the partial fulfilment of the award of **Master of Philosophy**. This work is original and has not been submitted, so far, in part or full, for any other degree or diploma of any University.

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CHAPTER I

INTRODUCTION

There has been a growing realization that the environment has limited assimilative and carrying capacity. This means that pollution control is essential in order to safeguard the environment and hence the quality of life. Waste, if not properly treated and handled, not only threatens human life in short term, but the environment as a whole in the long run. Unless we have a clear picture of the type and the quantity of the waste, which is generated from various sources, its proper management, including its handling, recycling, reuse, treatment as well as safe disposal, can not be planned.

Industrialization is generally believed to be the universal remedy for economic backwardness. Mounting pressure on industrialization to withstand in the context of advancement towards economic stability is constantly degrading the environment through air, water and soil pollution.

The increasing sophistication of society has resulted in the production of large quantities of hazardous wastes that are generally far more concentrated and harmful to the receiving environment. Such wastes may be in the form of solids, liquids or gaseous emissions. It should be noted that these wastes are either a by-product of initial production process, or may arise when objects or materials are discarded after they have been used.

Hazardous wastes could be defined as any material or mixture of materials, that is corrosive, flammable, reactive, toxic, or irritable. The chemical is also capable of causing serious injury, illness or damage to humans, animals and plants.

The term 'waste' implies that it is of no concern and is of no use to anyone. It is a problem because it is a material that is not wanted by the producers or consumers who, of course, seek to dispose it off at the lowest possible cost i.e. it is disposed to the land, water and air causing environmental pollution.

The industrial wastes are practically more troublesome than other wastes. It consists of toxic inorganic, organic materials and heavy metals which are harmful in degradation causing hazards to human health, living organisms and ecosystem. (Table-1) shows hazardous materials from some industries.

Microbial versatility is outstanding in the tolerance for extreme conditions as well as in the rapidity with which microbes adapt and modify their enzymatic machinery to face new challenges in the environment. It is among micro-organisms that we can find catabolic activities of great interest, which allow bio-remediation of environments heavily contaminated with recalcitrant inorganic and organic substances toxic to higher life forms (Young and Cerniglia, 1995). Microorganisms by virtue of their unique qualities viz. the small size, ubiquitous distribution, high specific surface area, potentially high rate of metabolic activity, physiological responsiveness, genetic diversitty, rapid growth rate and enzymatic and nutritional diversity, are able to inhabit extreme environment. They derive their carbon and energy requirement from inorganic and organic wastes, thus acting as agents of recycling of contaminants. However, they are also affected by the nature and concentration of

The following table (1) below shows Hazardous waste with their respective point of source :

	As	Cd	CHc[a]	Cr	Cu	Cn	Pb	Hg	Other Organics[b]	Sn	Zn
Mining & Metallurgy	*	*		*	*	*	*	*		*	*
Paints & Dies		*	*	*	*	*	*	*	*	*	
Pesticides	*		*			*	*	*	*		*
Electrical Electronics			*		*	*	*	*		*	
Chemical manufacturing			*	*	*			*	*		
Explosive	*				*		*	*	*		
Rubber & plastic			*			*		*	*		*
Batteries		*					*	*			*
pharmaceuticles	*							*	*		
Textile				*	*				*		
Petrolium & Coal	*		*				*				
Pulp & paper								*	*		
Leather				*					*		
Printing & Duplicating	*		*	*	*		*		*	*	
Electroplating & Metal finishing		*		*	*	*					*

Table (1) Some Hazardous material in industrial waste .

a = clorinated hydrocarbon

b = Aerolein, chloropicerin dimithyle, sulphate, dinitrobenzin dinitrophenol, nitroanaline Pentachlorophenol

toxic substance present in the waste. Therefore in contaminated environments, their abundance, diversity and composition vary significantly (Medigan et al., 1971; Rayner, 1995 ; Zehnder and Stumm 1988).

Microorganisms, with their different component members live as a community in the environment. Each member of community has a distinct role to play. In nature, a mixed microbial community may exist as discrete integrated units. In recent years, pollution induced changes in microbial communities structure have been widely recognized, but it is often difficult to define or quantitatively describe microbial community structure in natural environment (Senior et al., 1976 ; Davison et al., 1993).

Soil, owing to its special feature. i.e. involving biotic system the relative abundance and diversity of micro-organisms in soil reflects the state of its health. Anthropogenic pollutants which interact with microbiota in the soil system reduce their number and diversity. However, some microorganisms have novel genetic mechanisms by which either they develop tolerance towards such toxic materials or are able to degrade and recycle these contaminants. So a study of micro-organisms vis-a-vis hazardous wastes is important with the view of examining its recycling and management possibilities.

The national capital Territory of Delhi, with a population of approximately 10 millions covering an area of 1483 square kms, is highly polluted due to a large number of Industries. It has one of largest clusters of small scale industries in India, with a spectacular growth during the period 1968 to 1996 (Office of the commissioner of Industries, Delhi. 1996). Out of its 28 industrial sites, 'Wazirpur

Industrial Area' is a major industrial area releasing a significant quantity of hazardous solid waste. These industries often dump their wastes on the road side, or even in the industrial premises. The waste water is swept away in open drainage and underground sewage system, which gets solidified after 2-3 days of discharge thereby clogging the drains. During monsoon season water gets accumulated over the streets and becomes a streamlet with the dirty and hazardous wastes material of industries. Thus, it affects the ground water quality, biota, and even the health of the people living in the nearby surrounding area, (Lenka et al., 1992; Rao et al., 1993).

Therefore, in order to have an understanding of the environmental problem in that area, the nature and types of wastes generated and their effects on the surrounding environment, the present investigation was undertaken with the following objectives -

(1) Characterization of physico - chemical properties of industrial wastes.

(2) To study the total microbial population, as well as to identify the individual isolates of microorganisms in the industrial wastes.

CHAPTER II

LITERATURE REVIEW

The problem of solid waste disposal has been magnified to an alarming level in the recent past. Due to increasing urbanization, surging population and growing industries, the wastes generated enter into the environment thereby causing injuries to both its abiotic and biotic components.

REVIEWS ON PHYSICO-CHEMICAL CHARACTERISTICS

Industrial pollution by heavy metals is instantly recognised with the Minamata disaster in Japan, when several thousands of people suffered mercury poisoning by consuming the fish caught in Minamata bay, which was the recipient of Mercury released from a Vinyl Chloride plant, between 1953 and 1960 (Smith and Smith, 1975). Similarly, the high level of Cadmium of the local food stuffs in parts of Japan, attributable to irrigation water from the soil heap of an abandoned mine, caused Itai-Itai disease in 1955, mainly in women over forty. In U.S.A., the hazardous industrial solid wastes dumped in Love Canal were analysed. It was reported that out of its 82 chemical contaminants, 11 were suspected to be human carcinogens. Appreciable level of As, Sb, Cd, Cu, Cr, Pb, Ni, Ag and Zn were also detected. Cases of severe skin diseases, birth defects and elevated level of miscarriages resulted from hazardous leachates in that area (Brown, 1981).

Several workers have reported the leachability of various toxic metals in the industrial solid wastes. The rate and extent of leaching depends on the extent to which soluble complexes are formed between the metals and other components of the

leachate, metal speciation and pH (Bloomfield and Pruden, 1975 ; Gerrites et al., 1982; Sauders and Adams, 1987 ; Lun and Christianson, 1989 and Blais et al., 1993). The mobility of Zn and Cd are reported to increase with soil acidity (Esser and Bassam, 1981).

Plant uptake of heavy metals is influenced by solubility, mobility, pH, and chemical forms of the metal, soil cation exchange capacity, redox potential, texture and organic matter (John et al., 1972 and Iwai, 1975). Chumbley (1982), studied the role of heavy metals in plants and their effects on vegitation and uptake by roots.

Acidity of soil solution and the solubility of some metals are closely related. Bergkvist (1987) and Taylor et al., (1987) reported that the relationship between the pH and total concentration of Zn, Cd and Ni in the soil column is very close, though non-linear. They also reported that a drop in soil pH by merely 0.2 units range results in 3 to 5 fold increase in metal concentration.

Jenne (1968) reported that out of the total metals present in soil, small amounts (less than 7%) are taken up by edible parts of the vegitation thus introducing the metals into the food chain. Xian (1989), Clevenges and Mullins (1982) studied the heavy metals in contaminated soil using sequential extraction techniques. They reported that metals in the water soluble and exchangeable fractions would be readily bioavailable in the environment. Ma and Rao (1997) evaluated the mobility and bioavailability of metals from contaminated soil, and reported four metals in decreasing order (Zn>Cu>Cd>Ni) of mobility and bioavailibility, as a function of solubility and geochemical forms.

Adriano (1986) reported that Pb mobility and bioavailability are controlled by several soil factors such as pH, redox potential, organic matter, chemical form and species of Pb. Bramhley (1990) and Loganathan (1995) reported that most of the Cd applied to undisturbed soils remains in the top few cm of soil where plant roots are active. It is believed that Cd is unlikely to pollute the ground water because of its low mobility in soil.

The interaction of components of electroplating waste and its toxicity was studied by Dive et al., (1989) and Du et al., (1990). The elimination of phosphate from domestic sewage has been proved to be the most important step in the control of eutrophication. Gerrites (1993) observed that disposal of waste water in soil makes condition favourable for precipitates of PO_4^{3-} in $Ca_3(PO_4)_2$.

Reviews On Effects Of Wastes On Microbial Population And Diversity

There has been a thorough study made by different workers about the heavy metal toxicity towards micro-organisms and microbially mediated processes (Tyler, 1981; Duxbury, 1985; Babich and Stotozky 1986).

Bacterial and fungal abundance have usually been estimated as colony forming units (CFU) using plate count techniques. Freedman and Hutchinson (1980) did find a decrease in fungal CFUs near the Sudbury Smelter, though not very significant from the non-polluted soil samples. Greszta et al., (1979) added Pb-Cu sludge, Pb-Cu dust, Pb-Zn dust, and Cd-Pb-Zn dust to forest soils in a field experiment; they studied CFUs of fungi and bacteria, as well as numbers of cellulolytic, ammonifying and denitrifying bacteria. Negative effects were found but

at much higher levels. Zibilske and Wanger (1982) found bacterial CFUs to be less affected by the addition of Cd, Cu and Cr than soil ATP, which is an indicator of soil microbial biomass.

A reduction in the abundance and biomass of fungi and bacteria due to higher concentrations of heavy metals has been detected in numerous investigations. Fungi appear to be generally more tolerant than bacteria. Among bacteria gram negative bacteria appear to be more tolerant than gram positive ones, while selected groups, like *Azatobacter* or nitrifiers, have been reported to be especially sensitive to heavy metal pollution (Pancholy et al., 1975; Maliszewska et al., 1985).

The changes in the physico-chemical properties of soils by the application of sewage sludge were studied by Glauser et al., (1988), William et al., (1985), Shu & Bradshaw (1995). They had noticed reduction in micro-organism growth with high concentration of metals in the sub soil.

Nordgen et al., (1985) noticed only minor effects in microfungal diversity, except at contamination levels of about 1000 μg Cug^{-1} soil in Gusum area. A shift in the species composition was, however present at Cu level over 1000 μg Cug^{-1}. Other studies have also found an altered microfungal species composition due to metal pollution, for example with Cd, Cu, Pb and Zn (Hartman 1976) and Cu (Kendrick, 1982). At Sudbury, Carter (1978) reported a significant change in fungal community structure. The number of species and the species diversity of higher fungi decrease in highly metal polluted soil, and the production of sporophores appears to be one of the most sensitive measurements of biological effects of heavy metal pollution. Ruhling (1983) and Ruhling et al., (1984) found that the number of fruiting

bodies produced decreased in the vicinity of the Gusum smelter. In the control area (<100 μg Cu^{-1} organic matter) about 35 species occurred, decreasing to about 25 in moderately polluted sites (about 100 μ Cug^{-1} organic matter and 10 species near the mill (about 10000 μg Cug^{-1} organic matter).

Since bacteria are very seldom identified to a species level, few conclusions on the effect of heavy metals can be drawn. However, heavy metals appear to induce a shift towards more gram-negative bacteria compared to gram-positive (Baath, 1989). Doelman and Haanstra (1979) found more gram-negative bacteria tolerant and Barkay et al., (1985) found more *Pseudomonas* sp. in sludge amended soil with increased levels of Cd. Similar trends in soil to Cd, Co, Cu, Hg, Ni, Zn, were found by Duxbury and Bicknell (1983).

Barkay et al., (1985) studied not only the effect of sludge amendments with heavy metal Cd on the numbers of sensitive and tolerant bacteria, but also the species composition. They found no difference in species composition and diversity for the total bacterial community in the control and sludge amended soils. However, the Cd tolerant bacterial community had a higher diversity in sludge amended soils, with a predominance of *Pseudomonas* sp.

Soil pH is also of utmost importance for the effective metal toxicity on micro-organisms. Giashuddin and Cornfield (1979) studied the effect of Ni on nitrogen mineralization, nitrification and soil respiration in a sandy soil. At pH 5.8, an addition of 50 μg Ni g^{-1} soil caused a decrease in all three indices of microbial activities. By raising the pH to 6.9 or 7.6 a less negative effect of Ni addition on nitrification and soil respiration was found. Bewely and Stotzky (1983) found that the effect of an

acid treatment (lowered to pH 2.8) and addition of 1000 μg Cd g^{-1} soil exceeded the additive effect of individual treatments on CO^2 evolutions, from glucose supplemented soil. Similar results were found after addition of 1000 μg Zng^{-1}. Bhuiya and Cornfield (1974), found higher toxicity of Pb and Zn on nitrification in a soil adjusted to 7.7 than at the neutral and pH 6.0.

Baath (1996) reported that on an organic matter basis, bacteria are less abundant in low pH soils,high in organic matter content than in high pH soils with little organic matter. Nagele and Conard (1990) found the reduction of nitrate-reducing microbial population in low soil pH.

Reviews of Work Carried Out in India

In India, the research works on characterization of industrial wastes are very limited. However, the major concern is attributed to municipal solid wastes, garbage etc.

Rao and Santaram (1995) reported the applications of garbage in agricultural soils around Hyderabad resulting in enrichment of nutrients N, P, K, Ca, Mg, Na, SO_4 and Cl upto 30cm depth. However, they also noticed that application of garbage has also led to contamination of heavy metals in the same depth of soil.

Prusty et al., (1995) in their study have reported the generation, composition and existing disposal practice of various solid wastes in major industry of Rourkela (Orissa).

Olaniya et al., (1992) reported that wastes containing metals at low pH have high pollution potential on land. He noticed that high conductivity of the soil did not

allow the metal to easily leach out. Bhujpal et al., (1997) reported the accumulated levels of heavy metals in para grass plant in the order Co>Pb>Zn>Cr>Ni>Fe>Cd>Cu>Mn, while in the case of DTPA extractable metals it was Ca>Zn>Pb>Fe>Cu>Mn>Cr>Co>Ni>Cd

Ajmal (1985), Lenka et al., (1992) and Rao and Shantaram (1993) studied the metal movement in soils around industrial areas. They found that the biological activities in the soil are severely affected by polluted waste water.

Rao et al., (1993) reported that the effluents from the textile industries in Rajasthan have adversely affected the microbial population in the agricultural soil around the Bandi river. This has rendered the soil unproductive and degraded. They also reported a decrease in microbial population from 29% to 90% in irrigation of ordinary and polluted water.

Agrawal and Pandey (1996) also reported that acidic effluent from sulphuric acid and galvanizing plant has completely destroyed bacterial population in paddy field of Chattissgarh (M.P.).

Several workers have suggested various methods for removal of metals in waste management. Ajmal et al., (1983), Surender et al., (1993) and Singh et al., (1996) used soil as precipitates, cation exchanger and absorbent for removal of Cr, Zn, Ni, Pb, Cu and other heavy metals in the aqueous solution of electroplating wastes. Sharma (1992) used china clay for removing the heavy metals from several wastes. Several other workers used fly ash, bottom ash se wage sludge and saw dust respectively. Tiwari et al., (1989) reported activated carbon adsorption technique as effective up to 100% removal of metal

depending upon pH (pH 5.5 or less for Cr and pH above 7.5 for Cu and Np). Tyagi et al., (1987) studied metal removal from sludge by chemical and microbiological methods.

CHAPTER III

DESCRIPTION OF STUDY AREA

Wazirpur Industrial Area is located in the North-west part of Delhi, covering an area of 210 acres (Fig. 1). The area is surrounded by two ring road system, one, the north-western ring road and another, the north-western ring railway system.

There are approximately 1000 industries in this area, out of which 424 are registered. The main polluting industries are electroplating, rolling, pickling, and textiles. The other industries include rubber, plastic, soap, electronic goods etc. (table 2 & 3). Due to its large number of small scale industries and their unmonitored level of pollution, Wazirpur area has emerged as one of the major polluted industrial sites of Delhi.

The entire area is divided into three Industrial blocks A, B and C (fig. 2). During the last decade there has been a three fold increase of industries in this area. Every day huge amounts of toxic wastes are spewing out of these units.

Fig. 1 DELHI SHOWING WAZIRPUR INDUSTRIAL AREA

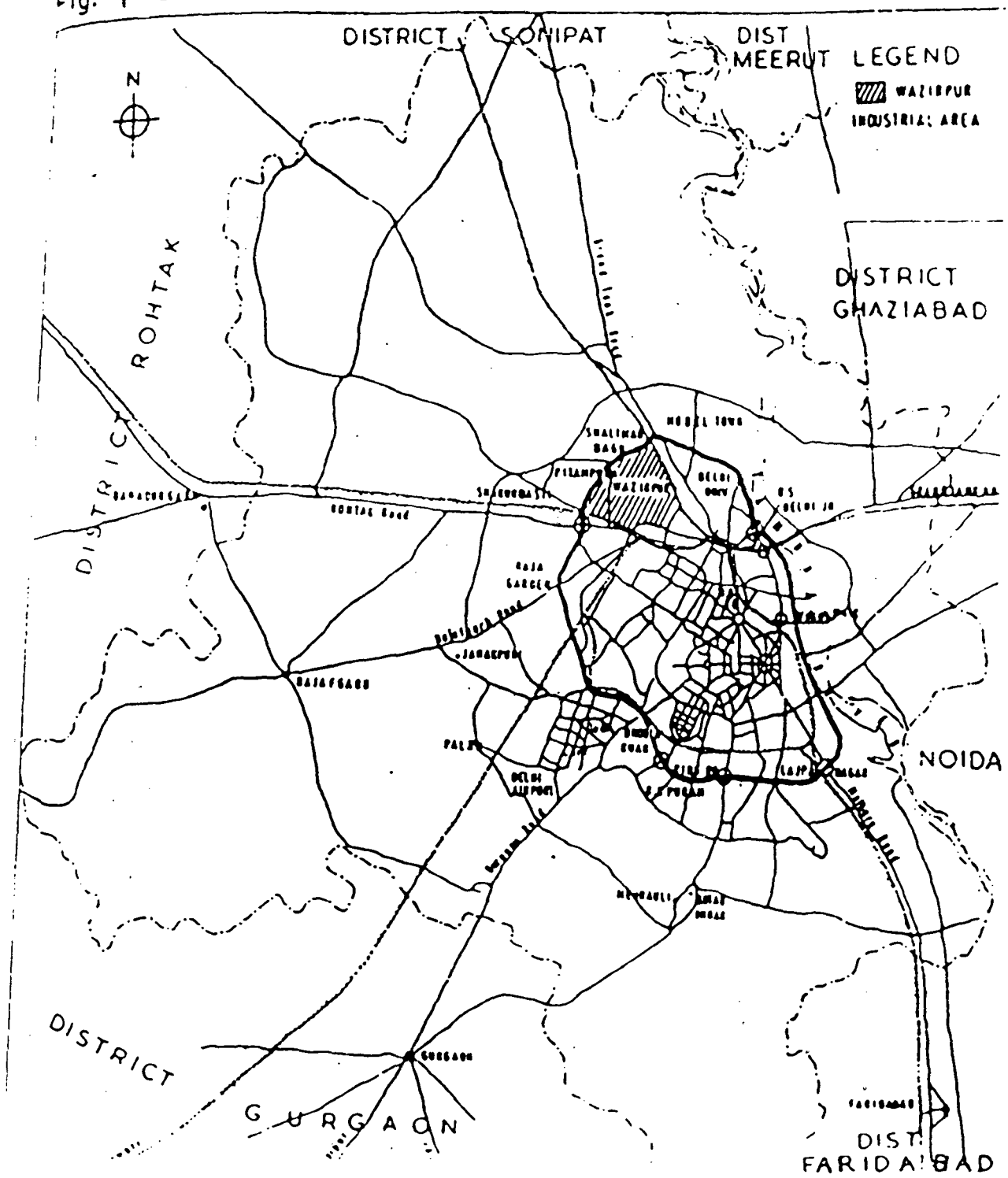


Fig 2 WAZIRPUR INDUSTRIAL AREA SHOWING SAMPLING SITES

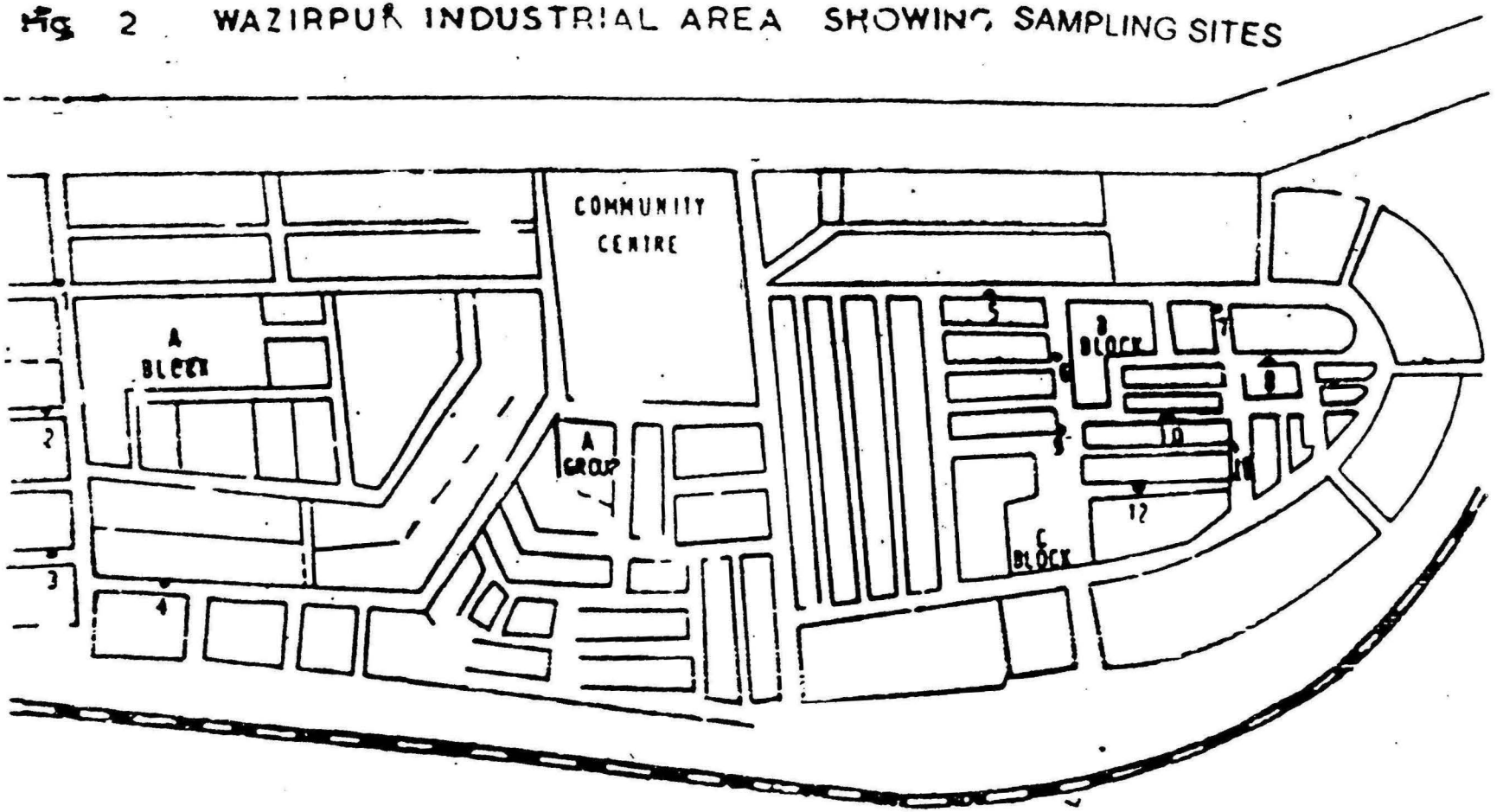


Table 2. List of Registered Industries in Wazirpur

Blocks	No. of Industries
A	253
A-group	089
B	036
C	046
Total	424

Source : Small scale Industries Association Wazirpur (1995)

Table 3. List of various industries in Wazirpur

1	Textile	46
2	Electroplating and Anodizing	20
3	Rolling and pickling	50
4	Soap	10
5	Others (Rubber, Plastics, Candle and Engineering etc.	30
Total		156

Source : CPCB publication (1986-87)

Since the main polluting industries are, rolling, pickling, electroplating and textiles, therefore major raw materials which are used in the processing are strong acids, HNO_3, H_2SO_4, HF, coating materials like Cr and Zn, bleaching powder, dyes, Iron sheets, charcoal etc. So these outputs mainly govern the characteristics of the output wastes. However, nitrogenous wastes, garbage and rubbish released from labour dwellings and mixed with industrial wastes inside the industrial area also influence the properties of wastes.

At present, industrial wastes generated from this area do not have a sound and satisfactory disposal system. The solid wastes are usually dumped on the road side

along the industrial premises. Blocking of drains and canals by these solid wastes result in flooding of liquid wastes all over the locality. Therefore, it affects the health of the workers very badly. Even drained waste water carrying the toxic materials with it, ultimately empties into the river Yamuna, a main source of drinking water forthe people of Delhi.

CHAPTER IV

MATERIAL AND METHODS

1 Work Plan

The work plan was prepared to highlight the physico-chemical and Microbiological study of industrial wastes of proposed area. Different parameters analysed under each head were as follows:-

2 Physico-chemical Characterization

- Measurement of pH, electrical conductivity, moisture content and water holding capacity.
- Study of organic carbon.
- Study of available and total nitrogen.
- Study of available and total phosphorus.
- Study of total concentration of nutrient elements (Na, K, Ca,Mg, Mn, Zn, Fe and Cu).
- Study of total concentration of some toxic metals (Cr, Cd, Ni and Pb).

3 Microbiological Characterizations

- Study of total microbial counts.
- Structural characterization of microbial colonies on agar plates and agar slants.
- Microscopic study of individual isolates.
- Biochemical characterization of Bacterial isolates.
- Based on above study, identification of micro-organisms.

4 **Sampling Site**

Four samples were taken from each block (A.B and C) of Wazirpur Industrial Area, comprising twelve representative sites.

5 **Sample Collection**

Samples were randomly collected from each block. For each sample wastes from the surface to 30 cm below were taken and mixed. Four replicates were collected from different places of one site to overcome the spatial variability. Stainless steel trowel was used for collection of samples and thereafter samples were packed in air tight polythene bags.

6 **Frequency of Sampling**

Samples were collected three (3) times during the course of study. It was done on following dates.

12.4.95 (Summer season)

28.10.95 (Winter season)

12.8.97 (Monsoon season)

7 **Storage of Sample**

Samples were stored in cold room at 4°C to avoid further contamination and chemical changes in it.

8 **Processing of Samples**

Except the determination of pH, E.C., moisture content and microbiological experiments, the soil samples were air dried. Aggregates were lightly crushed and filtered through 0.2 mm sieve. Then again samples were packed in air tight polythene bags and kept at 4°C.

PHYSICO-CHEMICAL ANALYSIS

Table No. 4 : Parameters of physico-chemical analysis and their specific methods used

S.No.	Parameter	Method and Instrument Used
1	pH	Digital pH meter (Zenar)
2	E.C.	Digital conductivity meter
3	Moisture Content	Water loss from the fresh sample at 105°C during 48 hours
4	Water Holding Capacity	Water loss from saturated sample at 105°C during 48 hours
5	Available Nitrogen	Modified Subbaiah and Asija (1956) method
6	Total Nitrogen	Micro Kjeldahl
7	Available Phosphorus	Bray No. 1 method for acidic samples and olsen method for alkaline & neutral samples
8	Total Phosphorus	Block digestion followed by ascorbic acid procedure with no pH adjustment
9	Organic Carbon	Walkley and Black rapid titration method
10	Total Metal Analysis	AAS (PU 9200 x Phillips)

I. pH :

Principle

The pH of a sample is a measure of the hydrogen ion activity and depends largely on relative amounts of the adsorbed hydrogen and metallic ions. Thus, it gives a good indication of the acidity and alkalinity of a sample.

However pH is conventionally defined as the reciprocal of hydrogen ion concentration :

$$pH = \log \frac{1}{a_H^+}$$

or - $\log_{10} a_{H+}$

in which the activity of H^+ in suspension a_{H+} is expressed in gm ions per litre.

The pH of solid waste in suspension highly depends on solid waste : water ratio and increases with dilution. Different laboratories follow different dilution, but 1:5 & 1:10 sample : water ratio are the most common.

Apparatus :

Apparatus used for the experiment were one glass electrode, pH meter with calomel reference electrode and salt bridge, 150ml beakers, magnetic stirrer, spatula, tissue paper, thermometer, digital balance, 250ml beaker, measuring cylinder, wash bottle etc.

Reagents :

Reagents for the experiment were buffer solution of pH 4.0, 7.0 & 9.2 and saturated KCl (40 gm/l) for the bridge distilled water

Procedure :

(a) Preparation of sample and water suspension:

Sample : distilled water of 1:10 (w/v) ratio were taken in 150ml beakers and stirred by magnetic stirrer upto 10 min. and kept for pH measurement.

(b) Measurement of pH :

First the pH meter was kept on at room temperature for about 30 min. Then the pH meter was calibrated with different buffer solution. After calibration of instrument solution of the sample were taken for pH measurement. Sample : KCl (1N) was also taken for measurement. Each sample was repeated 4 times to get a concordant reading. Standard error of the instrument was also calculated for correct reading.

II. Electrical Conductivity :

Principle :

Conductivity is a measure of current carrying capacity, thus gives a clear idea of soluble salts present in samples. Conductivity is non-specific and varies with the proportion of various species in the solution. It express the resistance of a $1cm^3$ of water to the passage of current, usually at 25°C. The resistance (R) is defined as:

$$R = \frac{E}{I}$$

Where E = Electrical potential in volts
I = Current in ampere
R = Resistance in ohms

Electrical conductance is reciprocal of resistance which can be expressed as :

$$C = \frac{1}{R} = \frac{I}{E}$$

Where C = Electrical conductance (EC) in milli siemen

Conductivity values depend on dilution of the sample. However sample water ratios 1:5 & 1:10 are most common for conductivity study. To reduce microbial influence on conductivity, measurement should be done within few hours of preparation of solution.

Apparatus :

Apparatus used for the experiment were electrical conductivity meter, magnetic stirrer, wash bottle, thermometer, digital balance and measuring cylinder.

Reagents :

Reagents used for the experiment were 0.02N KCl (1.4912 gm KCl/l) and CO_2 free distilled water.

Procedure :

a) Preparation of sample & water suspension :

sample:distilled water of 1:10 (w/v) ratio taken in a beaker and stirred well for about 10 min. and kept constant for half an hour.

b) Measurement of Electrical Conductance :

First the electrical conductivity meter with cell was kept on at room temperature and the cell constant was adjusted. After half an hour the instrument was calibrated with 0.01 KCl and then the sample water suspensions were taken for EC measurement. For measuring EC, platinum electrode was dipped into the suspension and EC was measured in milli siemen. Samples were repeated 4 times to get concordant value.Standard error was also calculated for correct reading.

III. Moisture Content (M.C.)

Principle :

Solid waste generally gets moisture from the infiltration of precipitate water when dumped in the open places. However, the samples collected were mostly saturated with water and acids because these were dumped after extracting from acidified liquid wastes coming from the industries. The moisture content of a solid waste at any time, more or less depends on its water holding capacity and environmental conditions with time. The moisture content is the amount of water held by the fresh samples at the time of collection. It is generally expressed in percentage with respect to the initial fresh weight of the samples.

Apparatus :

Apparatus used for the experiment were oven, balance, petridish with covers, desiccator and thermometer. No reagent was used here.

Procedure :

First clean oven-dry petridish were taken and weighed accurately (upto ± 0.001 gm). Then about 10gm of waste from each fresh sample were taken and weighed. After taking the second weight, samples with petridish were kept inside the oven at 105°C. Weights were noted after 24 hours and once again after 48 hours. To get a concordant value samples were repeated 4 times in same way.

Calculation :

$$Moisture Content(\%) = \frac{Loss in weight on dying}{Initial fresh weight of the sample} x100$$

$$= \frac{W_2 - W_3}{W_2 - W_1} x100$$

Where W_1 = Wt of petridish

W_2 = Wt of petridish with fresh sampel

W_3 = Wt of petridish wuth dry sample.

IV. Water Holding Capacity :

Principle :

Water holding capacity is defined as the maximum amount of water of a freely drained sample can hold. It is estimated after a saturated sample under study has been allowed to drain without allowing its moisture stores to be depleted by evaporation. The water holding capacity of a sample always depends on its physicochemical nature. It is expressed as the weight of the water held by 100gm of oven dry sample.

Apparatus :

Apparatus used for the experiment were oven, balance, patridish desiccator, thermometer, water bottle with water, conical flask, funnel and beaker (100ml).

Reagent :

Reagent used for this experiment were filter paper and water.

Procedure :

First about 25gm of each processed sample was taken in 100ml beaker. The beakers containing samples were floodded with water and left for 2 hours for full saturation. Then the saturated samples from the beaker were taken on the filter paper in the funnel by adding few drops of water. Then about 10gm of saturated samples were taken in previously measured patridish when the last drop of water were funnelled out. After taking the second weight the plates with samples were kept inside the oven at 105°C, then weights were taken after 24 hours and once again after 48 hours.

Calculation :

$$Waterholdingcapacity(\%) = \frac{lossinweightondrying}{dryweightofthesample} x100$$

$$= \frac{(W_2 - W_3)}{(W_3 - W_1)} X\ 100$$

Where W_1 = Wt of petridish

W_2 = Wt of petridish with saturated sample

W_3 = Wt of patridish with dry sample.

V. Available Nitrogen : (Subbaiah and Asija, 1956)

Principle :

The principle involves distilling the sample with alkaline potassium permanganate solution and determination of ammonia liberated which serves as an

indec of the available/mineralizable nitrogen status.

Apparatus :

Apparatus used for the experiment were kjeldahl flask, measuring cylinder, beaker, burette, glass beads, balance, volumetric flask (1000ml and 500ml) etc.

Reagents :

Reagents used for the experiment were

a) 0.32%$KMnO_4$; 3.2g of $KMnO_4$ was dissolved in 1 litre of distilled water.

b) 2.5%NaOH : 25g of NaOH was dissolved in one litre of distilled water. c) 0.02N H_2SO_4 : First 1N H_2SO_4 (28ml of concentrated H_2SO_4/litre) then .02N H_2SO_4 (20ml of 1N H_2SO_4/litre) was prepared. Finally the exact strength was calibrated by standardizing against 0.2N Na_2CO_3

d) Mixed indicator : 0.066g methyl red and 0.099g bromocresol green was dissolved in 100ml of 95% alcohol to get mixed indicator.

e) 2% boric acid solution with indicator :

20 gm of boric acid was dissolved in one litre of distilled water. Then 20ml mixed indicator was added to it.

f) Liquid paraffin : Extra pure liquid paraffin was used.

Procedure :

In a kjeldahl flask 20gm of sample was taken. To this 100ml of water was added followed by 100 ml each of 0.32% $KMnO_4$ and 2.5% of NaOH solution. The frothing during boiling was prevented by adding liquid paraffin and bumping, by adding a few glass beads. The contents were distilled in the kjeldahl assembly at a steady rate and the liberated ammonia was collected in a conical flask containing

20ml of boric acid with mixed indicator. With absorption of ammonia the pinkish colour turned to green. Nearly hundred ml of distillate was collected which was titrated against 0.02 N H_2SO_4 to the original shade pink. Simultaneously one blank was also taken for correcting final calculation.

Calculation :

1 ml of 1N H_2SO_4 ≡ 14 mg of nitrogen

$$\text{Hence, ppm of Nitrogen} = (a-b) \times N \times \frac{14}{S} \times 1000$$

Where N = calibrated normality of standard H_2SO_4
a = sample reading
b = blank reading
S = Wt of sample in gm

VII. Total Nitrogen :

Principle :

Analysis of total nitrogen needs the complete oxidation of organic matter. However, complete oxidation can be achieved by several methods. But weight acid oxidation by kjeldahl method is most common practice in many laboratories. In this method oxidation finally leaves a sulphuric acid solution. Hydrogen peroxide is added as an additional oxidizing agent, selenium is used as catalyst while K_2SO_4 is added to raise the boiling point of the mixture. The main advantages of this method are that a single digestion is required (for both plants and soil material) to bring nearly all nutrient into solution; no volatilization of nitrogen takes place and the method is simple and rapid.

Apparatus :

Apparatus used for this experiment were micro kjeldhal distillation unit, 50 ml volumetric flask, digital balance , test tubes fitted with block of the digestion unit, measuring cylinder, conical flask (50 ml) etc.

Reagents :

Reagents used for this experiment were of 2 types

a) Reagents for digestion mixture

i) Selenium powder

ii) Potassium sulphate

iii) Hydrogen peroxide

iv) H_2SO_4 (concentrated)

Selenium powder 0.42gm and 14 gm K_2SO_4 were added to 350 ml of H_2O_2 (30%) & mixed well. Then 420 ml of concentrated H_2SO_4 was added slowly with care while cooling in an ice bath. The mixture was stored at 2°C.

b) Reagents for distillation and Titration :

i) 40% NaOH : 400gm NaOH was dissolved in one litre of distilled water

ii) 1% boric acid : (H_3BO_3) 10gm of H_3BO_3 was dissolved in one litre distilled water

iii) HCl N/140 : first 0.1 N HCl (8.1 ml concentrated HCl/l), then N/70 HCl (143 ml of 0.1 N HCl/litre) and N/140 (500ml N/70 HCl/litre) was prepared. Lastly the exact normality was calibrated by standardizing against N/140 Na_2CO_3 with $N_1V_1 = N_2V_2$ relationship.

iv) Mixed Indicator : Bromocresol green 0.099 gm and methyl red 0.066 gm and 0.011 gm thymol blue were dissolved with shaking in 100ml ethanol.

Procedure :

a) Digestion :

First 0.3 ± 0.001gm of each dry processed sample was taken in dry and clean digestion tube. Then 4.4 ml digestion mixture to each test tube of the samples and 2 reagent blanks for each batch of samples were added. Sample were digested at 360°C for 3 hours then the colourless sand white solution were taken out and cooled with adding about 25ml of distilled water. Lastly the volume of the solution was made up to 50ml after transferring into volumetric flask and allowed to settle.

b) Distillation & Titration :

First the steam distillation apparatus was setup with distilled water as the source of steam. Then steam was passed through the apparatus for 30min. and 50ml of distillate was collected which consume 0.2ml of standard acid. Then the 10ml of aliquot and 10ml of 40% NaOH was taken into the reaction chamber and the liberated steam was collected in 5ml of 1% boric acid containing 4 drops of mixed indicator. Distillation was continued upto 2min. from the time the indicator turned green. Lastly the distillate was titrated with N/140 HCl and the volume of standard HCl consumed was noted. A blank was also run by the same way. However 0.05 ml of required acid was subtracted from the burrete reading of blank to get the corrected volume of HCl required by it.

Calculation :

$$Total Nitrogen(\%) = (T-B)\, x N x \frac{1.4}{S}$$

Where T = Titation reading (calculated reading for 50ml of original disgested sample)

B = blank titration in ml of standard acid

N = normality of standard acid

S = weight of the sample

VIII. Total Phosphorus :

Principle :

Principle first involves the conversion of organic phosphorus into inorganic form (as in case of total nitrogen). The total phosphorus is then colorimetricaly determined by ascorbic acid reduced molybdenum blue in acidic medium.

Apparatus :

Apparatus used for the experiment were spectrophotometer, wash bottle, pipette, block digester, test tube, balance (sensitive upto ± 0.001 gm) measuring cylinder, volumetric flask (50 ml) and beakers etc.

Reagent :

Reagent used for this experiment were of 2 types :

a) Reagents for digestion mixture (same as for total nitrogen determination)

b) Reagents for Spectrophotometric Analysis by ascorbic acid method - no pH adjustment.

i) Sulphuric acid (H_2SO_4) 5N:148 ml of concentrated H_2SO_4 was added to 500ml of distilled water while cooling in an ice bath. Then the volume was diluted to 1 litre.

ii) Ammonium molybdate/Antimony potassium tartrate solution. 12gm of ammonium molybdate was dissolved in 250 ml of warm (50°C) distilled water. Separately

0.291gm antimony potassium tartrate ($KSb.C_4H_4O_6$) was dissolved in 100ml distilled water. Then both the solutions were added to 1000ml of 5N H_2SO_4 (above). Then the volume was diluted to 2 litre and kept in a cool and dark place.

iii) Ascorbic Acid Reducing Agent :

2.108gm of ascorbic acid ($C_6H_8O_6$) was dissolved in 400ml ammonium molybdate/antimony potassium tartrate solution (prepared earlier) and was mixed well. This solution was prepared on the day of analysis.

iv) Standard Phosphorus Stock Solution (1000ppm) :

1.0967 gm of oven dry KH_2PO_4 was dissolved in 250 ml of distilled water to get 1000ppm phosphorus. From this 10ppm of working solution was prepared by diluting into one litre.

Procedure :

a) Preparation of Working Standard :

Working solutions of 0, 1, 2, 3, 4, 5 & 6ml were pipetted into 50ml of volumetric flask. Then 10ml of ascorbic acid reducing agent was added to each flask and the volume was made upto the mark and left for one min. to develop colour. Standards were containing 0, 0.2, 0.4, 0.6, 0.8, 10.0 & 1.2 ppm respectively.

b) 5ml of the supernatant clear wet ashed digested solution was taken in a 50ml volumetric flask. Then 20ml of distilled water to each flask was added and followed by 10ml of ascorbic acid reducing agents to each flasks..Then the volume was made upto the mark. However the addition of the ascorbic acid to standard solution was done at the same time. Then after 1 hour absorbance was measured at 880nm wave length.

Calculation :

$$Total phosphorus(\%) = CdxVdx\frac{Vs}{Va}x\frac{10^{-}4}{w}$$

Cd = Concentration (ppm) in diluted aliquot

Vd = Volume of the diluted aliquot

Vs = Volume of the digested sample

Va = Volume of the aliquot

W = Weight of the sample in gms

IX. Available Phosphorus :

Principle :

The principle first involves the extraction of available phosphorus by means of extractants. The combination of NH_4F & HCl is designed to remove adsorbed and easily acid solible forms of phosphorus, largely the calcium phosphats and a portion of aluminium and iron phosphate. NH_4F dissolves aluminium and iron phosphate by complex formations with these matal ions. $NaHCO_3$ solution adjusted to pH 8.5 has been designed to control the ionic activity of calcium through solublity product of $CaCO_3$ in case of neutral and alkaline sample. In the second step the available phosphorus in the extracts is colorimetrically determined by chlorostannous - reduced molybdophosphoric blue in hydrochloric acid system

Apparatus :

Apparatus used for this experiment were spectrophotometer, wash bottle, pipette, test tube, balance, measuring cylinder volumetric flask, beakers, filter paper

(whatman no. 1) & conical flask.

Reagents :

Reagents used in this experiment were of 3 types.

a) Reagent for Olsen's method (for alkaline sample)

i) Reagents for sample extract

Sodium bicarbonate of 0.5 M (pH8.5) was prepared by dissolving 4.2gm of $NaHCO_3$ in one litre of distilled water. The pH (8.5) was adjusted by adding 10% NaOH.

ii) Dickman & Bray's Reagent for sample extract .

15gm of ammonium molybdate (AR) was dissolved in 300 ml of distilled water by warming at 60°C. After cooling, 350 ml of 10 N HCL was added and made upto 1 litre. Normality of HCl was adjusted correctly by titration.

iii) Stanous Chloride.

10gm of crystalline stannous chloride (AR) was dissolved in 25ml of concentrated HCl by keeping air tight while on warming and stored in amber coloured bottle carefully avoiding all contact with air. Just before use 1ml was diluted to 66ml with distilled water.

b) Reagents For Bray's No.1 Method (for acidic sample)

i) Reagents for sample extract.

2.775 gm of NH_4F was added to 2.5 litre of 0.025N HCl. The normality of HCl was checked with NaOH solution.

ii) Dickman and Bray's Reagent for Sample Analysis Extract :

15gm of ammonium molybdate was dissolved in 300ml of distilled water by warming at 60°C. Then 350 ml of 10N HCl was added and made upto 1 litre. The normality of HCl was adjusted using NaOH by titration.

iii) Stannous Chloride (same as Olsen's method)

c) Phosphorus Stock Solution (100ppm)

0.439gm of dried KH_2PO_4 was dissolved in 500ml distilled water by adding 25ml of 7N H_2SO_4. Then the total content was diluted to 1 litre.

Procedure :

a) Sample Extract for Olsen's Method :

2.5gm of sample in 100ml of conical flask with a little activated carbon (free of phosphorus) was added followed by 50ml of Olsen's reagent. A blank was run without the sample. The flasks were shaken for 30 min. on a platform type shaker and the contents filtered immediately through Whatman no.1. paper into dry clean beaker.

b) Sample Extract for Bray's Method :

5gm of soil and 50ml of reagents were taken in 100 ml of flask and shaken exactly for 5 min. and filtered by Whatman no.1 filter paper. To avoid interference 7.5ml of 0.8N boric acid was added to 5ml of extract.

c) Preparation of Phosphorus Standard Solution :

First 2ppm of working standard was prepared by dissolving 20ml of 100ppm phosphorus stock solution in 1000ml. 0,1,2,3,4,5 and 10 ml of 2 ppm phosphorus were pipetted respectively into 50 ml volumetric flasks. To each of them, added 2 ml $SnCl_2$. Addition of $SnCl_2$ to standards & sample were done simultaneously and then

within 10 minutes absorbence were measured by spectrophotometer.

d) Sample Analysis :

First 5ml of the extract was taken in a 50ml of volumetric flask. Then 5ml of Bray's reagent was added. Olsen's reagent was added drop wise to remove CO_2. Then the neck of the flask was down and the content was diluted and 20ml and was added with 2ml of $SnCl_2$ solution. Lastly the volume was made up to the mark and the intensity of the blue colour was measured by spectrophotometer at 660nm wavelength the concentration with absorbance was noted for further calculation.

X. Calculation :

$$ppmofphosphorus = CdxVdx\frac{Vs}{Va}x\frac{1}{W}$$

Where
Cd = Concentration (ppm) in diluted extract
Vd = Volume of diluted aliquot
Vs = Volume of Sample extract
W = Wt of Sample in gms
Va = Volume of aliquote (5 ml)

Organic Carbon Analysis :

Principle :

Determination of organic Carbon by Walkley and Black (1934) rapid titration methods is based on oxidation of organic matter by chromic acid (potassium dichromat plus concentrated H_2SO_4 utilizing the heat of dilution of sulphuric acid). The unreacted dichromat is determined by back titration with ferrous ammonium sulphate (redox titration).

Apparatus :

The apparatus used for this experiment were balance, burrete, pipette, conical flask, beaker, volumetric flask etc.

Reagents :

Reagents used were :

a) 1 N potassium dichromate; 49.04gm of $K_2Cr_2O_7$ was dissolved in 1 litre distilled water.

b) 0.5N (approximately) ferreous ammonium sulphate : 196gm of hydrated crystalline salt of ferreous ammonium sulphate and 20ml concentrated H_2SO_4 was dissolved in 1litre distilled water.

c) Diphenyl amine indicator :

0.5gm of diphenyl amine was dissolved in a mixture of 20ml of water and 100ml of concentrated H_2SO_4 was added.

d) Concentrated Sulphuric Acid with 1.25% Ag_2SO_4 : (Ag_2SO_4 was added to remove interference of chlorides) 6.24 gm of Ag_2SO_4 was dissolved in 500 ml of concentrated H_2SO_4 to get the required solution.

e) Orthophosphoric Acid (85%)

It was available in the laboratory

Procedure :

1.00gm of processed sample (sieved by 0.20 mm size) was taken into a clean dry 500ml conical flask. Then 10ml of 1 N $K_2Cr_2O_7$ was added and swirled a little. The flask was kept on asbestos sheet. Then 20ml of H_2SO_4 was added and swirled again for three times. The flask was then allowed to stand for 30min. and thereafter

200ml of distilled water was added. Before going for titration 10ml of phosphoric acid and 1 to 3 ml of diphenyl amine indicator was added to it and violet colour was observed. Then the content was titrated against ferreous ammonium sulphate. The end point of the titration was marked by green colour of the solution. Simultaneously a blank was also run. When more than 7 ml of dichromat was consumed, the determination was repeated by smaller quantity of samples (0.252 - 0.5gm).

Calculation

$$Organiccarbon(\%) = \frac{10(B-T)}{B} x \frac{0.003}{S} x100$$

Where B = Volume (in ml) of ferrous ammonium sulphate solution required for blank
T = Volume of ferrous ammonium sulphate needed for sample.
S = Wt. sample in gm.

XI. Analysis of Total Metal Concentration (K, Ca, Na, Mg, Ni, Pb, Cd, Cr & Zn)

Principle :

a) Digestion of samples (Lorring & Rantakes, 1992)

b) Principle of AAS :

Chemical analysis of AAS involves converting the sample at least partially, into an atomic vapour and masuring the absorption of the atom at a selected wave length which is characteristic for each individual element. The measured absorbace is proportional to the concentration and analysis are made by comparing this absorbance with that given under the same experimental conditions by reference samples of known exposition. Almost all analytical application of the atomic

absorption method at the present time involve spraying a solution of the sample into a flame.

Beer's law applies only for monochromatic radiation, a linear relationship between absorbance and concentration, however it can be expected only if the band width of the source is narrow with respect to the absorption peak. No ordinary monochromatic radiation is capable of yielding a band of radiation as narrow as the peak width of an atomic absorption line (0.002 to 0.005mm). The most common source for atomic absorption measurements is the hollow cathode which consists of tungsten anode and a cylindrical cathode sealed in a glass tubes that is filled with neon or argon at a pressure of 1 to 5 torr. The cathode is constructed of the metal whose spectrum is desired or serve the support a layer of that metal. In this method a majority of free atoms in the commonly used flames remain in the ground state and the flames also have not enough energy to excite these atoms except for the group 1 elements. Absorption i.e decrease in energy is then measured by the relation. The absorbance of a solution with relation to other factor can be expressed as follows :

$$Absorbence = Log\frac{Io}{It} = K.C.L.$$

Where Io = intensity of incident radiation
It = intesity of transmitted radiation
c = concentration of sample
L = path length.

Apparatus:

Apparatus used in this experiment were polypropylene volumetric flask,

polypropylene graduated cylinder, polypropylene funnel, Teflon Bombs, Atomic Absorption Spectrophotometer.

Reagents :

Reagents Used for this experiment were

i) Hydrofluoric acid - 49%

ii) Nitric acid (HNO_3) - 70%

iii) Aqua-Regia - ($1HNO_3$: 3HCl)

iv) Boric acid crystals (H_3BO_3)

v) Known Rock Standard

Procedure

a) Digestion of Samples :

0.5 gm finely grounded sample was weighed and transferred to a Teflon bomb. Then 1 ml of aqua-regia was added to the sample. 6 ml of HF was added slowly to avoid excessive frothing. Bombs were closed tightly and kept in a oven (130°C) for 1 1/2 hours. 5.6gms of boric acid was weighed and transferred into a 100 ml polypropylene volumetric flask./ Then 20ml of H_2O was added and shaken briefly. Then the bomb was removed from the oven, cooled and was opened. The bomb was rinsed several times with deionised water. The sample was allowed to settle for 7-14 days till a gelatinous precipitate of borosilicates settled bearing clear surface layer then was kept ready for analysis.

b) Digestion of Rock Samples :

Rock standards were prepared by digesting the known concentration of rock samples in the same process as for samples. Then samples and standard were taken

for AAS analysis.

c) Analysis of Samples by AAS. First the instrument was calibrated by aspirating the standard Rocks sample. Then concentration against absorbance of various elements were noted by fixing their specific wave length at various flame types. The wave length and flame type used for this analysis for various types of elements are, illustrated in the table below.

Table 5 : Procedure for the metal analysis by AAS

Metal	Flame Type	Wave Length
K	Emission mode Air-Acetylene, calcium was added as an ionisation buffer	589 n.m
Na	Emission mode Air-Acetylene, Calcium was added as an ionisation buffer	765.5 nm
1Ca	Absorption mode Air-Acetylene (oxidizing) K is added as ionisation buffer.	422.2 nm
Mg	Absorption mode air-Acetylene (oxidizing) K is added as ionisation buffer.	248.3 nm
Fe	Absorption mode Air-Acetylene (Oxidizing)	279.5 nm
Mn	Absorption mode Air-Acetylene (stoichiometric)	213.9 nm
Zn	Absorption modeAir-Acetylene (Oxidizing)	324.7 nm
Cr	Nitrousoxide (highly reducing)	357.9 nm
Ni	Absorption mode Air-Acetylene (oxidizing)	341.5 nm
Cd	Absorption mode Air-Acetylene (Stoichiometrics)	228.8 nm
Pb	Absorption mode Air Acetylene (Stoichimetrics)	217.0 nm

MICROBIOLOGICAL ANALYSIS

Different experiments and their methods used are tabulated below:-

Table 6. Different parameters of microbial analysis and their methods

S.No	Description of Experiment	Method Used
1.	Total Microbial Counts	Dilution Plate Count
2.	Structural Characterisation of Individual Isolates.	Agar Plate and Agar slant method.
3.	Microscopic study of Isolated organism.	Study through Negative staining and Grams Staining using compound microscope.
4.	Biochemical Characterization	Through Biochemical test as described in 'Microbiological Methods' Manual.
5.	Identification of Isolates	By relating results with" Vergese Manual of Systemic Bacteriology".

For microbiological analysis all chemicals, stains and culture media of "High Media" make were used.

I. Total colony Forming Units Count

Principle

When unit weight of soil is diluted serially and cultured on nutrient agar plate, it is assumed that each colony developing has grown from one viable cell of microorganisms. The number of colony forming units appearing on nutrient plates are directly proportional to the dilution of soil sample.

Apparatus - Petridiscs, 0.1 ml. pipettes, test tubes antichlor, laminar How, incubator

Culture media - Nutrient agar

Procedure - Dilution plate count method

9 ml. water was pipetted into each of the six test tubes and sterilized. 1 gm of soil was weighed and mixed well in first test tube. After 3-5 minutes 1 ml. of supernatant was taken out with the help of 1 ml pipette from the first tube and blown out to the second tube. This process was repeated till the sixth tube. In this way serial dilution of the soil was obtained from 10^{-1} to 10^{-6}.

Now from each tube, 0.1 ml of soil suspension was taken and spread on different nutrient agar plates. Triplicates were made for each dilution. Plates were then incubated in incubator at 30°C for three days. Appearance of colony forming units (CFUs) of different microorganisms were counter in suitable dilution plate and recorded.

II. Structural Characterization of Individual Isolates

The growth appeared on nutrient agar plates of individual isolates were characterized depending characterized depending upon the morphology of the colonies based on diameter, colour, opacity, form, elevation, margin, smoothness texture and spreading nature.

For partial Purification of Isolates, the different colonies appeared on nutrient agar plates were streaked on fresh nutrient agar plates. The process was repeated three times to ensure the purity of each isolates. The individual isolates were kept

on nutrient agar slants for further studies.

The characteristics feature of the isolates on nutrient agar slants were made depending upon growth form, chromogenesis, colour and consistency.

III. Microscopic study of microbial isolates

(i) *Negative staining*:

A loopful of liquid culture of each isolates was spread on separate clean grease free microscopic slides. A drop of nigrosine was added with culture on slide and made it into thin smear with the help of another slide. Slides were air dried for 40 seconds and examined under microscope and cells were characterized depending upon shape and arrangement.

(ii) *Gram's staining*

For this purpose a loopful culture was taken on slide, smeared and heat fixed. The slides were stained with crystal violet for 30 seconds, washed with water and then few drops of Gram's iodine was applied for 30 seconds. The slides were rinsed with water. The slides were decolourized with 95% alcohol and rinsed with water. The slides were counter stained then with Safranine for 20-30 seconds. Finally, the slides were rinsed with water, blot dried and then examined under compound microscope with ollmmerson lense.

(iii) *Staining with Lactophenol-cotton blue*

For fungal isolates Lactophenol-cotton blue was used. For this purpose a loopful culture of isolate was taken up into a glass slide. A drop of Lactophenol-cotton blue stain was poured on slide and the mycelium of culture was spread over.

with the help of a needle. Then the mycelium was covered with cover slip and observed under microscope for its shape, type and presence or absence of fruiting bodies.

IV. Biochemical characterization of microbial isolates

The biochemical tests were performed as described: in 'Microbiological Methods' by Collin and Lyne (1989). Culture media for different biochemical test are given in Appendix-I. Procedure of each test is as follows:-

(a) Indole production test

All the isolates were incubated in to tubes containing 1% tryptone broth, which was then incubated at 37°C for 72 hours. Three ml of Kovac's reagent was added into incubated tubes and were shaken. Formation of red ring layers were recorded.

(b) Methyl red test

All isolates were incubated into glucose phosphate broth tubes at 37°C for 5 days. 0.5 ml absolute alcohol and 0.5 ml of methyl red reagent was added to all tubes. Development of cherry red colour indicated MR positive and no change in colour showed MR negative test.

(c) Voges-proskauer test

All the isolates were incubated into glucose phosphate broth at 37°C for 5 days. Then 3 ml of alphanepthol and 3 ml of KOH (40%) were added into culture tubes, Tubes were shaken well and allowed to stand for 3 to 5 minutes. Presence or absence of dark red ring formation was recorded for positive and negative test respectively.

(d) Citrate test

All isolates were streaked in Simmon's Citrate agar slants and incubated at 37°C for 48 hours. The change of colour from blue to green was recorded feach citrate positive test isolate.

(e) Starch hydrolysis test

All isolates were incubated on starch-agar plates, at 37°C for 72 hours. Then Gram's iodine was flooded over incubated plates. Zones of clearance were recorded for each positive isolate.

(f) Casein utilization test

Plates of skimmed milk agar were prepared. All the test isolates were incubated on these plates separately at 37°C or 72 hours. Results were noted by observation of clear zones around bacterial growth for each positive test isolates.

(g) Gelatin liquification test

All test isolates were inoculated into nutrient gelatin tubes and incubatated at 37°C for 48-72 hours. The tubes were chilled in ice water for about 30 seconds. Solidification of gelatin-agar medium was noted for all negative test isolates.

(h) Carbohydrate fermentation test

For the carbohydrate fermentation test glucose, sucrose, and lactose were used as growth medium. All the isolates were inoculated in glucose broth, sucrose broth and lactose broth, respectively. Each broth tube containing Bromo Cresol purple as an indicator for acid production and an inverted Durham tube for gas production was used. Tubes were incubated at 37°C for 24-48 hours. Change in colour of medium from purple to pink and presence or absence of gas bubbles were recorded.

(i) Catalase test

Culture suspension was taken lon a clean, grease-free micro-slide and mixed with a drop of H_2O_2. Liberation of gas bubble for catalase activity was taken as positive reaction.

(j) Oxidase test

All isolates were streaked on nutrient agar plates and incubated at 37°C for 24 hours. Few ml of p-amino-dimethyl aniline was flooded over each plate. Change in colour of colonies from original to pink were recorded in positive test.

(k) Urease test

All isolates were incubated in tubes of urea broth at 37°C for 24 hours. Change in colour of broth from yellow to pink was recorded for all isolates of positive test.

(l) Nitrate reduction test

The cultures were inoculated in the tubes containing nitrate broth, and after 3 days the nitrate broth containing culture solution were tested for the presence of nitrite. One ml of nitrate broth was transferred in a clean test tube and 2-3 drops of alphanaphthylamine solution was added and the tube allowed to stand for 5 minutes. Development of pink colour was recorded for different isolates.

(m) Hydrogen sulphide production test

Thiosulphate-iron medium slants were inoculated with culture medium. A strip of filter paper, dipped in a solution of lead acetate, was placed in the top of the tube. The tubes were incubated for 48 hours. Blackening of filter paper was recorded for each isolates.

CHAPTER V

RESULTS AND DISCUSSION

RESULTS

On the basis of the physico-chemical analysis of 12 samples collected from three blocks (A, B, and C) of Wazirpur Industrial Area for each three seasons i.e. summer, winter and monsoon, the results obtained are tabulated in tables 7, 8 and 9.

For each individual parameter the analysis was performed for each sampling site. Then the average value has been computed for each block on seasonal basis. Total average for each block has also been calculated. Tables 10 and 11 show the correlation coefficient of different parameters which indicate correlation among the respective sources of different variables.

For the microbiolaogical study, the analysis was performed on block basis for each season. The results of microbiological counts and identification of individual members have been tabulated in tables 12, 13, 14, 15 and 16. Finally the correlation between different chemical parameters and microbiological counts have been discussed.

Table 7: Physico-chemical Characteristics of Industrial Wastes

Block	No.	pH			E.C. (milli S / cm)			M.C.%			W.H.C.%			O.C.%			A.N.(ppm)			T.N.%			A.P(ppm)			T.P.%		
		a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
A	1	9.08	3.80	2.84	2.12	1.03	1.10	1.30	5.10	70.00	48.88	29.55	65.50	2.90	2.08	2.82	175.6	628.3	322.7	0.37	0.10	0.12	37.61	69.19	38.20	0.37	0.54	0.49
	2	8.28	5.52	3.49	1.06	1.40	0.35	0.20	2.80	21.90	31.81	30.91	35.64	3.05	2.52	2.45	145.4	259.9	57.1	0.27	0.27	0.07	10.19	29.96	83.90	0.26	0.13	0.43
	3	5.93	4.30	2.53	0.63	1.25	1.63	1.50	9.00	42.20	43.63	46.88	97.36	2.59	3.27	2.23	97.2	188.5	616.9	0.43	0.63	1.33	59.17	44.52	41.66	0.39	0.30	0.45
	4	7.58	3.75	2.67	0.70	1.41	1.20	1.30	0.10	47.98	25.58	25.00	77.38	3.19	2.89	2.01	94.5	499.8	214.2	0.07	0.53	0.13	54.50	48.98	7.21	0.10	0.09	0.41
	Avg	7.72	4.34	2.88	1.13	1.27	1.07	1.08	4.25	45.52	37.48	33.09	68.97	2.93	2.69	2.38	128.2	394.1	302.7	0.29	0.38	0.41	40.37	48.16	42.74	0.28	0.27	0.45
B	1	3.28	2.78	5.24	1.18	1.20	0.44	0.20	2.00	24.27	38.10	41.18	49.78	2.45	3.19	2.53	232.0	676.9	219.9	0.53	0.67	0.37	81.82	42.52	49.35	0.44	0.23	0.18
	2	3.50	4.02	3.38	0.70	1.15	0.72	0.30	0.50	15.81	47.36	47.37	30.33	1.85	2.67	1.78	75.4	211.3	179.9	0.20	0.17	0.03	20.57	99.20	2.20	0.31	0.38	0.19
	3	8.76	3.90	2.90	1.04	1.36	0.84	2.00	10.00	49.20	33.33	45.56	83.16	3.04	2.08	2.23	588.7	634.0	291.3	1.50	0.23	1.23	89.70	22.43	46.69	0.47	0.21	0.54
	4	3.80	6.20	2.66	1.60	1.09	1.22	0.40	2.00	21.90	39.02	43.88	46.78	3.27	2.23	0.93	545.2	471.2	237.0	0.63	0.80	0.01	50.96	64.75	15.98	0.18	0.16	0.29
	Avg	4.84	4.23	3.55	1.13	1.20	0.81	0.73	3.63	27.80	39.45	44.50	52.51	2.65	2.54	1.87	360.3	498.4	232.0	0.72	0.47	0.41	60.76	57.23	28.56	0.35	0.25	0.30
C	1	4.38	7.80	2.90	0.55	0.65	0.80	0.10	1.00	26.54	45.95	29.73	42.21	4.46	2.74	1.39	110.2	80.0	248.5	0.07	0.07	1.00	22.67	5.08	35.82	0.55	0.12	0.49
	2	4.42	7.90	2.90	1.35	1.19	1.15	0.20	6.00	62.50	31.58	29.06	95.01	2.15	1.86	2.23	623.5	197.1	662.6	0.27	0.30	1.33	10.99	8.04	40.97	0.34	0.24	0.62
	3	4.31	8.65	7.27	0.62	0.40	0.12	0.40	9.00	28.22	31.43	41.86	7.46	1.34	1.11	1.71	174.0	62.8	31.4	0.07	0.17	0.27	23.13	4.94	40.70	0.19	0.25	0.39
	4	4.12	3.85	5.94	0.45	0.50	0.25	3.60	1.90	8.94	41.46	38.24	36.06	2.82	2.67	2.97	191.4	317.0	62.8	0.03	1.40	0.43	0.73	25.07	39.00	0.04	0.19	0.31
	Avg	4.31	7.05	4.75	0.74	0.69	0.58	1.08	4.48	31.55	37.61	34.72	45.19	2.69	2.10	2.08	274.8	164.2	251.3	0.11	0.49	0.76	14.38	10.78	39.12	0.28	0.20	0.45
	T.Avg	5.62	5.21	3.73	1.00	1.05	0.82	0.96	4.12	34.96	38.18	37.44	55.56	2.76	2.44	2.11	254.4	352.2	262.0	0.37	0.45	0.53	38.50	38.72	36.81	0.30	0.24	0.40
	Max	9.08	8.65	7.27	2.12	1.41	1.63	3.60	10.00	70.00	48.88	47.37	97.36	4.46	3.27	2.97	623.5	676.9	662.6	1.50	1.40	1.33	89.70	99.20	83.90	0.55	0.54	0.62
	Min	3.28	2.78	2.53	0.45	0.40	0.12	0.10	0.10	8.94	25.58	25.00	7.46	1.34	1.11	0.93	75.4	62.8	31.4	0.03	0.07	0.01	0.73	4.94	2.20	0.04	0.09	0.18
	S.D.	1.90	2.19	1.70	0.49	0.37	0.44	1.25	3.53	18.77	6.73	7.37	28.43	0.98	0.67	0.65	209.0	231.9	199.6	0.51	0.45	0.49	32.62	34.04	21.20	0.17	0.11	0.16

a = Summer
b = Winter
c = Monsoon

Table 8: Total Concentration of Nutrient Elements in Industrial Waste

Block	Sample No.	Total Metal Concentration (%)																				
		Na			K			Ca			Mg			Fe			Cu			Mn		
		a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
A	1	0.203	0.263	0.450	0.004	0.043	0.028	0.335	1.100	0.190	0.222	0.230	0.153	5.010	6.375	5.223	0.102	0.053	0.030	0.875	1.433	0.443
	2	0.283	0.373	0.380	0.030	0.030	0.028	0.370	0.650	0.218	0.325	0.215	0.230	6.825	6.800	6.565	0.088	0.085	0.035	0.533	1.205	1.198
	3	0.268	0.380	0.343	0.008	0.025	0.000	0.305	0.725	0.290	0.180	0.193	0.275	4.520	5.200	4.563	0.211	0.105	0.033	1.150	0.728	0.703
	4	0.388	0.295	0.290	0.025	0.015	0.003	0.325	0.303	0.192	0.150	0.135	0.188	6.250	5.450	5.230	0.058	0.650	0.005	0.575	0.525	0.588
	Avg	0.286	0.328	0.366	0.017	0.028	0.015	0.334	0.695	0.223	0.219	0.193	0.212	5.651	5.956	5.395	0.115	0.223	0.026	0.783	0.973	0.733
B	5	0.328	0.355	0.395	0.050	0.080	0.045	0.403	0.625	0.368	0.425	0.303	0.325	4.802	4.350	6.090	0.013	0.011	0.020	0.550	0.425	0.800
	6	0.380	0.525	0.333	0.062	0.110	0.035	0.205	0.275	0.303	0.323	0.355	0.303	5.208	4.950	6.770	0.020	0.025	0.085	0.653	0.553	1.623
	7	0.423	0.360	0.500	0.035	0.065	0.208	0.300	0.800	0.295	0.193	0.215	0.195	6.210	5.325	4.800	0.011	0.005	0.013	0.723	0.628	0.353
	8	0.365	0.393	0.315	0.040	0.070	0.027	0.323	0.450	0.208	0.250	0.198	0.212	5.520	4.835	6.498	0.015	0.016	0.043	0.433	0.413	1.133
	Avg	0.374	0.408	0.386	0.047	0.081	0.079	0.308	0.538	0.294	0.298	0.268	0.259	5.435	4.865	6.040	0.015	0.014	0.040	0.590	0.505	0.977
C	9	0.450	0.280	0.285	0.038	0.010	0.015	0.390	0.250	0.153	0.050	0.375	0.303	7.125	7.005	5.650	0.006	0.014	0.023	1.200	0.475	0.598
	10	0.382	0.213	0.255	0.053	0.015	0.040	0.320	0.325	0.198	0.225	0.400	0.325	6.025	6.023	4.000	0.121	0.017	0.143	0.850	0.115	0.288
	11	0.321	0.155	0.415	0.020	0.020	0.110	0.385	0.150	0.278	0.175	0.210	0.280	5.050	5.050	5.245	0.005	0.020	0.018	0.753	1.250	0.283
	12	0.408	0.185	0.333	0.033	0.005	0.035	0.413	0.300	0.348	0.130	0.195	0.293	6.800	5.525	5.120	0.152	0.018	0.038	1.533	0.355	0.723
	Avg	0.390	0.208	0.322	0.036	0.013	0.050	0.377	0.256	0.244	0.145	0.295	0.300	6.250	5.901	5.004	0.071	0.017	0.056	1.084	0.549	0.473
	Min	0.203	0.155	0.255	0.004	0.005	0.000	0.205	0.150	0.153	0.050	0.135	0.153	4.520	4.350	4.000	0.005	0.005	0.005	0.433	0.115	0.283
	Max	0.450	0.525	0.500	0.062	0.110	0.208	0.413	1.100	0.368	0.425	0.400	0.325	7.125	7.005	6.770	0.211	0.650	0.143	1.533	1.433	1.623
	T.Avg	0.350	0.315	0.358	0.033	0.041	0.048	0.340	0.496	0.253	0.221	0.252	0.257	5.779	5.574	5.480	0.067	0.085	0.041	0.819	0.675	0.728
	S.D	0.067	0.100	0.065	0.016	0.032	0.053	0.053	0.265	0.063	0.094	0.078	0.055	0.794	0.760	0.785	0.063	0.166	0.034	0.304	0.373	0.375

a- Summer samples b- Winter samples c- Monsoon samples

Table 9: Total Concentration Of Toxic Metals in Industrial Waste

Block	Sample	Total Metal Concentration (mg/Kg)											
		Cr			Ni			Cd			Pb		
		a	b	c	a	b	c	a	b	c	a	b	c
A	1	3205	1625	2750	925	1125	900	50	75	150	30.5	55.5	16
	2	2250	1250	3275	1025	925	1675	100	100	150	50	30	11.5
	3	5425	1850	9925	825	875	850	100	100	150	42	80.5	24.5
	4	4275	1300	2300	1250	1825	1725	75	75	150	20.5	52.5	15.5
	Avg	3788.75	1506.25	4562.5	1006.25	1187.5	1287.5	81.25	87.5	150	35.75	54.63	16.88
B	1	4250	2550	2750	1800	825	1975	150	100	75	15.5	20.5	35
	2	3250	2625	10325	1725	1225	2700	100	75	125	20.5	40.5	28.5
	3	2675	2225	2200	1625	950	975	100	75	300	24.5	60.4	102
	4	2200	1950	2525	1925	1050	100	75	150	75	22	72.4	20.5
	Avg	3093.75	2337.5	4450	1768.75	1012.5	1437.5	106.25	100	143.75	20.63	48.45	46.5
C	1	1825	6750	4150	2050	1850	1075	75	150	75	15.5	15.8	39.5
	2	1975	8350	2925	2025	2075	1075	100	125	150	20	20.5	83
	3	2200	5350	8375	1825	2375	3725	75	75	75	18.5	50	24.5
	4	2650	7250	1225	2175	2050	875	75	75	75	30	40	29.5
	Avg	2162.5	6925	4168.75	2018.75	2087.5	1687.5	81.25	106.25	93.75	21	31.58	44.13
	Min.	1825	1250	1225	825	825	100	50	75	75	15.5	15.8	11.5
	Max.	5425	8350	10325	2175	2375	3725	150	150	300	50	80.5	102
	Total Avg.	3059.82	3351.34	4409.82	1567.86	1382.14	1455.36	90.18	97.32	131.70	26.13	45.83	35.24
	S. D.	1043.13	2456.14	2951.59	464.28	531.03	898.23	23.48	26.93	59.87	10.32	19.33	26.41

a- summer ; b- winter ; c- monsoon.

Table 10: Co-Relation Matrics for Physico-chemical Parameters of Industrial Wastes

	pH	EC	MC	WHC	AN	TN	AP	TP	OC
PH	1								
EC	-0.3123	1							
MC	0.2815	0.6208	1						
WHC	-0.4631	0.6682	0.5859	1					
AN	-0.2838	0.7673	0.6129	0.6772	1				
TN	-0.2117	0.1738	0.2668	0.7004	0.5573	1			
AP	-0.4571	0.5871	0.2023	0.4585	0.4972	0.1785	1		
TP	0.1810	0.4216	0.7266	0.5310	0.3411	0.2448	0.1764	1	
OC	-0.4128	0.1673	-0.0582	0.3168	0.0644	0.2498	0.3253	0.0419	1

EC (Electrical Conductivity); MC (Moisture Content); WHC (Water Holding Capacity); AN (Available Nitrogen); TN (Total Nitrogen); AP (Available Phosphorus); TP (Total Phosphorus); OC (Organic Carbon).

Table 11: Co-Relation Matrics for Different Metal Concentration of Industrial Wastes

	Na	K	Ca	Mg	Fe	Mn	Cu	Cr	Ni	Cd	Pb
Na	1										
K	0.7456	1									
Ca	0.1380	0.1182	1								
Mg	0.1763	0.2984	-0.0924	1							
Fe	-0.0874	-0.2771	0.2032	-0.1822	1						
Mn	-0.2261	-0.3388	-0.3240	-0.3278	0.2047	1					
Cu	-0.3425	-0.5170	-0.0557	-0.2916	0.8620	0.3542	1				
Cr	-0.926	-0.2186	-0.4548	0.2941	-0.1793	0.0663	-0.1074	1			
Ni	-0.3266	0.0616	-0.7436	0.1444	0.0894	-0.1486	-0.0731	0.5938	1		
Cd	0.4077	0.3358	0.1172	0.4777	0.0090	-0.6084	-0.0913	-0.0984	-0.3224	1	
Pb	-0.2116	-0.1600	0.6296	-0.3061	-0.0629	0.5073	0.2506	-0.3294	-0.4458	-0.4155	1

Na (Sodium); K (Potassium); Ca (Calcium); Mg (Magnesium); Fe (Iron); Mn (Manganese); Cu (Copper); Cr (Chromium); Ni (Nickel); Cd (Cadmium); Pb (Lead).

Fig.3 Seasonal Average Variation of pH in Different Blocks

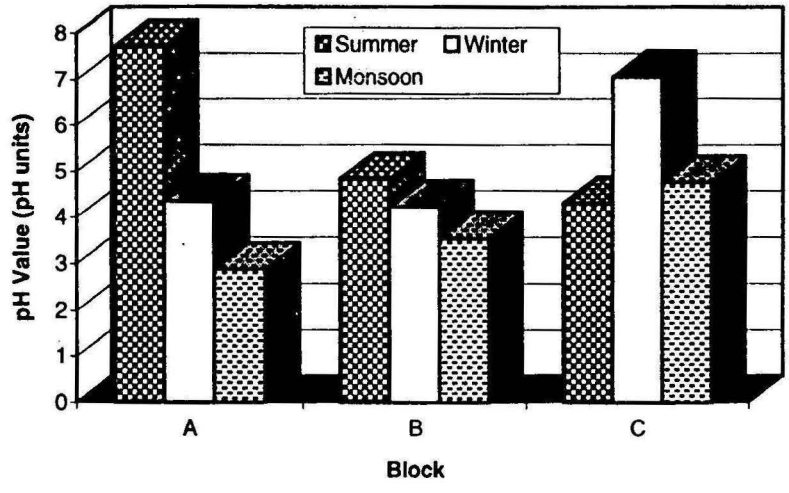


Fig. 4 Seasonal Average Variation of E.C. in Different Blocks

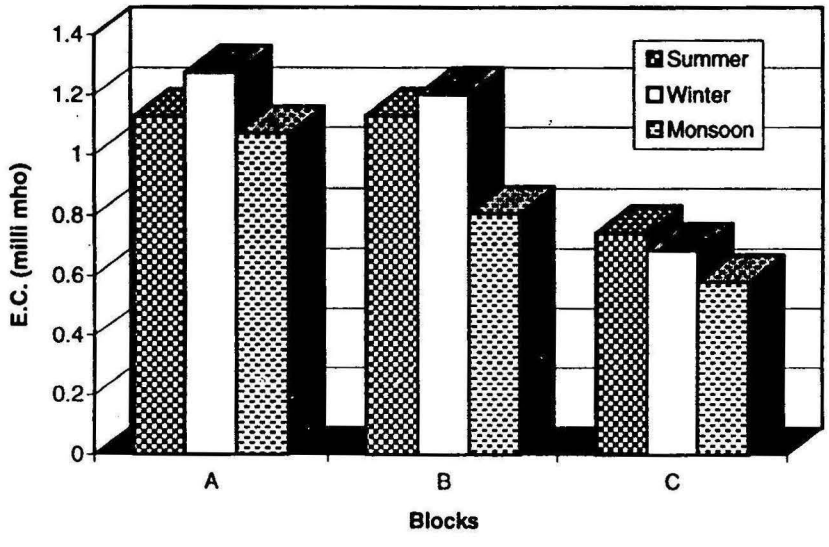


Fig. 5 Seasonal Average Variation of Moisture Content in Different Blocks

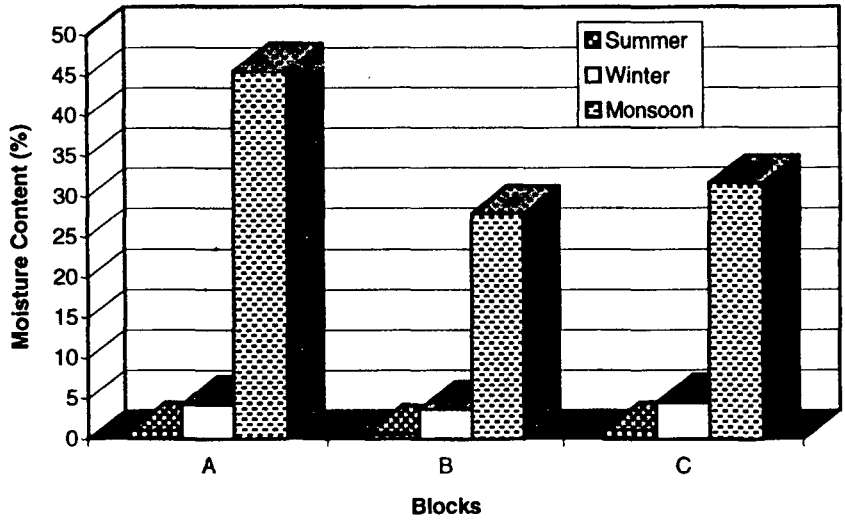


Fig. 6 Seasonal Average Variation of WHC in Different Blocks

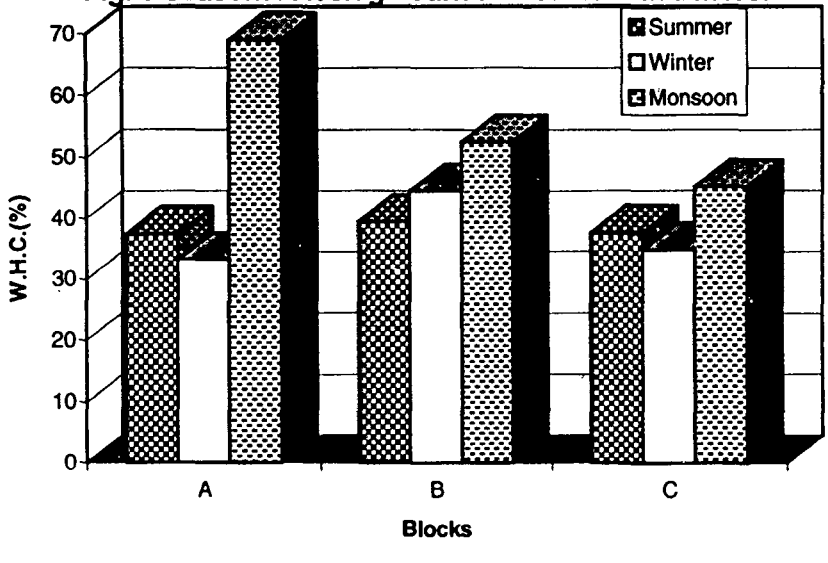


Fig. 7 Seasonal Average Variation of Available Nitrogen in Different Blocks

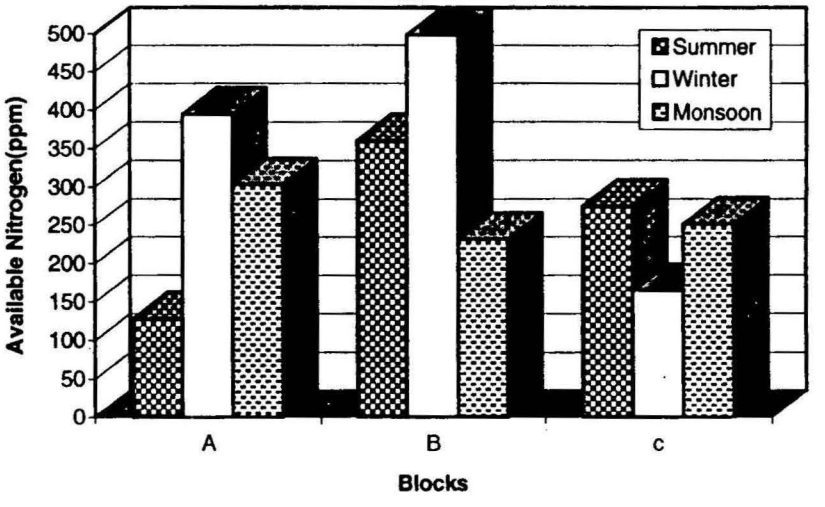


Fig. 8 Seasonal Average Variation of Total Nitrogen in Different Blocks

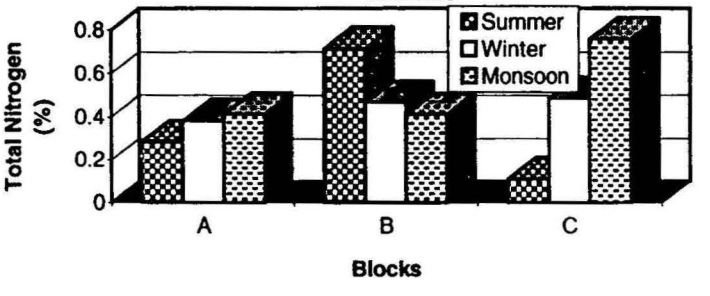


Fig. 9 Seasonal Average Variation of Available Phosphorus in Defferent Blocks

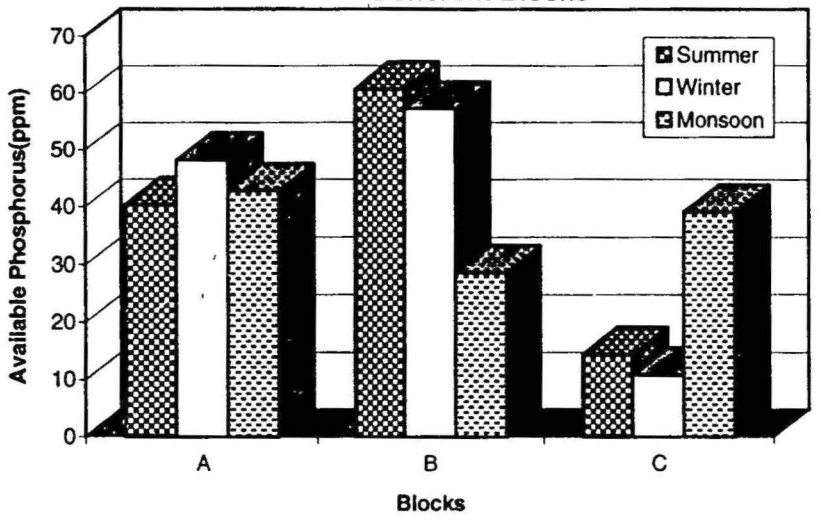
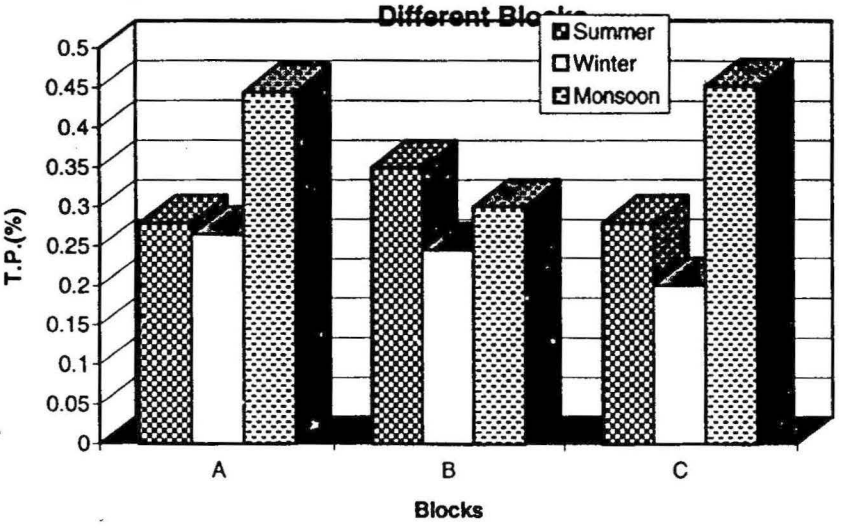


Fig. 10 Seasonal Average Variation of Total Phosphorus in Different Blocks



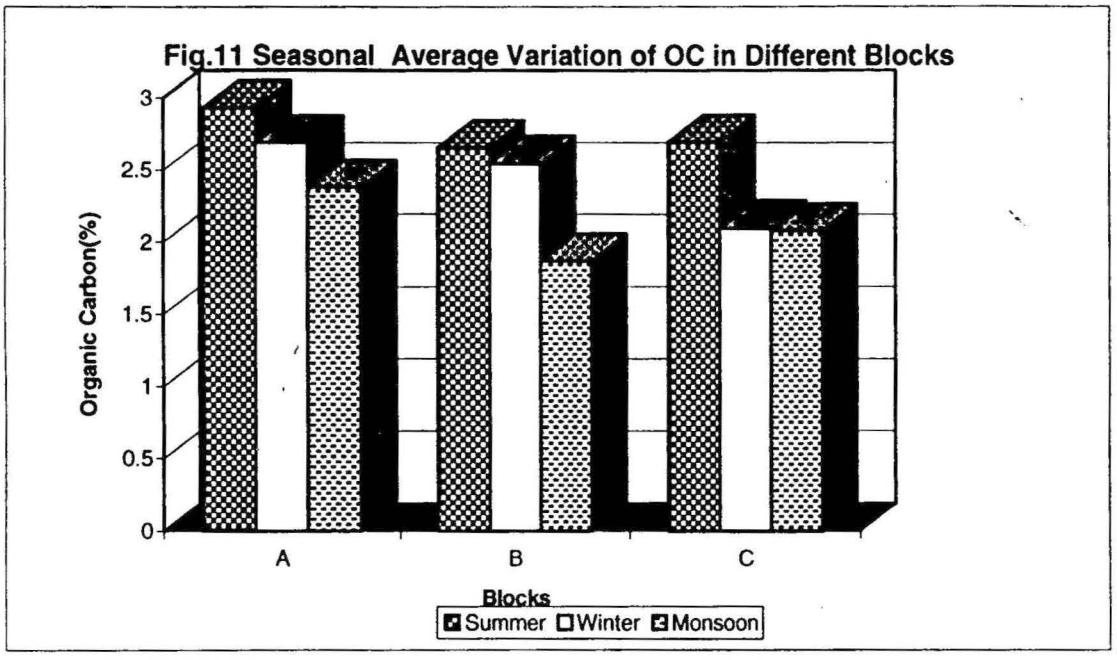


Fig.11 Seasonal Average Variation of OC in Different Blocks
Organic Carbon(%)
3
2.5
2
1.5
1
0.5
0
A
B
C
Blocks
Summer
Winter
Monsoon

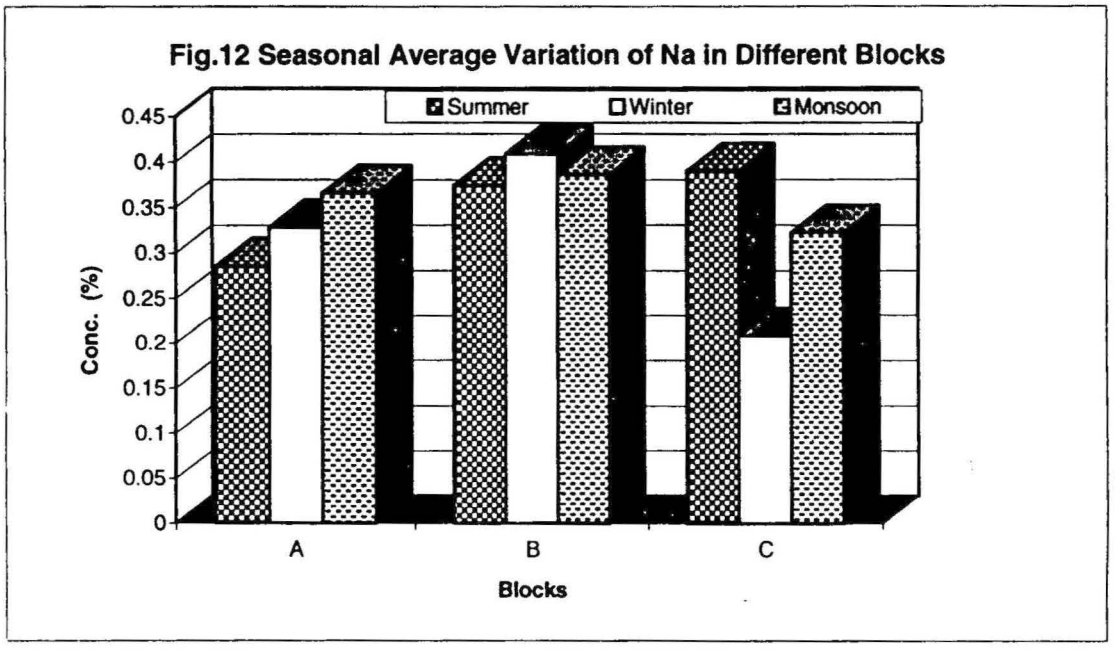


Fig.12 Seasonal Average Variation of Na in Different Blocks
Summer
Winter
Monsoon
Conc. (%)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
A
B
C
Blocks

Fig. 13 Seasonal Average Variation of K in Different Blocks

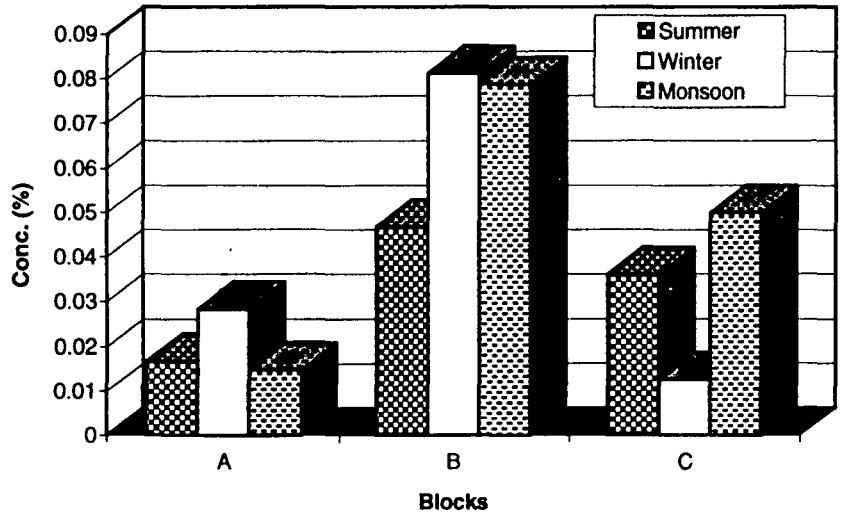


Fig. 14 Seasonal Average Variations of Ca in Different Blocks

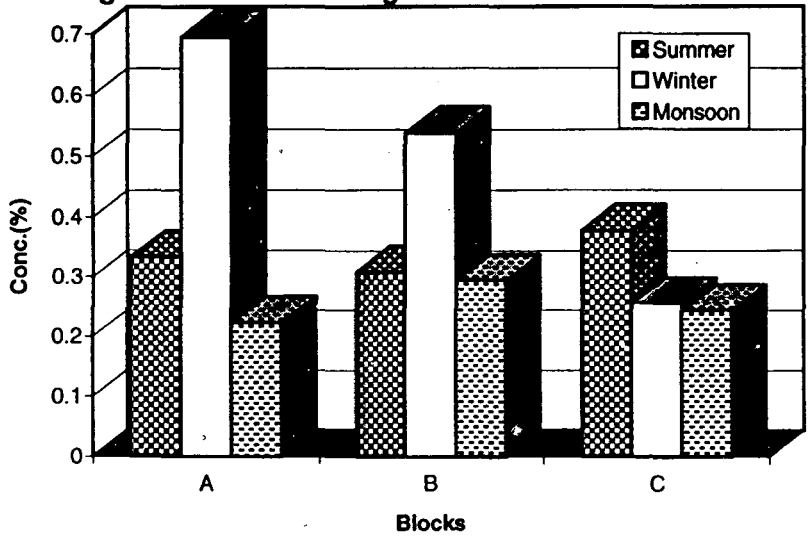


Fig.15 :Seasonal Avearage Variation of Mg in Different Blocks

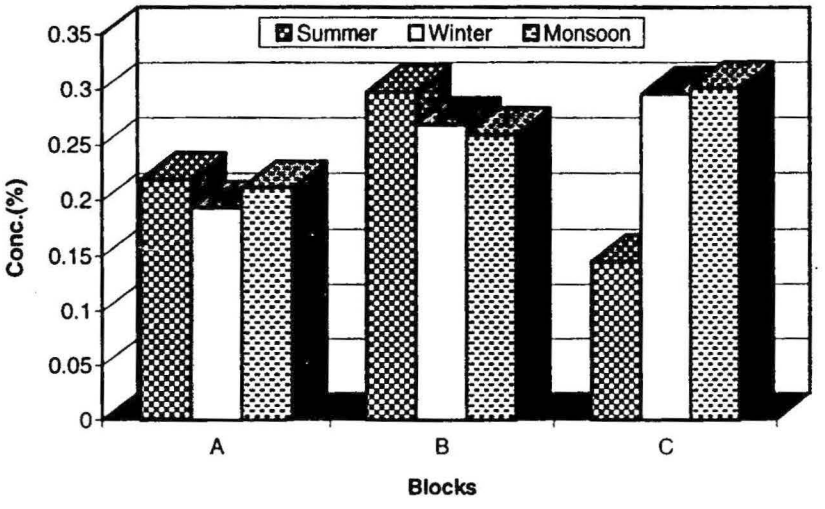


Fig. 16 Seasonal Average Variation of Iron in Different Blocks

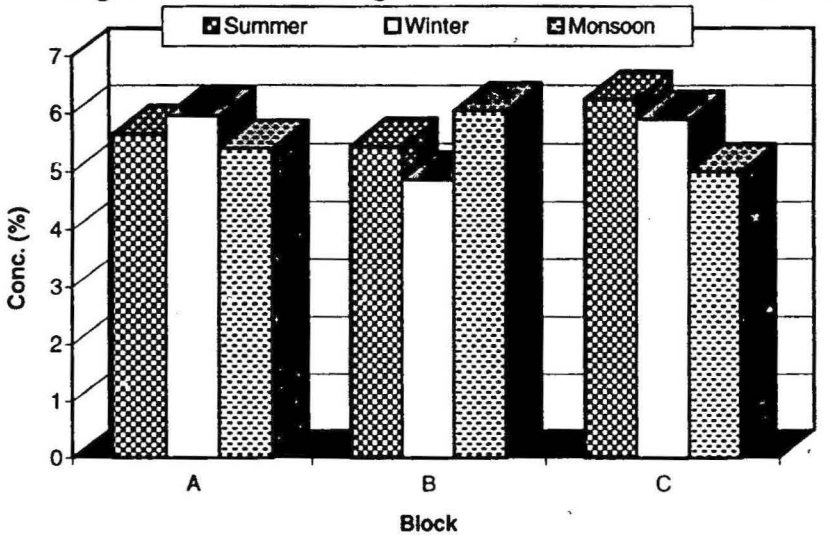


Fig. 17 Seasonal Average Variation of Copper in Different Blocks

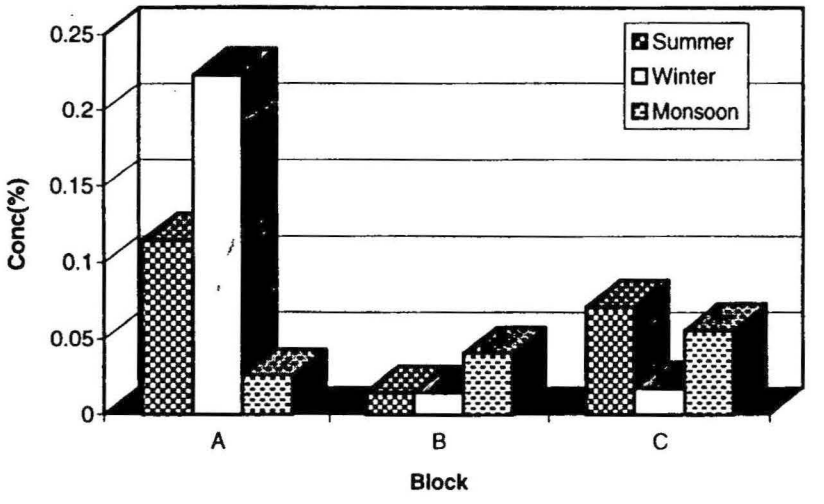


Fig. 18 Seasonal Average Variation of Manganese in Different Blocks

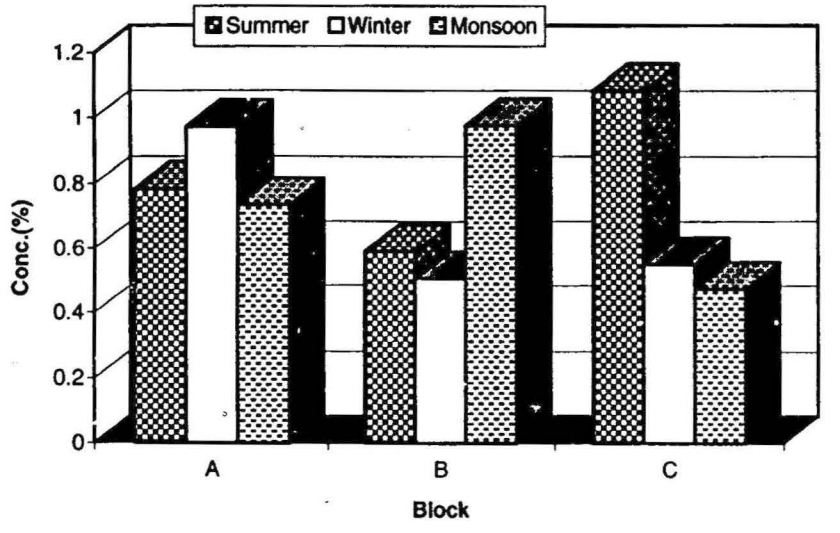


Fig. 19 Seasonal Average Variation of Chromium in Different Blocks

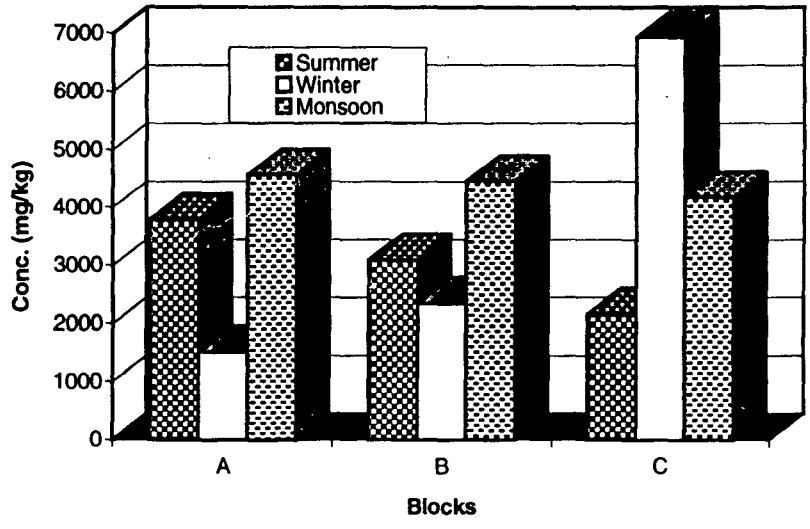


Fig. 20 Seasonal Average Variation of Nickel in Different Blocks

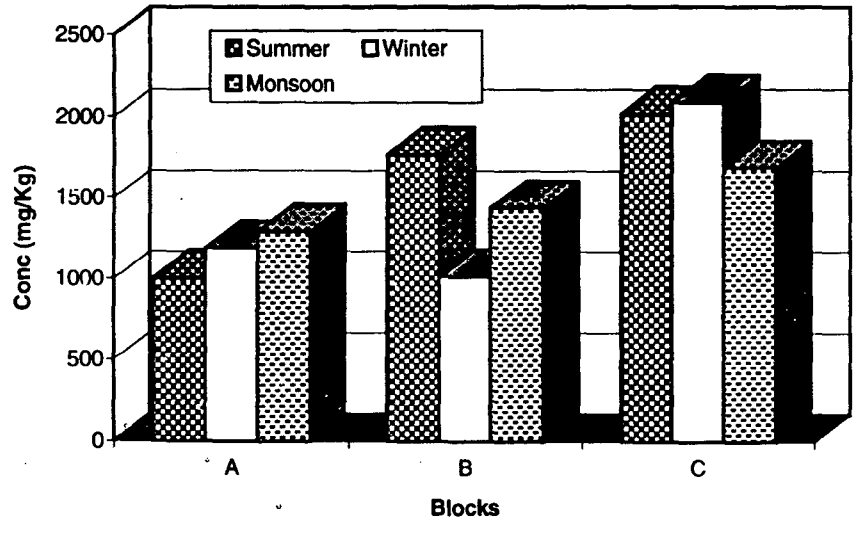


Fig. 21 Seasonal Average Variation of Cadmium in Different Blocks

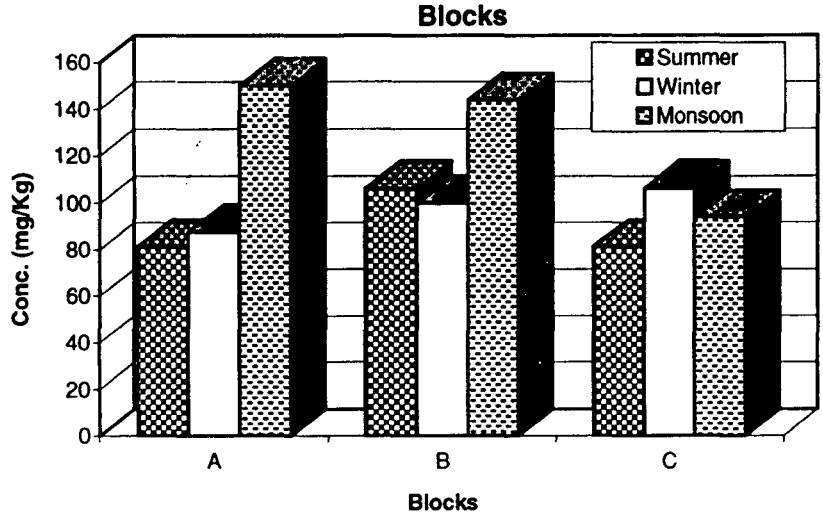


Fig.22 :Seasonal Average Variation of Pb in Different Blocks

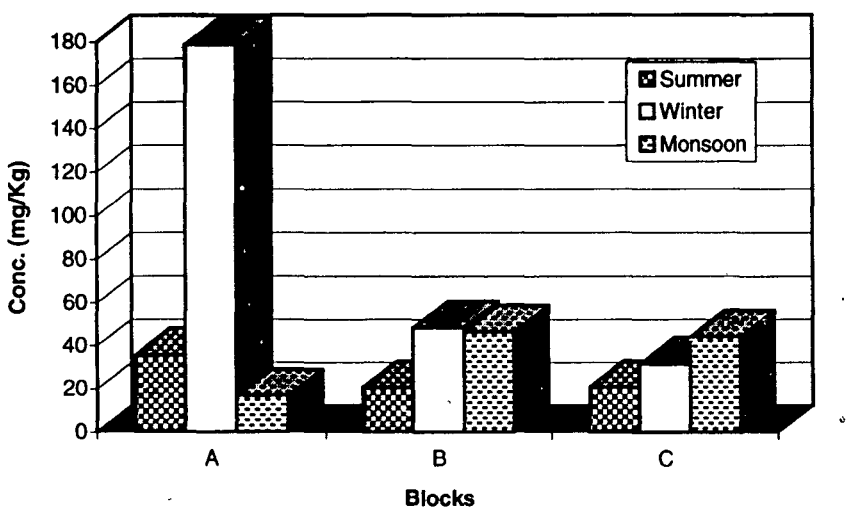


Table 12: Structural Characterization of Microbial Isolates on Nutrient Agar Plates

Season	Block Sample	CFU's	Isolate Type	Diameter (mm)	Colour	Opacity (a)	Form (b)	Elevation (c)	Margin (d)	Smooth-ness (e)	Texture (f)	Spreading Nature
Summer		32,000	1 (Bact-6)	3.0	Brownish Yellow	S	C	F	E	S	S	Yes
	A		2 (Bact-24)	2.0	White	O	C	F	E	S	S	Yes
			3 (Fungi-2_	5.0	White	O	C	R	E	D	D	Yes
	B	3,000	4 (Bact-2)	2.0	White Grey	O	C	R	E	S	S	Yes
			5 (Fungi-1)	5.0	BrownishWhite	O	C	R	E	D	D	Yes
	C	1,300	6 (Bact-11)	3.5	Brown	S	C	F	E	S	S	Yes
			7 (Bact-1)	6.0	Pink	O	C	F	E	S	S	Yes
			8 (Fungi-1)	4.0	White	O	C	R	E	D	D	Yes
Winter	A	5,200	9 (Bact-51)	3.0	Yellowish Brown	S	C	R	E	S	S	Yes
			10 (Bact-1)	3.0	Light Pink	O	C	R	E	S	S	Yes
	B	3,500	11 (Bact-34)	1.5	White	O	C	R	E	S	S	Yes
			12(Bact-1)	1.0	Dark Yellow	O	C	R	E	S	S	Yes
	C	29,000	13 (Bact-27)	0.5	Grey	S	C	R	E	S	S	Yes
			14 (Bact-1)	1.5	Brownish Yellow	S	C	R	E	S	S	Yes
Monsson	A	1,000	15 (Bact-1)	2.0	Yellow	O	C	R	E	S	S	Yes
	B	1,400	16 (Bact-7)	3.0	Creamish Grey	T	C	R	E	S	D	Yes
			17 (Fungi-7)	3.0	White	O	C	R	E	S	S	Yes
	C	10,000	18 (Bact- 5)	7.0	Grey	O	C	R	E	S	S	Yes
			19 (Bact-3)	5.0	White	T	C	R	E	S	S	Yes
			20 (Bact-2)	4.0	White	O	C	R	E	S	S	Yes

Legends:- (a) Transparent -T; Translucent-S; Opaque-O; (b) Circular-C (c) Flat-F ; Raised-R (d) Entire-E (e) Dull-D; Shiny-S; (f) Dry-D; Viscous-V;

Table 13: Isolates Characteristics on Nutrient Agar Slants

Isolate No.	Growth	Form	Chromogenesis	Colour	Consistancy
1.	Effuse	C	-	Grey	Yes
2.	Beaded	C	-	Grey	Yes
4.	Effuse	I	+	Whitish brown	Yes
6..	Beaded	C	-	Cream	Yes
7.	Effuse	C	+	Light pink	Yes
9.	Effuse	C	-	Dull cream	Yes
10.	Effuse	C	+	Pink	Yes
11.	Effuse	C	-	Whitish grey	Yes
12.	Beaded	C	+	Dark yellow	Yes
13.	Effuse	C	+	Creamy	Yes
14.	Effuse	C	+	Brownish yellow	Yes
15.	Effuse	C	-	Creamish	Yes
16.	Effuse	C	-	Light yellow	Yes
18.	Effuse	I	-	Creamish	Yes
19.	Filliform	I	-	Grey	Yes
20.	Beaded	I	-	White transparent	Yes

Legends: Form - Circular (c), Irregular (I)
Chromogenesis: Present (+), Absent (-)

Table 14: Microscopic study of microbial isolates

Isolate No.	Shape	Arrangement	Gram Reaction
1.	Medium rod	Single and double	+VE
2.	Large rod	Single and double	+ VE
4.	Coccus	Single and chain	+ VE
6.	Coccus	Single and chain	+ VE
7.	Medium rod	Single and chain	- VE
9.	Large rod	Single and chain	+ VE
10.	Medium rod	Single	- VE
11.	Coccus	Clumped	- VE
12.	Coccus	Double, Tetrad	- VE
13.	Coccus	Single double and clumped	+ VE
14.	Coccus	Single	+ VE
15.	Coccus	Single and chain	+ VE
16.	Large rod	Single double and chain	+ VE
18.	Medium rod	Single	- VE
19.	Medium rod	Single and double	- VE
20.	Medium rod	Single and Chain	- VE

Table 15: Results of Bio-Chemical Tests for Bacterial Isolates

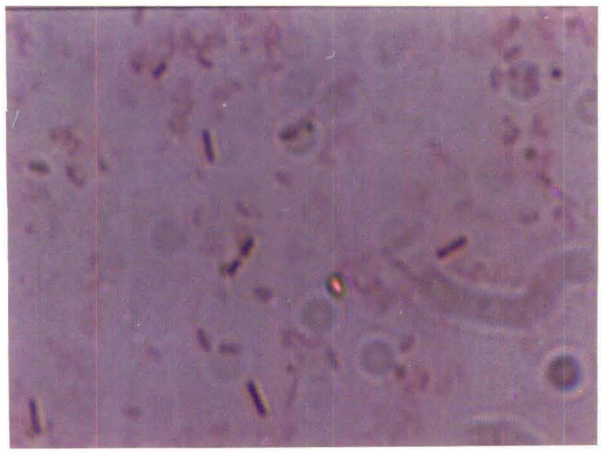
Isolate No.	Indole Test	MR Test	VP Test	Starch Hydrol.Test	Citrate Test	Carbohydrate Fermentation Test						H2S prodn. Test	Urea Prodn. Test	Nitrate Test	Catalase Test	Oxidase Test	Casien Util. Test	Gelatin Test
						Glucose		Sucrose		Lactose								
						F	G	F	G	F	G							
1	+	-	-	+	-	-	-	-	-	+	-	+	+	+	-	-	-	+
2	-	-	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-
4	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
6	-	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-
7	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	+
9	-	-	-	+	+	+	-	+	-	+	-	+	-	+	-	-	+	-
10	+	-	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+
11	+	-	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-
12	+	-	-	+	-	+	-	+	-	-	-	+	-	-	-	-	+	-
13	-	-	+	-	+	+	-	+	-	-	-	+	+	-	-	+	+	+
14	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	+	-
16	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-
18	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	+
19	+	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+
20	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+

Legend:- Positive response +ve; Negative response -ve; Fermentation-F; Gas Production-G.

Table 16: List of Identified Micro Organisms

Isolate No.	Genus
BACTERIA	
1.	*Pseudomonas*
2.	*Bacillus*
4.	*Nitrosococcus*
6.	*Mycococcus*
7.	*Pseudomonas*
9.	*Bacillus*
10.	*Pseudomonas*
11.	*Micrococcus*
12.	*Streptococcus*
13.	*Bacillus*
14.	*Micrococcus*
15.	*Arthobacter*
16.	*Ferrobacillus*
18.	*Pseudomonas*
19.	*Sideromonas*
20.	*Bacillus*
FUNGUS	
3.	*Penecillium sp*
5.	*Geotrichum sp*
8.	*Fusarium sp*
17.	*Corynespora sp*

PLATE-I



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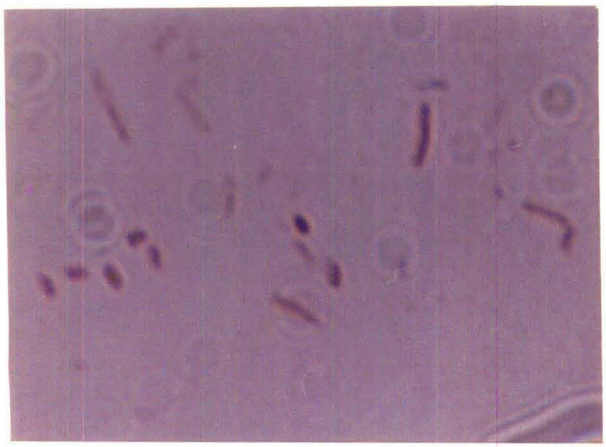

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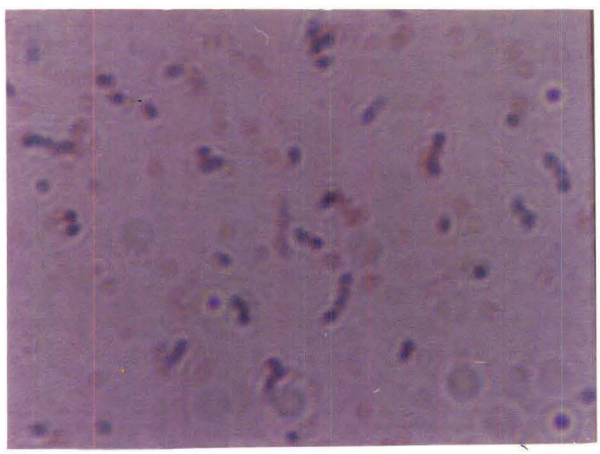

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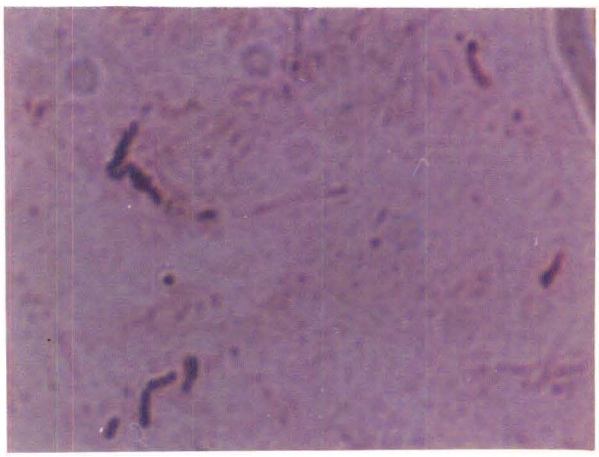

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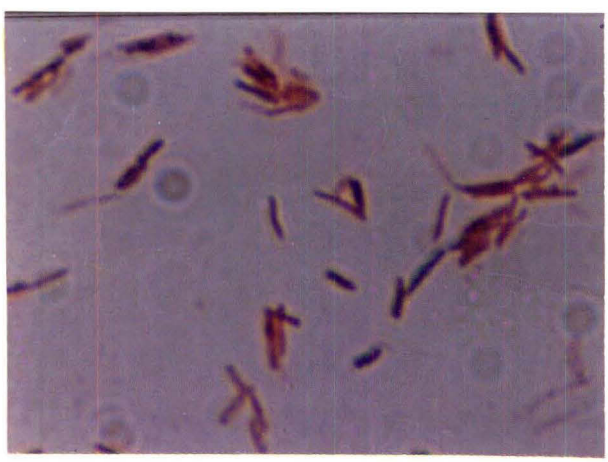
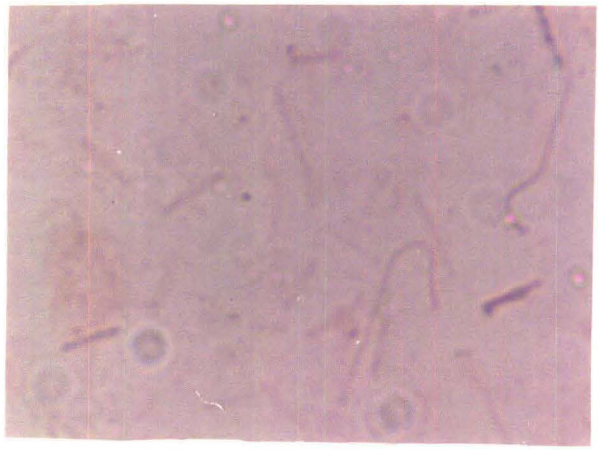


7

Bacterial Isolates
(100 X magnification)

1) *Pseudomonas sp.*
2) *Bacillus sp.*
4) *Nitrosococcus sp.*
6) *Mycococcus sp.*
7) *Pseudomonas sp*

PLATE-II



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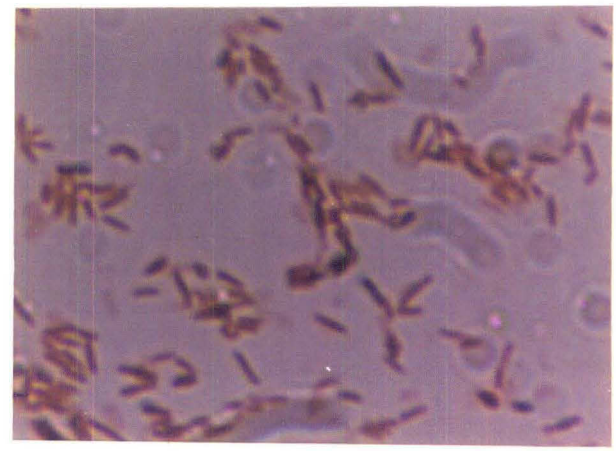

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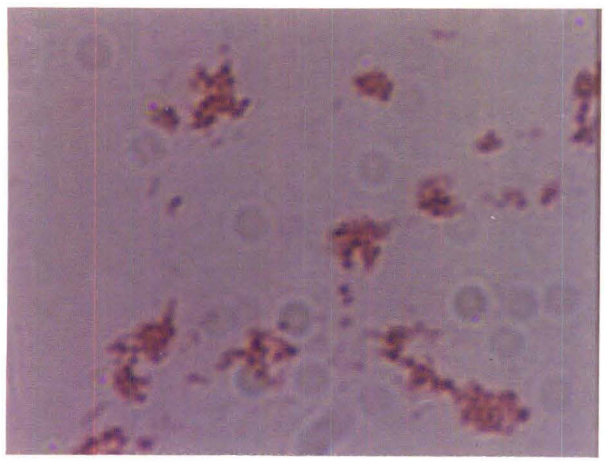

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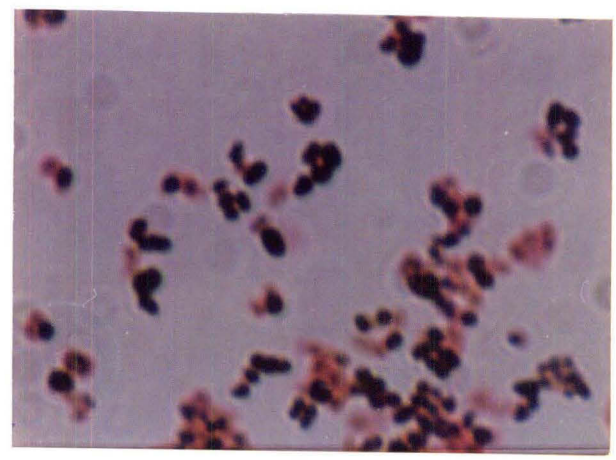

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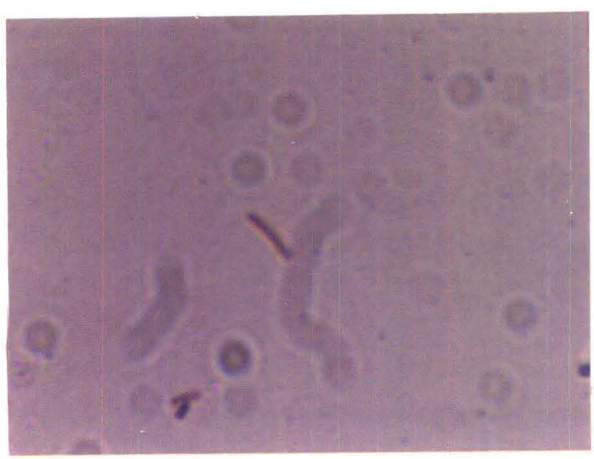
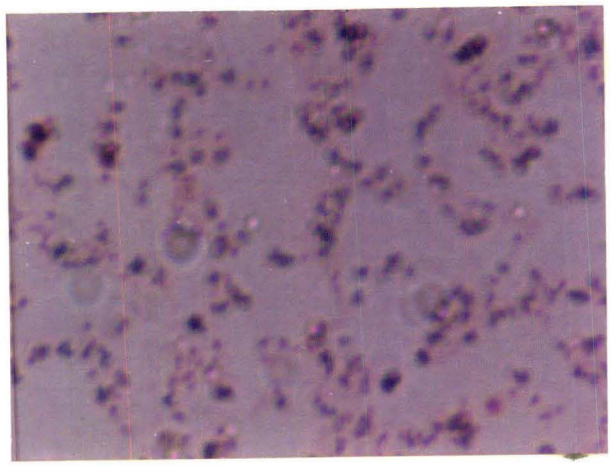


13

Bacterial Isolates
(100 X magnification)
9) *Bacillus sp.*
10) *Pseudomonas sp.*
11) *Micrococcus sp.*
12) *Streptococcus sp.*
13) *Bacillus sp.*

PLATE-III



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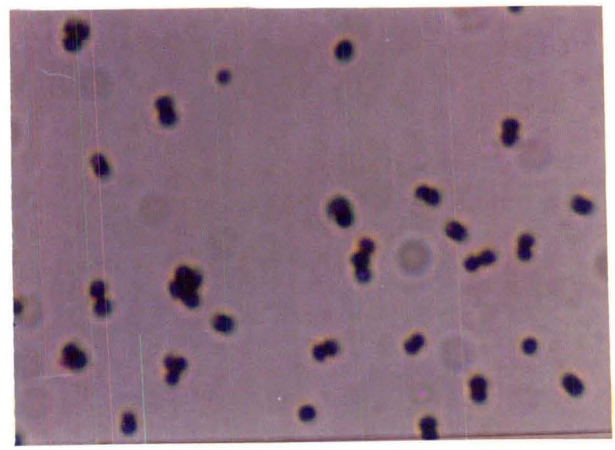

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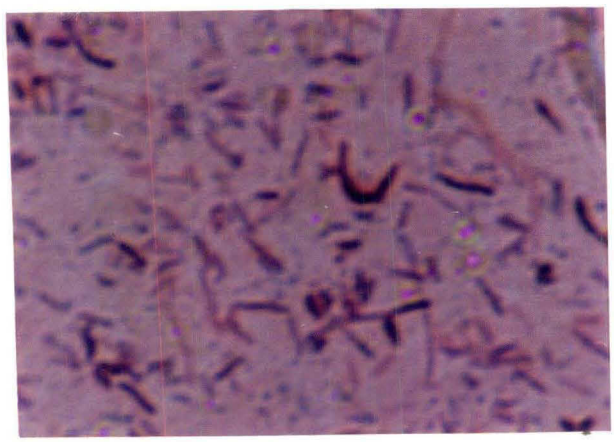

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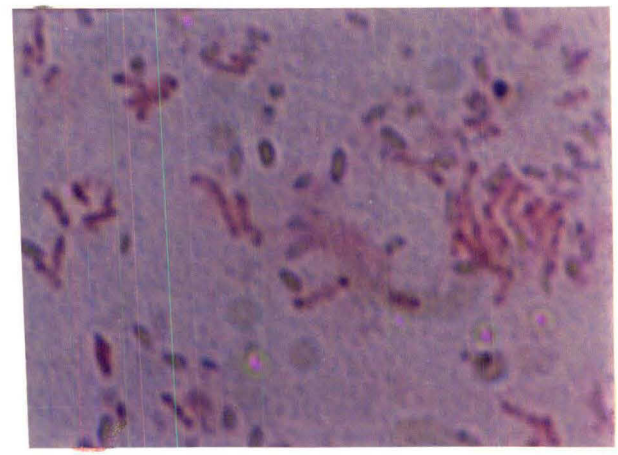

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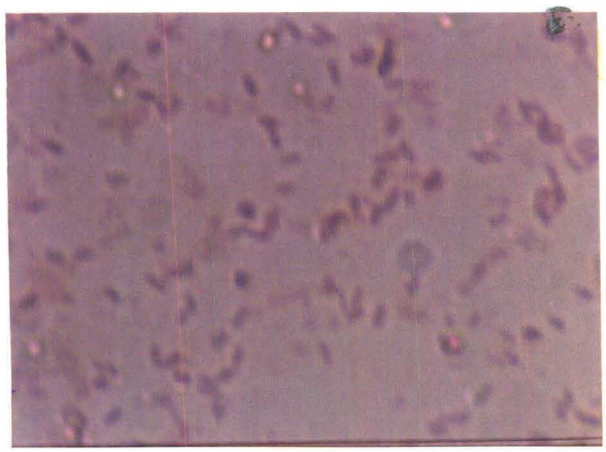
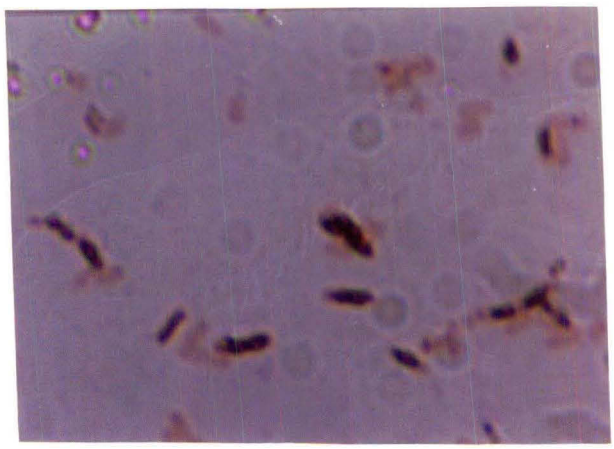


19

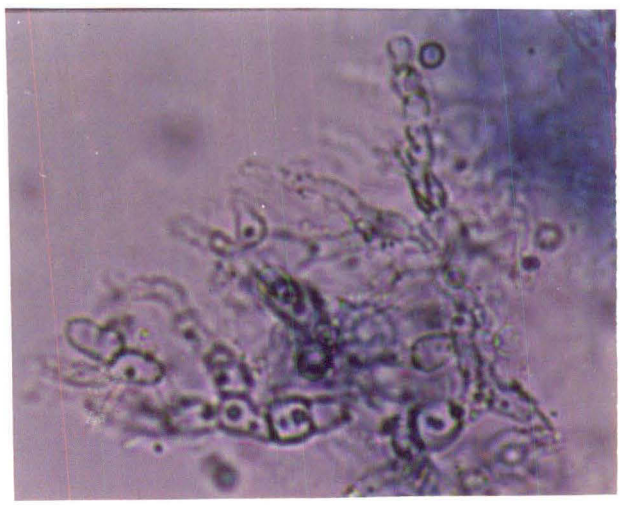
Bacterial Isolates
(100 X magnification)
14) *Micrococcus sp.*
15) *Arthobacter sp.*
16) *Ferrobacillus sp.*
18) *Psudomonas sp.*
19) *Sideromonas sp.*

PLATE-IV



20

Bacter ial Isolate
(100 X magnification)
20) *Bacillus sp.*
Fungal Isolates
(40 X) magnification)
3) *Penecillium sp.*
5) *Geotrichum sp.*
8) *Fusarium sp.*
17) *Corynespora sp.*



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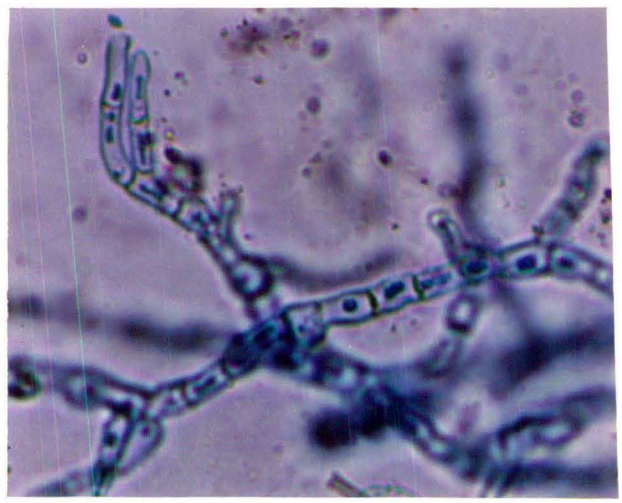

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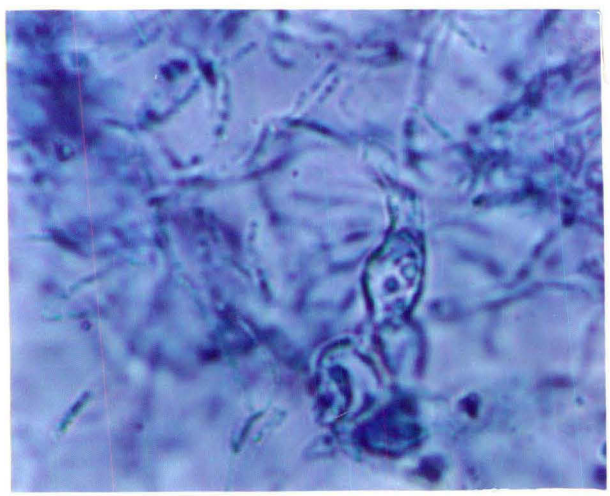

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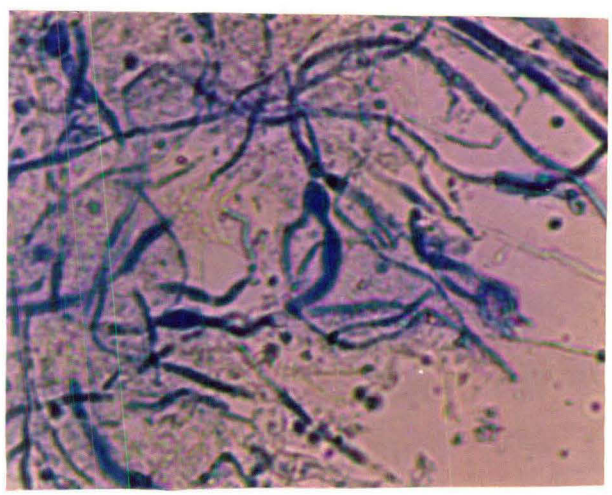


17

Fig. 23 Seasonal and Blockwise Variations in Microbial Counts

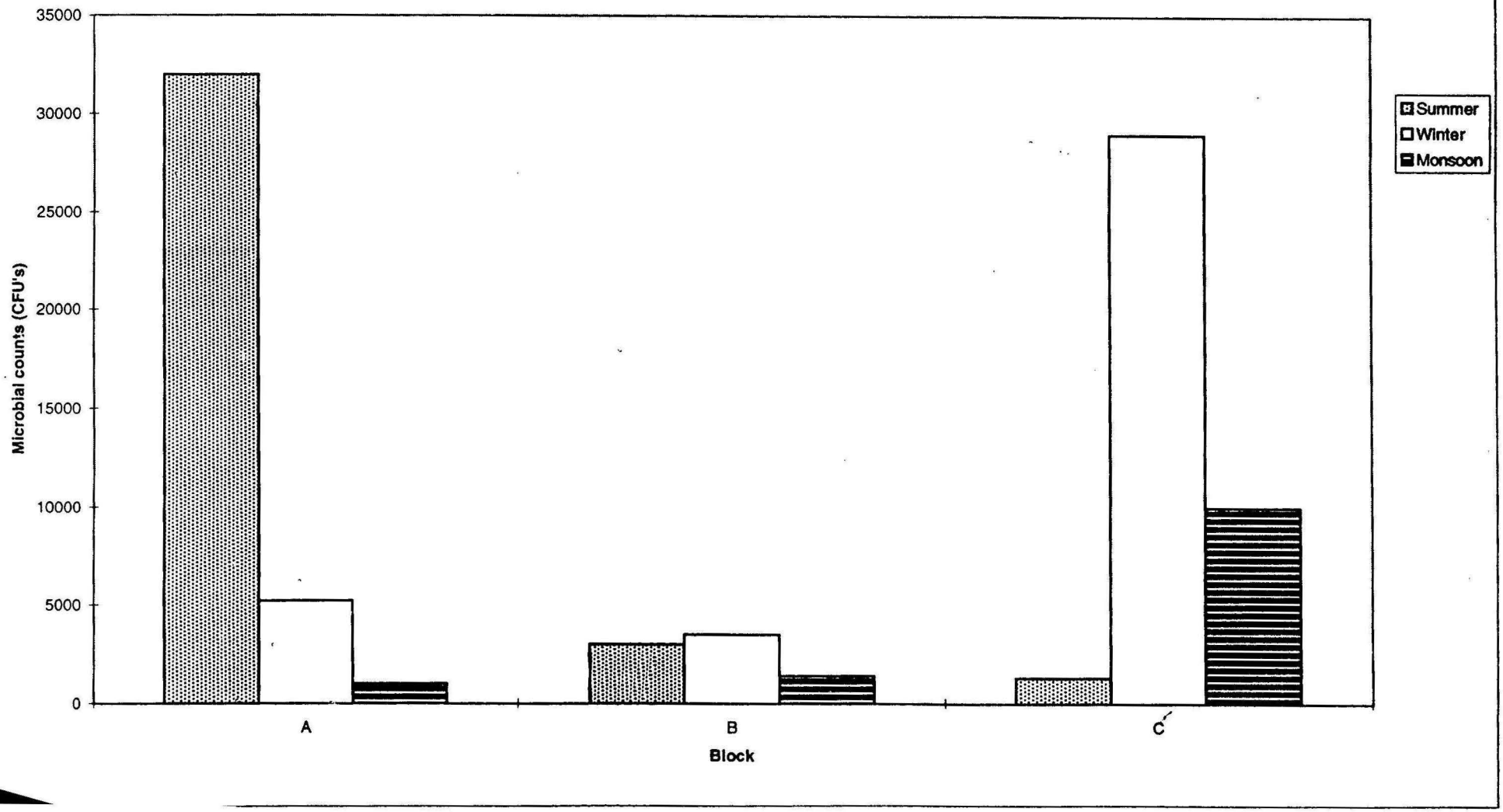


Fig. 24 Variation in pH, EC, Organic Carbon and microbial counts

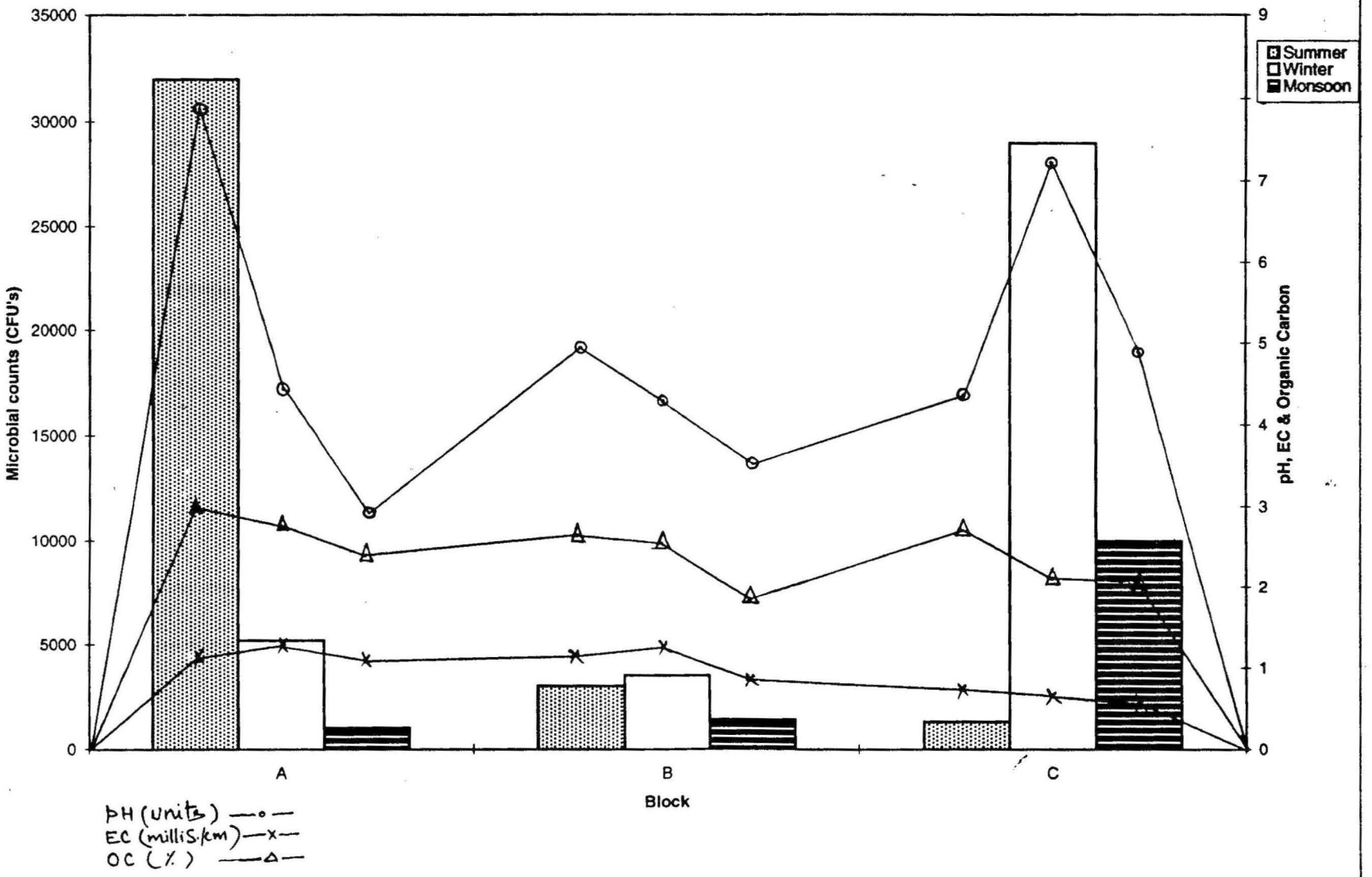


Fig. 25 Variation in Moisture Content, Water Holding Capacity & Microbial Counts

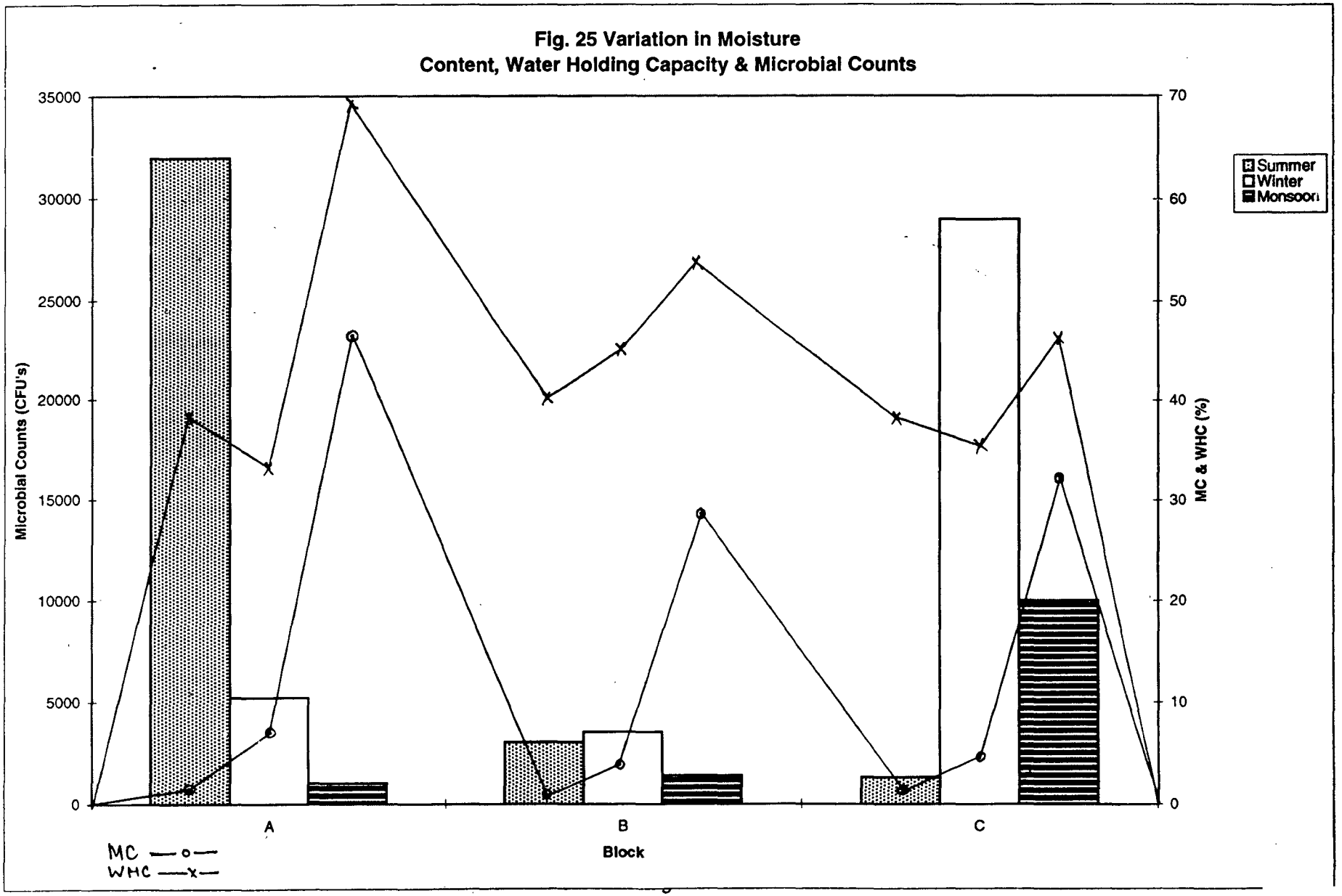


Fig. 26 Variation in Total Nitrogen, Phosphorus & Microbial Counts

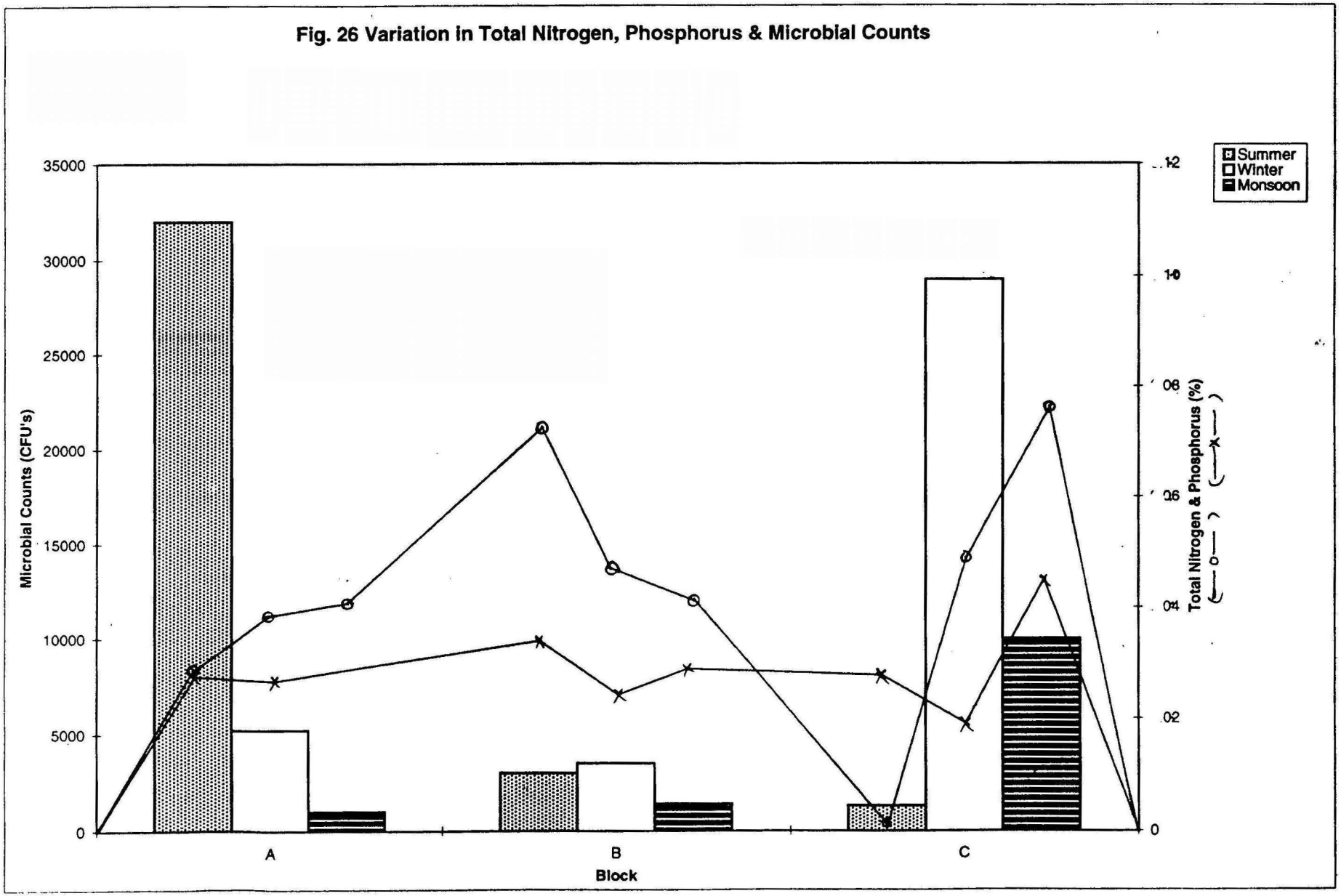


Fig. 27 Variation in Na, Ca, Mg & Microbial Counts

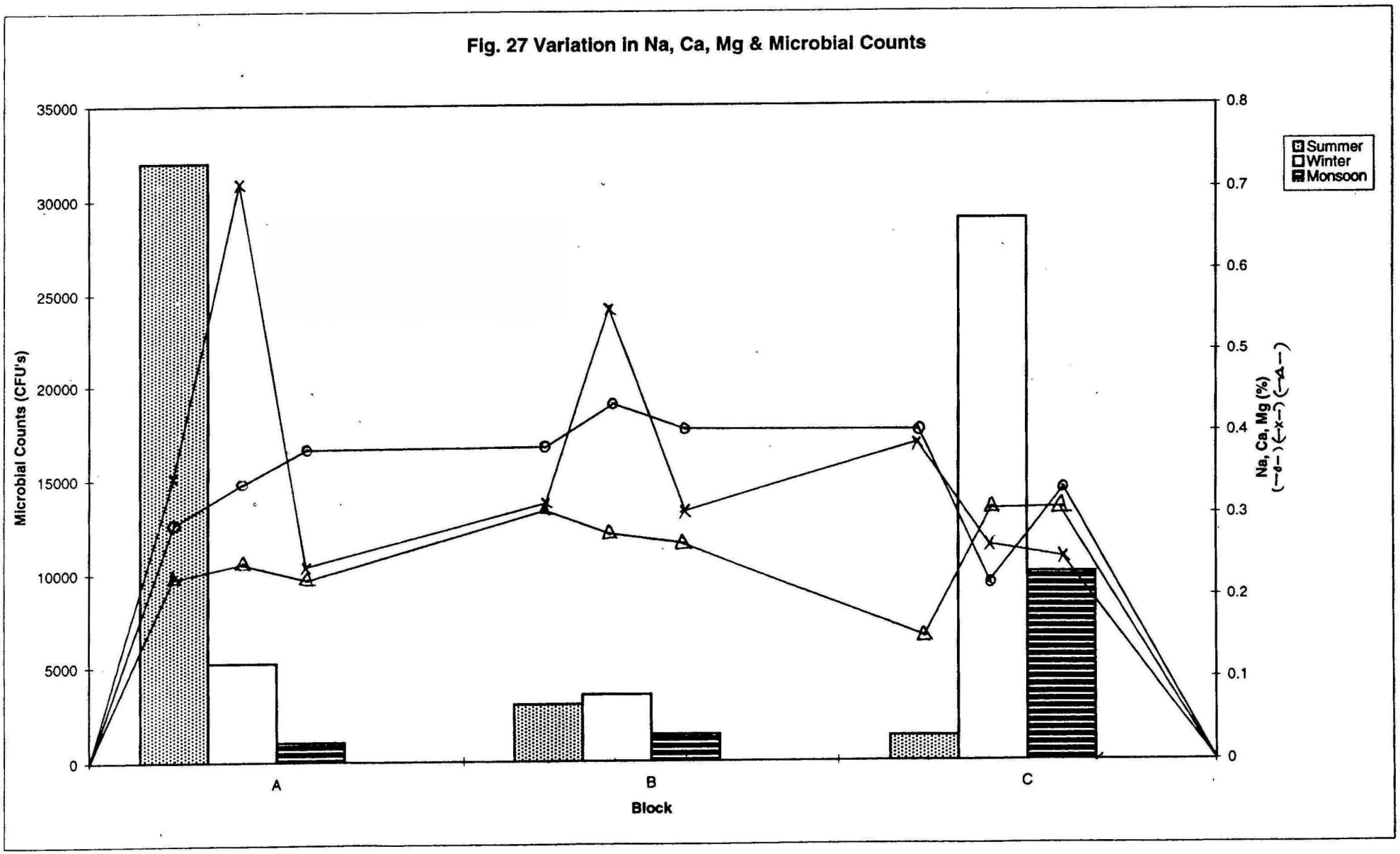


Fig. 29 Variation in Fe, Mn & Microbial Counts

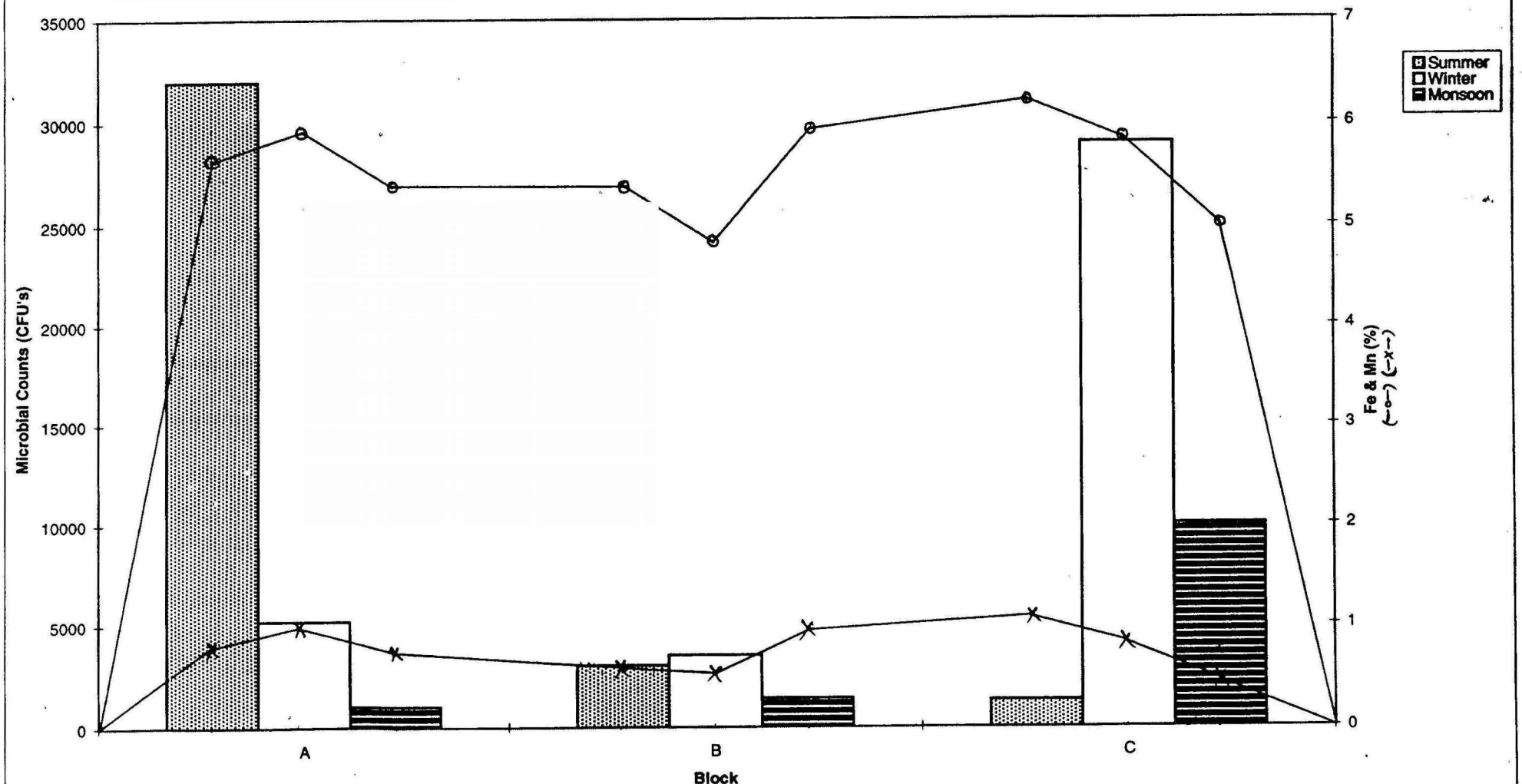


Fig. 28 Variation in K, Cu & Microbial Counts

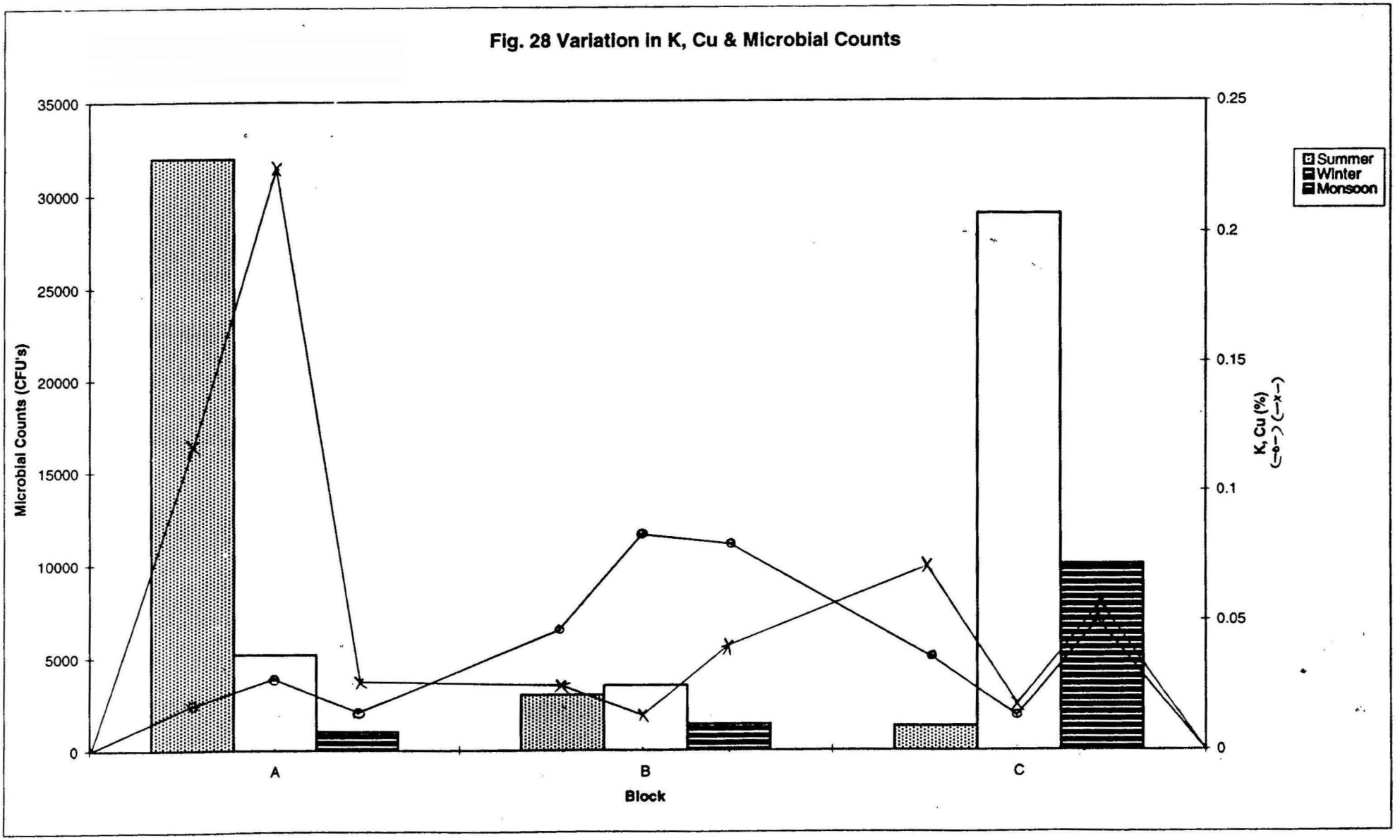


Fig. 30 Variation in Cr, Ni and Microbial Counts

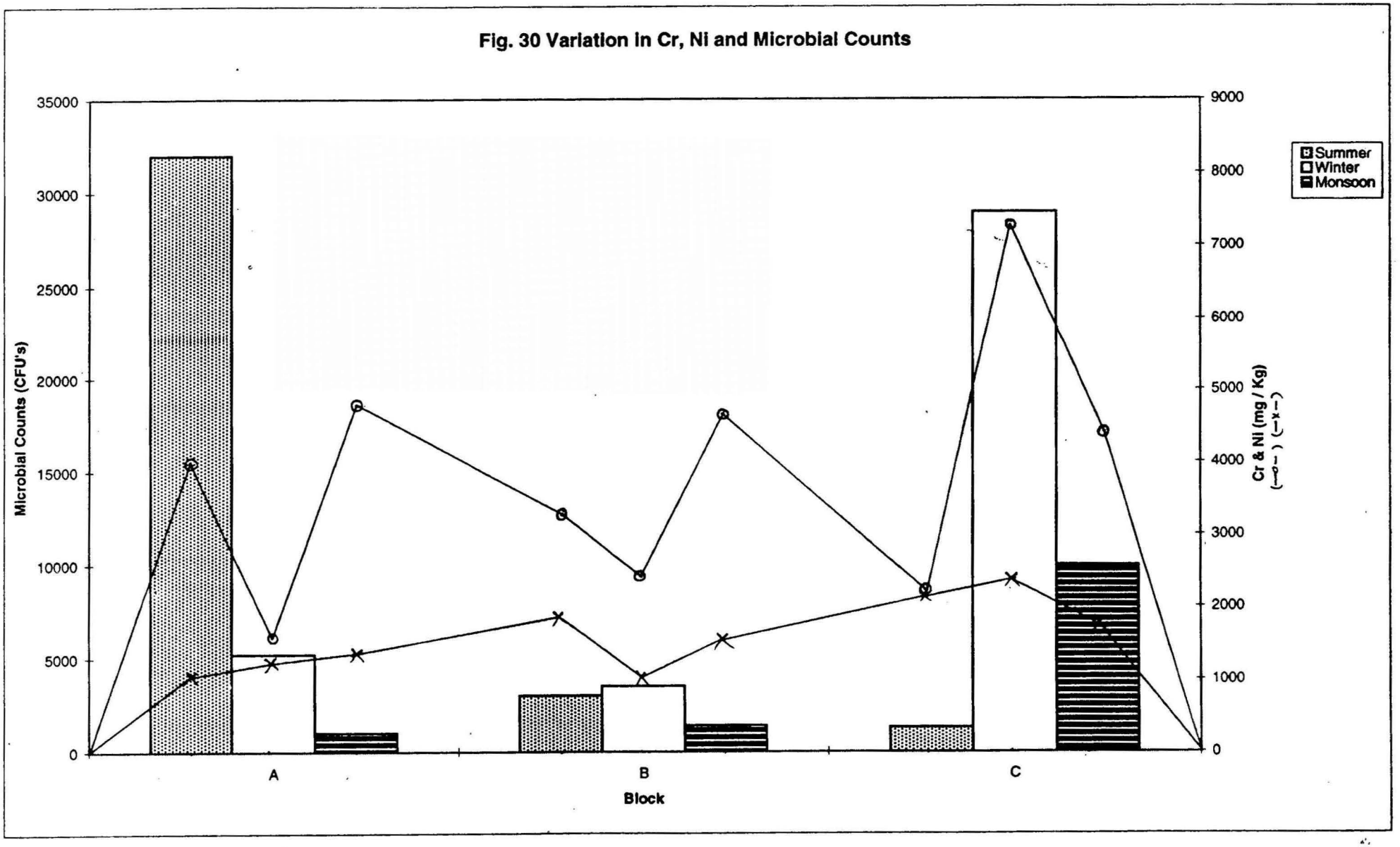
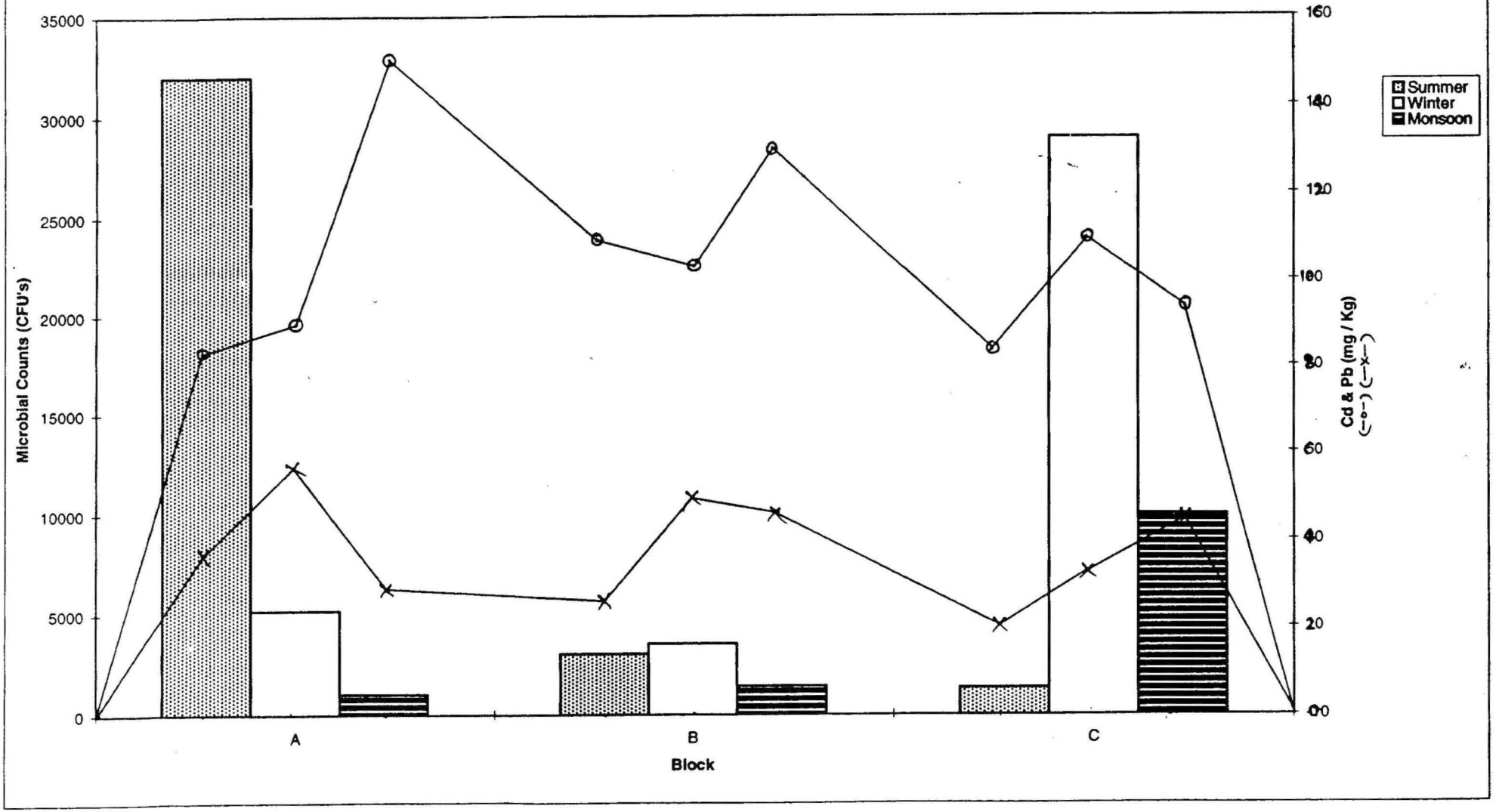


Fig. 31 Variation in Cd, Pb and Microbial Counts



DISCUSSION

pH

The results obtained for the pH values of collected samples varied from 2.53 to 9.08. The average value of samples was found to be in decreasing order in summer from block A to block C. In winter the average pH value of block C was found to be around neutral which corresponds in values for A and B blocks are 4.34 and 4.23. During monsoon season the block average pH value was found to be in the increasing order: C>B>A. It is observed that in Monsoon, the total average pH value of all the blocks showed lowest value (viz. 3.73) in comparison to those of summer (5.62) and winter (5.21) (table 7, fig. 3)

The extremely acidic nature of industrial wastes confirmed the exclusively excess activities of pickling industries in that area which use HNO_3, H_2SO_4 and HF for cleaning the metals. However, neutral and alkaline nature of some samples showed the addition of alkaline wastes from textile industries located in that area.

Electrical Conductance (E.C.)

The electrical conductance reflects the total soluble salts which might be occurring due to : i) Basic nature of materials; ii) the environmental factors changing the stability; and iii) anthropogenic activities, E.C. for individual samples analysed were ranged from 0.12 to 2.12 milli siemens/cm. For block A and B the average E.C in summer was found to be equal (1.13) and more than the block C (0.74). While average E.C of blocks A, B and C for winter and monsoon season was found to be in the decreasing order (table 7, fig. 4). The lower E.C of samples in monsoon as compared to summer and winter referred to leaching of available nutrients due to

raifall in presence of excessive acidic effluent.

Moisture Content (M.C.) and Water Holding Capacity (W.H.C)

The moisture content of waste sample varied from 0.1 to 70%. The total average moisture content of each block was found to be in increasing order form summer (0.96%) to Monsoon season (34.96%) (table 7, fig. 5). This would be attributed to the change in seasonal temperature, evaporization and rainfall in that area.

The W.H.C. of sample showed values from 7.46 to 97.36%. During summer the average WHC of all three blocks were found to be around same values (viz. 37.48%, 39.45%, 37.61%). Whereas in winter, it was found to be more in B block (44.5% as compared to A (33.09%) and C block (34.7%). The average WHC in monsoon season was found to be in the order of A (68.9%) B (52.51%) > C (45.19%) block (table 7, fig. 6).

The variation in water holding capacity in each block samples refers to the textural and chemical characteristics of industrial wastes. Presence of organic wastes and environmental factors may also have brought in the variation in water holding capacity of the area.

Available Nitrogen (A.N.) and Total Nitrogen (T.N.)

Available nitrogen of industrial wastes analysed varied from 31.41 to 676.87 ppm. The total average value of available nitrogen was found to be maximum (352.2 ppm) in winter season, then monsoon (262 ppm) and summer (254.4 ppm) (table 7, fig. 7). During summer, the average A.N. value for block B samples (360.3 ppm) was higher than block A (128.2 ppm) or block C (274.8 ppm). Similarly in winter the

corresponding value for block B (498.4 ppm) was also higher than those concerned value of block A (394.1 pppm) and block C (164.2 ppm). Whereas in monsoon season, the said value of block A registered highest one (302.7 ppm), next was the value for block C (251.3 ppm) and lowest value (232.0 ppm) was found for block B. This could be due to the moisture content, microbial activities and other associated environmental factors.

Total nitrogen content of samples ranged from 0.01 to 1.50%. A critical analysis of total nitrogen values for the samples from all the blocks will reveal that the average of total nitrogen value during summer varied from 0.11% (block C) 0.072% (block B), the corresponding value for block A being 0.29%. The corresponding figures for winter months would be 0.49% for block C, 0.47% for block B and 0.38% for block A. During monsoon season the said values are 0.6% for block C, 0.41% each for blocks A and B. Total average of total nitrogen content of all the blocks were found to be varying in different seasons, in tthe increasing order : monsoon (0.53%) > winter (0.45%) > summer (0.37%). The total nitrogen content of blocks A and C were found to be in the increasing order for seasons : monsoon (0.41%)>winter (0.38%)>summer (0.29%). While it was found to be opposite in block B for all three seasons (table 7, fig. 8). An increased level of total nitrogen in samples from block C during monsoon season might be due to use of more concentrated HNO_3 in treatment of metals. Mixing of residential garbage and organic wastes might also have attributed to the more total nitrogen content in the monsoon samples. The high values of available nitrogen and total nitrogen indicates that the wastes could be used effectively for agricultural purposes after proper treatment.

Avalable Phosphorus (AP) and Total Phosphorus (T.P.)

It will be clear from table 7 & fig. 9, when we compared the data of available phosphorus, the maximum average value was shown (60.76 ppm) by block B, incomparision to the respective values for block A (40.37 ppm) and block C (14.38 ppm) during summer season. During winter season the increasing order was block B (57.25 ppm) > block A (48.16 ppm) > block C (10.78 ppm). Whereas in monsoon season a block registered highest values (42.74 ppm), next comes C block (39.12 ppm), while lowest (28.56 ppm) was shown by block B. It is to be noted that the total average for block A, B and C blocks in summer, winter and monsoon registered almost equal values viz. namely 38.50, 38.72 and 36.81 ppm respectively.

The values for total phosphorus varied in the range of 0.04% to 0.62%. It is evident from the table 7 & fig. 10 that average values of total phosphorus during summer was highest (0.35%) in block B in comparision to block A (0.28%) and C (0.28%). The corresponding data for winter months showed; a block (0.27%) > B block (0.25%) > C block (0.20%). During monsoon period the said values for A and C were same (0.45%) against the value for B block (0.30%). Total average value for all the blocks in different seasons, namely summer, winter and monsoon come to 0.30%. 0.24% and 0.40% respectively.

Maximum total average was found to be in the monsoon samples (0.40%) (table 7, fig. 10). Total phosphorus and total nitrogen were found to be correlated well as thes two parameters showed their maximum concentration in monsoon. This amy be due to their uniform source of supply in the industrial wastes.

Organic Carbon (O.C.)

The organic carbon content of samples varied from 0.93 to 4.46%. Average block concentration of organic carbon in summer season was found to be in the order of A (2.93%) > C (2.69%) > C (2.65%). Similar pattern was observed in monsoon season samples i.e. Block A (2.38%) > block C (2.08%) > block B (1.87%). Whereas winter season the concentration of organic carbon recorded as 2.69% for block A, 2.54% for block B and 2.10% for block C. Seasonal average values for all the blocks showed summer (2.76%) > winter (2.44%) > monsoon (2.11%) pattern (table 7, fig. 11).

The variation in average organic carbon values in different blocks suggested mixing of organic wastes in industrial wastes. No definite interrelation can be traced from the values of organic carbon and total nitrogren, because of the use of conc. HNO_3 treatment process. This may be further explained that nitrogen was being used in the industries as constituent input in HNO_3 form whereas organic carbon was being mainly added from outside garbage and residential wastes and sewage.

Sodium (Na) and Potassium (K)

The total sodium concentrations of samples varied from 0.15 to 5.25%. Total average Na concentration for monsoon samples was found to be maximum (0.358%) than summer (0.315%) and winter (0.35%). Block B showed higher value of sodium (0.389%) than A (0.326%) and C (0.306%) (table 8, fig. 12) which would refer to the presence of soap manufacturing industries in that block.

Total average potassium concentration of samples varied from 0.004 to 0.208%. The total average showed an increasing values of potassium for summer

(0.033%), winter (0.041%) and monsoon (0.048%) season respectively (table 8, fig. 13). However, it was found to be varying in blocks samples with respect to season. A positive correlation between sodium and potassium (0.75) suggests possible common source, of both element which may be probably organic matter, fine earth and organic salts from textile industries.

Calcium (Ca) and Magnesium (Mg)

The average concentration of Ca and Mg of collected samples varied from 0.15 to 0.413% and 0.05 to 0.425% respectively. Seasonal total aveerage was higher in winter season for Ca (0.496%) and in monsoon for Mg (0.257%). Blockwise variation of Ca found to be in the increasing order of concentration i.e. A>B>C, while for Mg it was opposite C<B>A (Table 8, fig. 14 and 15). The low concentration of Ca & Mg in the industrial wastes may be due to leaching of these elements by acid and rains.

Iron (Fe), Manganese (Mn) and Copper (Cu)

In general, iron was found to be uniform in all blocks. The range of Iron content was recorded from 4.0 to 7.125% in wastes smaples. Though seasonal variation was not found to be varied substiantially, yet the trend for total average of Fe was summer (5.779%) > Winter 5.574%) > Monsoon (5.480%) (Table 8, fig. 16).

The Manganese in the analysed samples varied from 0.115 to 1.533%. In blocks the average concentration was found to be in the order of A>B>C. The seasonal total average variation followed the order of summer (0.819%) > Monsoon (0.728%) > Winter 0.675%) (Table, 8, Fig. 17).

The concentrtion of copper varied from 0.005 to 0.65% in wastes samples.

A block showed higher concentration (0.121%) then C (0.048%) and B (0.023%). In winter season Cu was maximum on total average basis, follwed by summer (0.067%) and Monsoon (0.041%) (Table 8, Fig. 18).

On an average Fe registered a high and uniform concentration in the waste samples follwed by Mn and Cu. This referred the presence of steel processing industries which have largely pickling and electroplating activities. Mn, though found to be less than the Fe concentration in industrial wastes, contributing metal population significanly. Cu has been detected in lowest concentration, but it showed a good co-relation (0.86) with iron, suggested a common source of their output (table 11).

Chromium (Cr), Nickel (Ni), Cadmium (Cd) and Lead (Pb)

The concentration of chromium and Nickel in analysed samples were varied from 1225 to 10325 mg/kg and 100 to 3725 mg/kg, respecively (Table 9). Seasonal total average concentrations of Cr was found to be in the order of summer (3059 ppm) < Winter (3351 ppm) < Monsoon (4409.8 ppm). Blockwise variation of average Cr concentration was in the order of A>B>C (Table 9, Fig. 19). Total average concentration for Ni was found to maximum in summer (1568 ppm), followed by monsoon (1455 ppm) and Winter (1382 ppm), whereas average concentration for blocks was in the order of A<B<C (Table 9, Fig. 20). The higher concentrations of Cr and Ni recorded in the industrial wastes have been associated with electroplating and pickling processes in industries which are in abundant numbers in that area. Co-relation coeffecient value of 0.59 for Cr and Ni indicated a common source of these two metals in the wastes (table 11).

Cadmium and Lead concentration in the solid wastes were found to be the range of 50 to 300 mg/kg and 11.5 to 102 mg/kg respectively (Table 9). The seasonal pattern of variation of total average Cd concentration was observed in the order of Monsoon (131.7 ppm) > Winter (97.3 ppm) > Summer (90.18 ppm), whereas bolck wise average variations was recorded as B>A>C (Table 9, Fig. 21). The seasonal trend for Pb was observed as higher total average values in winter (44.86 ppm) follwed by monsoon (35.24 ppm) and summer (26.1 ppm) season. Average block concentration was found in the increasing order of A>B>C (Table 9, Fig. 22). A significant co-relation (0.507%) has been found between Pb and Mn (table 11). The high concentration of Cd in industrial wastes are due to electroplating industries (Allen, 1995) and Ni-Cd batteries manufacturing industries (Alloway, 1990). A part from industries the other source for Pb in the samples may be due to automobile exhaust.

Heavy Metal Concentration of Industrial Wastes

The seasonal average concentration of different heavy metals in analyzed industrial wastes from Wazirpur industrial area has been given in Table 8 and 9.

From the table 8&9 the following total average concentration of heavy metals for all three seasons and blocks have been obtained.

Metal	Total Average Conc.(%)
Fe	5.610
Cu	0.064
Mn	0.743
Cr	0.361
Ni	0.147
Cd	0.011
Pb	0.004

The concentration of these heavy metals shows the following descending order of their concentration.

Fe > Mn > Cr >Ni > Cu > Cd > Pb

A comparison has been made in the Table 17 to show the recommended level of heavy metals and the level of metals analysed from Wazirpur Industrial Area

Table 17 : Analyzed level of heavy metals in Wazirpur Industrial wastes Vis-a-Vis the standard recommended level of metals in dry sludge.

Heavy Metals	Recommended Level (ppm)	Industrial Waste Conc. of Wazirpur (ppm)
Cu	100	643
Mn	1500	7406
Cr	1000	3612
Ni	180	1468
Cd	20	106.4
Pb	1200	50.4

Sources: Cu and Mn - Flynn et al., 1987.
Cd - Council of European Communities, 1987 (Alloway, B.J. 1990).
Pb - The Federal Republic of Germany, 1983 (Davis, B.E. 1990).

From the above table it is noted that Cu, Mn, Cr, Ni and Cd elements are in quite high concentration than the recommended level. The high level of these elements are bound to cause hazards to local biota.

Microbiological Study of Industrial Wastes

The microbiological study was performed on block sample basis for each season. The results of microbial analysis are given in tables 12,13,14,15, 16 and photo plates 1,2,3,4.

Microbial Counts (CFUs)

Table 18 : Seasonal adn blockwise variation in microbial counts (CFUs).

	Season		
Block	**Summer**	**Winter**	**Monsoon**
A	32000	5200	1000
B	3000	3500	1400
C	1300	29000	10000

The microbial counts of block average for block A samples showed the highest count (32000 CFUs) in summer season followed by 5200 CFUs in winter and 1000 CFUs in monsoon. In B block, the microbial counts were found to be 3000 CFUs in summer, 3500 CFUs in winter and 1400 CFUs in monsoon season. C block samples showed maximum counts in winter (29000 CFUs) followed by monsoon (10000 CFUs) and summer (1300 CFUs) (table 18, fig. 23).

Block wise C block registered maximum total counts (40300 CFUs) in all these three seasons as against block A (38200 CFUs) and block B (7900 CFUs). Whereas seasonal variation was found to be in the order of winter (37700 CFUs) > summer (36300 CFUs) > monsoon (25000 CFUs). The variation in microbial counts for different blocks would be due to phsico-chemical characteristics of industrial waste. Inter relation of microbial counts and the various physico-chemical parameters

analysed have been discussed in the following headings.

pH and Microbial Counts

In block A, for the summer season with neutral (7.72) pH value, highest numbers of microbial counts (32000 CFUs) were observed while in winter microbial couns (5200 CFUs) was reflected in lower pH value (4.34); but in monsoon lowest count (1000 CFUs) was obtained with the lowest pH value (2.88). In B block pH values 4.23 and 3.55 for winter and monsoon seasons were found to be associated with 3500 CFUs and 1400 respectively but pH 4.84 for summer season showed a less number of counts (3000 CFUs). C block also recorded low counts of microorganisms with low pH values i.e. microbial counts of 1300 CFUs, 29999 CFUs and 10000 CFUs were found during summer, winter and monsoon season samples of pH values 4.31, 7.05 and 4.75 respectively (fig. 24).

In general microbial population was found to be reduced with low pH. Nagele and Conard (1990) noted the similar findings for nitrate-reducing microbial population.

E.C. and Microbial Counts

From the fig. 24, it is noted that in C block, for winter and monsoon season the low E.C. values (0.69 and 0.58 millisiemens/cm) were found to be associated with hgih microbial counts (29000 and 10000 CFUs respectively). Except summer season samples of A and C block, all others have shown a high E.C. value with low microbial counts. This might be due to the fact that high soluble salt concentration would have harmful effects on the physiological functions of microorganisms. Rao, et al., (1993) have also reported the high salt concentration and reduced microbial population on agricultural land.

Moisture Content, Water Holding Capacity and Microbial Counts

Moisture plays an important role in microbial growth. In the analysed samples of Wazirpur, the moisture contents of block A in summer (1.08%), winter (4.25%) and monsoon (45.52%) were found to be associated with microbial counts of 32000 CFUs, 5200 CFUs, and 1000 CFUs respectively. In case of B block samples the moisture content was recorded as 0.73%, 3.63% and 27.80% for summer, winter and monsoon with the corresponding microbial counts of 3000 CFUs, 3500 CFUs and 1400 CFUs. C block registered microbial counts as 1300 CFUs, 29000 CFUs and 10000 CFUs for summer, winter and monsoon season respectively, with corresponding values of M.C. as 1.08%, 4.48% and 31.55% (fig. 25). In general in some samples a low microbial counts with high moisture content and vice versa were noticed and this variation would be abscribed to the variation of pH values of the block samples.

Water holding capacity refers to the texture of wastes and available pore space to be filled with water. In normal soil, a sandy loam texture favours microbial growth because of good aeration fig. 25, indicates that summer samples of block A with water holding capacity 37.48%, recorded the highest microbial counts (32000 CFUs). While winter monsoon samples of the same block with W.H.C. of 33.09% and 68.97% respectively, recorded lower counts, viz 5200 CFUs & 1000 CFUs, because of low pH of samples. In B block, the W.H.C. 39.45%, 44.5% and 52.51% for summer, winter and monsoon were found to be associated with corresponding microbial counts of 3000 CFUs, 3500 CFUs and 1400 CFUs. In C block, W.H.C. values of 37.61%, 34.72% and 45.19% for summer, winter and monsoon season were found to be associated with 1300 CFUs, 2900 CFUs and 10000 CFUs respectively. It is clear from the results that

low counts in some samples, even with high water holding capacity were noted. Obviously this variation would have been significantly affected by the influence of pH.

Organic carbon, Total Nitrogen, Total Phosphorus and Microbial counts

From the fig. 24, it is indicated that in A block, microbial counts were found to highest (32000 CFUs) with the highest organic carbon (2.93%) in summer and gradually decreasing with decreasing organic carbon content in winter and monsoon season samples. In B block, approximately the same concentration of organic carbon in summer (2.65%) and winter (2.54%) were found to be associated with 3000 CFUs and 3500 CFUs respectively, while the lowest concentration (1.87%) in monsoon sample recorded microbial counts of 1400 CFUs. In block C, high organic carbon content (2.69%) in summer was noticed with low microbial counts (1300 CFUs). But relatively low organic carbon contents in winter (2.10%) and monsoon (2.08%) were recorded with corresponding high microbial counts (29000 CFUs and 10000 CFUs). Baath (1996) reported lesser numbers of bacteria in high organic matter, low pH soil than low organic watter, high pH values in his study. The results of this study are in confirmity with the findings of Baath (1996) to some extent.

From the results in fig. 26, it is indicated that in A block the total nitrogen values for summer (0.29%), winter (0.38%) and monsoon (0.41%) were in increasing order but the corresponding microbial counts were recorded in decreasing order as 32000 CFUs, 5200 CFUs and 1000 CFUs respectively. In B block the total nitrogen concentration 0.72%, 0.47% and 0.41% were recorded for summer, winter and monsoon seasons respectively, while the microbial counts for the same order of season were found to be 3000 CFUs, 3500 CFus and 1400 CFUs. In block C, the total

nitrogen values for summer (0.11%), Winter 0.49% and monsoon (0.76%) were found to be associated with 1300 CFUs, 29000 CFUs and 10000 CFUSs respectively. The variation in total nitrogen content and microbial population may be due to the influence of various physico-chemical and environmental factors.

Total phosphorus concentration values and microbial counts for the different blocks and season as such have not shown significant relationship (fig. 26). It has been observed that neutral pH and low total phosphorus values were found to be associated with high microbial counts. For example, 0.28% total phosphorus, 7.72 pH in summer season of block A and 0.20% total phosphorus with 7.05 pH in winter were associated with 32000 CFUs and 29000 CFUs respectively.

Nutrient Elements (Na, K, Ca, Mg) and Microbial Counts)

Average block concentration of nutrient elements (Na, K, Ca, Mg) had not been found in uniform variation (table 8, fig. 27 & 28). It was noticed that in some samples the nutrient elements concentrations were low but microbial counts were found to be high viz, 0.286% Na, 0.017% K, 0.334% Ca and 0.219% Mg in summer season of block A was found to be associated with high microbial counts (32000 CFUs). While in other cases a high nutrient element concentration was reocorded with corresponding low microbial counts. viz., 0.386% Na and 0.079% K in monsoon season of block B (1400 CFUs), 0.695% Ca with 5200 CFUs in winter season of block A and 0.259% Mg with 1400 CFUs in monsoon season of block B. However, winter and monsoon samples of block C for Mg with their higher concentrations values 0.295% and 0.300% showed higher counts of microbes (29000 CFUs) and 10000 CFUs respectively).

In general the microbial populations were not found to be related with nutrient elements. The interaction of pH with these elements would have supposed to be their variable influence on the microbial population.

Heavy Metals (Fe, Mn, Cu) and Microbial Counts

Average block concentrations of Iron were not found to be varying much along seasons and blocks, whereas CFUs had shown significant high and low counts irrespective of seasons and blocks (fig. 29). However, low concentrations of Iron in summer season (5.651%) of block A, winter (5.901%) and monsoon (5.004%) season of block C, registered the high microbial counts of 32000 CFUs, 29000 CFUs and 10000 CFUs respectively.

In case of Mn the average concentration was 0.783% (summer) , 0.973% (winter) and 0.733% monsoon for A block, whereas the corresponding microbial counts were found to be 32000 CFUs, 5200 CFUs and 1000 CFUs respectively. For B block the Mn concentration and microbial counts were recorded as 0.59% and 3000 CFUs for summer, 0.505% and 3500 CFUs for winter and 0.977% and 1400 CFUs respectively. C block recorded 1.084%, 0.549%, 0.473% Mn and 1300 CFUs, 29000 CFUs and 10000 CFUs respectively for summer, winter and monsoon seasons (fig. 29).

In case of copper the trend was noticed to be similar except two samples i.e. winter sample (0.223% Cu) of block A and monsoon sample (0.056% Cu) of block C which registered high microbial counts (5200 CFUs and 10000 CFUs respectively) (fig. 28).

The observation for Mn, Cu and microbial count indicated that high concentration of these two elements were found to associated with reduced number of

microbial population. Nordgren et al., (1985) also found a shift in microfungal species.

Heavy Toxic Metal (Cr, Ni, Cd, Pb) and microbial counts

In block A the concentration of Cr were recorded in summer (3743 mg/kg), winter (1506 mg/kg) and monsoon (4562.5 mg/kg) with corresponding microbial counts as 32000 CFUs, 5200 CFUs and 1000 CFUs respectively. B block registered Cr concentration as 3094 mg/kg in summer, 2338 mg/kg in winter and 4450 mg/kg in monsoon season with corresponding microbial counts as 3000 CFUs, 3500 CFUs and 1400 CFUs respectively. In case of C block the microbial counts were found to be 1300 CFUs (summer), 29000 CFUs, (winter) and 10000 CFUs (monsoon) against the corresponding Cr concentration 2163 mg/kg, 6925 mg/kg and 4168.8 mg/kg (fig. 30).

From (fig. 30) it is noted that a low concentration (1006 mg/kg) of Ni in summer season of block A was found to associated with high number of microbial counts (32000 CFUs). While a high concentration of Ni (2088 mg/kg) in winter season of C block was also recorded with an increased number of microbial population (29000 CFUs). Hence, it can be said that the toxicity of Cr and Ni on microbial population is pH dependent.

Cadmium in analysed samples showed a negative effect on microbial population as an increased level of Cd (150 mg/kg and 143.75 mg/kg) in monsoon samples of A and B block found to be associated with decreased microbial population (1000 CFUs and 1400 CFUs) (fig. 31).

Fig. 31 indicated that the increased concentration of Pb except in monsoon sample of block B found to be associated with increased number of microbial counts. It is evident from the fig. 31, for Pb concentration and microbial counts of summer

sample (35.8 mg/kg Pb and 32000 CFUs) of block A, winter sample (48.5 mg/kg Pb and 3500 CFUs) of block B and winter (31.6 mg/kg Pb and 29000 CFUs) and monsoon sample (44.13 mg/kg and 1000 CFUs) of C block.

From the study of heavy metals and microbial counts, it is clear that heavy metals in high concentration affect the microbial population but the degree of toxicity by and large depends upon pH and other environmmental factors.

Isolation and Identification of Microbial Isolates

A total of twenty (20) members of micro-organisms were isolated from industrial solid wastes. Seasonal and blockwise distribution of isolates is presented in table (19).

Table 19 : Isolation of Micro-organism on Seasonal and Block basis

SEASON	BLOCKS		
	A	B	C
Summer	3	2	3
Winter	2	2	2
Monsoon	1	2	3

Out of 20 micro-organisms, 16 were identified as bacteria and 4 as fungi.

Based on the results of i) Structural characterization of microbial isolates on nutrient agar plates; ii) Isolates characteristics on agar slants; iii) Microscopic study; and iv) Biochemical tests for bacterial isolates, the micro-organisms were identified to the genus level. Table 16 presents the list of identified micro-organisms.

The microbiological study of industrial wastes revealed that the soil of Wazirpur area has become highly polluted as evident from its low microbiological population. It contained 2.8×10^4 CFUs/gm of soil as compared to normal soil range

of 10^7 - 10^8 CFUs/gm. The diversity of microbial community was also found very reduced as only 1 to 3 types of organisms could be isoleted from the sample. However, the presence of genera like *Pseudomonas, Bacillus, Nitrosococcus* indicate recycling and utilization of some waste parameters like nitrogen, carbon, phosphorus, calcium, magnesium etc., which utlize them as their source of carbon and energy. The presence of genera like, *Arthobacter, Micrococcus, Ferrobacillus,* and *Sideromonas*, help in recycling of heavy metals like Fe, Mn and Cu. These organisms also accumulate other toxic heavy metals like Cr, Ni, Cd, and Pb and in turn they are also affected by their toxicity. Presence of *Streptococcus* group of organism reveals that some residential wastes have mixed with the industrial wastes.

CHAPTER VI

SUMMARY AND CONCLUSION

The increasing output of industrial wastes in Wazirpur area has been already taking its toll by causing severe health hazards the local inhabitants and environment of that area. Lack of proper management, inefficient technology and unwillingness to maintain minimum standard level of environmental pollution control measures have putforth a bleak future of the surrounding areas as well as industries.

In the present study the industrial wastes were analyzed for physico-chemical and microbiological parameters. The results of study undertaken reveal that wastes are of highly acidic nature and contain high concentration of nitrogen, phosphorus, organic carbon and heavy metals including toxic ones. The microbiological population of wastes investigated was far less as compared to normal soil. The diversity of microbial community was also found to be reduced. *Pseudomonas*, *Bacillus* and *Microcous* bacterial groups were found abundant in the wastes whereas fungi were only 20%of total mocrobial population. *Streptococus* sp. were also found in wastes, the origin of which is from residences.

Hence in the light of results of this investigation, it can be concluded that the industrial wastes are of highly toxic materials and can not be used directly for agricultural purposes to exploit their beneficial roles. The effective agricultural use of these wastes is possible only after the removal of excessive acid and toxic heavy metals. For this purpose addition of lime can be made to regulate the pH of this waste for removal of heavy metals. There are several methods which can be employed to remove various heavy metals. But only few methods which could be successfully employed are metal chelate (Wasay, et al. 1990), and soil precipitants (Singh et al.

1996). After removing metals the waste may be directly utilized in agriculture depending on the suitability for their use or may be taken for composting. It is also suggested that a common treatment plant should be set up for a number of small scale industries in one zone (CPCB report). Landfill is another method of disposal of industrial wastes, provided that it does not contaminate ground water source by leaching.

For industrial use of the wastes generated in this area, this wastes also could be converted into economically viable materials like bricks which can be used in repairing of road works. Some of the economically costly metals may be further treated, processed, recycled and reuse to the respective industries.

A detailed microbial study could also be useful in removal and recycling of this industrial wastes. Microbes inhabiting these wastes may have novel genetic make up which are able to detoxify the toxic heavy metals and other chemicals by bioleaching and catabolic activities and eventually the waste can be put into beneficial use. Some of effluent treatment plants also use microoranism as an agent of treatment of wastes.

But before deciding the extract method or methodologies, cost-benefit analysis also has to be taken into consideration. Hence, for proper and efficient management of industrial wastes, firstly a detailed survey should be carried out to assess the quantity and quality of the wastes generated. Then proper methods of collection and transport system should be worked out and finally a suitable method of disposal has to be ascertained. Research for new and improved methods of disposal, recovery, reuse and recycling would form an integral part of an efficient management of industrial wastes and it needs to be strengthened and continued in order to have sustained production of industries with better quality of life in Wazirpur area.

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APPENDIX - I

Microbiological Media

1. **B.C.P. Carbohydrate Medium (gm/lit)**

Tryptone	10
Yeast extract	5.0
K_2HPO_4	2.0
Carbohydrate	5.0
Brom-Cresol purple indicator	2 ml

2. **MR-VP Medium (gm/lit)**

Glucose	5.0
Protease peptone	5.0
K_2HPO_4	5.0

3. **Nitrate Broth (gm/lit)**

Peptone	5.0
Beef extract	3.0
KNO_3 or $NaNO_3$	5.0

4. **Nutrient Gelatin (gm/lit)**

Beef extract	3.0
Tryptone	5.0
Gelatin	4%

5. **Simmons Citrate Agar Medium (gm/lit)**

$MgSO_4$	0.2
$NH_4H_2PO_4$	1.0
K_2HPO_4	1.0
Sodium citrate	2.0
NaCl	5.0
Agar	15
Boromothymol blue	0.08

6. **Skimmed Milk Agar (gm/lit)**

Agar	15
Skimmed milk	20

7. **Starch-Agar Medium (gm/lit)**

Trypton	10.0
Yeast extract	10.0
K_2HPO_4	5.0
Soluble starch	3.0
Agar	1.5

8. **Thiosulfate-Iron Medium (gm/lit)**

Beef extract	3.0
Peptone	30.0
Peptonized iron	0.2

	Sodium thiosulfate	0.025
	Agar	3.0

9. **Urea Broth (gm/lit)**

Peptone	1.0
NaCl	5.0
Glucose	1.0
KH_2PO_4	2.0
Phenol red	0.012
Urea	20
pH	6.8-6.9