"Soil is the stomach of the plant".
- Aristotle

Can we hit at the stomach of the plant without hitting at our own stomach?

CHAPTER 2 THE EXPERIMENTAL SOILS

: THEIR TYPES AND DISTRIBUTION IN CHHATTISGARH REGION

: CHARACTERIZATION, STUDIES OF MINERAL CONSTITUENTS

: MICRO-NUTRIENTS AND MICRO-POLLUTANTS

: HUMIC ACID

: BACTERIAL POPULATION
2.1 THE SOILS OF CHHATTISGARH REGION

INTRODUCTION

The Chhattisgarh region located in the south-eastern part of the Madhya Pradesh State comprises the seven revenue districts of Raipur, Durg, Bilaspur, Raigarh, Rajnandgaon, Sarguja, Bastar and Balaghat as shown in Fig.2-1. The soils occurring in the region have been found to differ widely in their characteristics, colour, texture reactions etc. The soils of the region are divided between four locally named categories called Bhata, Matasi, Dorsa and Kanhar.

It will be relevant to describe some important parameters related to the physical properties of the soils are: soil texture and soil structure. Soil texture is concerned with the size of mineral particles present in the soil. Specifically, it refers to the relative proportion of the particles of various sizes in a given soil. Soil structure is the arrangement of the soil particle into groups or aggregates. These properties are helpful in determining not only the nutrient supply ability of soil solids but also the supply of water and air which are so important to plant life. As soils are composed of particles varying greatly in size and shape. Specific terms are needed to convey some idea of their textural make-up, and to give some indication of their physical properties. For this, soil textural class-names such as sand, sandy loams, and silt loam have been used. Three broad and fundamental groups of soil textural classes have been recognised as described below:-
Fig. 2-1 TEXTURE BASED DISTRIBUTION OF SOILS IN THE CHHATTISGARH REGION.
Sands : The sand group includes all soils whose sand contents make up 70% or more of the material by weight. The properties of such soils are, therefore, characteristically sandy in contrast with the stickier nature of the behaviour groups of the soil.

Clays : A soil to be designated a clay must carry at least 35% of the clay-separate, and in most cases not less than 40%. In such soils, the characteristics of the clay-separate are distinctly dominant, and the class name is sandy clay, silty clay, or, the most common of all, simply clay.

Loams : An ideal loam may be defined as a mixture of sand, silt and clay particles which exhibits light and heavy properties in about equal proportions. Roughly it is half-and-half mixture on the basis of properties. Most soils of agricultural importance are some type of loam. In most cases, however, the quantities of sand, silt or clay present require a modified textural class name. Thus, a loam in which sand is dominant is classified as sandy loam. In the same way there may occur silt loams, silty clay loams and clay loams (1).

One of the most significant indirect effects of soil texture is through its control of the structure. Roots require space for growth, and this space is provided by the pores and fissures in the soils. Plant roots extend into the soil in search of both water and nutrients. Since most roots are of the order of 1-5 mm in diameter, and the pores in the soil are rarely more than 1.0 mm in size, roots can only grow
by forcing their way into the soil. A critical factor, therefore, is the ability of the soil to give way in the face of limited pressure which can be exerted by the tips of the growing roots. This in turn depends upon the consistence and bulk density of the soil. The percentage of pores of different size reported in soils of different textures have been shown in Table 2-1 (2).

Table 2-1 PERCENTAGE OF PORES OF DIFFERENT SIZE IN SOILS OF DIFFERENT TEXTURES.

<table>
<thead>
<tr>
<th>Pore classes</th>
<th>Percent pores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sandy loam texture</td>
</tr>
<tr>
<td>Macropores (60 um)</td>
<td>33</td>
</tr>
<tr>
<td>Mesopores (2-60 um)</td>
<td>33</td>
</tr>
<tr>
<td>Micropores (2 um)</td>
<td>33</td>
</tr>
</tbody>
</table>

It has been shown that real roots can extend into a sandy soil only if the bulk density is less than 1.7-1.8 g cm\(^{-3}\), and into a clayee soil only at bulk density less than 1.5-1.6 g cm\(^{-3}\) (2). Compacted soil-horizons, therefore, present a formidable barrier to root growth. In the same way, high bulk densities limit seedling growths. The consistence of the soils tends to decrease as the moisture content increases; as the soil receives water, root- and seedling-growth become easier (2). The salient morphological features of the four major types of soils of Chhattisgarh region have been described in Table 2-2. The texture-based
<table>
<thead>
<tr>
<th>S.N.</th>
<th>Morphological features</th>
<th>Soil types</th>
<th>Bhata</th>
<th>Matasi</th>
<th>Dorsa</th>
<th>Kanhar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td></td>
<td>Reddish, dark reddish, brown</td>
<td>Yellow</td>
<td>Brownish grey</td>
<td>Dark grey, brown to black</td>
</tr>
<tr>
<td>2.</td>
<td>Texture</td>
<td></td>
<td>Gravelly coarse, loamy to sandy</td>
<td>Sandy loam</td>
<td>Silty clay</td>
<td>Clayey</td>
</tr>
<tr>
<td>3.</td>
<td>Structure</td>
<td></td>
<td>Massive (Structure-less)</td>
<td>Angular blocky</td>
<td>Sub-angular to angular blocky</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Consistence</td>
<td></td>
<td>Nonsticky &amp; non-plastic sticky</td>
<td>Slight sticky and very plastic</td>
<td>Sticky and very plastic</td>
<td>Very sticky and plastic</td>
</tr>
<tr>
<td>5.</td>
<td>Lime concretion</td>
<td></td>
<td>Absent</td>
<td>Absent or very few</td>
<td>Present</td>
<td>Abundant</td>
</tr>
<tr>
<td>6.</td>
<td>Other concretion</td>
<td></td>
<td>Ferruginous gravel</td>
<td>Few iron concretion</td>
<td>Numerous iron concretion throughout</td>
<td>Numerous black iron concretion throughout</td>
</tr>
<tr>
<td>7.</td>
<td>Reaction with HCl</td>
<td></td>
<td>Non effervescence</td>
<td>Efferescence in last horizon</td>
<td>Efferescence throughout</td>
<td>Efferescence throughout</td>
</tr>
<tr>
<td>8.</td>
<td>Cracks</td>
<td></td>
<td>Absent</td>
<td>Few line cracks</td>
<td>Wide vertical cracks</td>
<td>Bharka or sink hole</td>
</tr>
<tr>
<td>9.</td>
<td>Depth</td>
<td></td>
<td>Very shallow</td>
<td>Moderate</td>
<td>Medium</td>
<td>Deep</td>
</tr>
<tr>
<td>10.</td>
<td>Internal drainage</td>
<td></td>
<td>Rapid</td>
<td>Moderate</td>
<td>Moderate to slow</td>
<td>Slow</td>
</tr>
</tbody>
</table>
distribution of soils in the Chhattisgarh region has been shown in Fig. 2-1.

The district-wise distribution of the four principal types of the soils reported in the Chhattisgarh region have been shown in Table 2-3 (3).

The physical characteristics of soils which have significant role in supporting the plant life have been explained below:

**Bulk density**: This is the mass of the dry soil per unit bulk volume (g cm\(^{-3}\)) including the air space. The bulk volume is determined before drying the soil to constant weight at 105°C.

**Infiltration rate**: It is a soil characteristic describing the maximum rate at which water can enter the soil (cm hr\(^{-1}\)) under specified conditions including an excess presence of water.

**Field capacity**: It is the percentage of water remaining in soil 2 or 3 days after having been saturated, and after free drainage from the soil has practically ceased.

**Wilting point**: It is the moisture content of the soil, on oven-dry basis, at which plant (specifically sunflower plants) wilt and fail to recover their turgidity when placed in a dark humid atmosphere.
Table 2-3  DISTRICT-WISE DISTRIBUTION OF DIFFERENT SOILS IN CHHATTISGARH REGION* (in lakh hectares)

<table>
<thead>
<tr>
<th>District</th>
<th>Bhata</th>
<th>Matasi</th>
<th>Dorsa</th>
<th>Kanhar</th>
<th>Total cultivated area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raipur</td>
<td>1.50</td>
<td>4.09</td>
<td>2.04</td>
<td>2.55</td>
<td>10.22</td>
</tr>
<tr>
<td></td>
<td>(15)**</td>
<td>(40)</td>
<td>(20)</td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td>Durg</td>
<td>1.12</td>
<td>1.37</td>
<td>1.11</td>
<td>1.90</td>
<td>5.51</td>
</tr>
<tr>
<td></td>
<td>(20.4)</td>
<td>(25)</td>
<td>(20.2)</td>
<td>(34.5)</td>
<td></td>
</tr>
<tr>
<td>Bilaspur</td>
<td>1.56</td>
<td>2.59</td>
<td>2.07</td>
<td>1.90</td>
<td>10.37</td>
</tr>
<tr>
<td></td>
<td>(15)</td>
<td>(25)</td>
<td>(20.2)</td>
<td>(34.5)</td>
<td></td>
</tr>
<tr>
<td>Raigarh</td>
<td>1.16</td>
<td>2.90</td>
<td>1.45</td>
<td>0.29</td>
<td>5.81</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(50)</td>
<td>(25)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Surguja</td>
<td>0.40</td>
<td>2.06</td>
<td>1.37</td>
<td>1.03</td>
<td>6.86</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(30)</td>
<td>(20)</td>
<td>(15)</td>
<td></td>
</tr>
</tbody>
</table>

* Statistics of Balaghat and Bastar Districts have not been shown.
** The parantheses show the percentage of the total land of the region.
Available water: It is the portion of water in soil that can be readily absorbed by plant roots. This is defined by most workers as that water held in the soil against a pressure up to 15 bars (1).

The physical characteristics reported (1) to be assigned to the four types of the soil have been shown in Table 2-4.

2.2 SOILS OF THE CHHATTISGARH REGION: THEIR TESTING AND CHARACTERIZATION

INTRODUCTION

The soil testing gives a measure of the availability of the nutrients to the crops. It is multi-purpose in nature. Among other things, it aims at: (i) Grouping the soils into classes relative to the level of nutrients. (ii) Predicting the probability of getting profitable responses in terms of the crop yield. (iii) Helping to evaluate soil properties, and (iv) Determining the specific soil conditions like alkalinity, salinity and acidity which limit crop yield (4). The significance of the physico-chemical characteristics experimentally determined here has been described below:-
### Table 2-4. SELECTED PHYSICAL PROPERTIES OF THE PRINCIPAL SOIL TYPES OF CHHATTISGARH REGION.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Physical properties</th>
<th>Soil types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bhata</td>
</tr>
<tr>
<td>1.</td>
<td>Mechanical composition (3)</td>
<td>Matasi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dorsa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kanhar</td>
</tr>
<tr>
<td>1.</td>
<td>Sand</td>
<td>60-80</td>
</tr>
<tr>
<td></td>
<td>Silt</td>
<td>15-22</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>9-20</td>
</tr>
<tr>
<td>2.</td>
<td>Bulk density (g/cm³)</td>
<td>1.76-1.80</td>
</tr>
<tr>
<td>3.</td>
<td>Soil depth (cm)</td>
<td>5-30</td>
</tr>
<tr>
<td>4.</td>
<td>Infiltration rate (cm/hr)</td>
<td>5.0-7.0</td>
</tr>
<tr>
<td>5.</td>
<td>Field capacity (%)</td>
<td>5.52</td>
</tr>
<tr>
<td>6.</td>
<td>Wilting point (%)</td>
<td>3.40</td>
</tr>
<tr>
<td>7.</td>
<td>Available water (%)</td>
<td>2.20</td>
</tr>
</tbody>
</table>
pH: The supply of the plant nutrients, and thus the fertility of the soil is significantly affected by pH. The solubility of most nutrients varies in response to pH. Most of the nutrients become more soluble as pH falls, and thus they are released more rapidly in acid conditions. As acidity increases, however, the losses of the nutrient by leaching increases also, and thus their availability to plants may actually be decreased. In other cases, the quantities of some nutrients may rise to such large extents under acid conditions that they become toxic to plant. In addition, we may note that soil acidity also affects the activity of soil organisms—a factor which indirectly influences soil fertility. For these reasons, one of the main aims of soil management is to control the pH, and maintain a slight acidity in the otherwise neutral soils (2).

**Electrical conductivity**: The measurement of electrical conductivity is used as means of apprising soil salinity. The electrical conductance, expressed in mS cm\(^{-1}\) increases with soluble salt contents, and thus allows simple interpretation of the readings (5). Saline soils include those soils containing salts in quantities sufficient to interfere with the growth of most crop plants, but not containing enough exchangeable sodium to alter the soil characteristics appreciably. Technically, a saline soil is defined as a soil having a conductivity of the saturation extract greater than 4.0 mS cm\(^{-1}\) and an exchangeable sodium percentage less than 15 (5).
**Organic carbon**: Carbon is a common constituent of all organic matter. Consequently, its movements during the microbial digestion of plant tissues are extremely significant. Much of the energy acquired by the fauna and flora within the soil comes from the oxidation of carbon. As a result, its oxide is evolved continuously in large amounts. The various changes this element undergoes within or without the soils are collectively described in what is known as the carbon cycle. The soil is the main source of CO\textsubscript{2} gas, although small amount of it are excreted by plant roots, and are brought down in rain water. Under optimum conditions, as much as 45.5 Kg of carbon dioxide per acre per day may be evolved. The carbon dioxide from the soil ultimately escapes to a large degree into the atmosphere where it may again be used by plants thus completing the cycle. The degradation of the organic matter also results in the formation of other carbon products. Elemental carbon is found in soils to a certain extent, and its presence in soils is considered significant. Under certain conditions, methane and carbondioxide may be produced in small amounts in soils. But, of all the simple products, carbondioxide is by far the most abundant (1).

**Available nutrients**: The plant growth depends upon the supply of a range of plant nutrients, many of which are derived from the soils. Numerous factors influence the availability of plant nutrients. In the case of those nutrients, such as nitrogen supplied by biological process of fixation and mineralization, the biological properties of the
soils are particularly important. In the case of minerals, such as phosphorus and potassium, a more crucial factor is the rate of ions released from their source minerals. A pool of readily available nutrients occurs in the soil solutions, and these are supplied to the plants directly. When these are drawn out from the soil solutions, more nutrients are released from the reserves held by adsorption on the colloidal particles. Thus, most nutrients occur in three forms: (i) available nutrients which are dissolved in the soil water, (ii) exchangeable nutrients which are held on the surface of colloidal particles (iii) labile nutrients which are held within the soil minerals, and released by weathering. The rate of release of nutrients from the exchangeable reserve into the soil solution is sufficiently rapid to meet the needs of the plants. The rate of replenishment of the exchangeable reserves by minerals from the labile pool is slow, however. Consequently, over time the exchangeable reserves tend to be depleted, and the soil become exhausted (2).

**Cation Exchange Capacity**: The plants need C, H, O, P, K, N, S, Ca, Fe, Mg, Mn, Cu, B, Zn, Co, Mo, Cl, Na, and probably several other elements which are yet to be confirmed. A small but an important fraction of the total of the elements present in soil minerals occurs as readily exchangeable or adsorbed ions. These forms of the elements are of vital importance to the nutrition of the plants growing on different soils. The mineralogical composition of soils thus
has an important bearing on soil productivity. The rate of release of ions slows down as the soil minerals become highly weathered, often becoming too slow to support intensive crop production. The electric charge on the soil particles is neutralised by an equivalent amount of oppositely charged ions, the so called exchangeable ions held to the surface by mainly Coulomb forces.

The most common exchangeable cations of soils are $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{K}^+$, $\text{H}^+$, $\text{Na}^+$, and $\text{NH}_4^+$. $\text{Ca}^{2+}$ generally is the dominant ion. In very acid soils, $\text{Al(OH)}_2^+$ may constitute a considerable part of the counter ions, the proportion increasing with falling $\text{pH}$. In alkali soils, the content of $\text{Na}^+$ is exceptionally high. Common anions are $\text{SO}_4^{2-}$, $\text{Cl}^-$, $\text{NO}_3^-$, $\text{H}_2\text{PO}_4^-$, $\text{HPO}_4^{2-}$, $\text{HCO}_3^-$ and anions of humic acids. Though some of these anions do not always function as exchangeable ions, but are nearly always present in the soil solutions. The capacities of soils to adsorb and exchange cations and anions vary greatly with the content of clay, organic matter and the mineralogical composition. The Cation Exchange Capacity (CEC) is defined as the amount of cation species bound at $\text{pH}$ 7.0 or another suitable $\text{pH}$, depending on the method used for its measurement. It varies slightly with the bonding strength of the ions, and increases with the content of clay and organic matter. The CEC of mineral soils may range from a few to 50-60 meq/100 g, whereas the CEC of organic soils may exceed 200 meq/100 g (5).
Silica: Free silica occurs in the soil mainly in the form of quartz ($SiO_2$), consisting of a continuous frame-work of silica tetrahedra. Free silica also occurs in some soils. It also occurs in some soils as cristobalite. Substituted cristobalite occurs with varying substitutes (NaAl, $SiO_2$), in which the Na is exchangeable. In certain soils, cristobalite may take up nearly 50% of the clay, the other half being quartz (6). Exchange capacity of the clay arises almost entirely through the substitution in cristobalite. Free silica particles provide the frame-work of soil structure, and influence the soil formation. Quartz occurs in the majority of the soils, and makes up from 50 to 90% of the sand coarser silts of many soils.

Iron oxide: The most common iron oxides in the soils are hematite ($Fe_2O_3$) which gives pink to bright red colour to the soils, and goethite ($Fe_2O_3.H_2O$) which gives brown and dark reddish colour to the soils. Iron oxides provide an extremely important reflection of the chemical properties of the soil and the genetic processes that have governed the soil formation (7-9). In poorly drained organic soils, the occurrence of FeOOH, an isomer of goethite, has been reported (8). It gives a brown orange colour to the soils. Iron oxides of the soils are usually the products of weathering of iron-bearing minerals. Hematite may also be inherited from the parent rocks.
Aluminium oxides: The crystalline mineral, gibbsite \( \text{Al(OH)}_3 \)
(or \( \text{Al}_2\text{O}_3 \cdot 3\text{H}_2\text{O} \)), is the most abundant hydrous oxide of aluminium in soils occurring primarily in soils of tropical and sub-tropical region which have undergone intensive leaching of silica. Clay formation is enhanced by \( \text{Al}_2\text{O}_3 \)-content of the parent material of non-resistant minerals. High porosity which enhances the water holding capacity was found to enhance clay formation (5).

The physico-chemical characteristics of the four soil-types (Bhata, Matasi, Dorsa, and Kanhar) have been determined and described below.

MATERIALS AND METHODS

Soil Sampling from Farm-Fields: Three farm-fields (2 to 4 hectares each) were identified in each of Chandkhuri, Lakholi, Arang and Kurud villages in the Raipur Tehsil and District of the Chhattisgarh region of Madhya Pradesh. These villages are known for the occurrence of Bhata, Matasi, Dorsa, and Kanhar types of soils respectively. Thin slices of soils were taken out with the help of a spade from the plow-layer of each field at intervals of 20 steps, and a total of 30 samples were collected from each field. The samples collected from each village were composited and brought to the laboratory for chemical analysis (10). The collected samples were first air-dried. The bulk samples were then passed through a sieve (6 mm), partitioned, and quantities (about 1 Kg each) were retained. The samples were then further subjected to the processes of grinding, mixing,
sieveing (4mm and 2mm) and partitioning, and representative samples of about 100 g in each case were obtained. The samples were then heated in oven at 110°C for 3 hours, and used for analysis.

Procedure: The determinations were carried out as described below:

\textbf{pH:} A soil-water suspension (1:5 by wt.) was prepared, and the pH was directly recorded using a pH meter (Systronic Model 324) (11).

\textbf{Electrical conductivity:} The electrical conductivity of the soil suspension (1:5 by wt.) prepared in conductivity water was measured using a conductivity meter (Century Model CK-710) (11).

\textbf{Organic carbon:} Weighed quantities (1 g each) of the samples were transferred to Erlenmeyer flasks after pretreatment with sulphuric acid to decompose the carbonates present. 10 ml of chromic acid solution (34% CrO$_3$ in H$_2$O - H$_3$PO$_4$ medium) was added, followed by the addition of 50 ml of H$_2$SO$_4$ - H$_3$PO$_4$ (1:1 mixture). The mixture was boiled for 5 minutes using a flow of CO$_2$-free air through the reaction mixture. The evolved CO$_2$ was absorbed in 25 ml of 0.5 N NaOH solution placed in a conical flask. 15 ml of 0.1 M BaCl$_2$ solution was then added to precipitate the carbonates. The excess NaOH was back titrated with standard 0.5 N HCl using phenolphthalein solution as an indicator. From the weight of CO$_2$ formed, the amount of total organic carbon was calculated (10).
Total nitrogen : Weighed quantities (5.0 g each) of the finely powdered form of the samples were placed in Kjeldahl digestion flasks. 20 g of sodium sulphate and a pinch of CuSO$_4$.5H$_2$O were then added. This was then followed by an addition of 35 ml of conc. H$_2$SO$_4$. The mixture was digested for 30 minutes in low flame, and then for another one hour at full flame. The flask was then cooled, and 300 ml of ammonia-free water was added cautiously, and the mixture was further cooled. The contents were transferred to a distillation flask, and ammonia was distilled after the addition of NaOH solution (40%) into the distillation flask. The evolved ammonia was absorbed in 25 ml of boric acid solution (4%) placed in a 500 ml conical flask with 4 drops of bromocresol green methyl red indicator solution. The boric acid was then back-titrated with standard hydrochloric acid solution (0.788 N) (10).

Total phosphorus : Weighed quantities (2 g each) of the finely powdered samples were transferred to a 300 ml conical flask. The sample was heated on a hot plate with 20 ml of conc. HNO$_3$ for the oxidation of the organic matter. Then 30 ml of HClO$_4$ solution (60%) was added and the mixture digested in a fume cupboard for 40 minutes. After cooling, 50 ml of distilled water was added, and the mixture was filtered into a 200 ml volumetric flask. An aliquot of this solution was used for the analysis by the phosphovanadomolybdate method. For this purpose, the solutions were prepared by the dissolution of 25 g of ammonium molybdate in 400 ml of water,
and 1.25 g of ammonium metavanadate in 300 ml of water acidified with 250 ml conc. HNO₃. The two solutions were mixed and diluted to 1 litre. An aliquot (10 ml) of the test solution was placed in a 50 ml volumetric flask. The HNO₃ acidity was adjusted to about 0.3 N. 10 ml of vanadomolybdate reagent was added and the solution diluted to 50 ml with distilled water. The absorbance was measured at 470 nm. A calibration graph was prepared using standard solutions of KH₂PO₄, and the concentration of phosphorus was found out using the calibration graph (10).

**Total potassium**: Weighed quantities (0.1 g each) of finely ground soil were placed in 30 ml of platinum crucible. Few drops of distilled water were added followed by the additions of HF (5 ml), HNO₃ (3 ml), and HClO₄ (1 ml). The mixture was heated on a sand bath till the contents were evaporated. The crucible was cooled and the acid treatment was further repeated till all the organic matter was completely removed. The residue was dissolved in 5 ml of HCl (6 N) and 5 ml of distilled water. The solution was filtered into a 50 ml flask. Potassium was then determined using a flame photometer (Systronics, Model 305), and a calibration graph prepared by using standard solutions of KCl (12,13).

**Cation Exchange Capacity**: Weighed quantities (5 g each) of samples were placed in 100 ml centrifuge tubes, and stirred with 50 ml of sodium acetate solution (1 N) at pH 5.0. The soil-suspension was digested in water bath for 30 min. The salts were removed by centrifugation. Two additional...
using the same sodium acetate solutions and the same bath treatment were also done. Thereafter, the samples were given five washings using 1 N CaCl₂ solution. The excess salt was removed by washings with 80% acetone until the washings were free from chloride. Finally, the calcium ions were replaced by giving five washings with a neutral solution of ammonium acetate (1 N). The calcium was then determined in the solution using a flame photometer (Systronic, Model 305) (10).

Exchangeable cations (Ca, Mg, K & Na): Weighed quantities (5 g each) of the samples were taken in 250 ml conical flasks and mixed with 100 ml of ammonium acetate solution (1 N) and allowed to remain in the suspension form for 10 min. The suspension was then filtered and washed with the ammonium acetate solution. The filtrate was made up to 250 ml using the same extracting solution. The extract was transferred to a 400 ml beaker, and evaporated to a small volume on a hot plate. The contents were then transferred to a platinum crucible and heated first slowly and then to full red heat for 20 min. The residue was taken up in 50 ml HCl solution (0.1 N), and boiled to obtain a clear solution. Aliquots of the solution were used for the estimation of Ca, Mg, Na, and K. Na and K were determined flame photometrically, whereas Ca and Mg were determined titrimetrically using EDTA (disodium salt) solution (0.01 M), NH₃/NH₄Cl as buffer (pH 10.0) and Eriochrome Black T solution as indicator. The use of Ratton & Reeder's indicator was made to determine calcium, and the value of Mg was found out by taking the difference between the titre values (10).
Silica: Weighed quantities (5 g each) of soil samples were repeatedly treated with an acid mixture of HCl and HNO₃ (3:1) and evaporated to dryness. The residue was then treated with HCl (1:10), filtered, washed, dried and ignited in a platinum crucible and weighed. The residue was further treated with few drops of H₂SO₄ and then with HF, and ignited to a constant weight. The loss in weight was taken as that SiO₂ (13).

Fe₂O₃: The filtrate obtained after the removal of silica was made up to 250 ml in a volumetric flask. An aliquot of the sample solution, after adjusting acidity between 5-6 N with respect of HCl, was treated with a concentrated solution of SnCl₂ until the colour due to ferric ions nearly disappeared. Few drops of stannous chloride solution were added in excess. The excess of stannous chloride was removed by adding mercuric chloride solution. The solution was then cooled, 25 ml of Zimmermann Reinhardt reagent solution was added, and the mixture titrated with standardized KMnO₄ solution (0.05N) (13).

Aluminium oxide: Weighed quantities (0.1 g each) of finely powdered sample were fused with 0.1 g sodium carbonate in a platinum crucible. After cooling, 8 ml of HClO₄ (60%) was added and the contents were heated till white fumes of HClO₄ were removed. The crucible was cooled, 5 ml of distilled water was added and boiled to dissolve the salts. The contents were transferred to a centrifuge tube, mixed with 2 ml of HCl (6 N) and 60 ml of distilled water and then centrifuged to throw down the silica. To the filtrate, ...
solution (1%) was added and the mixture centrifuged. 10 ml of hot NaOH solution (25%) was added and the tube was placed in hot water bath for 5 min., diluted with 50 ml of water and centrifuged to throw down the Fe(OH)$_3$ precipitate. An aliquot of the supernatant liquid was transferred to a 100 ml beaker, the contents diluted to 75 ml with 50 ml of distilled water, pH adjusted between 2-3 using NaOH or HCl, and the aluminium estimated spectrophotometrically using aluminon reagent (0.8% in ammonium acetate HCl medium). The absorbance was recorded at 520 nm. A calibration graph was prepared by using series of standard solutions of $K_2SO_4.Al_2(SO_4)_3.24H_2O$ in water. The standard solutions were treated with aluminon reagent as described in the case of sample solutions (12,14).

The results obtained for the four types of the soils have been shown in Table 2-5.

RESULTS AND DISCUSSION

Distinct natures based on the physico-chemical characteristics have been observed in the four types, namely, Bhata, Matasi, Dorsa and Kanhar soils of the Chhattisgarh region. A gradation amongst the soil-types is evident from the data obtained. While the Bhata and Matasi types have indicated weakly acidic to almost neutral nature, the Dorsa and Kanhar types have indicated neutral to a slightly alkaline nature. The gradual increase in electrical conductivity from Bhata to Kanhar types indicated corresponding increase in the electrolyte contents of the
Table 2-5 PHYSICO-CHEMICAL CHARACTERISTICS OF FOUR PRINCIPAL SOIL TYPES OF CHHATTISGARH REGION.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>Soil types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bhata</td>
</tr>
<tr>
<td>1.</td>
<td>pH</td>
<td>5.70-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.50</td>
</tr>
<tr>
<td>2.</td>
<td>Electrical conductivity</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>(m mhos/cm)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>organic carbon (%)</td>
<td>1.40</td>
</tr>
<tr>
<td>4.</td>
<td>Available nutrients (ppm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrogen</td>
<td>193.00</td>
</tr>
<tr>
<td></td>
<td>Phosphorous</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td>Potas</td>
<td>451.00</td>
</tr>
<tr>
<td>5.</td>
<td>Cation Exchange Capacity</td>
<td>8.50</td>
</tr>
<tr>
<td></td>
<td>(m eq/100 g)</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Exchangeable cations (m eq/100 g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
<td>0.40</td>
</tr>
<tr>
<td>7.</td>
<td>SiO₂ (%)</td>
<td>48.20</td>
</tr>
<tr>
<td>8.</td>
<td>Fe₂O₃ (%)</td>
<td>36.00</td>
</tr>
<tr>
<td>9.</td>
<td>Al₂O₃ (%)</td>
<td>8.00</td>
</tr>
</tbody>
</table>
soils. The organic carbon which can be taken as an indicator of humus substances of the soils was also found to be increasing from Bhata to Kanhar types. Available nutrients (N,K,P) showed highest values in the Kanhar type indicating it to be the most fertile soil. The Cation Exchange Capacity and the exchangeable cations (Ca, Mg, K and Na) have also been found to be highest in the case of Kanhar soil.

On the basis of the highest values of electrical conductivity, organic carbon, available nutrients, cation exchange capacity, and exchangeable cations observed in the case of the Kanhar soil, it can be considered to be fully equipped and the richest of all the soils examined here. Such a soil when exposed to interactions with the effluents of diverse nature and compositions is expected to exhibit more pronounced effects on its characters. Any damage undergone by this type of superior soil through the exposures of industrial effluents shall be of great concern. In the work carried out and described in the subsequent chapters, the impacts of industrial effluents of a wide range of nature mainly on the Kanhar soil have been investigated. However, the basic principles underlying the damage-causing reactions shall be applicable to any category of the soils.
2.3 THE CHHATTISGARH SOILS: STATUS OF MICRO-NUTRIENTS AND MICRO-POLLUTANTS

INTRODUCTION

Out of all the elements known, seventeen elements have been found to be essential to the plant growth. These elements may be divided into two groups: macro-nutrients (C, H, O, N, P, K, Ca, Mg, and S) and micro-nutrients (Fe, Mn, Cu, Co, Zn, B, Mo and Cl) (5).

MICRO-NUTRIENTS: Amongst the micro-nutrients, boron has been found essential for the green algae and the higher plants (15). Manganese is important to activate a number of metalloenzymes such as arginase (urea formation), puruvate, carboxylase. It is preferably accumulated in mitochondria, and hence it acts as a co-factor in the respiratory enzymes. While Mn (II) state is found in acid soils, Mn(III) and Mn(IV) states are favoured by high pH and oxidising conditions (16). Bromfield and Skirman (17) found that MnSO₄ could be oxidised to MnO₂ in the microbial presence of two microspecies, Corynbacterium and Flavobacterium or Chromobacterium. Although Mn(II) is oxidised to Mn(IV) in soils, a concentration of 0.02 M of Mn(II) introduces manganese toxicity resulting in the deminution of the oxidation rate (18). The majority of the Indian soils contains 300 to 1600 ppm of manganese (19). Organic matter in the soils affects the manganese transformations through such processes as complexation, diminution in the oxidation potential of the soil or stimulation of microbial activity that results in the incorporation of manganese in biological tissues (16).
Copper is important in maintaining proper functioning of metalloenzymes such as ascorbic oxidase, cytochrome oxidase, celluloplasmine, lysine oxidase etc. (17). Zinc deficiency symptoms are reported to be acute where top soils were removed for levelling purposes (5). Zinc is among the micro-nutrients which are made available to the plants through the supply of fertilizers. Its total absence can cause crop failure. It can also become toxic to crops if present in large quantities (20). Although significant quantities of iron ore present in soils, this element is regarded as micro-nutrient in view of the very small amount of this element being taken up by the plants. The approximate weight of iron removed by average crops has been reported to be 0.5 kg/hactare, although iron may be present in the soils in the range 2000-10,000 kg/hactare (20). Plants differ in their ability to take up iron from soils. Very little is known about the concentration of iron in the soil solutions but it is probably present as inorganic complexes. The roots of certain categories such as water culture plants have been reported to excrete soluble organic compounds into the soil, that form soluble complexes with iron available at the surface of iron(III) hydrous oxides (21). Suitable iron chelates such as ferric citrate and tartarate and ferric-EDTA have been reported to be added to soils to ensure the supply of this element to the plants (22). The role of iron in the formation of chlorophyll was discovered long ago. In India, the deficiency of iron in soils has been reported from Punjab, Haryana, Uttar Pradesh, Rajasthan and Delhi. The iron
deficiency is called iron chlorosis which results in the yellowing of the plants due to lack of chlorophyll (23).

The reservoir of cobalt in many soils is reported to be that adsorbed on the surface of manganese oxides, the availability of cobalt is decreased by any soil-treatment which converts the manganous ions to insoluble oxides, and is increased by those treatments which convert manganese oxides to manganous ions (24). It is reported that biological nitrogen fixing system needs cobalt along with iron and molydenum (25, 26). Cobalt in the form of its sulphate is usually added to correct the cobalt deficiency of soils (27). Molybdenum is needed for symbiotic nitrogen fixation. It is also required for the reduction of nitrates in plant tissues. It is taken up as either $\text{HMnO}_4^-$ or $\text{MoO}_4^{2-}$ (28). The reservoir of molybdate is that adsorbed on the surface of ferric oxides or hydrated oxides which have a very strong affinity for molybdate (29). Molybdenum deficiency is normally found in acid soils (30). The molybdenum content varies from 0.2 to 5.0 ppm with a mean value of 2 ppm (31).

Chlorine has in recent years been established as one of the essential nutrients for plant growth. Wilting is considered the most generally symptom of chlorine deficiency. Considerable work has been done on the toxicity symptoms caused by excess presence of Cl. Generally, these symptoms include burning of the leaf tips or margins, bronzing, premature yellowing and abscission of leaves, and less frequently chlorosis (23). There is no strong evidence that chloride has any specific effect on plant growth, though it
may some time hasten maturity. Its main function, for which, however, it is not specific, is on osmotic pressure regulator and cation balancer in the cell sap and in the plant cells (32).

Excess supply of one or more of the above stated micronutrients often results in depression in growth rate, and visual pathological effects which at time are characteristics of elements supplied in excess (23).

MICRO-POLLUTANTS: THEIR INDUSTRIAL SOURCES AND UPTAKE BY PLANT SPECIES

Apart from micro-nutrients, the soils may also receive some elements which have no established role in the plant growth. No beneficial effects of these elements on the plant growth have been reported. On the contrary, these elements have been reported to result in damage to the plants in one or more ways. Some elements of this nature are being described below.

Arsenic has been described has poisonous to the plants (35). Arsenic exists in nature in -3, 0, +3, and +5, state, is stable in aerated water, whereas elemental arsenic and arsine (AsH₃) exist in reducing sediments. In moderately reducing environment, the trivalent state is found to occur (34). As(III) is known to be highly toxic, while As(V) is known to be less toxic. Arsenic can be removed from solutions in the As(V) state by adsorption on activated solids. Arsenic is very common element present in nearly all soils, and hence in plants and animal products. At 1 ppm an below, there is no
known harmful effect of arsenic (35). Sodium arsenite is reported to have been used for many years as a weed killer (36,37). More recently organic arsenic compounds such as cacodylic acid has been found useful as general contact herbicides to control, weeds, particularly grasses (38,39). Soils near some smelters are often polluted with large amount of arsenic (39). Methylation of dimethylarsenic acid, which is also used as a pesticide, is evident in soils treated with this pesticide (40). A strain of methanobacterium is reported to produce dimethylarsine from arsenate (41).

Extensive work has been done about the presence of lead in air, water and soils. It has been remarked that modern man contains 100 times more lead than pre-historic man (42). The average concentration of lead in soils is reported to be 16 mg/kg. Brown algae and phytoplankton have been reported to show an affinity for lead (43). It will be useful to take into account the environmental pollution by cadmium, zinc and lead attributed to the largest lead-zinc smelter plant of Japan located at Annaka city. Here about 500 samples of soil and 80 samples of agricultural products were analysed for Cd, Zn and Pb. A high correlation was found to exist between the content of Cd, Zn, and Pb of soils and the occurrences of these elements in the farm products (44,45). In other words, the closer the soil and vegetation to the smelter plants, the greater was the contamination found. The concentrations of these metals in leaf vegetables were found to be substantially higher than those in root vegetables, fruits and cereals. The flour from wheat and barley were found to contain much more Cd, Zn, and Pb than rice in the
same polluted area. The soil-profiles near the above-stated smelter stack were also examined. The upper soil (0-10 cm) was found to be most polluted and soils deeper than 40 cm showed approximately the same values as in unpolluted soils (44, 45). The toxic effects of lead on human body have been described in Chapter 1 (Section 1.3). It has been estimated that in USA, the man has a daily intake of approximately 300 ug of Pb (46), of which about 60% comes from the ingestion of contaminated food (47). The lead-content of food has been largely influenced by environmental contaminations. This may have been through surface contamination as well as plant tissue incorporation of lead that was deposited on soils or crops grown in areas near the highways or lead smelters (48). When such crops were used as forage for farm animals, the lead was found transported into milk or meat (48, 49). Amongst birds, ducks have been shown susceptible to poisoning from the consumption of marsh soils contaminated with lead (50). The soils near smelters contain far greater amounts of lead than does the forage. It was found that horses which have a marked tendency of pulling grass along with the roots and soils could digest far greater quantities of lead than would be estimated from the analysis of forage alone (51). The extent to which lead contamination in corn leaves from lead smelter situated 75 meters away can occur is evident by the reported presence of lead at a level of 3200 ug/g of the corn leaves on dry weight basis (52). The lead presence in the range 1 to 7000 ppm in the ash of a variety of plants from the whole of USA was found in 761 out of 912 samples (53). In
contaminated areas, the Pb-content may be much higher; cedar trees in Missouri on a roadside contaminated with lead-ore dusts contained as much as 20,000 ppm in ash (54), and Spanish moss near heavily travelled roads contained as much as 15,000 ppm in ash (56). There is also report of biomethylation of lead in environment. Any aqueous saline medium would produce mono- and dimethyl lead(IV) species in the soils. These species are known to decompose rapidly (56). According to Schroeder (57), tetraethyllead is 100 times more toxic as inorganic lead.

Mercury compounds in the crust of earth are believed to degrade to the metallic form which in turn is volatilized to the atmosphere. The atmosphere contributes to the world-wide distribution in the environment. Rain fall acts as a scrubber of the atmosphere, and may deposit upto 500 mg annually/acre on the arth surface (58,59). The levels of mercury have been reported as follows: Swedish soil 50-510 ppb, England and Northern Ireland soils 5000-15,000 ppb, French and Sudan soils 10-60 ppb, (58,59). Plant and animal tissues also ahve been reported to have substantial amounts of mercury with a wide range of levels (60). Some values are as follows : apples (New Zealand) 11-135 ppb, apples (U.K.) 20-120 ppb, rice (Japan) 227-1,000 ppb, rice (U.K.) 5-15 ppb. Schacklette (61) reported mercury in trees and shrubs that grew over a cinnabar deposit in Alaska-range between 0.5-3.5 ppm on dry basis. As the absorbed mercury passes into blood, one half of it is firmly bound to the albumine of plasma and the other half is associated with the red cells (62). It is.
therefore, readily redistributed to the tissues, and in a few
hour found in highest concentration in the kidneys. By the
end of a week, 85-95% of all the mercury in the body is
stored in the kidneys (63). Other details of the toxic
effects of mercury have been described in Chapter I (Section
1.3). In sulphide-rich anoxic sediments, mercury
concentration becomes vanishingly small as a result of the
formation of HgS. In natural systems, bacterial methylation
of mercury occurs in sediments, mobilising the mercury and
making it available for biota (64,66). The evidence of the
formation of mercury humate with purified humic acid
extracted from the river sediments has been reported (66).
Mercury is methylated in aquatic environment by biological
and non-biological processes that were discovered several
decades ago. Dimethyl mercury \((\text{CH}_3)_2\text{Hg}\) and methyl mercury
cation \(\text{[CH}_3\text{Hg]}^+\) are both formed in nature (67). Methyl- and
ethyl mercury which are soluble are carried and stored for
extended periods in the blood cells (68). Extremely high
toxicity of methyl mercury to man and its concentration in
the environment have been reported to be of particular
concern (69). Most of the mercury in the Ji Yun river
sediment was found associated with humic acid and other
organic substances (70). Humic acid present in the sediments
has also been found to dissolve mercury (II) sulphide. Thus,
mercury sulphide in the sediments might be mobilized and
cycled between the sediments and water column (71). The
adsorption capacity of different adsorbents for mercury was
found to decrease in the following order: humic acid > \(\text{MnO}_2\) >
clay minerals > \(\text{Fe}_2\text{O}_3\) > \(\text{SiO}_2\). The adsorption of mercury is
the main process for the enrichment of mercury in soils and sediments (72,73). Humic acid in the river water thus enhances the transport of mercury in form of soluble complex. The stability of mercury humate in river was found to be greater than the stabilities of mercury chloride, hydroxide, sulphate, or phosphate complexes (74). The emission of methyl mercury from the sediments into the water is reported to be in the range of 0.4 to 5.0 ug Hg m\(^{-2}\) day\(^{-1}\). (75).

The concentration of cadmium in most soils is reported in the range 0.5-1.0 mg kg\(^{-1}\) although concentrations greater than 20 mg kg\(^{-1}\) occur naturally in some places. Raised concentration results from smelting and mining activities, and from the use of sewage sludge on land. The average concentration of cadmium in the earth crust is 0.15 mg kg\(^{-1}\) (76), and the background concentrations found in soils depend upon parent material from which they are derived (77). Soils derived from igneous rocks are reported to contain 0.1-0.3 mg Cd kg\(^{-1}\) (dry matter), from metamorphic rocks 0.3-11.0 mg kg\(^{-1}\) (76). Cadmium is a contaminant of the zinc ores, calamine (ZnCO\(_3\)) and sphalerite (ZnS). The contamination of soil resulting from mining activities in U.K. have been found to contain up to 800 mg Cd kg\(^{-1}\). (78). Soils near the large zinc smelting complex in 1100 kg\(^2\) area in U.K. is reported to have Cd-levels in excess of 4 mg kg\(^{-1}\) (79). Phosphatic fertilizer is another important source of Cd in agricultural soils. Williams and David (80) reported Cd-concentrations of 5-100 mg kg\(^{-1}\) in rock phosphate, most or all of which enters the fertilizer prepared from it. The background values of cadmium in a selection of 50 different
plants have been reported in the range 0.02-1.00 mg kg\(^{-1}\) (dry matter) \((81,82)\). Jarvis et al. examined the distribution of cadmium between the roots and shoots of 23 plants species after exposure to a nutrient solution containing 0.01 mg Cd L\(^{-1}\). In all, except three species, more than 50% of the Cd taken-up was retained in the roots \((83)\). In the case of rye grass, approximately 80% of Cd was retained in the roots. John \((84)\) examined the distribution of Cd in a range of crops grown in soils containing 40-200 mg Cd kg\(^{-1}\) soil. He found that root-concentrations of Cd were found to exceed leaf concentrations for all the crops tested. Using Cd\(^{109}\) as a tracer, Stenstrom and Lonsjo \((85)\) found that 0.33% of Cd in sludge-treated soil was recovered in wheat tops, but only a quarter was found in the grain. Cadmium is a non-essential element for both plants and animals, so there is no lower critical concentration below which deficiency would occur. Upper critical concentrations marked the onset of toxicity. Cd may be described as zootoxic since it is more toxic to the animals than to the plants. So the zootoxic upper critical concentration in plant-tissue precedes the phytotoxic concentrations, and animals could conceivably be harmed by eating apparently healthy plant material. Beckett and Davis \((86)\) found that at a median value of 80 mg Cd kg\(^{-1}\) in tissues of young barley plants, the yield was found reduced. For practical purposes, Yield is taken to be a sensitive indicator of plant health, and reductions in yield precede visual symptoms of Cd-toxicity as shown by chlorosis, red brown patches on leaves, stunted stems and, in severe
cases, leaf curling and abscission (87,88). The long-term exposure to enhanced level of Cd which is a cumulative poison may be significant. Long lived animals such as humans are more at risk to chronic Cd-toxicity than farm animals whose life is comparatively short. Under normal circumstances, food constitutes the most important source for the general population. Over 50% of the total dietary intake of Cd is derived from the cereals and vegetable portions of the diet (89). WHO and US environmental agency have independently estimated the safe limit of daily intake of Cd at about 70 ug (90,91). Cadmium is an element which is highly toxic to mammals. In 1969, Tsuchiyia (92) reported that Itai itai syndrome was associated with the ingestion of Cd-contaminated rice together with other pre-disposing factors (93). It is now well established that kidney dysfunction following proximal tubule damage is the most characteristics symptom of chronic poisoning by cadmium (94,95). Details of other toxic effects of cadmium have been described in Chapter I (Section 1.3). There are reports of cadmium undergoing biomethylation. The dimethyl cadmium hydrolyses quickly to a gelatinous precipitate of polymeric $\text{CH}_3\text{Cd(OH)}_x$. This polymer is capable of methylaing aquated metal electrophiles such as Hg(II) (96,97). Studies have also shown that cadmium is adsorbed on clay minerals, hydrous oxides of iron and manganese, humic substances which are all normal constituents of the soil (98,99). It is reported that the uptake and transport of cadmium in plants is reduced at increased zinc or potassium concentrations (100). The cadmium presence in farm products
near Annaka city where Japan's largest zinc smelter is located has been reported as follows (46): rice 0.4-0.95; wheat 0.5-8.6; green vegetables 2.6-56.0; root crops 0.9-17.0 fruit vegetables 0.3-0.6; ppm (dry matter basis). In 1968, Fukushima et al (101) analysed rice samples collected from 77 farm houses in different parts of the endemic area of Itai-itai disease in Japan, and also from control areas, and obtained a correlation between the geographical distribution of the prevalence of disease and the cadmium contents in the soil and rice in the endemic area (102). The results of pot culture experiments on rice plants and wheat preferred by Kobayashi and Muramoto (103) in which different amounts of cadmium and zinc were added to the soil indicated that the more cadmium oxide added to the soil the greater was the uptake of cadmium especially in wheat, and that the yield of wheat was reduced at a level of 0.003% cadmium in the soil, while in rice a significant decrease in yield was not seen until 0.1%. There is also a report of severe chlorosis of the leaves of sweet potatoes which turned bright yellow due to cadmium poisoning in a cadmium mining district in Japan (104).

Chromite (FeO.Cr$_2$O$_3$) is the only important ore of chromium, and approximately 0.03% of the igneous rock in the earth crust is chromium. The absorption, distribution and of excretion chromium by animal and man at levels beyond normal ingestion have been well investigated (105). At these levels, orally administered trivalent chromium is absorbed to the extent of only about 1% or less (106). Hexavalent chromium...
better absorbed than trivalent chromium (106), and readily passes through the membrane of red blood cells and becomes bound to the globin fraction of hemoglobin (107). The other adverse effects of chromium have been described in Chapter I (Section 1.3). The occurrence of chromium in plant tissues of food and feed plants have been reported as follows: the element has been detected in the range of less than 1 to 700 ppm in ash of a variety of native species found throughout USA in 1096 samples out of 1139 samples (108). Chromium salts, particularly chromates, even at very low concentrations, have been found to be toxic to plants (109). Chromium (III) has been reported as an essential element for man and animals required as part of the glucose tolerance factor (110,111).

Nickel is widespread in its occurrence, and distributed widely in foods. Estimates of daily human intake are from 0.24-1.0 Ni mg/day (112,113). Vegetable materials contain much more nickel than material from animal origin. Estimates between 0.15-0.35 ppm Ni have been made for foods, tubers and grains (114,115). Some items, high in Ni, were tea 7.6 ppm, buck wheat seed 6.4 ppm, oysters 6.0 ppm, on dry weight basis (115,116). Significant concentrations of Ni are present in DNA and RNA (117,118). The harmful effects of nickel on human health have been described in Chapter I (Section 1.3). The occurrence of nickel in tissues of food and feed plants in USA has been reported from 5 to 500 ppm in 82% samples out of 912 samples on dry weight basis (108). In ash, Ni occurs in most samples of food plants in concentration of
5 ppm, and rarely exceeds 100 ppm (119). An exception is provided by soya bean seed samples in which Ni is found to occur in the range of 30-500 ppm (in ash), in contrast with corn grains from the same area in which Ni ranged between 5-70 ppm (in ash) (120). There is a report that cyanobacteria brown and green algae and yeast cells all bioconcentrate nickel (121).

The determinations of the micronutrients and micro-pollutants in the principle soil-types of Chhattisgarh region have been described below.

**MATERIALS AND METHODS**

**Sample collection**: The method of the collection of the samples of the four principal soil-types (Bhata, Hatasi, Dorsa, and Kanhar) and the methods of sample preparations have been described earlier (Section 2.2).

**Method**: Manganese, copper, zinc, molybdenum, cobalt, lead, cadmium, chromium, and nickel were determined in the soil samples using an atomic absorption spectrophotometer. As and Mg were determined spectrophotometrically.

**Sample preparation**: Weighed quantities (5 g each) of the samples were dried at 110°C in an oven for three hours, and thereafter reduced to a powder form. Weighed quantity (0.5 g) of samples were treated in a teflon digestion bomb using 10mL acid mixture of HCl, HF, and HNO₃ and placing in an oven at 180°C for three hours (122). Sample solution for the
determination of Pb were made in a medium of 0.1 M EDTA to suppress the interference of phosphate and fluoride (123).

**Reagent solutions**: For the AAS determinations, the standard solutions of the respective metals were prepared by the prescribed procedures (123) as follows:

**Manganese**: 1 g of the metal (wire form) was dissolved in minimum volume of nitric acid (1:1), and the solution was diluted to 1 L (1 ml = 1,000 ug Mn).

**Copper**: 1 g of the metal wire was dissolved in minimum volume of nitric acid (1:1), and the solution diluted to 1 L (1 ml = 1000 ug Cu).

**Zinc**: 1 g of the granule was dissolved in 40 ml HCl (1:1), and the solution diluted to 1 L (1 ml = 1,000 ug Zn).

**Molybdenum**: 1 g of the metal wire was dissolved in HCl acid (1:1) with gentle heating. The solution was cooled and diluted to 1 L (1 ml = 1,000 ug Mo).

**Cobalt**: 1 g of metal wire was dissolved in minimum volume of nitric acid (1:1), and the solution diluted to 1 L (1 ml = 1,000 ug Co).

**Lead**: 1 g of the metal-wire was dissolved in nitric acid (1:1) and the solution diluted to 1 L (1 ml = 1,000 ug Pb).

**Cadmium**: 1 g of the metal was dissolved in minimum volume of nitric acid (1:1), and the solution diluted to 1 L (1 ml = 1,000 ug Cd).
Chromium: 1 g of the metal wire-form was dissolved in HCl acid (1:1) with gentle heating. The solution was diluted to 1 L (1 ml = 1,000 ug Cr).

Nickel: 1 g of the metal strip was dissolved in nitric acid (1:1), and the solution diluted to 1 L (1 ml = 1,000 ug Ni).

All the metals and chemical reagents used were of spectroscopic grade. The water used was distilled and deionised. The glassware used were superior quality borosilicate.

Apparatus: The analysis was carried out by using an atomic absorption spectrophotometer (Varian Techtron Model AA 575). The operating conditions of the instrument (123) have been shown in Table 2-6.

During calibration the standard solutions of the metals were suitably diluted to match the concentrations of the sample solutions within the measurement sensitivity (124). The physical properties of the sample and standard were matched closely to avoid matrix effect (124). The methods were also suitably modified to remove the effects of interference.

The results obtained have been shown in Table 2-7.

The determinations of arsenic and mercury were made spectrophotometrically using the following procedures.
<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Lamp Band Pass (nm)</th>
<th>Fuel</th>
<th>Support</th>
<th>Flame Stoichiometry</th>
<th>Optimum Working Range (µg/ml)</th>
<th>Interferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>324.7</td>
<td>0.5</td>
<td>3.5</td>
<td>C₂H₂/N₂O</td>
<td>Air</td>
<td>Oxidizing</td>
<td>2-8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Interference with high Zn/Cu ratio, removable by nitrous oxide - acetylene flame.</td>
</tr>
<tr>
<td>Co</td>
<td>240.7</td>
<td>0.2</td>
<td>7.0</td>
<td>C₂H₂</td>
<td>Air</td>
<td>Oxidizing</td>
<td>3-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ni interferes if more than 1500 µg/ml</td>
</tr>
<tr>
<td>Ni</td>
<td>232.0</td>
<td>0.2</td>
<td>3.5</td>
<td>C₂H₂</td>
<td>Air</td>
<td>Oxidizing</td>
<td>3-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No Chemical interference.</td>
</tr>
<tr>
<td>Pb</td>
<td>217.0</td>
<td>1.0</td>
<td>EDL</td>
<td>C₂H₂</td>
<td>Air</td>
<td>Oxidizing</td>
<td>5-20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No cationic interferences, PO₄³⁻, CO₃²⁻, I⁻, F⁻ and CH₃COO⁻ suppress absorbance which is removable by use of 0.1M EDTA.</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>1.0</td>
<td>5(or EDL)</td>
<td>C₂H₂</td>
<td>Air</td>
<td>Oxidizing</td>
<td>0.4-1.6</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>0.2</td>
<td>5.0</td>
<td>C₂H₂</td>
<td>Air</td>
<td>Oxidizing</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No Chemical interference.</td>
</tr>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.5</td>
<td>3.5</td>
<td>C₂H₂ (or EDL)</td>
<td>Air</td>
<td>Oxidizing</td>
<td>0.5-2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No chemical interference in air-acetylene flame</td>
</tr>
<tr>
<td>Cr</td>
<td>357.9</td>
<td>0.2</td>
<td>7.0</td>
<td>C₂H₂/N₂O</td>
<td>Air</td>
<td>Reducing</td>
<td>2.0-8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Co, Fe and Ni depress absorbance. Effect removable by the use of acetylene/nitrous oxide</td>
</tr>
<tr>
<td>Mo</td>
<td>313.3</td>
<td>0.5</td>
<td>7.0</td>
<td>C₂H₂</td>
<td>N₂O</td>
<td>Reducing</td>
<td>15-60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No chemical interference.</td>
</tr>
</tbody>
</table>
**Arsenic**: A weighed quantity (5 g) of the soil sample was transferred to a Kjeldahl flask and mixed with 20 ml concentrated sulphuric acid, 5 ml concentrated nitric acid and 0.1 g of potassium chlorate. After completion of digestion, mixture was cooled, filtered and diluted to 250 ml. An aliquot (25 ml) was placed in an arsine evolution flask, treated with 5 ml concentrated HCl, 2 ml KI solution (15%), and eight drops of SnCl\(_2\) solution. After 15 min., 5 g of pure granulated zinc was added to the solution. The arsine evolved was absorbed in a silver diethyldithiocarbamate solution (5% in pure pyridine). The absorbance of the complex was measured at 540 nm. A blank was also run. A calibration curve was prepared by using standard solutions of arsenic prepared by dissolving arsenious oxide in NaOH solution (13, 125).

**Mercury**: A weighed quantity (1 g) of the sample was transferred to a Kjeldahl flask, 30 ml of 1:1 mixture of conc. sulphuric and nitric acid was added, and heated for two hours. The mixture was cooled, filtered and diluted to 250 ml. An aliquot (25 ml) was taken in a separatory funnel, and 50 ml of dilute hydrochloric (20%) was added. It was then treated with 10 ml of dithizone solution (0.25%) in chloroform. Two more extractions were done. The organic layer was treated with 50 ml of hydrochloric acid (1:70) and 5 ml of KBr solution (40%) and shaken vigorously. The chloroform layer containing copper was discarded. The aqueous phase containing mercury was used for the spectrophotometric
determination using dithizone reagent at pH 1-2 by measuring absorbance at 485 nm (14).

The results obtained have been shown in Table 2-7.

RESULTS AND DISCUSSION

The results obtained (Table 2-7) show that the four principal soil-types (Bhata, Matasi, Dorsa, and Kanhar) of the Chhattisgarh region contain the selected micronutrients (Zn, Cu, Mo, Co, Mn) at concentration levels which have a reasonable agreement with those reported for the soils of Madhya Pradesh State and in the neighbouring area (25). The status of the selected micronutrients in the soils here can thus be taken as normal.

Apart from the micronutrients, the soils have indicated the presence of a number of metallic elements which have no direct role in the plant growth. On the other hand, some of these elements e.g. Cd, Ni, Cr have been reported to have a tendency of getting into the plant body, and farm part of the food chain. However, cadmium which has been found to be very sensitive element from the point of view of plant uptake, particularly by the paddy crops, has been found to be present at low concentration levels (1.0-2.0 ppm).
Table 2-7 CONCENTRATIONS OF MICRO-NUTRIENTS AND MICRO-POLLUTANTS (ppm) IN PRINCIPAL SOIL-TYPES OF CHHATTISGARH REGION.

<table>
<thead>
<tr>
<th>Elements analysed</th>
<th>Bhata soil</th>
<th>Matasi soil</th>
<th>Dorsa soil</th>
<th>Kanhar soil</th>
<th>Concentration ranges reported in M.P. soils (Ref.23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>78.0</td>
<td>62.0</td>
<td>52.0</td>
<td>55.0</td>
<td>39.0-60.0*</td>
</tr>
<tr>
<td>Cu</td>
<td>89.0</td>
<td>71.0</td>
<td>60.0</td>
<td>63.0</td>
<td>22.4-63.8</td>
</tr>
<tr>
<td>Mo</td>
<td>3.5</td>
<td>3.0</td>
<td>2.5</td>
<td>2.5</td>
<td>Trace-1.20*</td>
</tr>
<tr>
<td>Co</td>
<td>22.5</td>
<td>18.0</td>
<td>15.0</td>
<td>16.0</td>
<td>50.0-64.0*</td>
</tr>
<tr>
<td>Mn</td>
<td>962.0</td>
<td>764.0</td>
<td>644.0</td>
<td>682.0</td>
<td>484.0-1540.0</td>
</tr>
</tbody>
</table>

**MICRO-NUTRIENTS**

| As | 5.0 | 5.0 | 5.0 | 5.0 | - |
|Cd | 2.0 | 1.5 | 1.0 | 1.5 | - |
|Pb | 85.0| 67.0| 57.0| 60.0| - |
|Hg | Nil | Nil | Nil | Nil | - |
|Ni | 69.0| 55.0| 46.0| 49.0| - |
|Cr | 56.5| 45.0| 38.0| 40.0| - |

* Correspond to black clay of Maharashtra.
** Nil denotes undetectable.
INTRODUCTION

Humus has been defined as the substance left after the soil organisms have modified it from the original organic matter to a rather stable group of decay product. In other words, humus is the colloidal remains of organic matter. Soil organic matter has been defined as any living or dead plant or animal materials in the soil. Organic matter is often confused with humus, because organic matter consists of either living or dead plant or animal materials. Hence it must contain all the nutrients needed for the growth of these organisms. The amount and type of these nutrients are directly dependent on the original source. In raw (undecomposed) organic matter, there are few major groups of organic compounds which are contributed to the soils. These are: carbohydrates, lignins, proteins, fats, waxes and resins. Of these, carbohydrate and proteins are the most important and most readily decomposed. These two constituents are also the greatest contributors of soil nutrients such as nitrogen, sulphur and phosphorus. Lignin is a very resistant compound which persists in the soil as one of the main component of the humus. Fats, waxes and resins are resistant compounds which contribute sulphur and phosphorous to the soil. Alongwith these major compounds, there exists a host of other minor compounds. Unless the organic matter is decomposed, these nutrients will remain in the soil, but in unavailable form. The decomposition of organic matter is carried-out by soil organism (22). The nuclei of humus arise both from altered lignin compounds and through the synthesis of aromatic
compounds by micro-organisms. Micro-organisms are thus implicated in all phases of humus formations. They affect the decomposition of the original plants and animal residues to simpler compounds, and at the same time are responsible for the alteration of lignin and tanin molecules which will become parts of the structural units of humus. Several hypotheses have been proposed about the nature of humic substances. Most of these hypotheses have several aspects in common: humic substances are amorphous, three-dimensional, polymeric, acidic substances, of high molecular weight, with more or less aromatic nature. It is also generally agreed that no single specific structural formula will adequately represent the humic substances. Rather most hypotheses suggest a type or a skeleton structure in which only general aspects are included. The hypotheses differ primarily in the nature of structural nucleus whether it is benzenoid, phenolic, quinonic, or heterocyclic in nature, whether the N is a fundamental part of such nucleus or an accidental contaminant or whether there is a reasonable degree of uniformity as reflected in a number of structural units randomly distributed throughout the nucleus (25). Humus was regarded as a soil constituent of great significance in soil-forming processes, and in soil fertility; its presence in the soil was the qualitative feature distinguishing the soil from the parent rock. Efforts to quantitatively isolate humus matter in a form suitable for analysis have had only a limited success. Most schemes of separation involved the
solubilisation of organic matter through the alkaline, neutral extracting reagents and various organic solvents (25).

Humic material primarily consists of negatively charged colloids. Some of it is bound to the clay through polyvalent cations such as calcium or aluminium, one of whose charges neutralise a negative charge on the clay and the other on a humus. Some is bound to positively charged surfaces of iron or aluminium hydrated oxides, and some is held at clay surfaces through hydrogen bonding or by van der Waals forces. The classical way of separation of humus part from soil that is most widely used is to extract the soil with sodium hydroxide (0.1-0.5 N). This replaces the polyvalent ions by sodium and precipitates them as hydroxides, or converts the alumina to the aluminate anion. It increases the negative charge on humus colloid and decreases the positive charge on any sesquioxide surfaces. So far as the humus is bonded to the clay through polyvalent cations, a treatment with chelating agent such as sodium pyrophosphate will be an effective dispersing agent (126). The classical method for fractionating the humic colloid dispersed in sodium hydroxide extract is to acidify the suspension with sulphuric or hydrochloric acid, which causes a part of dispersed organic matter to precipitate. The part that stays in solution is known as fulvic acid, that which precipitates out as humic acid, and that part of the organic matter which does not disperse in the alkali but remains in the soil as humin. Although these fractions are given
definite names, they are not homogenous. Each contains particles with a wide range of molecular weight and constitution, nor there is a clear cut division between the fractions. Also, the proportion of humic matter that is precipitated by the acid depends on the type of acid used and its strength.

The humic colloids (or humic acids) are built-up of carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus; and, as far as is known, no other element forms an integral part. The elemental composition of humus from different soils varies within quite wide limits, but for many agricultural soils, the ratio C:N:S:P is of the order 100:10:1:2 on a weight basis. The dispersed humic particles have a wide range of molecular weight, but there is still some difficulty in separating the humic suspension into fractions having a molecular weight in a given range. The molecular weights of humic acids have been found to be above 5000 and going to several millions (127). Humic colloids contain a range of polysaccharides, proteins or polypeptides and substances of unknown compositions, but rich in aromatic rings, as well as a large range of substances present in small or very small quantities. This includes waxes and asphalts on the one hand (127), and substances likely to be present in the living cells in the soil organisms such as purin and pyrimidine bases and nucleic acids (128) on the other.

Humic substances are able to form complex linkages of various kinds with metals by ion-exchange, adsorption on
surfaces and formation of chelates. Cation exchange reactions are important in plant nutrition. According to the different types of linkages between functional groups of humic acids and the inorganic soil constituents, three main types of metal-organic derivatives can be distinguished: (i) the ionic type with participation of carboxyl and phenolic hydroxic groups leading to the corresponding humates by the known reaction of salt formation, (ii) the semi-polar type, formed by co-ordination linkages with participation of amino, imino, keto and thioether groups, leading to the complex compounds of chelate types, (iii) a type that is formed by polarisation effects and hydrogen bridge linkages with special participation of terminal functional groups forming compounds of the adsorption type (129).

The infrared spectroscopy of humic acid preparations and their fractions is used by many authors for characterizing humic substances of different soil origins (129). The assignment of different specific bands is limited by the fact that soil organic matter preparations represent in most cases mixture of more or less complex molecules containing different types of linkages and functional groups. This leads to an overlapping of the absorption bands. The infrared absorption spectra of humic acid, therefore, shows only some bands which are characteristic for the chemical nature of the molecule. The infrared absorption properties of the samples are also strongly influenced by different methods of sample preparation (130).
The isolation of the humic acid from one variety of
the paddy soils (Kanhar type), and its subsequent characteri-
zation by IR-spectroscopy has been described below.

MATERIALS AND METHODS

Sample collection: The method of the sample collection of
the Kanhar variety of the paddy soil has been described
earlier (Section 2.2).

Isolation of humic acid: A weighed quantity (200 g) of the
soil was extracted with 2 litres of sodium pyrophosphate
solution (0.1 M) in 0.1 M sodium hydroxide medium (pH 13.0).
The supernatant was collected by centrifugation. The extract
was acidified with \( \text{H}_2\text{SO}_4 \) to a pH of 1.5 which yielded
precipitated humic acid.

Purification of humic acid: The humic acid obtained was
purified by repeated flocculation and peptisation using
dil.\( \text{H}_2\text{SO}_4 \) and \( \text{NaOH} \) till free form metal and sulphate ions.
The product was further purified in a batch of flasks
containing Dowex-15 cation exchange resin until no further
decrease in its pH was noticed. The ash content in a small
portion of the purified humic acid was determined, and the
value of the same was found to be 0.25%. The total weight of
humic acid after oven drying at 105\(^\circ\) C was recorded (131,
132).
IR-spectra of isolated humic acid: The IR-spectra of the isolated pure humic acid were recorded in the spectral range of 4000 to 400 cm\(^{-1}\) in the nujol medium using an IR-spectrophotometer (Nicolet FT-IR). The spectra obtained has been shown in Fig.2-2. The observed bands and their assignment to different functional groups have been shown in Table 2-8.

RESULTS AND DISCUSSION

The results obtained (Fig.2-2, Table 2-8) have confirmed the presence of a number of functional groups in the skeletal network of the humic acid. The notable functional groups whose presence is confirmed in the humic acid structure include hydroxy, aromatic, carboxylate, carbonyls of aldehydes, ketones and quinones, and C=C unsaturated groups, apart from stretching frequencies of N-H and S-H groups.

The response of these groups towards different reacting cationic species has been studied in detail, and described in subsequent chapters.

2.5 BACTERIAL PRESENCE: STANDARD PLATE COUNT STUDY OF PADDY SOIL (KANHAR VARIETY).

INTRODUCTION

The Standard Plant Count (SPC) procedure provides a standardised method of determining the density of aerobic and facultative aerobic and heterotrophic bacteria in water as
Fig. 2-2 THE IR SPECTRA OF PURE HUMIC ACID.

Table 2-8 THE PROMINENT IR BANDS (cm\(^{-1}\)) OF HUMIC ACID AND THEIR ASSIGNMENTS. (REF. 133, 134).

<table>
<thead>
<tr>
<th>Wave numbers</th>
<th>Band assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3600 (B)</td>
<td>(\nu(\text{O-H}))</td>
</tr>
<tr>
<td>1740 (S)</td>
<td>Characteristic band of COO(^-) group</td>
</tr>
<tr>
<td>1680 (S)</td>
<td>Amide band 1</td>
</tr>
<tr>
<td>1550 (S)</td>
<td>Thioamide band 1</td>
</tr>
<tr>
<td>1450 (M)</td>
<td>Characteristic band of COOH group</td>
</tr>
<tr>
<td>1355 (M)</td>
<td>Characteristic band of COOH group</td>
</tr>
<tr>
<td>1030 (M)</td>
<td>Thioamide band 11</td>
</tr>
<tr>
<td>680 (W)</td>
<td>Amide band 11</td>
</tr>
</tbody>
</table>

B - broad, S - strong, M - medium, W - weak;
* due to \(\nu(C=O)\);
** due to \(\delta(H-H) + \nu(C-H)\);
*** due to \(\nu(C-H) + \nu(C-S)\).
well as in soil. Soil is a unique medium that contains a diverse community of organisms representing many morphological and physiological types. In attempting to characterise these organisms, one has to take into consideration both their numbers and activities. Organisms in soils are never static in number and activity. Therefore, the enumeration of the population or microbial activity represents a point in time for that particular population that is in dynamic equilibrium with its physical, chemical and biological environments. Variation of microbial numbers and activities can occur with depth and soil types (135, 136). Soil structure, texture and moisture levels can drastically affect aeration within the soil environment attributing aerobic to anaerobic properties. The season of the year is as important as soil physical properties for the microbial diversity (137). Hagedron (138) observed differences in numbers and diversity of actinomycetes depending on soil acidities. Schmidt (139) considers the plate count method acceptable particularly because in this method media that are specific for fixed physiological groups are developed, and the incubation conditions approach the natural environment. It will have to be kept in mind that organisms are not uniformly distributed throughout the soil environment, but rather are found in a point distribution depending on a localised feature that allows maximum expression of that particular organism.
MATERIALS AND METHODS

Sampling: The soil samples of the Kanhar type of paddy soil was collected by standard procedure as described earlier (Section 2.2). The samples were shifted to the laboratory soon after collection. During the short period of storage of the samples, a room cooler was put on operation to provide a static and moderately cool temperature.

Procedure: The freshly collected soil sample (1 g) was taken in a sterilized bottle. 10 ml of aseptic distilled water was added to obtain a suspension. The supernatant liquid was decanted into another sterilized bottle and used for Standard Plate Count study. Reagents required for the plate count were prepared as follows:

Dilution water: (a) 3.4 g of potassium hydrogenphosphate was dissolved in about 50 ml of distilled water. pH was adjusted to 7.2 by using 1 N NaOH and finally the volume was made upto 100 ml by distilled water. (b) 3.8 g of magnesium chloride was dissolved in 100 ml of distilled water. 1.25 ml fo the above stated phosphate buffer solution and 5.0 ml of magnesium chloride solution were mixed in a volumetric flask (1,000 ml) and the volume made upto 1 litre using distilled water. After 15 to 20 min., this dilution water (1 L) was sterilized in an autoclave.

Nutrient agar medium: 5.0 g of trypton, 2.5 g of yeast extract, 1.0 g of glucose and 15.0 g of agar were added to
1 litre of distilled water and sterilized in an autoclave.

**Method**: Using the soil-extract solution, more diluted samples were prepared using sterilized dilution water in sterilized bottles in the following ratios (i) 1:1, (ii) 1:10 (iii) 1:100, (iv) 1:1,000, (v) 1:10,000, (vi) 1:1,00,000. 1ml aliquots of the above diluted samples were transferred separately to sterilized Petri plates in three replicates. 12 ml of sterilized nutrient agar medium, (temp.45°C) was poured to each of these Petri plates. The medium and the samples were mixed by gently moving the Petri plates in circular order. When the medium was solidified, Petri plates were inverted and incubated at 37°C for 48 hours in an incubator. On completion of incubation period, bacterial colonies were counted in each plate and calculated as No./ml by dividing it with dilution factor (140). The average value was recorded and shown in Table 2-9.

**RESULTS AND DISCUSSION**

The primary environmental variables influencing soil bacteria include: moisture, aeration, temperature, organic matter, acidity and inorganic nutrient supply. Some lesser variables such as cultivation, season, and depth have also been described to influence the density and composition of the bacterial flora. The plate count of the bacteria as found here (Table 2-9) was $20 \times 10^6$/g soil. The value of a typical soil-profile in USA for a depth of 3-8 cm is reported to be $7.8 \times 10^6$/g soil (39). The count found here thus indicated a correct order.
Table 2-9. THE PLATE COUNT AND THE ENVIRONMENTAL CONDITIONS* OF THE SOIL.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Environmental Parameters</th>
<th>Values found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Soil depth (cm)</td>
<td>2.0</td>
</tr>
<tr>
<td>2.</td>
<td>Moisture (%)</td>
<td>5.3</td>
</tr>
<tr>
<td>3.</td>
<td>Oxygen level (ppm) (10% slurry)</td>
<td>79.0</td>
</tr>
<tr>
<td>4.</td>
<td>Temperature (°C)</td>
<td>23.5</td>
</tr>
<tr>
<td>5.</td>
<td>pH</td>
<td>7.2</td>
</tr>
<tr>
<td>6.</td>
<td>Organic matter (%)</td>
<td>6.4</td>
</tr>
<tr>
<td>7.</td>
<td>Inorganic nutrients (ppm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>63.0</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>11,10,000.0</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>682.0</td>
</tr>
<tr>
<td></td>
<td>Mo</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Co</td>
<td>16.0</td>
</tr>
<tr>
<td>8.</td>
<td>Season of soil collection</td>
<td>Winter (Month-January) (after harvest)</td>
</tr>
<tr>
<td>9.</td>
<td>Plate count found (/g)</td>
<td>20 x 10^6</td>
</tr>
</tbody>
</table>

* Parameters at S.No. 5-7 were determined earlier and described in Section 2.2 & 2.3.
SUMMARY

This chapter was devoted to having a close acquaintance with the soils of the local areas which formed the central object for studying the impacts of the effluents. For this purpose, the soil types, their morphological features and their distributions in different districts of the Chhattisgarh region were studied. Some important terms related to soil structure (bulk density, infiltration rate, field capacity, wilting point) were also explained. The importance of key-parameters (pH, electrical conductivity, organic carbon, available nutrients, cation exchange capacity, silica, iron oxide, aluminium oxide) which affects the functionality of the soil was explained. The four principal soil types (Bhata, Matasi, Dorsa, and Kanhar) were collected from farm fields as per prescribed procedures and their physico-chemical characteristics (pH, electrical conductivity, organic carbon, available nutrients, cation exchange capacity, exchangeable cations, SiO$_2$, Fe$_2$O$_3$, Al$_2$O$_3$) were determined. A gradation amongst the four soil types used here was found evident from the data obtained. While the Bhata and Matasi type indicated weakly acidic to neutral nature, the Dorsa and Kanhar types were neutral to slightly alkaline. On the basis of available nutrients (N,K,P,CEC) and the organic carbon, the Kanhar type appeared to be the most fertile of the four types. It was found to be more useful to select this particular soil type for the study of the effluents' impacts.
The status of micronutrients (Zn, Cu, Mo, Co, Mn) and micro-pollutants (As, Cd, Pb, Hg, Ni, Cr) were studied in detail. The role of the micronutrients in the growth of the plant species was studied. A literature survey to identify the channel of entries of toxic metals from different industrial sources and the resulting harmful effects on crops and vegetations was carried out.

The humic acid contents of the paddy soil (Kanhar type) were studied in detail. For this purpose, the genesis and the structural aspects of the humic acid were studied and described. The reactivity of the humic acid with metal ions were also studied. For the experimental studies, the humic acid was isolated from the paddy soil and purified with standard procedures. The IR spectra of the pure humic acid were recorded in the range of 4000 to 400 cm⁻¹. The bands observed in the spectra were assigned to respective functional groups. On the basis of the IR spectra, the presence of -OH, -COO⁻, amide, and thioamide groups were indicated in the humic acid.

The bacterial count in the Kanhar variety of paddy soil was determined by the standard plate count method. Prior to the plate count determination of the soil bacteria, the environmental conditions of the soil (depth, moisture, oxygen level, temp., pH, organic matter, inorganic nutrients, and the season of soil collection) were also determined. The plate count of the bacteria was found to be 20 x 10⁶/g soil. The order of the count was found to be in agreement with that reported elsewhere.
REFERENCES


