WS2	Wet Season 1999
DS3	Dry Season 2000
WS3	Wet Season 2000
DS4	Dry Season 2001
WS4	Wet Season 2001

3.3 Sampling procedure

From each site, soil samples were collected at two depths (0 – 15 cm and 15 – 30 cm) to ascertain the impact of paper mill operation not only on the topsoil but also on the subsoil.

Collection of soil samples was done by using an auger. In each case, a triangular block was cut with the help of the auger. Eight samples were collected from a single station at small distances from one another and these were then mixed together to obtain a composite, representative sample.

Soil samples were brought to the laboratory in sterile containers. Before analysis, the samples were spread out thinly on a piece of hard paper for drying in air in a shade. Soil samples were brought to the laboratory in sterile containers and were spread out thinly on a piece of stout paper for drying in air in a shade. Entry of dust particles from air is prevented by covering the soil samples with superfine wire net. The big lumps were broken down, and plant roots, pebbles and other undesirable matter were removed.

After the soil became completely dry, it was sieved through a 2 mm sieve. The samples were preserved in clean polythene bags for analysis.

3.4 Selection of parameters

A large number of parameters are generally used to characterize the soil quality criteria. The most important consideration should be those properties of soil, which influence the movement, and retention of water that contribute to store and supply of nutrients. The soil samples were analyzed for almost all the parameters of importance with respect to its suitability for crop production. Taking into account the available experimental facilities and the possible soil contaminants entering into soil due to the discharge of the pulp and paper mill effluent and dumping of solid wastes, the following parameters were chosen for the present work:

i) Soil texture,

ii) Bulk density,

iii) Water holding capacity,

iv) Hydraulic conductivity,

v) pH,

vi) Electrical conductance,

vii) Organic carbon,

viii) Anions: chloride and sulphate

ix) Nutrients: nitrogen, phosphorous and potassium, and also boron,

x) Metals: Sodium, Potassium, Calcium, Magnesium, Iron, Manganese, Chromium, Lead, Nickel, Cadmium, and Mercury,

xi) Soil composition with respect to major and minor oxides, with the help of XRF measurement,

xii) Identification of the soil clay fractions with XRD and IR measurements.

3.5 Importance of the selected parameters and methodology for their measurement

The significance and importance of each parameter along with the method of determination in soil samples is described below. Analysis for all the parameters was done following standard procedure [Jackson, 1967; APHA, 1995; Boruah and Borthakur, 1997]. All chemicals used in the analysis were of analytical or equivalent grade and were used without further purification.

3.5.1 Soil texture

soil texture determination helps in indirect benefits in terms of characterizing water and nutrient retention and transmission properties of soil, in evaluating structural condition and dispersibility, it also provides information on an estimate of ion absorption and the exchange behavior of soil [Tamhane et al., 1964].

Soil texture is determined by relative percentage of clay, silt and ~~loam~~ Sand. The hydrometer method was used for this purpose. 50g of the air dry soil was taken in a 500 ml beaker and was treated with 50-60 ml of 6 % H_2O_2. The beaker was covered with a watch glass and was placed on a water bath at about 70 oC until the organic matter was oxidized. The beaker was allowed to cool. The above process was repeated three times and finally the beaker was put on the water bath again for about two hours to remove the excess H_2O_2. About 400 ml of distilled water and 100 ml of calgon (sodium

hexametaphosphate) solution was added into it. The suspension was stirred with an electric stirrer for about 10 minutes. The suspension was then transferred into a settling cylinder and the volume was made up to one liter with distilled water. The mixture was shacken vigorously back and forth for 1 minute by placing a rubber stopper over the mouth of the cylinder. Time was recorded immediately. The hydrometer was inserted into the suspension. The first hydrometer reading was taken after 4 minutes. The temperature of the suspension was recorded. The hydrometer was removed carefully and washed with distilled water. The hydrometer was calibrated at 19.45 °C. The suspension was allowed to remain undisturbed for two hours and hydrometer reading was taken by reinserting the hydrometer into the suspension.

The sand, silt and clay percentage were then calculated from the following expressions:

$$\text{Sand \%} = 100 - P_4$$

$$\text{Silt \%} = P_4 - P_{120}$$

$$\text{Clay \%} = P_{120}$$

where

$$P_4 = \frac{(R_4 \pm r) \times 100}{W}$$

$$P_{120} = \frac{(R_{120} \pm r) \times 100}{W}$$

and R_4 = hydrometer reading at 4 min, R_{120} = hydrometer reading at 120 min

r = temperature correction = $\pm (t - 19.45) \times 0.2$

W = oven dry wt of soil sample

t = temperature in oC at the time of measurement.

If the working temperature is more than 19.45 °C, the temperature correction, r, is positive and if it is less than 19.45 °C, r is negative.

3.5.2 Bulk density

Bulk density of a soil is required to be known for determining the degree of compactness. It also helps to determine the soil structure, to calculate the soil pore space etc. It is also an indicator of aeration status of soil and it provides information on the environment available to the many soil microorganisms, which live within them.

Soil bulk density (ρ_b) is defined as the oven dry weight of soil per unit of its bulk volume. The bulk volume comprises volume of soil solids and of pore spaces. The density ρ_b is expressed in the unit of g cm^{-3}. As its value depends upon the amount of air space, it is therefore affected by several factors, viz., texture, arrangement of particles, organic matter content, state of packing or compaction, etc.

Bulk density was determined in the laboratory in repacked cubes as per the procedure of Chopra and Konwar [1986] and the values were given by –

$$\text{Bulk density } (\rho_b), \text{ g cm}^{-3} = \frac{W_2 - W_1}{V}$$

where W_1 = Weight of the empty bottle.

W_2 = Weight of the bottle packed with oven dry soil

and V = Volume of the bottle, obtained by measuring the volume of water required to fill it completely.

3.5.3 Water holding Capacity

The knowledge of water holding capacity is essential to determine soil water depletion pattern which is required for scheduling of irrigation, to determine available water capacity of soil, to determine soil strength, rate of movement of water in the soil and the plant response to water.

Water is held in soils because of its polar character with the two hydrogen atoms forming the positive end of the dipole and oxygen making the negative end. The water dipoles are held strongly due to attraction of positive and negative ions present in soil. The hydrogen atoms of the water dipole are attracted towards the nearby negatively charged ions, such as oxygen, even to the oxygen of another adjacent water molecule. Most soil minerals are composed of 70-85 % by volume of oxygen. Hydrogen atoms of water molecules bond strongly to these surface oxygen atoms by adhesive bonding.

Water holding capacity of the collected soil samples was determined by using circular brass box of known weight (a). The perforated bottom plate of the box was supported on a Whatman No. 1 filter paper. Approximately 10g of the soil was added to the brass box and weighed (b). The box with the soil was kept in a petri dish containing water, so that about one fourth of the box remained under water. The box was kept overnight. Next day, the box was removed and the excess water was allowed to drain off. When there was no more water dripping from the bottom of the box, it was weighed again (c). The weight (m) of a moist Whatman No. 1 filter paper was also measured. The values of water holding capacity were calculated from the following expression:

$$\text{Water holding capacity \%} = \frac{C - (b + m) \times 100}{b - a}$$

3.5.4 Hydraulic Conductivity

Hydraulic Conductivity is significant in determining the internal drainage of the soil. As the final infiltration rate of a soil is equal to Hydraulic Conductivity, it is also important in determining what proportion of rainfall or irrigation will run off the soil. The importance of Hydraulic Conductivity eventually lies in predicting behavior of soil under irrigation and in land classification.

The mathematical expression for the vertical water flow through soil is called Darcey's law. Darcey stated that the rate of flow increased with an increased depth of water above the soil through which it flowed. The flow decreased with an increased depth of soil. Each soil has different combination of pore sizes and number of pores and each soil has a different flow rate constant, which is called hydraulic conductivity.

Hydraulic conductivity was measured as follows. A soil core of 15.4 cm height was made inside an aluminum ring and the core was supported on a filter paper placed on a perforated aluminum plate, this arrangement was placed on a funnel clamped to a rack. Water is delivered to the core with an aspirator bottle maintaining a constant head of 2.5 cm above the core and water flowing down the core was collected in a beaker in an interval of 30 minutes. The hydraulic conductivity was calculated from the formula:

$$\text{Hydraulic conductivity (K), cm/min} = \frac{QL}{HAT}$$

where,

Q = Quantity of water collected in cm^3

A = Cross sectional area of the inside of the ring in cm^2

L = Length of the soil core in cm

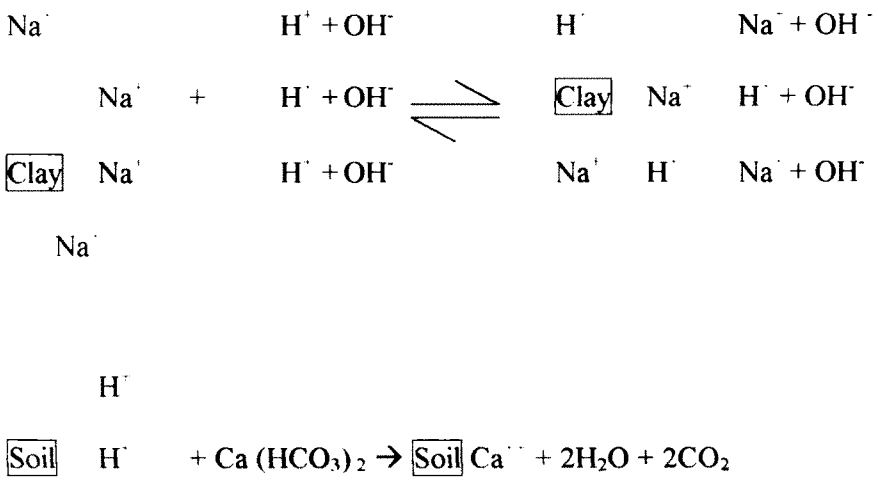
H = Total height of water column (i.e. core height + water head) in cm

T = Time of flow in minutes.

3.5.5 pH

The pH determination is an indispensable means for characterizing soil from the standpoints of nutrient availability and physical condition, viz., structure, permeability, etc. It gives information about the status of microbial environment and its net effect on the mineralization of organic residue and immobilization of available nutrients. Determination the soil pH provides the most rational basis for managing soil for selective agricultural crops, pasture cultivation, forestry, etc. It also provides information on the potency of toxic substances present in soil

The acidity, neutrality or alkalinity of a soil is measured in terms of hydrogen ion activity (active concentration) of the soil water system. The negative logarithm of the hydrogen ion activity i.e. pH of soil, is a measure of only the intensity of acidity and not the amount of the acid present. Soil becomes acidic by the leaching effect of rainwater which replaces basic cations (Ca^{++}, Mg^{++}, Na^{+}, K^{+}) with H^{+} from the carbonic acid formed from water and dissolved carbon dioxide. The exchange mechanism may be as follows [Townsend, 1973]:



Soil pH was determined by using digital pH meter (Elico LI 120) in 1:5 soil/water suspension using standard buffers for calibration.

3.5.6 Conductance

The electrical conductance of the soil is the measure of the soluble salts, which indicates the salinity of the soil. The high electrical conductivity value is a reflection of large amount of anions and cations. Most of the soluble salts are composed of cations like sodium (Na^+), Calcium (Ca^{++}) and Magnesium (Mg^{++}) and anions chloride (Cl^-) sulphate (SO_4^{-2}) and bicarbonate (HCO_3^-) and smaller quantity of potassium (K^+), ammonium (NH_4^+), nitrate (NO_3^-) and carbonate (CO_3^{2-}).

Soil conductivity was determined by using a conductivity bridge (Elico CM180) by using a conductivity cell of cell constant 1.0 in 1:5 soil/water suspension.

3.5.7 Organic Carbon

The role of organic matter in soil in relation to soil fertility and physical conditions is widely recognized [Stevenson, 1986, Johnston, 1986]. The organic matter is the source of plant nutrients, which are released in assimilable forms during microbial degradation. A major proportion of nitrogen (95-99% of the total), phosphorous (33-67% of the total) and sulphur (75% of the total) in soil occur in organic combination, which mineralize them to release the nutrients in inorganic form to be used by plants. It serves as a reservoir of plant nutrients and promotes water storage as well as regulates microbial activity.

The organic carbon (%) of the soil samples was determined by titrimetric method (Walkley and Black, 1974). 1g of the soil sample was taken in a 500 ml conical flask. 10 ml of 1N potassium dichromate solution was added and shaken. 20 ml of concentrated sulphuric acid (containing 1.25 % Ag_2SO_4) was added and the flask was swirled 2 or 3 times. The flask was allowed to stand for 30 minutes for the reaction to complete. 200 ml of distilled water was poured to the flask to dilute the suspension. 10 ml of 85 % H_3PO_4 and 1 ml of diphenylamine indicator were added and the solution was titrated with 0.5 N ferrous ammonium sulphate till the colour flashed from violet through blue to bright green. Volume of the ferrous ammonium sulphate solution was noted. A blank titration without soil was carried out in a similar manner.

Calculation

Weight of the sample = W (1.0 g)

Volume of 0.5 N $Fe(NH_4)_2(SO_4)_2$ solution used = B ml for the blank titration.

Volume of 0.5 N $Fe(NH_4)_2(SO_4)_2$ solution used = S ml for the sample titration.

Volume of the 1N $K_2Cr_2O_7$ used for oxidation of C = 0.5 x (B-S) ml

[1 ml of 1N $K_2Cr_2O_7$ (=1meq) = 0.003 g of org C]

[Walkley averaged a 77 % recovery of org. C by this method. Thus the correction factor is 100/77 = 1.3]

% of org. C in the soil (uncorrected) = 0.5 x (B-S) x 1N x 0.003 x (100/W) = Q

% of Org. C in the soil (corrected) = Q x 1.3 = R

3.5.8 Total Nitrogen

Of the total amount of nitrogen present in soil, nearly 95 - 99 % is in the organic form and 1 – 5 % in the inorganic form as NH_4^+ and nitrate (NO_3^-) [Troeh and Thompson 1993].

Total nitrogen is merely an indicator of the soil potential for the element, but not the measure in which it becomes available to the plant. During growth and development an average of only 0.5 - 2.5 % (rarely 5 %) of the total nitrogen is converted into forms accessible to plants [Rao, et al.1997]. Total nitrogen content of soil is needed for the evaluation of C/N ratio of soil, which gives an indication of the process of transformation of organic N to available nitrogen like ammonical nitrite and nitrate N.

The most common method for estimating nitrogen in soil is Kjeldahl method, which measures organic and ammonium forms excluding nitrate. In the present work, soil nitrogen was estimated by micro Kjeldahl method [Jackson, 1967], the brief procedure for which is as follows:

10 g of soil sample in a 300 ml kjeldahl flask was mixed with 25 ml of distilled water to make a suspension. 20 g of the digestion catalyst mixture (20 g copper sulphate, 3 g mercuric oxide, 1 g of selenium powder were mixed together and 1g of this was mixed with 20g of sodium sulphate to obtain the catalyst mixture) and 35 ml of concentrated $H_2 SO_4$ were added to the suspension and were mixed by gentle swirling motion. The content was heated at low heat for about 10-30 minutes until the frothing stops. The heat was then raised and the flask was rotated at every five minutes interval for about two hours. Then the digested solution was cooled and the supernatant liquid was transferred to 100 ml volumetric flask. The residue was washed several times with distilled water and after each washing, the supernatant liquid was transferred to the flask.

25 ml of the above solution was taken in a round bottom flash of the micro kjeldahl apparatus. 25 ml of 40 % NaOH solution and pieces of Zn were added to the flask and swirled gently. A 500 ml conical flask containing 25 ml of boric acid and mixed indicator (Mixed indicator was prepared by mixing alcoholic solutions of bromocresol green (0.5 %) and methyl red (0.1 %) in 2:1 ratio. 5 ml of this solution is added to 100 ml of 4 % boric acid solution) was placed below the condenser. Distillation was commenced and about 100 ml of distillate was collected. This was then titrated with O.1 N HCl until the colour changes from blue to light pink.

A blank titration was run with distilled water using other chemicals in same quantities.

Total nitrogen was calculated from the formula

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

where

a = ml of HCl acid required for titrating sample solution

b = ml of HCl acid required for titrating blank

N = Normality of acid solution

V = ml of total solution after digestion (= 100ml)

v = ml of digested solution taken for distillation (= 25 ml)

S = weight of the soil taken (10 g)

3.5.9 Available Phosphorous

Both inorganic and organic forms of phosphorous occur in soils, both are important to plants as sources of this element, and the relative amounts in the two forms vary greatly from soil to soil (Zhang and Karathanasis, 1997) The term available Phosphorous (P) refers to the inorganic form occurring in the soil solution, which is almost exclusively orthophosphate. This orthophosphate occurs in several forms and combination, and only a small fraction of the total amount present may be available to plants.

The phosphate concentration in solution is governed by heterogeneous equilibrium in which it takes part. The situation can be represented as:

P adsorbed in solid phase ⟷ P in soil solution.

⟷ P Precipitated.

The available P is considered to be a fairly good indicator of the P-supplying capacity of a soil.

Phosphorus in soil is generally determined as available phosphorus, which can be extracted from soil with 0.002 N H_2 SO_4. After extraction, the phosphorous was estimated spectrophotometrically by Dickman and Bray [1940] method.

10 g of air-dry soil sample was taken in a 500 ml conical flask and 200 ml of 0.002 N H_2SO_4 was added. The suspension was shaken for about half an hour and filtered though Whatman No. 50 filter paper to get a clear solution. 2 ml of ammonium molybdate solution and 5 drops of stannous chloride reagent were added to 50 ml of the extract and a blue colour developed. The intensity of the blue colour was measured by using spectrophotometer (Perkin-Elmer UV-visible Lambda EZ 201) at 690 nm. A standard curve was prepared with potassium hydrogen orthophosphate solutions in the range of 0.0 to 10 mg dm^{-3} following the same procedure.

The available phosphorus is given by

$$P\ mg/kg = \frac{mg\ P/dm3\ in\ soil\ extract \times V}{S \times v}$$

Where V = total volume of the soil extract prepared (200 ml)

S = wt of soil taken in gram.

v = volume of the aliquot taken for analysis (50ml)

3.5.10 Boron

Boron is unique in soil by its narrow range between deficiency (for plant growth) and toxicity. Less than 1 ppm of B may lead to deficiency and 5 ppm may be toxic.. It is often useful to measure boron-calcium ratio. The most useful measure of available boron is the water-soluble form. Knowledge of water-soluble B in soil is of considerable agricultural significance in the context of its narrow limits between deficiency and sufficiency, its interaction with calcium and its precipitation as calcium metaborate in extreme cases.

Water soluble Boron was estimated by curcumin oxalic acid reagent method [Jackson 1967] by extracting it from the soil samples with water. 10g of air-dry sample were boiled with 50 ml of distilled water. The suspension was filtered through Whatman No. 44 filter paper and the volume was made up to 100ml. 0.1 ml HCl followed by 5.0 ml of concentrated H_2SO_4 was added to 50 ml of this solution. The solution was allowed to cool and 5 ml of curcumin reagent was added. The intensity of the colour of the B-complex was measured spectrophotometrically (Perkin-Elmer UV-visible spectrophotometer Lambda EZ 201) at 585 nm. The water-soluble boron is given by

$$B, \text{mg / kg} = \frac{\text{mg B/dm3 of soil extract} \times V}{S \times v}$$

where V = total volume of the soil extract prepared (100ml)

S = wt of soil taken in grams

v = volume of aliquot taken for analysis.

Several standard boron solutions were prepared from boric acid to obtain a calibration plot.

3.5.11 Chloride and Sulphate

Estimation of soluble salt like Cl^-, SO_4^{-2} is necessary to examine soil for saline constituents for irrigation purposes. Knowledge of soluble salt is also important to establish limits for essential elements in particular which can indicate deficiencies and physiological disturbances and especially for the determination of excess chloride, which is just as detrimental as a deficiency.

Chlorine exists in soil almost entirely as chloride ion (Cl^-), a very soluble and mobile ion. Sulphur has long been recognized as essential for plant and animal growth. Plants that are sulphur deficient are characteristically small and spindly. The younger leaves are light green to yellowish, and in the case of legumes, nodulation of the roots is reduced. The maturity of fruits and seeds is delayed in the absence of adequate sulphur.

The most accessible form of sulphur is sulphate. Despite the fact that plants absorb S almost exclusively as SO_4^{2-}, mobility of SO_4^{2-} in soil may not always yield satisfactory results in accordance with the time of sampling while assessing SO_4^{2-} availability. Development of chemical tests for the estimation of available sulphur (as SO_4^{2-}) is of recent interest in soil testing work, particular in some areas and for certain crops, whose requirement of this nutrient is high enough, often, exceeding that of P.

Measurement of Chloride. The best-known reaction for chloride determination is based on the formation of nearly insoluble Ag salt:

$$Cl^- + Ag^+ \rightarrow Ag\,Cl$$

$AgNO_3$ solution in the presence of the indicator K_2CrO_4 was used for precipitating Cl^-

$$Cl^- + AgNO_3 \rightarrow Ag\,Cl + NO_3^-$$

$$K_2CrO_4 + 2\,Ag\,NO_3 \rightarrow Ag_2\,CrO_4 + 2KNO_3$$

Reddish brown ppt.

First, the most stable salt (AgCl) is formed and then the excessive $AgNO_3$ react with K_2CrO_4 forming a reddish brown precipitate of Ag_2CrO_4, which indicates the end point of the reaction.

40 g of the soil was dissolved in 200 ml of water. 50 ml of the aliquot from the soil water extract was taken. To it, 5-6 drops of K_2CrO_4 indicator was added. The solution was titrated with 0.02 N $AgNO_3$ solution (with stirring) till the first reddish brown tinge appears. The volume of $AgNO_3$ (titre value) required refers to the amount of chloride present.

Calculation

[1ml of 0.02 N $AgNO_3$ (0.02 meq $AgNO_3$) = 0.00071 g Cl^-]

Volume of aliquot taken (from soil extract) = V ml (50 ml)

Volume of $AgNO_3$ solution used for titration = T ml (titre value)

Wt of the soil taken = 40 g.

Normality (N) of $Ag\ NO_3$ = 0.02

meq of $AgNO_3$ used for titration = 0.02 x T

meq of Cl^- per litre of soil extract or water sample = (0.02 x T) x (1000 / V)

meq of Cl^- per kg of soil = [(0.02 x T) x (200/V) x (1000/40)]

Estimation of Available sulphur. The turbidimetric procedure is widely used in the estimation of available S in the soil because of its rapidity. The extraction was made with monocalcium phosphate [Ensminger, 1954].

In this method, soil is shaken with a solution of monocalcium phosphate. During this extraction, phosphate ions displace the absorbed sulphate. Calcium ion depresses the extraction of soil organic matter, thus eliminating contamination from extractable organic sulphur. This method extracts soluble SO_4^{2-}, plus a fraction of the absorbed SO_4^{2-}. The filtrate is, then, analyzed for S by the turbidimetric method.

Turbidimetric Procedure. The filtrate is treated with barium chloride in the presence of gum acacia solution, and the turbidity produced by the precipitation of SO_4^{2-} as $BaSO_4$, is measured colorimetrically. Gum acacia, in this determination, helps to prevent rapid settling of $BaSO_4$ precipitate.

$$Ca(H_2PO_4)_2.2H_2O \rightarrow Ca^{2+} + 2H_2PO_4^- + 2H_2O$$

$$\text{Soil colloid}]SO_4 + 2H_2PO_4^- \rightarrow \text{Soil colloid}]\begin{matrix} H_2PO_4 \\ \\ H_2PO_4 \end{matrix} + SO_4^{2-}$$

$BaCl_2 \rightarrow Ba^{2+} + 2Cl^-$

$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4$ (precipitate)

20 g of the air-dried soil sample was taken in a 200 ml flask. 100 ml of extracting solution was added and was shaken for about 30 minutes. The suspension was filtered through Whatman No. 42 filter paper. 20 ml of the aliquot of the extract was transferred to a 25 ml volumetric flask. 1 g of $BaCl_2$ crystals and 1 ml of 0.25 % solution of gram acacia was added and shaken for 1 minute. The volume was made up to the mark with distilled water. Transmittance was measured using colorimeter (Systronic Model 101) using 420 nm wavelength.

Several standard sulphate solutions were prepared from potassium sulphate salt to obtain a celebration curve.

Calculation

Wt of the soil taken = 20 g

Volume of the extractant added = 100 ml

Volume of the aliquot taken = 20 ml

Final volume = 25 ml

Transmittance (%) as read from the colorimeter = T

ppm of S from the standard curve = C

First dilution = 100 / 20 = 5 times

Second dilution = 25 / 20 = 1.25 times

Total dilution = 5 x 1.25 = 6.25 times

Now, available S in the soil (ppm) = C x 6.25.

3.5.12 Common metals (Ca, Mg, Na, K and Fe)

Calcium and magnesium. Determination of exchangeable Ca and exchangeable Mg is essential to characterize the soil with special reference to the momentary state of Ca and Mg supply. The active Ca value is an indication of potentially active quantities, which maintain its concentration through the solubility product principle. Most economic crops yield best in soils when Ca^{2+} dominates the exchangeable cations.

Sometimes high Ca/Mg ratio hampers the uptake of Mg, but most commonly encountered antagonistic ion is K. Addition of K to soil may decrease the ease of displacement of Mg and result in less available Mg. High concentration of K in the plant may prevent Mg from functioning properly. So measurement of all the three cations (Ca, Mg and K) is important. Since a balanced supply of these cations is possible only when their ratio in the soil solution is maintained within the range of some threshold values, and thus may necessitate tempering the soils with amendments under acidic and sodic soil condition.

Sodium. The measurement of sodium is important because soils with high exchangeable sodium level crust badly and swell and disperse, greatly decreasing the soil's hydraulic conductivity or water permeability. Clay particles disperse and plug the soil water flow channels, as does swelling of clay particles. Decreased permeability

interferes with the drainage required for salinity control and with the water supply and aeration required for plant growth.

Potassium. Soil contains about 0.05 – 2.5% of total potassium It is distributed in mineral form (lattice K, 90 – 98 %), fixed non-exchangeable K (1 – 10 %) and exchangeable plus water soluble K (1 – 2 %) [Baruah and Borthakur, 1997]. Both water soluble and exchangeable potassium are most accessible to plant. The neutral normal ammonium acetate extract contains both water soluble and exchangeable K. Potassium extraction by this method is considered as a suitable index of K availability in most soils. It gives information about the dynamics of exchangeable K and the supply status of the plant.

Iron. Iron is one of the micronutrients necessary for the maintenance of chlorophyll in plants. It is an important part of the plant's oxidation-reduction reaction. The exchangeable Fe is the one that is available to the plants. The major problem with iron availability is how to keep iron sufficiently soluble for plants to absorb enough of it. Information in the solubility relationship of the forms of iron is helpful in predicting possible incidence of deficiency and toxicity of iron, which depends upon the soil pH, textural gradation, etc.

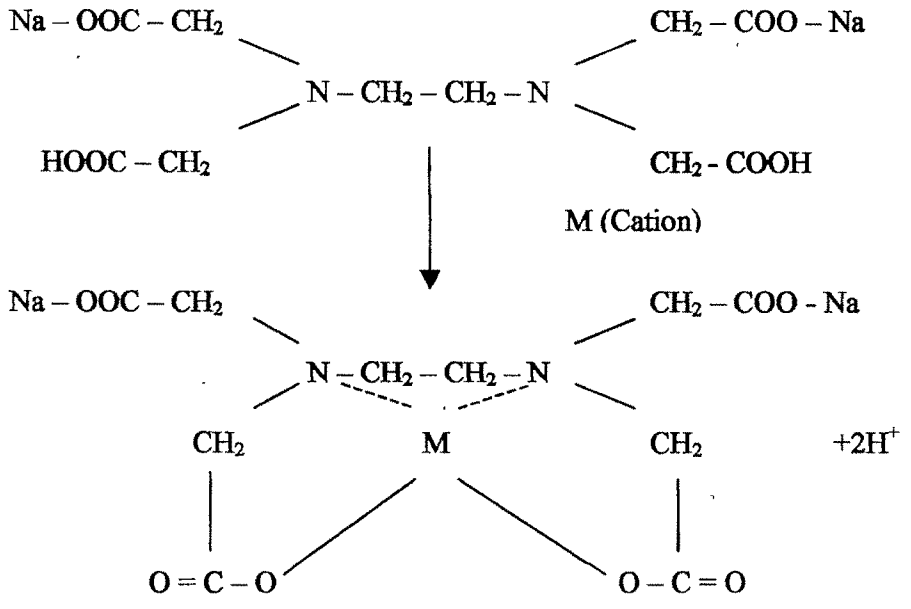
Method of estimation of Ca, Mg, Na and K. The exchangeable Ca^{2+} and Mg^{2+} ions in soil are extracted with a neutral 1.0 N NH_4OAC solution when the cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) are replaced with NH_4^+ ions. In the extracted solution, the Ca^{2+} and Mg^{2+} were determined by the complexometric titration method involving

ethylenediaminetetraacetic acid (EDTA) and the Na^+ and K^+ were determined by the flame photometric method.

The most widely used salt of EDTA is the disodium salt with the formula $Na_2H_2Y.2H_2O$, where Y is the tetravalent anion of EDTA. When Ca^{2+} is treated with $H_2Y^{-2,}$ a very stable complex is formed. The generalized reaction of EDTA with Ca^{2+} ion is shown below.

$$Ca^{2+} + H_2Y^{-2} \rightarrow CaY^{2-} + 2H^+$$

Mg^{2+} ion forms a similar complex, MgY^{-2}, which is far less stable than the Ca-complex. The characteristic reaction showing the complex formation of EDTA with a metal cation M is as follows [Hesse, 1971]



Preparation of the Ammonium Acetate extract. 50 g of the air-dried sample was treated with 40 % alcohol and filtered through Whatman No. 50 filter paper. The soil

was washed 4-5 times with 50 ml portion 40 % alcohol. Then the soil was treated with 100 ml 1.0 N NH_4OAC solution and kept overnight. The suspension was filtered through Whatman No. 42 filter paper and the volume was made up to 500 ml with distilled water. A portion of the NH_4- acetate extract was evaporated to dryness to eliminate the interference of organic matter. The residue was dissolved in aqua regia. Again, it was evaporated to dryness. This time the residue was dissolved in distilled water to make up the original volume of the extract evaporated.

Calcium and magnesium. 50 ml of the aliquot was taken in a conical flask. 1ml of the buffer solution (NH_4Cl – NH_4OH) and 100 mg of Eriochrome Black T indicator were added. The wine red solution was titrated with 0.01 N EDTA solution till the colour changes to blue.

Calcium. 50ml of the aliquot was taken in a conical flask. 2 ml of 10 % NaOH solution and 100 mg murexide indicator were added. The pink colour solution was titrated with 0.01 N EDTA solution until the pink colour changes to dark purple.

Calculation

$$\text{Ca, meq/kg} = \frac{A \times 400.8 \times V}{v \times 20.04 \times S}$$

$$\text{Mg, meq/kg} = \frac{(B-A) \times 400.8 \times V}{v \times S \times 1.645 \times 12.16}$$

where

A = volume of EDTA (ml) used for Ca^{2+} determination

B = Volume of EDTA (ml) used for Ca + Mg determination.

V = Total volume of soil extract prepared (500ml)

v = Volume of the soil extracts titrated (50ml)

S = weight of the soil sample (50 g).

Sodium and Potassium. The Na^+ and K^+ in the filtrate (NH_4-acetate extract) were measured by the flame photometric method (Elico Model CL361).

Estimation of Iron. Extraction of Fe is complete only if the pH is lowered to 3; but under such conditions some non-exchangeable iron is also extracted. The extraction procedure, normally followed for the available iron in soil and its subsequent estimation, is given below.

25 g of the soil sample was extracted with 250 ml 1.0 N ammonium acetate solution and the pH was made 3.5 by adding an appropriate volume of 1 M sodium acetate-acetic acid buffer. 50 ml of the aliquot was taken in a flask, 2 ml of concentrated HCl and 1 ml $NH_2OH.HCl$ solutions were added. The volume was reduced to about 20 ml by boiling to ensure dissolution of all the iron. 4 ml of 0.4 % phenanthroline solution was added and the volume was made up to 50 ml. The intensity of colour developed was measured by UV-visible spectrophotometer (Perkin-Elmer Lambda EZ 201) at 515 nm.

Several standard Fe-solutions were made from $Fe(NH_4)_2(SO_4)_2.6H_2O$ salt, which were used to obtain a calibration plot and the iron concentration (mg dm^{-3}) in the aliquot was

determined. The iron content of the soil sample was calculated using the following formula:

$$\text{Fe, meq/kg} = \frac{(\text{mg Fe/dm}^3 \text{ of soil extract}) \times V}{v \times S \times E}$$

Where V = Total volume of the soil extract prepared (250 ml)

S = Weight of the soil taken in gram (25 g)

v = Volume of aliquot taken for analysis(50 ml)

E = Equivalent weight of iron.

3.5.13 Trace elements

The ecological problem of soil contamination by toxic heavy metals is of extreme importance in the present global scenario. The contamination of soil with heavy metals from sewage can potentially result in phytotoxicity [Chaney et al., 1978] and increased movement of metals into the food chain. Some of the trace metals and other elements present in soil act as micronutrients for plants while others may be toxic in nature. Even some micronutrients can be toxic if the concentration exceeds a certain critical limit. The effectiveness of various heavy metals such as Mn, Pb, Cd, Ni, Hg, Cr, etc. in soil is governed by the nature and extent to which they are bound to clay minerals and soil organic matter. It has been observed that the presence of these heavy metals in agricultural soil greatly influences the availability of other nutrients for plant growth Among these, manganese is a micronutrient white chromium, nickel, lead, mercury are

hazardous and create complication in the utilization of other nutrients [Khan and Khan, 1983].

Method of determination of Ni, Cr, Mn, Pb, Cd, Hg. Air-dried soil samples were grounded and screened through 80-mesh sieve. The metals were extracted from the soil as per procedure given by Pinta [1975]. 1.0 g of the sieved sample was digested with 30 ml of acid mixture (4 parts of concentrated H_2SO_4, 2 parts of concentrated HCl, and 1 part of concentrated HNO_3); the mixture was heated gently at first, and then more strongly until white fumes were no longer evolved. The digested soil was taken up with hot dilute HCl (1:1), filtered through a filter paper (Whatman No. 42) and was washed several times with distilled water. The volume of the filtrate was made up to 100 ml. The concentration of the metals was measured with the atomic absorption spectrophotometer (Varian Spectra AA 220) and the content in the soil samples was found using the calculation formula:

$$\text{Metal concentration, mg/kg} = \frac{P \times Q \times R}{W}$$

Where P = Concentration of metal in digested solution

Q = Final volume of digested solution, ml

R = Further dilution ratio

W = the weighed amount of the sample.

The detailed experimental conditions for AAS analysis are given in Table 3.2.

Table 3.2: Analytical conditions for atomic absorption analysis. Only air-acetylene flame was used with a burner height of 13.5 mm.

Element	Wavelength (nm)	Slit width (nm)	Working range (ppm)	Lamp current (mA)
Mn	279.5	0.2	0.02 – 5.00	5
Cd	228.8	0.5	0.02 – 3.00	4
Ni	232.0	0.2	0.1 – 20.00	4
Cr	357.9	0.2	0.06 – 15.00	7
Hg	253.7	0.5	2.00 – 400.00	4
Pb	217.0	1.0	0.1 – 30.00	5

3.6 XRD and IR analysis

XRD measurements were done to identify the clay fraction of the soil at the University Science Instrument Centre, Gauhati University using Phillips X-ray spectrometer (PW 1710) using with Cu anode. The scanning range was from 8.0 to 59.9^0 (2θ) in the continuous scan mode. The identification of clay minerals was done by using standard technique [Jackson 1975, Moore and Reynolds Jr., 1989, Imam 1994].

The infrared measurements have always been used as a complementary technique to XRD. The IR spectra were recorded with a Perkin-Elmer FTIR spectrometer (Model Spectrum RXI, Range 4400 – 450 cm^{-1}), using nujol as the mulling reagent. The