MANUAL OF LABORATORY EXERCISE FOR SOIL SCIENCE AND AGRONOMY COURSES

DEPARTMENT OF AGRONOMY
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NAME:_____

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SOIL SCIENCE
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SECTION A
EXERCISE 1 (WEEK 1)

Examination of Soil in the Field, Sampling and Preparation of Soil Samples

Lecturer: Dr. S. Y. Alasinrin and Prof. J. O. Olaniyan

The soil profile may be examined most conveniently in the field but a full description of the soil can only be given after laboratory measurements of the properties of various parts of the profile have been made. Thus, the examination of soil falls into three (3) parts.

A. Field examination
B. Collection of Samples
C. Laboratory examination

Field Examination

Site Characteristics

As the character and development of a soil is highly influenced by drainage, plant cover, cropping history and erosion, the first point to be noted are those dealing with the geography of the site being examined.

Profile Number:

Date of Examination:

Author:

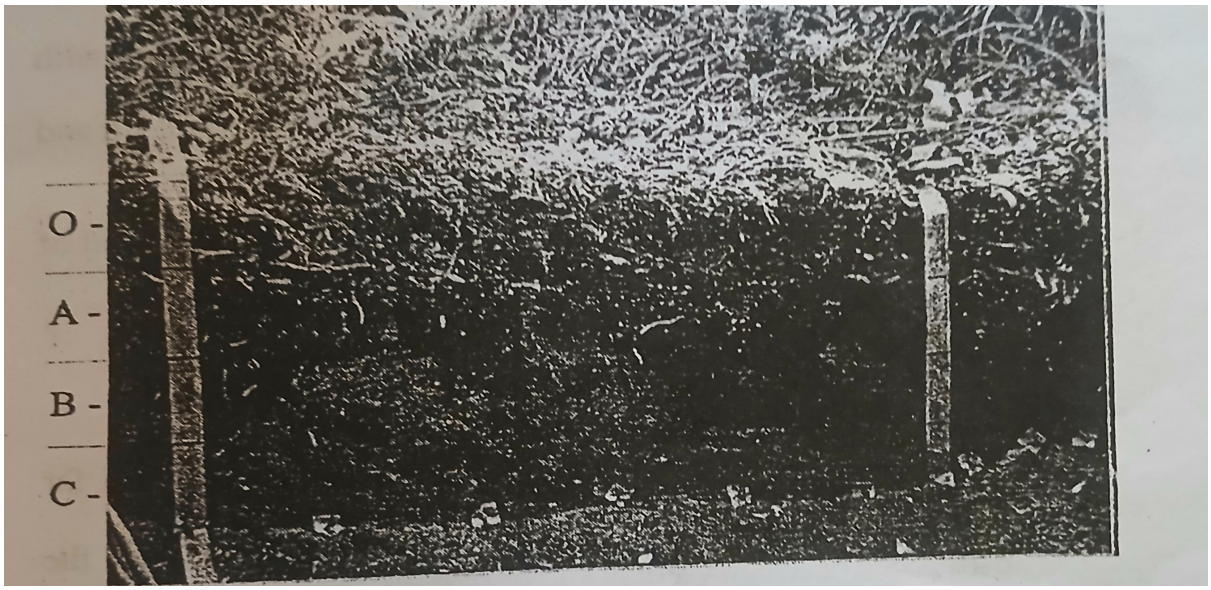
Note:

a. Locality of the site e.g. approx. 30 [unclear] 21°E or approx. 25km NE of Ilorin.
b. Type of relief (topography) e.g. plain, rolling, hilly, mountainous and plateau.
c. Location of the site e.g. hill top, hill slope, valley bottom.
d. Altitude and type of slope e.g. north facing, south facing etc.., regular, irregular, concave, convex, height above sea level in feet.
e. Plant cover e.g. grass (tall or short) savannah, forest swamp. If cultivated, mention "nature" vegetation if possible.
f. Fauna e.g. presence or absence of warm casts, termitaria, termite tubes, ant holes or heaps.
g. Human interference e.g. in cultivation (mention crop) abandoned recently, grazing ground.
h. Drainage e.g. well-drained, poorly drained.
i. Erosion e.g. normal, accelerated, sheet, gully.
j. Slope gradient: state % slope e.g. 3-4% on gently sloping etc.

1. Profile Description

The soil is best studied by examining a vertical section known as soil profile. A profile is a vertical section of the soil from the surface down to the parent rock so; a depth of 1.5m is taken as an adequate exposure of the soil profile features. A profile pit is not necessary for estimating the fertilizer requirement of an area. Here, samples are taken at selected points by boring holes with the auger.

It is important that a fresh surface of a profile should be used for description. A profile consists of a number of horizons. These are horizontal layers of soil which are formed as a result of a number of processes taking place within the soil system.



A shallow soil pit with a face that has been cleaned and prepared for describing the soil profile.

2. Steps in Profile Description

Identify (mark out) the horizons, measure their depths, number and name them from the top downward. The following description should be made for each horizon:

1. Colour – use the Munsel colour chart to describe
   - The colour of the soil matrix
   - Motle colour (if any)
2. Texture – by feel, including gravel content
   - Boulder – contains much of particles of 25cm diameter
   - Stony – contains much of particles of 7.5cm – 25cm diameter
   - Gravelly - contains much of particles of 0.5cm - 7.5cm diameter
   - Coarse sandy - contains much of particles of 0.2 – 2mm diameter
   - Fine sandy - contains much of particles 0.02 – 0.2mm diameter
   - Silty – contains material intermediate in size between clay and fine sand and with floury feel.
   - Clayey- contains materials finer than 0.002mm diameter and with plastic feel.
   - Loamy – compose largely of friable materials of fine sand, silt and clay grades.
3. Structure – describes the degree of soil aggregation i.e. the kind of lumps into which the soil breaks naturally.
   (a) Structureless – no observable aggregation
      - Massive if together e.g. in heavy clays
      - Single grain if not coherent e.g. in dune sand
   (b) Platy-plate like (i.e. flat) with one dimension
   (c) Prismatic – like a prism vertical dimension angular and far more than horizontal
   (d) Columnar-similar to prismatic but not angular and the difference between vertical and horizontal is less i.e more rounded
   (e) Angular blocky-block-like with distinct angular contact
   (f) Sub-angular-blocky-block-like but vertices not sharply angular i.e. more spheroidal
   (g) Granular- mainly granules, each particles on its own and partially roundish
   (h) Crumby- particles of non-uniform shape i.e. breaking into various forms like bread crumbs.

4. Consistence – the degree of cohesion of soil particles. It is reflected in the behavior of the soil when handled or pressed together.

   (a) Wet stickiness 0- non sticky; 1- Slightly sticky; 2- Sticky; 3- Very sticky.

   Plasticity 0- Non plastic; 1- Slightly plastic; 2- Plastic; 3-Very plastic

   (b) Moist 0- loose; 1- very friable material crushes easily but coheres when pressed together.

   Friable: requires little more pressure to crumble than for

   Firm: more resistance to crumbling pressure Very firm: requires strong pressure to crumb

   Extremely firm: soil material crushes only under very strong pressure

   (c) Dry 0- loose; 1- soft breaks into powder under very slight pressure; 2- slightly hard-weakly resistant; breaks easily between thumb and forefinger; 3- Hard; barely breakable between forefinger and thumb but can be broken in the hands; 4- Very hard; can be broken in the hands; 5- Extremely hard – cannot be broken in the hands.

5. Root concentration and Size

   (a) Concentration

      i. Very few 5
      ii. Few 5-10
      iii. Common 10-25
      iv. Many 25

   (b) Size

      i. Very fine 1mm diameter
      ii. Fine 1-2mm diameter
      iii. Medium 2-5mm diameter
      iv. Coarse 5mm diameter

6. Artefacts

   The presence of artefacts (pieces of brick, pattery, flint tools etc.) or other evidence of the activity of man (e.g. charcoal) occurring below normal plough depths like 20cm should be recorded as evidence of disturbance of very long periods of cultivation or separate levels of deposition.

7. Nature of boundary with horizon below
   a. Width of boundary
      I. Abrupt – width less than 2cm
      ii. Clear –width 2-5cm
      iii. Gradual –width 5-12cm
      iv. Diffuse- 12cm wide
   b. Topography of boundary
      i. Smooth: near a plain surface
      ii. Wavy: pockets wider than depths
      iii. Irregular: pockets deeper than widths
      iv. Broken: boundary not continous e.g. separated by cracks

3. A. Type Description

Pit No. 1

Site: University Farm, Ilorin. Pit half way up north face of hill at about 650feet. Irregular surface, generally convex at a point of sampling, slope of 12%. Secondary scrub about two years old, many old worm casts, some surface termite tunnels, large termitarium about 200yards eastward along the hill crest. There is little evidence of recent cultivation. Well drained and normal erosion.

Profile: Very thin surface layer of dead leaves.

0-2" Greyish brown (10YR5/2) coarse sandy loam, structureless, friable, very porous. Many fibrous, dead roots, some branched and tap roots, very dry. Merging into

2-15" Yellowish brown (10YR5/4) fine sandy loam, structureless, slightly compact, porous, some branched and tap roots, a little humus, dry, clear, irregular boundary with

15-30" Reddish grey (5YR5/2) sandy clay with rock and mineral fragments. Fragments increasing in size and number downwards. Structureless, occasional tap roots. No humus, moist above, wet below. Merging into

30" Bedrock. Biotite gneiss

B. Soil Sampling

1. Sampling equipment

A number of tools are exhibited in the laboratory. The tool used depends upon the purpose for which the sample is being taken and the nature of the soil being sampled. Difficulty in sampling occurs particularly in coarse-textured sols (when dry), in stony soils and in very fine textured soils.

(a) Spades, towels, picks e.t.c.

(b) Screw augers: soil must be sufficiently fine textured and moist to adhere to the screws. The sample is very disturbed. Of use in

(c) Post-hole augers: Consist of closed cylinder with 2 cutting edges which loosen the soil and feed it back into the cylinder when the handle is turned. Generally more satisfactory than screw augers. The sample is less disturbed and it is possible to lift dry samples. Usually, the diameter is between 2 and 4 inches.

(d) Core samplers: consist of tubes of varying diameter which are pushed or driven into the ground to the desired depth and then removed. The king tube is a seamless steel tube (which may be extended to any length by screwing sections together) with a tools-steel cutting point of approximately one-inch internal diameter. It is used frequently for obtaining moisture samples. For the accurate determination of bulk density, a cylinder 3-4" in diameter and 32 long is used.

2. Sampling technique

a. Sampling from a pit

i. This method is usually used for obtaining type samples in the soil surveys. It has the advantage that the different horizons in the profile may be easily examined and clearly differentiated. This method also avoids contamination of samples with soil of overlying horizons. Its principal drawback is obvious- the large amount of time and labour involved in digging a pit.

For most physical, chemical and biological determinations, the oven dried soils should not be used as soil properties change with over drying. Nevertheless, determinations made on air-dry soil samples are usually adjusted to an oven-dry basis. In the soil science practical classes, results will be expressed on air-dry basis for the sake of simplicity unless otherwise stated.

Exercise 1

1. Identify the number of horizons in the dug profile pit
2. Measure the depth of the pit
3. Measure the thickness of the different horizons identified.

EXERCISE 2

Mechanical Analysis of Soil by the Bouyoucos or Hydrometer Method

Lecturer: Dr. Kehinde O. Affinnih

Principle

The particle size analysis of a soil estimates the percentage sand, silt and clay contents of the soil and often reported as percentage by weight of oven- dry and organic matter-free soil. The analyses are usually performed on air- dried soil. Based on the proportions of different particle sizes, a soil textural category may be assigned to the sample.

Reagents and apparatus

1. **Calgon (sodium hexametaphosphate) solution, 10%.** Dissolve 100g of calgon in 1 liter of distilled water. Note: this solution should not be kept over one month, when too old it loses its dispersing efficiency.
2. Thermometers
3. Hydrometer with Bouyoucos scale in gram per liter.
4. Soil dispersing strirrer.
5. Reciprocating shaker/multi-mix machine with baffled “Milkshake” cups.

Procedure

i. Weigh 50g of air-dry < 2mm soil (100g in case of very sandy soil) into a 400ml beaker.

ii. Add distilled water to saturation and 10ml of 10% calgon solution.

iii. Stir and let stand overnight (8h).

iv. Transfer the suspension to the dispersing cup and add about 300 ml of tap water.

v. Mix the suspension for 2 minutes with an electric high speed stirrer (ordinary bottles if cup is not available and shake overnight on reciprocating shaker if stirrer is not available).

vi. Transfer the suspension into a graduated cylinder and rinse remaining soil into the cylinder with distilled water.

vii. Insert the hydrometer into the suspension and add water to 1130 ml mark, then remove the hydrometer.

viii. Cover the cylinder with a tight-fitting rubber bung and mix the suspension by inverting the cylinder carefully ten (10) times. Note the time.

ix. At 40 seconds, take a hydrometer reading and measure the temperature of the suspension.

x. Repeat step (viii) and allow the cylinder to stand undisturbed for 2 hours. After two hours, take both hydrometer and temperature readings.

xi. Make necessary temperature corrections.

Temperature (°c)	Hydrometer correction (g/liter)
15	-2.0
16	-1.5
17	-1.0
18	-1.0
19	-0.5
20	Nil
21	+0.5
22	+1.0
23	+1.0
24	+1.5
25	+2.0

Calculations

To calculate sand as %: after 40 seconds, the sand has settled and the hydrometer reading reflects the grams of silt + clay in 1 litre of the suspension. To calculate the amount of sand present in 1 litre of the suspension, subtract this value from the original sample weight.

Sample Calculation

If the hydrometer reading after 40 seconds corrected for temperature is 18.0g per litre, then silt + clay weigh 18.0g in the 1 litre soil suspension. Therefore, the sand weighs 50.0 – 18.0 = 32.0g in the 1 litre suspension (of the original 50.0g of air-dry soil sample). The percentage sand is calculated by dividing the sand content (32g) by the total (50g) and multiplying by 100 as follows.

Sand % = (32.0/50.0) x 100 = 64%

Clay: After 2 hours, the silt has settled. The hydrometer reading now reflects the clay content of the original suspension. For example, if the hydrometer reading after temperature correction is 4.7g/litre, then the percentage of clay in soil is:

Clay% = (4.7/50.0) x 100 = 9.4%

Silt: The silt content is calculated by subtracting the sum of the clay and sand contents from 100%:

Silt% = 100 - (9.4% clay + 64% sand) = 26.6%, OR

The assumption made here is that the organic matter is negligible. However, if soil is found to be high, treat the soil with hydrogen peroxide until the frothing reacting subsides. Alternatively, one may determine the organic matter (OM) content separately and calculate as follows:
Silt% = (100 – OM) – (9.4% clay + 64% sand) = 26.6%

Soil texture

Once the sand, silt and clay distribution is measured, the soil may be assigned to a textural class based on the soil textural triangle. Within the textural triangle are the various soil textures which depend on the relative proportion of soil particles. In the example above (64% sand, 27% silt and 9% clay), the corresponding soil texture is a sandy loam.

Mechanical Analysis by the Pipette Method

Moisture:

Weigh out 100g of air-dry 2mm sieved soil into an evaporating dish or a can. Dry in the oven at 105°c for 24hrs, cool and re-weigh. Discard the soil and weigh the can empty. Express the loss in weight of the air-dry soil as a percentage.

LOSS ON SOLUTION

1. Weigh out accurately 10g of air-dry 2mm sieved soil into a 250ml beaker.

2. Add about 30ml of 6% (10 vol.) hydrogen peroxide and warm the contents, stirring frequently to reduce frothing. When frothing has subsided, bring the liquid to boil and keep boiling for about 5mins. If much organic matter is present, more hydrogen peroxide should be added and the contents boiled again.

   Note: Manganese dioxide decomposes hydrogen peroxide catalytically. If the soil contains manganese dioxide before starting the water treatment, add 50ml of water and 0.5 – 1g of sodium bisulphate and boil until the volume is reduced to about a half. Then, add water and proceed as above.

3. Cool the liquid and dilute to about 100ml with distilled water.

4. Add about 2.5ml of conc. HCl with stirring (if the soil contains much calcium carbonate, conc. HCl should be added slowly till all the carbonate is decomposed and then a further 2ml of HCl is added).

5. Stir on and off for about an hour and then filter under suction using a hardened filter paper (Whatman No. 50 – this should be dried and weighed before use).

6. Wash the residue with water till the filtrate is nearly neutral tolitmus.

7. After draining, transfer the soil and filter paper to a weighed evaporating dish and dry in the oven at 105°c for 24hrs. Cool in the desiccators and weigh.

8. Determine the weight of oven-dry soil by subtracting the weight of the dish and the filter paper. Express the losses in weight of the air-dry soil as a percentage and call this; loss on solution.

Removal of Organic Matter and Soluble Constituents

The procedure is exactly the same as that for loss on solution except that all weights and volumes are double, i.e. 20g soil warmed with 60ml of 6% water then cooled and made up to 200mls. 5ml conc. HCl are added with stirring and then left for 1hr the filtration under suction and washing with water are done till nearly the filtrate is nearly neutral.

Dispersion

1. After washing the soil free of acid, transfer it quantitatively to the dispersion bottle and bring the volume up to about 250ml with water. Add 10ml of 1N NaOH, mix and allow to stand for 15mins.
2. Insert the baffle into the bottle and lower the stirrer blade into the suspension making sure it is not in contact with the baffle or the bottom of the baffle.
3. Stir the contents for 15mins in routine analysis, the suspension is then shaken overnight (12-16hrs) on a mechanical shaker.

SILT +CLAY

1. Transfer the suspension to a 1L measuring cylinder and make up to volume with water.
2. Mix the suspension properly by inverting the cylinder a number of times. Take the temperature of the suspension.
3. Allow to stand for the appropriate length of time and then take a 20ml sample using a pipette with the tip 10cm below the surface. The sedimentation time for “silt+clay” and “clay” are given in the table below. A special pipette holder may be constructed from an asbestos mat. Run the contents of the pipette into a weighed evaporating basin.
4. Evaporating to dryness on a water bath and dry in the oven at 105°C for 24hrs. Cool in a desiccators and weigh. The above operations are carried out for “clay+silt” and “clay”. The “silt” figures are obtained by subtracting the figures for clay from those of “silt+clay”.

Sedimentation Time for "Clay+Silt" and Clay fractions

Temp. °C	Clay hrs.	mins	"Clay+Silt" mins	Secs	Temp. °C	Clay hrs.	mins	"Clay+Silt" mins	Secs
11	10	10	6	10	23	7	25	4	30
12	9	50	6	0	24	7	15	4	20
13	9	35	5	50	25	7	5	4	15
14	9	20	5	40	26	6	55	4	10
15	9	5	5	30	27	6	45	4	5
16	8	50	5	20	28	6	40	4	0
17	8	35	5	10	29	6	30	3	55
18	8	25	5	0	30	6	20	3	50
19	8	10	4	55	31	6	15	3	45
20	8	0	4	48	32	6	5	3	40
21	7	50	4	40	33	5	55	3	35
22	7	40	4	30					

Coarse and fine sand

1. After pipette sampling for the clay fraction, pour off the supernatant suspension and transfer the material at the bottom of the cylinder quantitatively to a 1L beaker with water (store till the next practical period).
2. Mark the beaker at a height of 10cm above the base and add water to this mark. Stir well with a glass rod.
3. After the appropriate time depending on the temperature, pour off the supernatant liquid carefully without disturbing the sediment.
4. Fill up to the mark again with water, stir and pour off after sedimentation. The process of filling to 10cm mark, stirring, settling and decantation is carried out until the supernatant is quite clear.
5. Finally, pour off the clear liquid. Dry the residue (coarse + fine sand) at 105°C for 24hrs. Cool and then weigh.
6. Separate the fine sand using a 70 I.MM sieve (which has holes 0f 0.2mm diameter) and weigh. Obtain the weight of coarse sand by difference.

RESULT

Tabulate your results as follows;

Coarse sand	%
Fine sand	%
Silt	%
Clay	%
Moisture	%
Loss on solution...	%

EXERCISE 3

Bulk Density and Water Holding Capacity

Lecturer: Dr. H. E. Ahamefule

The bulk density of soil is the mass per unit volume expressed as g cm^{-3}. Once the bulk density is known, measurement of soil mass, volume or percentages can be expressed interchangeably or expressed in absolute terms (e.g. from g kg^{-1} soil to kg ha^{-1}). The water holding capacity of a soil is expressed as a moisture content of a freely drained, oven dried soil.

APPARATUS

1. Metal tubes/PVC cores
2. Oven
3. Cutlass/hoe
4. Knife

PROCEDURES (for non-stony soils)

a. Remove 1-2 cm of surface soil from the spot where samples will be taken, and level the spot using cutlass or hoe.

b. Drive a 5cm diameter thin sheet metal tube or PVC core of known weight (W1) and volume (V) into the soil surface.

c. Excavate the soil from around the tube/core and cut the soil beneath the tube bottom.

d. Dry at 105°C for 2 days, and weigh (W2).

CALCULATION

Bulk density (g/cm^3) = (W2 – W1)/V

PROCEDURE (for stony soils)

a. Excavate an intact clod of soil (about fist size).

b. Air-dry the clod, tie a thin thread around it and weigh it (Ws).

c. Dip it briefly in melted paraffin wax (60°C) to waterproof it.

d. Weigh the coated clod (W_{sp}) and calculate the weight of the paraffin coating ($W_p = W_{sp} – W_s$).

e. Suspend the clod from the balance arm and submerge it completely in a beaker of water. Record the weight (W_{spw}). If it leaks air, discard it.

f. Break open the clod, take a subsample of the soil, weigh it, oven-dry it, and re-weigh it.

CALCULATION

Correct W_s to its oven-dry mass, W_{dry}:

Wdry = Ws x (weight of subsample after drying /weight of subsample before drying)

Bulk density (g/cm^3) = W_{dry} / [{($W_{sp} - W_{spw}$)/ρ_w} – (W_p/ρ_p)]

Where

ρ_w = density of water at temperature of determination (1.0) ρ_p = density of paraffin wax (approximately 0.9)

PROCEDURE (for shrink-swell soils–vertisols)

Approach and calculation

In shrink-swell soils, the wet bulk density and the dry bulk density differ. The It is difficult to measure when the soil is either very wet or very dry. The minimum bulk density, which occurs when the soil is fully wet and expanded, is estimated by assuming that the particle density is 2.65 gcm^{-3} and air content when field is saturated is 0.03 $cm^3\ cm^{-3}$ (Shaw and Yule, 1978). Hence:

Bulk density (gcm-3) = 1/(Wmax + 0.4046)

Where W_{max} is the maximum gravimetric water content of the horizon in question, the field saturated water content.

Water holding capacity determination

PROCEDURES

a. Get a crucible of known weight (W1).
b. Weigh 10grams of the soil sample into the crucible in (i), record the weight (W2).
c. To W2, add distilled water until the soil is saturated. Record the weight (W3).
d. Oven-dry the saturated sample (W3) at 105°C for 48hours.
e. Record the weight of the oven-dried sample (W4).

Calculation

Water Holding Capacity = (W3–W4) / (W2–W1) x 100

EXERCISE 4

Soil pH and Electrical Conductivity Determination

Lecturer: Dr. Kehinde O. Affinnih

Principal: Measurement of pH is expressed as the inverse logarithm of the hydrogen ion (H^+) concentration. The pH of the soil solution controls the form and solubility of many plant nutrients. Soil pH is measured on 2.5: 1 water to soil suspension. The electrical conductivity measurement identifies soils which are potentially saline.

Apparatus/Reagents:

1. Glass electrode pH meter
2. Conductivity meter
3. 50ml beakers
4. Glass rod
5. Distilled water
6. **1N potassium chloride (KCl):** Weigh 74.56g KCl into a 1000ml volumetric flask and bring to mark with distilled water.

Procedure

a. Soil pH in water (1:2.5 soil/water ratio)
   i. Weigh 10g of air-dried soil (passed through 2mm sieve) into a 50ml beaker.
   ii. Add 25ml of distilled water and stir for 10minutes, allow standing for 30 minutes and stirring again for 2 minutes with the glass rod.
   iii. Allow standing for 15 minutes and insert the electrode of the pH meter into the partly settled suspension
   iv. Record the pH of the suspension after standardizing the pH meter with buffer solution of known pH.
   v. Allow to settle for one hour then measure the conductivity of the supernatant liquid.

b. Soil pH in 1N KCl (1:2.5 soil/KCl ratio)
   i. Weigh 10g of air-dried soil (passed through 2mm sieve) into a 50ml beaker.

ii. As described in a (ii) above.

iii. Allow standing for 15 minutes and insert the electrode of the pH meter into the partly settled suspension.

Measure the pH of the suspension and record result as soil pH in 1N Kcl

Exercise

1. Define active acidity and reserve acidity
2. Was there a difference in the pH as measured by water and Kcl.
3. How much larger is the reserve acidity compared to the active acidity for the soil?

(Hint: Measurement of pH using unbuffered salt solutions (like KCl) is a measure of exchangeable acidity. Look at the difference in the water and KCl pH.)

SECTION B
EXERCISE 1

Plate culture technique for isolating soil microorganisms

Lecturer: Dr. J. U. Yahaya

One routine method for determining the microbial content of soils in the soil dilution plate method. The method is based on the assumption that each viable cell will develop into a single colony and therefore, the number of colonies that develop is regarded as the number of microbes in the sample. While the method helps to give an approximate number of microbes present in a soil sample, it is fraught with some lapses. Most soil fungi are filamentous and they produce asexually by spores. The soil dilution method is therefore biased towards these moulds, aerobic bacteria, yeast and actinomycetes, leaving out the few that do not sporolate heavily and those which may be slow in growing.

Nevertheless, the method is convenient and has also been found helpful in establishing that different soil types have characteristics microbial populations. A serial dilution of the soil suspension is obtained and a total viable microbial count of the dilution made using either the pour plate or the spread plate method. It is advisable that the dilution is done such that the final plates will contain between 30 and 300 colonies.

I. Plating out a soil dilution series by the pour plate method **Materials required per group of four students**

1. 12 sterile 1ml pipette
2. 8 test tubes each containing 9ml sterile water
3. A suspension of 10g dry soil particle in 90ml sterile water. Shake well for 2 mins.
4. 10 sterile petri dish
5. 10 tubes of sterile nutrient agar at about 48°C

Procedure

1. Make a serial 10-fold (decimal) dilution of the soil suspension up to 10^{-7}
2. Label sterile plate (in duplicate) with the dilution 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. The lower dilutions of 10^{-1} to 10^{-3} are likely to contain too many colonies within the incubation period for accurate counting.

3. Using a sterile ml pipette aseptically withdraw 1 ml of appropriate dilution into 9ml of molten agar in the test tube. Mix rapidly and pour into the appropriately labeled sterile plate. Make a duplicate.
4. Allow medium to set on the bench.
5. Incubate all plates inverted at 35° for 48hrs.
6. After the incubation period, count the total bacteria colonies on plate that contain 30-300 colonies. If available, use a colony counter but if not use a tally hand counter.
7. The number of colonies on a plate multiplied by the dilution factor gives the plate count per ml of the soil sample. Eg. If the counts for 2 plates of the 10^{-6} dilution were 55 and 65, the average is 60. Therefore, the original soil sample contains $60 \times 10^6 = 6 \times 10^7$ cells per ml.

ii. Plaiting out a soil dilution series by the spread plate method

Materials required per group of 4 students

1. The soil serial-dilution for (A) above
2. 8 poured plates of nutrient agar
3. In each case, use a sterilized glass spreader to spread the suspensionon the surface of agar. (To sterilize the spreader dip in absolute alcohol, shake off excess and flame the rod over a burner to burn off any adhering
4. Incubate and count colonies as for A above.

Note to instructor

In this experiment, nutrient agar has been used. As this medium is particularly suitable for the isolation of bacteria, the malt extract agar or Czapek Dox agar may also be used, probably by a section of the class for the isolation of fungi. It may also be useful to suppress bacteria growth on this medium by introducing an antibiotic or by asceptic acidification on this medium of a pH of about 4 after autoclaving. Similarly special actinomycetes agar plates may be made for the isolation of the actinomycetes. In both cases of fungi, actinomycetes however lower dimension of 10^{-1} to 10^{-3} and longer incubation periods (3-5 days) at about 20° to 25°C must be used.

EXERCISE 2

Observation of Algae

Lecturer: Dr. J. U. Yahaya

The presence of algae in the soil may be demonstrated by inoculation into a suitable inorganic medium for algae growth. The cultures must be kept in a good light. Examine the green growth which may appear after one or two months. Make drawings

Algae medium

Sodium nitrate.....0.5g

Monopotassium phosphate..... 0.5g

Magnessium sulphate.....0.15g

Calcium chloride.....0.05g

Ferric chloride..... trace

Tap water..... 1000.00ml

Place about 50-60ml in small conical flasks, plug and sterilize.

EXERCISE 3

Isolation of the Rhizosphere Microbes

Lecturer: Dr. J. U. Yahaya

The rhizosphere is an ill-defined zone of soil immediately surrounding the plant root. Many soil microbes occur in higher numbers in the rhizosphere than in the soil distant from the root. The root surface itself, called the rhizoplane contains microbes which remain on the root after thoroughly washing the root to remove adhering soil.

Materials required per pair of students

i. Any common plants on the lawn or in the garden, preferably a weed

ii. Other materials except for the soil sample.

Procedure

i. Uproot the weed such that some soil comes out with the root. Receive the whole thing into a new polythene bag.

ii. For comparison, sample soil about 30cm away from the root.

iii. Suspend 10g of the rhizosphere in 90ml sterile water. Repeat for the sample distant from the root.

iv. Treat the suspension as described above.

v. If possible, use nutrient agar, Czapek Dox agar and actinomycetes agar and compare the bacteria, fungi and actinomycetes populations in both the rhizosphere and the soil away from the root.

EXERCISE 4

Characterization of Bacteria

Lecturer: Prof. Oluyemisi B. Fawole

Single bacteria cells are too tiny to be seen with the naked eyes. However, a bacteria colony could comprise 100 of cells and therefore be visible to solid media. Such a colony has some peculiar characters which help as a first step in the identification of the bacterium. Such characters include the following:

- The shape of the colony which could be circular, irregular or rhizoid.
- The elevation of the colony could be flat, raised, convex or unbornate
- The edge of the colony could be entire, undulate, lobate, denate or rhizoid.
- The optical characteristics which could be transparent, translucent or opaque.
- Consistency: when touched with a sterile loop, the colony surface could be describe as butyrous (i.e. has consistency of butter), viscid (i.e. growth tends to follow the loop when touched), granular (i.e. growth rather dry) or membraneous (i.e. growth thin and membrane like).
- Colony surface could also be smooth, rough, dull, wrinkled, glistering or granular.
- Pigmentation of colony could be white, red, pink, light yellow, straw yellow, deep yellow etc.

Although the above name characters of the colony would be a pointer to the type of the bacteria. Further tests are required for actual identification. As a next step therefore, a stained preparation of the bacterium could be made and the structure studied under the microscope. Bacteria can be placed under 3 broad groups on the basis of shape. These are;

- The rod shaped ones (straight or curved) termed the bacilli
- The spherical or ellipsoidal ones termed the cocci, some of which occur singly and others stick together in pairs, tetrads, packets or chains.

- The spiral ones termed spirilla, the presence or absence of flagella is another characteristics.

In other to fully characterize a bacterium, the colony characters and the cell morphology have to be supplemented with routine bio-chemical tests. Such tests include, reaction of the bacterium to carbohydrates, proteins, lipids and whether or not it produces certain gases and enzymes.

Test for motility

Some bacteria possess flagella and can therefore move. Such motility can be demonstrated by the 'hanging-drop' technique.

Materials required per student

- One cavity slide plus a covership
- Immersion oil or liquid paraffin
- A young (12-24 hours old) agar slant culture of *pseudomonas fluorescens and staphylococcus aureus.*
- A microscope with both the low power (x10) and the high dry (x40) objectives. The soil immersion objective should not be used because the focusing movement of the objective could be mechanically transferred to the hanging drop and result in turbulence that may give misleading result.

Procedure

- Place a little immersion oil or liquid paraffin around the edge of the depression of a cavity slide.
- Transfer a loop full of *Proteus* or other motile bacterium to the centre of a clean dry cover slip placed on a flat sheet of paper. Avoid spreading the drop of culture.
- Invert the cavity slide over the cover slip such that the culture drop is in the centre of the slide depression. Press the slide down carefully but firmly so that the oil seals the cover slip in position.

- Quickly and carefully invert the slide and the culture drop now appears 'hanging'.
- Examine immediately for motility under the microscope. In doing this reduce the light either by lowering the sub-stage condenser or closing the diaphragm. This is necessary only not because illumination renders unstained preparations invincible but also because the heating effect may make them loose their motility.
- First use the lower power objective to focus the edge of the drop.
- Turn to the high power objective, raising it gently with the fine adjustment until the motile cells come in view, moving rapidly in the field. This true motility should be distinguished from the Brownian movement of other none motile cells. The Brownian movement is a bombardment of small particles suspended in a liquid following unbalanced impacts with molecules of the surrounding medium.

Action on simple carbohydrates

A large number of simple carbohydrates can be utilized by bacteria. In some cases, the pattern of utilization is specific and can therefore be used to characterize the bacteria. E.g., the utilization may be accompanied by production of acid alone or by both acid and gas. In order to test for this, a carbohydrate broth to which an indicator has been added is inoculated with the bacterium. The test tube also bears an inverted Durham tube (a tiny test tube) which would trap any gas if it is produced.

The mode of utilization of the carbohydrate by the bacterium could also be tested i.e. whether it is done oxidatively or fermentatively. The standard test medium for this is that of *Hugh and Leifson (1953).*

Production of acids from sugar with or without gas formation

Materials required per group of four students

- Nutrient broth containing 0.5% of glucose, lactose or sucrose
- Eight tubes in each case
- Indicator incorporated into medium in form of either Andrade's indicator (1%), phenol red (0.01%) or bromocresol purple (0.0025%).

- Cultures of the unknown bacteria
- Cultures of known bacteria e.g. *E.coli* and *Bacilusubtilis*
- Durham tubes inverted in the broths

Procedure

- Sterilize the media set up by steaming for 30mins on three successive days.
- Inoculate each indicator sugar broth with each of the bacteria.
- Leave one tube of each medium uninoculated as a control.
- Incubate tubes at 35°C for 2-7 days; watch daily to decide when to terminate the experiment.

Observation

- Growth occurs in the inoculated tubes while none occurs in the controls.
- If there is acid production, this will be shown in change to the colour of the indicator as follows.

  In the presence of acid, Andrade's indicator changes to pink, phenol red to yellow and bromocresol purple to yellow.

  Note: On prolonged incubation of some bacteria, a pH inversion may occur due to the production of ammonia from the peptone contained in the nutrient broth.
- If there is gas production, this will be shown by medium displacement in the Durham tube, Record observation clearly indicating whether or not there is fermentation and acid formation with or without gas production for each organism and for each sugar.

<u>Oxidative and fermentative utilization of sugars</u>

Materials required per pair of student

- 10ml of the sterile basal Hugh and Leifson's medium in each of 8 test tubes

- Separately autoclave a 10% solution of glucose. This will be aseptically added to the sterile basal Hugh and Leifson's medium to a final concentration of 1%
- Broth cultures of *Eshecherichia coli, Staphylococcus aureus, Alcaligenes faecalis, Bacillus subtillis* and unknown bacterium.

Procedure

- Stab-inoculate medium with the bacterium in duplicate
- Retain an uninoculated tube as the control
- Cover the surface of one of the inoculated tubes with sterile liquid, paraffin, vaspar of agar. Leave the other without such covering
- Incubate all cultures at 35°C for up to 14days making observations daily.

Observation

Acid production in the cultures is indicated by a change in colour of the medium from blue to yellow. Acid production in both sealed and open tubes suggests a fermentation reaction; acid formation in the open tubes only suggests an oxidative utilization of the sugar.

Starch hydrolysis

Starch is a polysaccharide. Many bacteria possess enzymes called amylase which can hydrolyse complex molecules of starch to sugars.

Action on litmus milk

Milk can serve as a rich culture medium for microbes because it contains both protein (casein) and carbohydrate (lactose) substrates in addition toother components. The mode of microbial attack on casein and lactose in milk can be used for the biochemical characterization of certain bacteria.

Materials required per pair of student

- 8 tubes of litmus milk medium
- Nutrient broth cultures of e.g. *streptococcus lactis, lactobacillus acidophilus, streptococcus faecalis and pseudomonas aeruginosa.*

Procedure

- Inoculate tubes of sterile litmus milk with a loop full of the bacteria, leaving an uninoculated tube as a control
- Incubate at 35°C for up to 14days making observations daily for any changes in the medium.

Observation

The types of changes that are likely to occur are listed below;

- Lactose fermentation: Acid production shown by a change of litmus pink; acid cloth (cord) may occur if a considerable acid is produced; gas may be produced shown as bubbles on the medium.
- Casein: may be solubilized as a result of proteolytic enzyme activity
- Reduction reaction: the litmus may be decolorized
- Consistency: copious production of capsule or slime may cause the milk to be viscous or ropy.
- Utilization of other constituents of the milk: litmus may change to a deep blue colour because utilization of the citrate in the milk results in alkaline reaction.

<u>Lipid hydrolysis</u>

The complex molecules of lipids can be broken down by many bacteria. The effective enzymes are called lipases and the products are fatty acids and glycerol.

Materials required per student

- Three plates of tributyrin agar medium
- Nutrient broth cultures of two of e.g. *Serratia marcescens, Bacillus cereus, Pseudomonas fluorescens* and *E. coli*

Procedure

- Streak a culture on the surface of the medium. Repeat for another bacterium and leave one plate uninoculated.
- Incubate cultures at 35°C for 2-7 days and examine daily

Observation

Tributyrin is normally dispersed in the medium as an emulsion and therefore makes it look opaque. If it is hydrolysed, there is a clear zone around the bacteria streak on the medium because of the formation of the soluble products-fatty acids and glycerols.

Protein hydrolysis

Protein is one of the most important food substrate to micro-organisms. The ability of an org. both to synthesize and hydrolyse the complex molecules of proteins is therefore of vital significance. The hydrolyzing enzymes are collectively called 'proteolytic enzymes' or 'proteases.'

Materials required per student

- Three plates of milk agar
- Nutrient broth cultures of two of e.g. *Bacillus subtilis, Staphylococcus aureus, Enterobacter aerogenes, E. coli* and *Pseudomonas fluorescens.*

Procedure

- Streak a culture on the milk agar. Repeat for another bacterium and retain one medium uninoculated as control.
- Incubate plates at 35°C for 2-7days and examine daily

Observation

Casein, a protein occurs in a colloidal sate in milk. It gives whiteness to the milk. Clear zones which occur on the culture medium following incubation indicate evidence of casein hydrolysis since the products of hydrolysis as soluble.

Hydrogen sulphide production

Hydrogen sulphide is produced by certain bacteria following the decomposition of organic sulphur compounds such as cystein, methionine or through the reduction of inorganic sulphur compounds such as sulphate, thiosulphate and sulphite. The resulting hydrogen sulphide can be detected by incorporating a heavy metal salt in the medium with which the gas reacts to form black metal sulphides

Materials required per student

- Three tubes of lead acetate agar or peptone-iron agar
- Agar slants of *Bacillus subtilis, Staphylococcus aureus, Proteus vulagris, E. coli and serratia marcescens.*

Procedure

- Stab medium in tube with a heavy inoculums using a bacterium for each tube.
- Leave one tube uninoculated as a control Incubate tube 35°C for 7days and examine daily
- Record results; a black line along the line of inoculation indicates production of hydrogen sulphide.

Nitrate reduction

A biochemical characteristic of many bacteria is the ability to reduce nitrates. The product, nitrite is then tested by a special reagent.

Materials required per student

- 7 tubes of nitrate peptone water
- Nutrient broth culture of *Bacillus subtilis, Pseudomonas fluorescens, Serratia marcenscens, and E. coli.*
- The modified Greiss-Ilosvay's reagents
- Zinc dust

Procedure

- Inoculate 6 tubes of nitrate peptone water with one of the bacteria suggested. Set aside one tube uninoculated to serve as control.
- Incubate tubes at 35°C for 2-7days and test daily for colour changes

Observation

- After incubation for 2 days and daily thereafter, test for nitrate reduction as follows: add 1ml each of reagents 1 and 2 of the modified Greiss-Ilosvay's reagent. The presence of nitrite is indicated by the development of a pink, purple or maroon colour within a few minutes. The control tube should show little or no colour change.
- A change result in the incubated tubes could mean either
  i. That nitrate is not reduced, or
  ii. That the reduction has gone beyond nitrite formation to ammonia or gaseous nitrogen.
- In order to test for the possibility (ii) above, add a trace of zinc dust. This will reduce any residual nitrate to nitrite and lead to a development of colour. However note the weakness of this step as it does not clearly distinguish between possibilities (i) and (ii) above. It therefore appears that the most objective method is to test the cultures daily and thus prevent nitrate reduction beyond the nitrite stage.
- The formation of gas in the Durham tube suggest production of gaseous nitrogen.

<u>Catalase production</u>

Most aerobic micro-organisms are capable of producing the enzyme catalase although to different extents. Others, like the obligate anaerobes are catalase negative.

Materials required per student

- Three plates of sterile nutrient agar
- Nutrient broth or agar cultures of *Aerobacter aerogenes, Serratia marcescens and E. coli*

- Hydrogen peroxide (3%)

Procedure

- Inoculate 2 of the bacteria onto agar plates, leaving the third uninoculated to serve as control.
- Incubate for 24-48 hrs.
- Add several drops of 3% hydrogen peroxide

Observation

- Effervescence, caused by the liberation of oxygen as gas bubbles indicates the production of catalase by the bacterium.
- No such bubbles with the control Urease production

Biochemical characteristic to distinguish between certain members of the Entero-bacteriacease, particularly when the Christensen's urea agar medium is used.

Materials required per student

- Three slants of the Christensen's urea agar medium
- Two slants of the control basal medium that lacks urea
- Nutrient broth cultures of *Proteus vugaris, E. coli* and *Salmonella* sp. (*Salmonella* is pathogenic; handle with special care).

Procedure

- Inoculate 2 of the complete media slants with 2 of the bacteria leaving one slant uninoculated to serve as control.
- Inoculate the 2 urea-free slants medium with the same bacteria to act as another control
- Incubate all slants at 35°C for up to one week, watching daily for any colour changes

Observation

- Where there is an active urease, urea is hydrolysed to ammonia, which increases the pH as indicated by colour change in the

EXERCISE 5

Identification Fungi

Lecturer: Dr. J. U. Yahaya

Fungi (singular: fungus) are examples, eukaryotic organisms which lack chlorophyll. Their vegetative structure is called a *mycelium* which in turn comprises highly branched tube-like filaments called *hyphae* (singular: hypha). A hypha normally develops from a germinated fungal spore. The hypha branches repeatedly into the *mycelium*. Most fungi reproduce both sexually and asexually by producing spores, usually in large numbers. While most fungi are microscopic, some can have large fruit-bodies e.g. mushrooms and puff-balls. Since fungi lack chlorophyll, they live either as saprophyte or parasites on plants or animals. Many of them are of importance to man. The major groups include phycomycetes, the ascomycetes, basidiomycetes and the Deuteromycates (fungi imperfecti).

Septate and Non-septate hyphae

While some fungal mycella (especially members of the phycomycetes) have aseptate hyphae, others have septate hyphae.

Materials required

- Agar cultures of *Rhizopus, Mucor, Aspergillus, Penicillium, Sordaria, Curvularia, Pleurotus, Sclerotium.*
- Lactophenol
- Cotton blue-in-lactophenol stain
- Clean slides and coverslips
- Filter paper
- Two mounted needles
- A microscope

Procedure

- Put a drop of lactophenol on a clean slide
- With the aid of a mounted needle, remove a small portion of the mycelium from any of the above fungal cultures and place it in the drop of lactophenol and cotton blue in lactophenol.

- Spread the mycelium very well on the slide with the aid of two mounted needles
- Gently lower a coverslip on it
- Wipe off excess liquid by putting the slide between 2 filter papers and applying a gentle pressure around the coverslip
- Examine slide under the microscope
- Repeat for all specimens mounting each in lactophenol and cotton blue-in-lactophenol respectively

Observation

- Note that hyaline appearance mounted in lactophenol only
- Note the blue colour of the hyphae in cotton blue-in-lactophenol
- Note which of the fungi have aseptate and those with septate hyphae
- From the stained slides draw portions of the hyphae from each specimen under the x40 objective to show any septation and branching of the hyphae.

Mucorales

These are members of the Zygomycetes

1. **Asexual Reproductive Apparatus**

Material required

Agar cultures of *Mortierella, Mucor; Rhizopus, Absida, Pilobolus, Blakesla, Choanephora, Radiomyces, Helicostylum, Thamnidium, Syncephalastrum, Syncephalis, Piptocephalis, Cunninghamella, Chaetocladium*

Incubated cow dung on which *Pilobolus* is growing

Procedure

- Mount portions on the mycelium from each of the cultures in a drop of cotton blue-in-lactohenol on a clean slide
- Put a coverslip and examine under the microscope
- Observe each slide under the x40 objective and make drawings to show the structure of the hyphae (coenophytic) and the asexual reproductive apparatus – sporangiosphore, different types of sporangia, sporangiospores/conidia.

Observation

- Mortierella – Sporangium with many or few sporangiospores and absence of columella
- Mucor, Rhizopus, Absidia, Phycomyces, Pilobolus – Large columellae sporangia bearing reduced numbers of sporangiospores
- Helicostylum, Thamnidium – Sporangiospores which bear a terminal sporangium with numerous spores and lateral branches bearing sporangia with few spores
- Syncephalastrum, syncephalis, Piptocephalis – Cylindrical anerosporangia bearing very few linearly arranged sporangiospores.
- Cunninghamella, Choanephora, Chaetocladium – one spored sporangia which have thus become conidia

2. **Sexual reproductive Apparatus**

Materials required

Agar cultures of the following fungi showing sexual reproduction; *Rhizopus sexualis* (homothallic), *Mucor hiemnalis* (hetherotallic), *Absidia spinosa* (homothallic), *Zygorhynchus molleri* (homothallic).

Procedure

- Mount portions of the mycelium from the homothallic culture in a drop of cotton blue-in-lactophenol on clean slide and examine under the microscope.
- Mount portions of the mycelia from the point of contact of the two strains in each of the heterothallic culture in a drop of cotton blue-in-lactophenol on a clean slide. Examine under the microscope
- Observe and draw the different stages of development of the zygospore in each specimen in order.

Observation

Note the features peculiar to the sexual reproductive apparatus of each species as follows;

Rhizopus sexualis – suspensors equal in size

Mucor hiemalis - suspensors equal in size

Mortierella parvispora -unequal suspensors (i.e heteromametangic), zygospores not surrounded by sterile hyphae unlike *Mortierella rostafinskii*, *Absidia spinosa* – zygospores

surrounded by curved unbranched suspensore appendages, *zygorhynchus molleriheterogametangi* zygospores. Saprolegniales These are true aquatic fungi belonging to the group Oomycetes.

Materials required

Agar culture of Saprolegnia, Achyla, Dictyuchus, Tharaustotheca

Procedure

- Mount a portion of the mycelium of each of the above fungi in cotton blue-in-lactophenol on a clean slide.
- Cover with coverslip and wipe off excess liquid from slide
- Examine under the microscope
- Draw a few hyphae under the x40 objective to illustrate the structure and mode of branching. Note that these are aseptate.
- Observe the terminal cylindrical sporangia and the primary oospores
- Under the x40 objective, draw an intact sporangium and one which has discharge its spores
- Draw a few discharged primary zoospores
- make notes on your observations

Yeast

The yeast are fungi which belong to the order Endomycetes. They usually do not form mycelium. They are single-celled but larger than bacteria. Yeasts are the most important microbes involved in alcoholic fermentation. Therefore, they are of economic importance especially in the industrial production of beer and wine.

Materials required

- Palm wine, tapped at least 12 hours previously to act as a source of yeasts
- Agar cultures of *Saccharomyces cerevisiae, Shcizosaccharomyces octoporus* and *Diploascus albidus*

Procedure

- Put a drop of the palm-wine on a clean slide

- Place a coverslip and examine under the microscope.
- Note the presence of both bacteria and yeast cells in the fermentating palm-wine
- Compare the size of both types of organisms
- Under the x40 objective, observe and draw the types of yeast cell found
- Observe and make drawings of budding yeast cells at different stages of budding
- Place a coverslip and examine under the microscope
- Observe and draw somatic and sexual reproductive structures under the x40 objective

Eurotiales

This order belongs to the Plectomyces. Members include those fungi whose imperfect (conidial) stages are more common and prominent than the prefect (sexual) stages. The imperfect stages have therefore been given generic names different from their perfect stage. Examples of these are *Aspergillus* whose perfect stages are *Eurotium, Sartoya* and *Emericella* and *Penicillium* whose perfect stages are *Talaromyces* and *Carpenteles.*

Procedure

- Using a scapel, cut a small portion of the agar plus mycelium
- Without using a coverslip, examine under the x10 objective of the microscope
- Make sketches to illustrate the shape of the asexual reproductive structures
- Mount a tiny portion of the mycelium in a drop of cotton blue-in-lactophenol on a clean slide, cover with coverslip and examine under the x40 objective
- Observe and draw vegetative hyphae (septate) and asexual reproductive apparatus.

Observation

Note

- In *Aspergillus* – conidiospore, foot cell. Vesicle, sterigmata (whether one row or two rows) and chains of conidia
- In *Penicilium* – conidiospore, ramus, metula and phialide and chains of conidia.
- In *Ceratocysis*-
- In *Talaromyces* and *Eurotium* – clestothecia at different stages of development with ascopores.

I. Vegetative hyphae (septate) ascocarp as well as fringe of hairs at the tip ostiole of the neck.

ii. Swollen base and long neck of ascocarp as well as the fringe of hairs at the tip ostiole of the neck.

EXERCISE 6

Non–Symbiotic Nitrogen Fixers

Lecturer: Prof. Oluyemisi B. Fawole

The purpose of this experiment is to demonstrate the presence of *Azotobacter* in soil and to determine the nitrogen fixing ability of this organism in solution culture.

A. **The Occurrence of Azotobacter and Beijerinkia in soil**

To 30g, dry soil in each of four small evaporating dishes, add 0.72g mannitol and mix thoroughly with the soil. Treat the separate sample as follows:

1. Add 0.1g $CaCO_3$
2. Add 5ml of 3% Na_2HPO_4 solution
3. Add both the $CaCO_3$ and Na_2HPO_4
4. No addition.

After these additions have been made, sufficient water should be added to make a thick paste. Too much water should be avoided. Divide the soil into duplicate halves of small petri dishes and smooth off surface with a wet glass slide or spatula. Make the top as smooth and even as possible. Incubate the plates in a moist chamber for a week then examine for the presence of *Azotobacter* colonies which appear as small limpid, opaque or shining raised colonies on the surface of the soil.

Make a slide preparation of some of the colonies and stain with erythrosine or gentian violet. Examine under the microscope. Morphologically, the cells are larger than the majority of bacteria and vary from rod to spherical shape. Round or oval types usually predominate. Prepare a mount in dilute iodine solution and examine carefully for the presence of starch granules in the cell.

B. **Nitrogen fixation in solution culture**

Place 50ml of N-free manitol in each of two conical flasks, plug with cotton and sterilize. Inoculate the flasks with bacteria from two of the colonies of Azotobacter obtained in part A. Place the flasks on a wrist action shaking machine to facilitate aeration and incubate for 1 week then, analyze for organic nitrogen as follows:

Transfer the medium to a 100ml Kjeldahl flask with a minimum amount of water, add 5ml of conc. H_2SO_4 and mix then, drop in a small boiling stone and place on the digestion rack. Heat until SO_4 fumes no longer come off and the remaining solution is clear. A small amount of $FeSO_4$-$CuSO_4$ added to the digestion mixture will speed up the reaction. After digestion is complete, cool the contents of the flask, add 25ml distilled water then, transfer the contents to the steam distillation apparatus and distill over the ammonia into boric acid. Calculate total weight of nitrogen fixed per gram of mannitol.

EXERCISE 7

MICROBIAL BIOMASS, CARBON AND NITROGEN DETERMINATION

Lecturer: Dr. J. U. Yahaya

Principle. Microbial biomass as determined by the fumigation-extraction technique subjects a fresh soil to chloroform fumigation that results in cell wall lyses, allowing the cellular contents to become extractable in 0.5 M K_2SO_4. This is not a measure of soil microbial activity because no differentiation is being made between quiescent and active organisms, or between different classes of microorganisms. Care must be exercised when comparing soils collected from different locations as microbial biomass fluctuates greatly within a single soil in response to litter inputs, moisture availability and temperature.

If different agricultural soils are being compared at a single time, the fresh soil should be at or near moisture holding capacity. If soils from different natural ecosystems are being compared, samples should be collected toward the middle of the wet and dry seasons. The following procedures are based on that of Anderson and Ingram (1993), Brooks *et al.* (1985) and Vance *etd al.*(1987). Many refinements to measurement of microbial biomass and its components are available, including substrate-induced incubation and the extraction of ATP (see Horwath and Paul, 1994). Powlson (1994) published a concise review of the concept of microbial biomass measurement that contains numerous useful citations.

Reagents

1. Chloroform (alcohol-free). Wash chloroform with 5% concentrated H_2SO_4 in a separation funnel, separate the acid and then rinse repeatedly (8-12 times) in distilled H_2O. Store in a dark bottle.
2. Potassium Sulphate, 0.5M. Prepare by dissolving 87.13g K_2SO_4 in 1000ml distilled water.

Procedure

1. Place 15g of fresh soil samples into a 50ml beaker. Conduct a moisture determination on soil sub-samples so that the results can be expressed on a dry weight basis.
2. Place the beakers into the two paired desiccators. Into one of the desiccators, place a 100ml beaker containing 25ml of chloroform (alcohol-free) into the centre of the desiccators. Adding boiling chips to the chloroform assists in rapid volatilization of the chloroform. The second desiccator contains the non-fumigated control samples which apart from fumigation/evacuation are to be handled in the same fashion. Close the lids of the desiccators paying particular attention that the sealant is uniformly distributed.
3. Apply a vacuum to the fumigated treatment until the chloroform is rapidly boiling. Close the desiccator and store under darkened conditions for 72hours at room temperature.
4. Evacuate the fumigated treatments using the vacuum pump repeatedly (8-12 times). Remember that the chloroform is being trapped by the oil in the vacuum pump so the oil must be changed more often than normal. Alternatively, chloroform can be trapped by a cooling finger to prevent contamination of the vacuum oil. It is not necessary to evacuate the control desiccators.
5. Open the desiccators and transfer the soil samples to shaking bottles or flasks (125-250ml). Add 50ml 0f 0.5 M K_2SO_4 and shake on a wrist action shaker for 25 minutes.
6. Either filter the soil suspension using No 42 filter paper, or centrifuge, in order to obtain a clear extract.
7. Digest the sample and analyse for total N and, C using standard methods.

Figure 11.1 A DESICCATOR

Calculations

Microbial Biomass C = ($C_{fumigated} - C_{control}$) Microbial Biomass N = ($N_{fumigated} - N_{control}$)

Remarks. Some authors suggest that empirically derived correction factors should be applied to these results. These factors may be obtained by conducting the fumigation/extraction procedure on inert soils containing a known quantity of microbial biomass (e.g. mushrooms or washed bacteria cells). Vance et al. (1987) advocates a factor of 2.64 for microbial biomass C and Brooks et. al. (1985) recommends a factor for 1.46 for biomass N. If these factors are applied, this should be clearly indicated when reporting the results. Because of the tremendous variation in soil microflora and microfauna population in soils, we suggest that these factors not be applied.

SECTION C

EXERCISE 1

Field and Herbarium Techniques

Lecturer: Prof. Olabisi F. Adekola

A herbarium is a collection of dried, pressed and nicely preserved specimens arranged in the sequence of an accepted system of classification. Such dry specimens are used for the identification of fresh specimens, comparison between floras or palynological studies. The herbarium is the data bank of the plant taxonomist. Botanical gardens, Universities and Research institutions are the usual places of herbarium collections. The herbarium is a growing source of knowledge about the vegetation of an area. Its effectiveness and value depends on the care with which the specimens were preserved and mounted, the accuracy and completeness of identification ad labeling and the satisfactory arrangement and maintenance of the collections so that they do not deteriorate and can be readily referred to. A herbarium may contain millions of specimens, gradually accumulated by some large institutions (e. g. Royal Botanical Gardens, Kew) and may represent the flora of a continent or it may contain local collections.

Zthe primary objective of plant collection and preservation in the herbarium is to preserve for all time a series of specimens and note that will yield the maximum information about the plant concerned. As it is very expensive and difficult to explore the flora for collection, exchange of specimens among botanical institution and collectors is a standard means to

increase collection at a little cost. Exchange a specimens between research institution avoids additional cost and repetitions in the exploration. It is also helpful in the comparison of specimens and identification of several plants. On the basis of such studies a comparative account of ecological conditions of the floras can be prepared, understanding of which has a direct impact on taxonomy.

Specimens for exchange purposes are usually unmounted with quate labels. Specimens are loaned by the curators of herbal institutions or taxonomists requesting them. Loan of specimen usually made for a specified period of time and the borrower normally pay the transportation expenses.

Field Sampling and Plant Collection

Equipment for collection and collection methods

Field collection is a simple process and needs only careful handling of the selected specimens. As far as possible, select a specimen that has both flowers and fruits. A plant without flower is of little taxonomy value as it cannot be conclusively identified.

The following equipment are items are needed for plant collection on the field;

- A digger or trowel
- A secateurs
- Vasculum or polythene bags
- A plant press containing old newspaper or any blotting material
- A field note book

i. The digger or Trowel: In the case of collecting herbaceous plants and ferns, it is essential that the underground plant per part rhizome, corns, bulbs and tubers be made a part of the specimen. These parts add completeness to the specimen since they provide valuable clues for the identification of specimen. For this reason, it is necessary to carry a suitable digger to plant the field.

ii. Secateurs or a sharp pruning knife is necessary for collecting and cutting of branches of herbs, shrubs and trees. Sharp sickle with long handle is useful for obtaining branches and flowers from a high tree. For this purpose, small piece of cylindrical wooden rods measuring about ½ meters are selected. Each is fitted with screw and socket and can be joined together

to make a long supporting sickle at the extremity. After the use, it can be unscrewed. This screw and socket would facilitate easy carrying to the field.

iii. The vasculum and plastic bags

The Vasculum is an oval shaped metallic container which is 45 x 60cm in length, 20cm in width and 25cm in depth. It is provided with hinged door with screwed fastener in order to keep the vasculum air tight to avoid wilting of specimens by conservation of moisture. A pair of buckles is provided on the vasculum on either end to be attached with sharp to sling it on the shoulders. Large plastic bags have been found very useful in the collection of plants as they prevent loss of moisture when closed tightly. It is also very convenient to carry polythene bags on collection trips.

iv. The field note book: it is always necessary to carry bound pocket sized field note book while going on field excursions. The data on collection like the location (country, province or state, city or town or other major geographic point such as park or river), habitat (river bank, forest edge, field, swamp etc.), field observations about the plant (height if the entire plant not taken, flower colour, fruit colour, odour, nature of the stem juices – milky colours, sticky, red, clear sap, thick or watery), collection number, date of collection, name of the plant if known, association with other plants and its abundance etc. are noted down in the field note book. Each collection should be given a consecutive number starting with one for the first and ending with as many plants as you collect. This number should be the first entry in the field note book. This number should also be conspicuously written along with your last name on the outside of each folded newspaper containing a plant specimen and as far as possible use of slips or small pieces of paper should be avoided since the chances of losing the slips the more.

v. Plant press: The plant press consists of equal sized top and bottom frame measuring 30cm x 45cm they are composed of thin, light, strong pieces of plywood or metal laid at right angles to each other as to form a lattice work, the joints being securely screwed is riveted. Between the frames are the folders, in each of which the collector places the specimens. The blotters are the absorbents and the ventilators are the corrugated card- board sheets placed on either side of the blotters containing newspaper folders.

EXERCISE 2

Plant Preservation

Lecturer: Prof. Olabisi F. Adekola

a. Drying of specimen: The press containing the specimens is tightly bound together by strong straps of canvas or leather long enough for the expansion of the press and is placed in the hot sun or suspended over moderate heat. The folders should be changed daily according to the weather conditions.Larger specimens are bent into a V or W shape so as to fit them on a standard sized (30 x 45cm) mounting sheets. While arranging the specimens on the folders, leaves should not be crowded together and the ugly leaves should be removed. Thick organs may be trimmed from the bark and a cotton pad is applied on the side for the proper pressing. The corolla should be properly spread over. Completely dried specimens need not be stored tightly pressed. But once pressed flat and dried, they must be stored in a dry place free from insects.

b. Mounting of Pressed Specimens

Equipment for mounting specimen

The process of attaching dried and pressed specimens on suitably hard standard sheet on one side is referred to as mounting of the specimens. The standard size of the mounting paper is 41.25 x 28.75cm. The mounting paper should be fairly heavy to reduce flexing when mounted specimens are picked. The following items are required for mounting;

- A piece of glass plate or any other inert and water resistant material. The size required as about 45 x 60cm the surface must be smooth and can be easily cleaned.
- Adhesive: a neutral, opaque library paste has been found satisfactory. Various paste and mucilages may be used provided they do not become brittle with age and can be easily cleaned from the mounting board. The paste should be freely miscible with water since it is necessary to dilute it occasionally on the board especially when the air is dry and it may become very stiff.
- Brushes: a 4cm paint brush is useful for applying adhesive to the mounting board. A large brush is ideal for applying glue to individual leaves that are too flexous to place in the film of paste on the mounting board.

- Forceps: forceps with spatulate tips are excellent for picking up specimens from the mounting board.
- Gummed linen tape: gummed linen tape is cut into small strips about 8cm wide. The tape should be pliable and have good adhesive properties

The plastic is made of the following ingredient;

Ethylcellulose = 250g

Dow resin = 75g

Methanol = 200cc

Toluene = 800cc

The plastic is dispensed from a trigger type of oil gun with a small nozzle.

EXERCISE 3

Mounting Procedure

Lecturer: Prof. Olabisi F. Adekola

Moisten the brush and mounting board with water and apply thin coating of paste to the board. Place the specimen on the paste and gently press it down to make sure that the lower surface is well coated. Then carefully pick up the specimen and drop it gently on the mounting paper in the desired position. If any plated leaves tend to drop a slight upward flip just before the specimen is lowered into place will ensure that the leaves land fully extended. Some grasses and sedges droop too much to be handled by flipping. In handling such plants, let them hang vertically and bring the mounting sheet slowly into contact with very flaccid specimens, such as *Potamogeton*, spread a thin layer of paste on one surface and lower the mounting paper on top of them. When the sheet is lifted, the specimen will come with it.

The specimen should be located carefully on the sheet so that it does not come within 3cm of the edge at any point. The seeds, fruits and extra flowers may be placed in transparent envelopes and carefully pasted to a convenient part of the herbarium sheet.

EXERCISE 4

Herbarium Labels

Lecturer: Prof. Olabisi F. Adekola

The herbarium label is an important part of the finished specimen. Labels vary in format and size but are usually 6 x 10cm they are pasted with glue usually on the lower right hand corner of the mounting sheets. Do not paste down the whole label at this generally makes the corner of the sheet curl. A label should bear the following data:

- Banner headline to show the region of the collection, accompanied by the name of the institution.
- Botanical name of the plant with author citation
- The locality of collection
- The habitat
- The name of the collector and his field number
- Collector's field observations
- Date of collection and the name of the person who identified the specimen. Often, corrections and suggestions in the nomenclature of the plant are made. Such remarks are noted on a separate sheet along with the name of the person who suggested them. This is then pasted near the previous label. These labels are called annotation labels.

After the specimen has been pasted down the label attached cover sheet with a piece of wax paper, then with a heavy card board of convenient weight. Additional specimens, each covered by wax paper may be stacked under the cardboard and weight. The specimens should be left to dry overnight under the weight. After the specimens are dry, remove them gently and reinforce them at various points to ensure that the paste does not give way with flexing of the sheet. Use either linen tape or plastic stripping and reinforce the specimen every 7cm or so. Reinforce the large leaves near the tip and if wide, on the sides. The use of cellotape should be avoided because of its lack of permanency.

For neatness, strength and speed of application, plastic reinforcement it better than linen tape. The liquid is sprayed on the lower surface of the specimen and then it is placed on the sheet. It dries quickly. Sometimes, it is necessary to sew a very coarse stem, a cone, a heavy piece of bark or some other large specimen to the sheet. Heavy linen thread should be used. Pass it twice or more over the specimen and knot it on the back of the sheet and cover the threads on

the back of the sheets by strips of gummed paper tape. Sample of a herbarium label is presented below:

UNIVERSITY OF ILORIN, ILORIN

DEPARTMENT OF AGRONOMY

HERBARIUM COLLECTION NO.

NAME.....

FAMILY.....

NATURAL ORDER.....

LOCALITY.....

HABITAT.....

ALTITUDE.....

DESCRIPTION.....

.....

DATE.....

COLLECTOR.....

DETERMINED BY.....

EXERCISE 5

Filing

Lecturer: Prof. Olabisi F. Adekola

The arrangement of specimens in herbarium is termed as filling. All specimens belonging to one genus are placed within a genus cover. When there are several sheets of a s given species, they are placed in a genus cover different from the other species of that genus. In general, the genus cover has the name of the genus written on the lower left edge.

When the cover is registered to specimens of a single species (*Vigna unguiculata*, *Vigna unguiculata sesquipedalis*) the genus initials and species names are written on the lower right hand margin (e.g *V. unguiculata*). The genera and species should be arranged in alphabetical order under each family. In major herbaria, technicolour folder scheme is used depicting political areas. The colour of folder is indicative of the distribution of the plants. In some cases, specimens are kept in tight fitted steel cases with 2-3 steps. An idex card is displayed outside the colour.

EXERCISE 6

Care of Herbarium

Lecturer: Prof. Olabisi F. Adekola

Specimens handled with proper care can last indefinitely. Herbaria in the warmer and moist regions may have pest problems. Commonly herbarium pest should be identified (e.g. adult and larva of tobacco beetle) and Stegobium panicenum (Family Anobiidae). Both adults and larve eat plant materials if unchecked. Thus, it is essential to continue treatment of specimens until all eggs are destroyed.

Treatment of herbarium specimens

Insecticides such as dichlorophenyl trichloroethane, carbon disulphide, cyanide gas etc.

Poisoned specimen with 10% aqueous mercuric chloride before mounting

Insect repellant such as naphthalene balls paradichlorobenzene

Method of application

Spraying of insecticide daily

Filling muslin bags with a mixture of two parts naphthalene and one part paradichlorobenzene placed in the herbarium case. Refill bad periodically.

Placing small bottles of calcium chloride with mouth covered with loose cotton so as to keep the herbarium dry.

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