

SECTION 3

SAMPLE PROCESSING AND LABORATORY ANALYSES

3.1 CHARACTERIZING, SUBSAMPLING AND CRUSHING SAMPLES

3.1.1 Characterizing Samples

3.1.1.1 Principle--

Even when careful measures are taken, errors may result in the labeling and collecting of samples in the field. This procedure is a laboratory check list to verify that all information was gathered when samples were collected.

3.1.1.2 Comments--

Samples should be checked for errors as soon as possible after sampling. The time spent rechecking field data and samples may eliminate missing data, errors in data, or unnecessary trips back to the sample site to collect missing information.

3.1.1.3 Chemicals--

None required.

3.1.1.4 Materials--

1. Field record book.
2. Collected samples.

3.1.1.5 Procedure--

1. Verify the following:
 - a. Sample site has been located correctly on a topographic map and longitude and latitude have been recorded.
 - b. Surface elevation at sampling site has been recorded.
 - c. Name, depth, and thickness of coal seam(s) scheduled for mining have been recorded.

- d. Total overburden thickness has been recorded.
 - e. For each sample the correct site location, sample number, soil or rock type (if done in field), sampling interval represented by sample, depth from surface, and date sampled have been recorded in the record book. NOTE: Both the interval represented by the sample and the depth from the surface should be recorded even though one can be calculated from the other.
2. For each overburden column, check to make sure that each sample container has a sample, is numbered correctly, and is labeled correctly.
 3. Determine rock type in the laboratory if not completed in the field.
 4. Determine color in the laboratory on ground (less than 60 mesh) sample.

3.1.2 Subsampling and Grinding Rock and Native Soil Samples

3.1.2.1 Principle--

Crushing reduces the field sample into a convenient size range for use with various laboratory analyses. Samples are crushed, subsampled, and ground to pass a 0.25 mm (60 mesh) sieve.

3.1.2.2 Comments--

Crusher and pulverizer should be cleaned after each sample to avoid contamination between samples.

Native soils are soil horizons which are taken above and are treated as an extension of a core, blast hole, or hand sampled highwall column. Soil and rock samples should be air dried, not oven dried, before subsampling and grinding.

3.1.2.3 Chemicals--

None required.

3.1.2.4 Materials--

1. Crusher, chipmunk, motor driven, capable of crushing samples to less than 6.35 mm (0.25 in) (Cat. No. 5-60836, Sargent-Welch Scientific Company; or equivalent).
2. Pulverizer, capable of crushing samples to less than 60 mesh (Fen Corp., Wickliffe, Ohio, Model PA-M; or equivalent).
3. Mortar and pestle, cast iron (Cat. No. 12-976, Fisher Scientific Company; or equivalent).
4. Sieve, 0.25 mm openings (60 mesh).
5. Sieve, 6.35 mm (0.25 in) openings (optional).

6. Vials, plastic with snap caps, 148 cc (40 drams) capacity.
7. Container, plastic or waxed paper, 1 liter (32 oz) capacity.

3.1.2.5 Procedure (revised and updated from Smith et al., 1974)--

1. Spread sample evenly on a sheet of brown paper and allow to air dry.
NOTE: Sample may have to be mixed periodically to speed drying.
2. After drying, the field sample is split into two representative subsamples. One subsample is placed in a container, labeled, and stored for physical analyses or individual preference tests.
3. The other subsample is crushed to 6.35 mm (0.25 in) or smaller with a Chipmunk crusher. If a crusher is not available, the material can be crushed using a hammer or mortar and pestle until it passes through a sieve with 6.35 mm openings. NOTE: This step may be omitted on most native soil samples.
4. Place sample in 1 liter container and cover. NOTE: Containers should not be more than two-thirds full or mixing (step 5) will be impaired.
5. Tumble container end-over-end until material is thoroughly mixed.
6. Place three heaping teaspoons of the mixed material in the pulverizer. Material is pulverized until it passes a 0.25 mm (60 mesh) sieve. NOTE: A cast iron mortar and pestle can be substituted for the pulverizer.
7. Place pulverized material in plastic vial for laboratory use.
8. Label vial with the sample identification shown on the field container.
9. Mix sample thoroughly by tumbling the vial end-over-end before subsampling for laboratory procedures (primarily chemical analyses).

3.1.3 Subsampling and Grinding Minesoil Samples

3.1.3.1 Principle--

See 3.1.2.1

3.1.3.2 Comments--

Samples should be air dried before processing begins. Samples should never be oven dried before processing.

Pulverizer should be cleaned after each sample to avoid contamination between samples.

3.1.3.3 Chemicals--

None required.

3.1.3.4 Materials--

1. Wooden rolling pin (kitchen style).
2. Pulverizer, capable of crushing samples to less than 60 mesh (Fen. Corp., Wickliffe, Ohio, Model PA-M; or equivalent).
3. Sieve, 20 cm (8 in) diameter, 19 mm (0.75 in) openings.
4. Sieve, 20 cm (8 in) diameter, 6.35 mm (0.25 in) openings.
5. Heavy brown kraft paper.
6. Vials, plastic with snap caps, 148 cc (40 drams) capacity.
7. Containers, large enough to contain sample fractions.
8. Large spatula.

3.1.3.5 Procedure--

1. Pour field sample out onto a large square of brown paper. Spread material evenly and allow to air dry. NOTE: Sample may have to be mixed periodically to speed drying.
2. After drying, the field sample is split into two representative subsamples. One subsample is placed in a container, labeled, and stored for physical analyses or individual preference tests.
3. The other subsample is placed between two sheets of brown paper and crushed by moderately rolling over the top sheet with a rolling pin. This process is continued until the entire field sample has been processed. NOTE: Do not allow paper fragments to become incorporated with the soil sample. Do not crush rock fragments.
4. Pass the crushed material through a sieve with 19 mm openings and discard material retained on the sieve.
5. All material passing the 19 mm sieve is crushed to pass through a sieve with 6.35 mm openings.
6. Place sieved sample in a 1 liter container and cover. NOTE: Container should not be more than two-thirds full or mixing (step 7) will be impaired.
7. Tumble container end-over-end until material is thoroughly mixed.
8. Place three heaping teaspoons of the mixed material in the pulverizer. Material is pulverized until it passes a 0.25 mm (60 mesh) sieve. NOTE: A cast iron mortar and pestle can be substituted for the pulverizer.
9. Place pulverized material in a plastic vial for laboratory use.

10. Label vial with the sample identification shown on the field container.
11. Mix sample thoroughly by tumbling the vial end-over-end before subsampling for laboratory procedures (primarily chemical analyses).

3.2 CHEMICAL METHODS

3.2.1 Summary

Chemical methods for characterizing overburdens and minesoils are given. For a particular parameter, more than one method may be listed. This will allow the user of the manual some freedom of choice.

The determination of toxic or nontoxic materials due to acidity is overriding in importance in the Appalachian and Eastern and Western Interior Coal Provinces. The methods for determining toxic or potentially toxic materials are given high priority and are listed at the very front of the chapter. Methods 3.2.2, 3.2.3, 3.2.4, and 3.2.6 are used to determine the acid-base balance of minesoils and overburdens.

Next in importance is the nutrient status of the overburden materials. Nutrient status can be measured by using methods 3.2.5, 3.2.6, and 3.2.15. These methods give a measure of plant nutrients such as phosphorus, potassium, calcium, magnesium, and nitrogen. A knowledge of what plant nutrients are contained in an overburden material enables the mine operator to efficiently plan the mining operation so that full advantage can be taken of these nutrients in the resulting minesoil.

For more intensive study of minesoils and overburden materials, procedures for determining the cation exchange capacity (3.2.16 and 3.2.17) are given. Ways of estimating the lime requirement in minesols are presented in methods 3.2.7 through 3.2.10. Also, methods applicable to arid and semi-arid regions have been included.

3.2.2 Paste pH

3.2.2.1 Principle--

Perhaps the most commonly measured soil characteristic is pH. Soil pH was defined by Sorensen (1909) as the negative logarithm of the hydrogen-ion concentration. However, in actuality, hydrogen-ion activity is measured instead of hydrogen-ion concentration.

Soil pH is measured by a glass electrode incorporated with a pH meter for this procedure. Water is added to the sample forming a paste. The electrode is placed in the paste with pH being read directly from the meter.

3.2.2.2 Comments--

Six factors affecting the measurement of pH are: (1) drying the soil sample during preparation; (2) soil:water ratio used; (3) soluble salts content;

(4) seasonally influenced carbon dioxide content; (5) amount of grinding given the soil; and (6) electrode junction potential (Jackson, 1958; Peech, 1965).

Care must be taken to insure electrode life and accurate pH measurements:

(1) Electrode should not remain in the sample longer than necessary for a reading, especially if more alkaline than pH 9.0. (2) Electrode should be washed with a jet of distilled water from a wash bottle after every measurement (sample or buffer solution). (3) Electrode should be dipped in dilute (1 part acid to 3 parts water) hydrochloric acid for a few seconds and washed with distilled water to remove any calcium carbonate film which may form, especially from alkaline samples. (4) Drying out of the electrode should be avoided. Electrode is cleaned and suspended in distilled water (which is protected from evaporation) for storage. (6) Place pH meter in standby position when electrode is not in a solution (Jackson, 1958; Peech, 1965).

The pH meter and electrode should be standardized with buffers differing by 3 or 4 pH units, such as 4.0 and 7.0, before beginning a series of measurements. After every tenth measurement, recheck the standardization with both buffers. Care should be taken not to contaminate one buffer with the other buffer or with the test solution. Never return used standard buffers to their stock bottles. The procedure describes the technique for measuring pH with a glass electrode and meter. If pH is taken in the field using color paper strips or indicator solutions, modification will have to be made by qualified personnel to the procedure.

3.2.2.3 Chemicals--

1. Standard buffer solutions, pH 4.00 and pH 7.00.
2. Distilled water (H₂O).

3.2.2.4 Materials--

1. pH meter (Corning model 12 or equivalent) equipped with combination electrode.
2. Paper cups, 30 ml (1 oz) capacity.
3. Plastic cups.
4. Stirring rod.
5. Wash bottle containing distilled water.
6. Balance, can be read to 0.1 g.

3.2.2.5 Procedure--

1. Turn on, adjust temperature setting, and "zero" pH meter per instruction manual.

2. Place pH 4.0 and pH 7.0 standard buffers in two plastic cups (one buffer in each cup). NOTE: NEVER return used buffers to stock bottles.
3. Place electrode in the pH 7.0 buffer.
4. Adjust pH meter to read pH 7.0.
5. Remove electrode from buffer solution and wash with a jet of distilled water from a wash bottle.
6. Place electrode in the pH 4.0 buffer and check the pH reading. NOTE: If pH meter varies more than ± 0.1 pH units from 4.0, something is wrong with the pH meter, electrode, or buffers.
7. Weigh 10 g of less than 60 mesh material into a paper cup.
8. Add 5 ml of distilled water to sample. NOTE: Do not stir! Allow water to wet sample by capillary action without stirring. With most overburden and minesoils materials, the 2:1 (soil:water) ratio provides a satisfactory paste for pH measurements; however, for the very coarse textured and the very fine textured material, more material or water can be added to bring the soil near saturation. At near saturation conditions, water should not be puddled nor dry soil appear at the surface.
9. Stir sample with a spatula until a thin paste is formed adding more water or soil as required to keep soil at saturation point. NOTE: At saturation, the soil paste glistens as it reflects light and the mixture slides off the spatula easily. Wash the spatula with a jet of distilled water before stirring another sample.
10. Place electrode in paste and move carefully about to insure removal of water film around the electrode. CAUTION: Do not trap particles between electrode and inside surface of the sample container. Electrodes are easily scratched. Contact between paste and electrode should be gentle to avoid both impact and scratching damage, especially in sandy samples.
11. When reading remains constant, record pH and remove electrode from paste. Carefully wash electrode with distilled water to insure removal of all paste. If all pH measurements are completed, the electrode should be stored in a beaker of distilled water. NOTE: After every 10 samples, check meter calibration with standard buffers.

3.2.3 Neutralization Potential

3.2.3.1 Principles--

The amount of neutralizing bases, including carbonates, present in overburden materials is found by treating a sample with a known excess of standardized hydrochloric acid. The sample and acid are heated to insure that the reaction between the acid and the neutralizers goes to completion.

The calcium carbonate equivalent of the sample is obtained by determining the amount of unconsumed acid by titration with standardized sodium hydroxide (Jackson, 1958).

3.2.3.2 Comments--

A fizz rating of the neutralization potential is made for each sample to insure the addition of sufficient acid to react all the calcium carbonate present.

During digestion, do not boil samples. If boiling occurs, discard sample and rerun. Before titrating with acid, fill buret with acid and drain completely. Before titrating with base, fill buret with base and drain completely to assure that free titrant is being added to the sample.

3.2.3.3 Chemicals--

1. Carbon dioxide-free water: Heat distilled water just to boiling in a beaker. Allow to cool slightly and pour into a container equipped with ascarite tube. Cool to room temperature before using.
2. Hydrochloric acid (HCl) solution, 0.1 N, certified grade (Fisher So-A-54 or equivalent).
3. Sodium hydroxide (NaOH), approximately 0.5 N: Dissolve 20.0 g of NaOH pellets in carbon dioxide-free water and dilute to 1 liter. Protect from CO₂ in the air with ascarite tube. Standardize solution by placing 50 ml of certified 0.1 N HCl in a beaker and titrating with the prepared 0.5 N NaOH until a pH of 7.00 is obtained. Calculate the Normality of the NaOH using the following equation:

$$N_2 = (N_1 V_1) / V_2, \text{ where:}$$

V_1 = Volume of HCl used.

N_1 = Normality of HCl used.

V_2 = Volume of NaOH used.

N_2 = Calculated Normality of NaOH.

4. Sodium hydroxide (NaOH) approximately 0.1 N: Dilute 200 ml of 0.5 N NaOH with carbon dioxide-free water to a volume of 1 liter. Protect from CO₂ in air with ascarite tube. Standardize solution by placing 20 ml of certified 0.1 N HCl in a beaker and titrating with the prepared 0.1 N NaOH until a pH of 7.00 is obtained. Calculate the Normality of the NaOH using the equation in 3.2.3.3 No. 3.
5. Hydrochloric acid (HCl), approximately 0.5 N: Dilute 42 ml of concentrated HCl to a volume of 1 liter with distilled water. Standardize solution by placing 20 ml of the known Normality NaOH prepared in 3.2.3.3 No. 3 in a beaker and titrating with the prepared HCl until a pH of 7.00 is obtained.

Calculate the Normality of the HCl using the following equation:

$$N_1 = (N_2V_2)/V_1, \text{ where:}$$

V_2 = Volume of NaOH used.

N_2 = Normality of NaOH used.

V_1 = Volume of HCl used.

N_1 = Calculated Normality of HCl.

6. Hydrochloric acid (HCl), approximately 0.1 N: Dilute 200 ml of 0.5 N HCl to a volume of 1 liter with distilled water. Standardize solution as in 3.2.3.3.5, but use 20 ml of the known Normality NaOH prepared in 3.2.3.3 No. 4.

7. Hydrochloric acid (HCl), 1 part acid to 3 parts water: Dilute 250 ml of concentrated HCl with 750 ml of distilled water.

3.2.3.4 Materials--

1. Flasks, Erlenmeyer, 250 ml.
2. Buret, 100 ml (one required for each acid and one for each base).
3. Hotplate, steam bath can be substituted.
4. pH meter (Corning Model 12 or equivalent) equipped with combination electrode.
5. Balance, can be read to 0.01 g.

3.2.3.5 Procedure (revised and updated from Smith et al., 1974)--

1. Place approximately 0.5 g of sample (less than 60 mesh) on a piece of aluminum foil.
2. Add one or two drops of 1:3 HCl to the sample. The presence of CaCO_3 is indicated by a bubbling or audible "fizz."
3. Rate the bubbling or "fizz" in step 2 as indicated in Table 1.
4. Weigh 2.00 g of sample (less than 60 mesh) into a 250 ml Erlenmeyer flask.
5. Carefully add HCl indicated by Table 1 into the flask containing sample.
6. Heat nearly to boiling, swirling flask every 5 minutes, until reaction is complete. NOTE: Reaction is complete when no gas evolution is visible and particles settle evenly over the bottom of the flask.

TABLE 1. VOLUME AND NORMALITY OF HYDROCHLORIC ACID USED FOR EACH FIZZ RATING

Fizz Rating	HCl	
	(ml)	(Normality)
None	20	0.1
Slight	40	0.1
Moderate	40	0.5
Strong	80	0.5

7. Add distilled water to make a total volume of 125 ml.

8. Boil contents of flask for one minute and cool to slightly above room temperature. Cover tightly and cool to room temperature. CAUTION: Do not place rubber stopper in hot flask as it may implode upon cooling.

9. Titrate using 0.1 N NaOH or 0.5 N NaOH (concentration exactly known), to pH 7.0 using an electrometric pH meter and buret. The concentration of NaOH used in the titration should correspond to the concentration of the HCl used in step 5. NOTE: Titrate with NaOH until a constant reading of pH 7.0 remains for at least 30 seconds.

10. If less than 3 ml of the NaOH is required to obtain a pH of 7.0, it is likely that the HCl added was not sufficient to neutralize all of the base present in the 2.00 g sample. A duplicate sample should be run using the next higher volume or concentration of acid as indicated in Table 1.

11. Run a blank for each volume or normality of acid using steps 5, 7, 8, and 9.

3.2.3.6 Calculations--

1. Constant (C) = (ml acid in blank)/(ml base in blank).

2. ml acid consumed = (ml acid added) - (ml base added X C).

3. Tons CaCO₃ equivalent/thousand tons of material = (ml of acid consumed) X (25.0) X (N of acid).

3.2.4 Maximum Potential Acidity by Total Sulfur Determination

3.2.4.1 Principles--

This method measures the total sulfur in a sample. If all of the total sulfur occurs in pyritic forms, the calculation of maximum potential acidity from sulfur corresponds with actual potential acidity from sulfur. But if part of the sulfur occurs in other forms, the maximum as calculated will be too high. This is the reason that such calculations are referred to as maximums and in doubtful cases approximate determinations should be made which rule out other sulfur forms (see 3.2.6). These determinations are not necessary when the maximum acid from total sulfur is within safe limits.

A sample is heated to approximately 1600°C. A stream of oxygen is passed through the sample during the heating period. Sulfur dioxide is released from the sample and collected in a dilute hydrochloric acid solution containing potassium iodide, starch, and a small amount of potassium iodate. This solution is automatically titrated with a standard potassium iodate solution.

A trace amount of potassium iodate reacts with potassium iodide and dilute hydrochloric acid to yield free iodine, potassium chloride and water. The free iodine combines with the sulfur dioxide and water to yield sulfuric acid and hydroiodic acid. The amount of potassium iodate solution used during the titration is recorded. The calculation of the percent total sulfur is based on the potassium iodate measurement (Smith et al., 1974).

3.2.4.2 Comments--

Some samples, e.g. coal, when first placed in the furnace may change the color of the solution in the titration vessel to pink or purple (probably due to organic compounds). Some samples may contain halogens (iodine, chlorine, fluorine) which darken the solution in the titration vessel and will therefore produce results that are low. The halogen problem, if encountered, may be eliminated by the use of an antimony trap between the furnace and titration assembly. Interference may result with samples high in nitrogen; however, this does not appear to happen with rock samples. Additional information can be obtained by reading Leco Equipment Application 120 and Instructions for Analysis of Sulfur in Hydrocarbons by the Leco High Frequency Combustion Titration Procedure.

Materials with a low chroma (2 or less) may have a high (over 1.0%) sulfur content; therefore, use a 0.250 g sample when the chroma of the material is 1 or 2. If the chroma of the material is zero, a 0.100 g sample is used. If sulfur is not detectable or more accurate values are desired in this sample size, increase to next highest sample size and rerun.

Read entire manuals on both the Leco Induction Furnace and the Automatic Titrator.

Periodically clean titration chamber and associated glassware with acetone or concentrated hydrochloric acid and rinse thoroughly with distilled water.

The following procedure is for use with a LECO Induction Furnace, Model 521 with Automatic Sulfur Titrator, Model 532. Other similar or advanced models of this instrumentation may perform equally well; however, the following procedure will require detailed modifications by a qualified person for application to other instruments.

3.2.4.3 Chemicals--

1. Iron chip accelerator (Leco number 501-077).
2. Iron powder accelerator (Leco number 501-078).
3. Copper ring (Leco number 550-189).
4. Magnesium oxide (MgO).
5. Potassium iodate (KIO₃), 0.0052 N: Dissolve 1.110 g KIO₃ in distilled water and dilute to 1 liter.
6. Hydrochloric acid (HCl) solution: Dilute 15 ml of concentrated HCl to a volume of 1 liter with distilled water.
7. Arrowroot starch solution: Dissolve 4.0 g of arrowroot starch (Leco number 501-061) in 100 ml of distilled water in a 250 ml beaker. Stir on a mechanical stirrer with a stirring bar. While starch is stirring, boil 300 ml of distilled and deionized water in a 600 ml beaker. Remove from heat when boiling point is reached. Remove starch from stirrer. Place boiled water on mechanical stirrer with stirring bar. While water is continually stirring, add 5 ml of starch mixture in 20 second intervals until all starch solution has been added. Place a small amount of the solution in the 600 ml beaker back into the 250 ml beaker that contained the starch mixture. Wash beaker by hard swirling and then pour contents back into the 600 ml beaker. Continue stirring solution in the 600 ml beaker allowing solution to cool to 40°C. Add 12.0 g of potassium iodide (KI). Continue stirring for 15 to 20 minutes.
8. Potassium iodide (KI).
9. Sulfur standards (Leco number 501-502).

3.2.4.4 Materials--

1. Leco Automatic Sulfur Analyzer, package unit, number 634-700.
2. Scoops, 0.2 ml volume.
3. Ceramic crucibles with porous covers.
4. Carboys, 19 liters (5 gal).
5. Tongs.

6. Glass wool.
7. Oxygen regulators.
8. Mechanical stirrer.
9. Stirring bar.
10. Combustion tube, hydrocarbon (Leco number 519-004).
11. Hot plate.
12. Balance, can be read to 0.001 g.

3.2.4.5 Procedure (revised and updated from Smith et al., 1974)--

NOTE: Read entire manuals on Leco Furnace, Automatic Titrator and this entire procedure before starting.

1. Place one level scoop of iron chips in crucible.
2. Weigh 0.500 g of sample (less than 60 mesh) into the crucible.

NOTE: For samples that are suspected to contain over 1% sulfur or have a chroma of less than 2, see 3.2.4.2.

3. Add one scoop MgO.
4. Add one copper ring and then one scoop of iron powder.
5. Gently shake the crucible to evenly cover the bottom and place one porous cover on the crucible.
6. Turn on "Filament Voltage" grid tap to medium position.
7. Wait for one minute then turn "High Voltage" switch to ON.
8. Set "Titrate-Endpoint" switch to its middle position.
9. Turn on titrator (upper left switch above "Endpoint Adjust").
10. Drain "Titration Vessel" completely.
11. Set timer switch to ON, adjust timer to 10 minutes, or a time sufficient to satisfy steps 25, 26, and 27.
12. Slosh carboys containing HCl and KIO₃ to mix the condensate on the walls of the container.
13. Fill "Iodate Buret."

14. Fill "Titration Vessel" approximately one-third full with the HCl solution.
15. Turn on oxygen. Set the pressure to 15 psi, and the flow rate to 1.0 liter per minute. NOTE: Oxygen flow must be started before starch is added.
16. Raise the "Locking Mechanism Handle" WITHOUT a sample crucible on the pedestal, and lock in place. NOTE: Make sure there is an airtight contact between sample platform and combustion chamber by observing a vigorous bubbling in the "Titration Vessel" chamber.
17. Add one measure (5 ml) of starch solution. NOTE: If solution in "Titration Vessel" chamber turns turbid or yellow after starch solution is added, turn off the instrument following steps 33 through 39 and make NEW starch solution.
18. Set "Titrant-Endpoint" switch to "Endpoint."
19. After a few seconds when titrant level in "Iodate Buret" has stopped falling (Buret reading should be no more than 0.004) the solution in the "Titration Vessel" chamber should be a deep blue. NOTE: If the solution is a pale blue or almost black, turn off the instrument following steps 33 through 39 and make NEW starch solution.
20. Set "Titrant-Endpoint" switch to middle position and lower "Locking Mechanism Handle."
21. Refill "Iodate Buret."
22. Place sample crucible on pedestal, making sure it is centered, and carefully raise "Locking Mechanism Handle" and lock in place.

NOTE: Make sure there is an airtight contact between sample platform and combustion chamber by observing a vigorous bubbling in the "Titration Vessel" chamber.
23. Set "Titrant-Endpoint" switch to Titrant, or if it is known that sample will evolve SO_2 slowly, set switch at Endpoint. The Endpoint setting acts as a "Fine Control" allowing buret valve to discriminate smaller increments.
24. Push RED button on timer to start analysis.
25. Plate current must go to 400-450 ma for at least 2 minutes during the analysis; if not, reweigh and rerun sample.
26. Adjust rheostat to prevent plate current from exceeding 450 ma.
27. When buret reading does not change for 2 minutes, and Plate Current has achieved 400 to 450 ma, it can be assumed that all of the sulfur has been removed from the sample. If buret reading is still changing when timer shuts off instrument, set Timer Switch to OFF, which restarts furnace, leave furnace on until buret is stable for 2 minutes, then turn Timer Switch to ON.

28. Set "Titrate-Endpoint" to middle position. IMPORTANT: Record titration reading.
29. Lower sample platform, remove crucible using tongs, place fresh sample crucible in place, but do not close sample chamber.
- NOTE: Slightly drain titrating chamber to maintain original level. Drain, flush, and refill titrating chamber every 3rd sample, or more often if a large quantity of titrant was used by the previous sample (steps 16-22).
30. Refill KIO_3 buret.
31. Close sample chamber, making sure it is tight. Check endpoint (steps 18, 19 and 21).
32. Go to step 23 and continue until all samples have been processed.
33. Turn "Titrate-Endpoint" switch to mid position.
34. Turn off main O_2 valve on top of tank.
35. Turn off "High Voltage."
36. Turn off Automatic Titrator.
37. Drain titration chamber; flush twice with a chamber full of HCl solution or water, cover and leave chamber full of HCl solution.
38. If O_2 has stopped bubbling in the purifying train, turn off small knurled valve on gauge outlet.
39. Turn off "Filament Voltage."

3.2.4.6 Calculations--

1. Percent sulfur. NOTE: Percent sulfur is dependent upon the concentration of potassium iodate titrant and sample size.
- A. Using 1.110 g KIO_3/L and 0.500 g sample (0.005 - 1.00% sulfur range)
%S = Buret reading X 5.0.
- B. Using 1.110 g KIO_3/L and 0.250 g sample (0.010 - 2.00% sulfur range)
%S = Buret reading X 10.0.
- C. Using 1.110 g KIO_3/L and 0.100 g sample (0.025 - 5.00% sulfur range)
%S = Buret reading X 25.0.
2. To convert % sulfur to maximum CaCO_3 equivalents: Multiply % sulfur by 31.25 to get tons CaCO_3 equivalent/1000 tons of material.

3.2.5 Sodium Bicarbonate Extractable Phosphorus

3.2.5.1 Principle--

This method is a non-destructive extraction of phosphorus from the surfaces of particles. The pH of extracting solution remains nearly constant during the extraction procedure.

The concentration of phosphorous in solution increases in calcareous, alkaline or neutral soils containing calcium phosphates since the concentration of calcium decreases due to the precipitation of calcium as calcium carbonate. In the presence of the solid-phase calcite, the concentration of calcium is $6 \times 10^{-7} \text{ M}$ in the extracting solution at equilibrium. As the pH rises, the phosphorous concentration increases in acid soils containing aluminum and iron phosphates. Secondary precipitation reactions are reduced to a minimum in acid and calcareous soils because the aluminum, calcium, and iron concentrations remain at a low level in this extractant (Olsen and Dean, 1965).

3.2.5.2 Comments--

Temperature of the extracting solution and the shaking speed may cause variations in the results. Phosphorous increases approximately 0.43 ppm for each degree rise in temperature between 20° and 30°C for soils testing between 5 and 40 ppm of phosphorous.

Plastic containers should be used to store the extracting solution. If glass is used, a fresh solution should be prepared every month, since the pH tends to increase with time resulting in a higher value for extractable phosphorous.

This method is especially important for overburdens or minesoil because carbonates often are present, even when the paste pH is below 7.

A shaking speed of 2 should be used on the Burrell wrist-action shaker. Other shakers may be used, but when the speed increases greatly from that of the Burrell shaker, somewhat higher results may be obtained.

3.2.5.3 Chemicals--

1. Sodium bicarbonate (NaHCO_3), 0.5 M: Dissolve 42.0 g of NaHCO_3 and dilute to 1 liter with carbon dioxide-free water (see 3.2.3.3 No. 1). Adjust to pH 8.5 with 1 N NaOH. Protect from CO_2 in air with soda lime or ascarite in a guard tube. Store in polyethylene container and make fresh every 2 months.

2. Ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$): Dissolve 15.0 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 300 ml of warm distilled water. Filter if cloudy and allow to cool. Gradually add 342 ml of 12.0 M (37%) HCl. Dilute to 1 liter with distilled and deionized water.

3. Stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$), stock solution: Dissolve 10.0 g of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (large crystals) in 25 ml of 12.0 M (37%) HCl. Store in brown glass bottle in a refrigerator. Prepare fresh every 2 months.

4. Stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$), dilute solution: Mix 0.5 ml of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ stock solution with 66 ml of distilled water. Prepare dilute solution for each set of determinations.

5. Potassium phosphate (KH_2PO_4), standard phosphorus stock solution: Dissolve 0.4393 g of KH_2PO_4 with 500 ml of distilled water and dilute to 1 liter. Add 5 drops of toluene to reduce microbial growth. This is a 100 ppm P standard.

6. Potassium phosphate (KH_2PO_4), dilute solution: Dilute 20 ml of KH_2PO_4 stock solution to 1 liter with distilled water. NOTE: This solution contains 2 micrograms of P per ml (2 ppm).

7. Toluene ($\text{C}_6\text{H}_5\text{CH}_3$).

8. Hydrochloric acid (HCl), 12 M (37%).

9. Decolorizing charcoal, Darco G-60 (J. T. Baker Co. or equivalent).

3.2.5.4 Materials--

1. Flasks, Erlenmeyer, 50 ml with stoppers.

2. Flasks, volumetric, 25 ml, with caps.

3. Flask, volumetric, 1000 ml.

4. Funnels, 60 mm diameter.

5. Funnel rack.

6. Beakers, 50 ml.

7. Cylinder, graduated 25 ml.

8. Pipet, 20 ml.

9. Pipet, 10 ml.

10. Pipet, 5 ml.

11. Pipet, 1 ml.

12. Filter paper, 110 mm diameter, medium porosity, ashless (Whatman 40, S & S 589, or equivalent).

13. Balance, can be read to 0.0001 g.

14. Shaking machine, Wrist-Action (Burrell model BB or equivalent).
15. Colorimeter or Spectrophotometer, with filter or adjustment to provide 660 nm incident light.
16. Cuvettes or matched test tubes to fit above colorimeter.
17. pH meter (Corning model 12 or equivalent) equipped with combination electrode.
18. Measuring spoon, 1/4 teaspoon volume.

3.2.5.5 Procedure (revised and updated from Smith et al., 1974)--

1. Add 1.250 g of less than 60 mesh rock or soil sample, 1/4 teaspoon decolorizing carbon, and 25 ml of NaHCO₃ solution to the 50 ml Erlenmeyer flask. Stopper the flask.
2. Shake for 30 minutes using a shaking speed of 2 on a Burrell wrist-action shaker.
3. Filter the suspension. NOTE: Shake flask before pouring suspension into filter funnel. If filtrate is yellow, add 1/4 teaspoon carbon, mix well and refilter. If filtrate is cloudy, filter using fine porosity filter paper.
4. Pipet 10 ml of filtrate into a 25 ml volumetric flask. Pipet 10 ml of H₂O into a separate 25 ml volumetric flask (blank). NOTE: If necessary to interrupt work, stop here, stopper and refrigerate.
5. Slowly add, with a pipet or calibrated dispenser, 5 ml of ammonium molybdate solution and mix immediately holding the top of the flask tightly closed. NOTE: Gases are generated during this mixing. The pH of the solution after adding molybdate should be between 3.0 and 4.0. With some alkaline soils it may be necessary to add more acid in order to assure the indicated pH for consistent color development. However, with minesoils studied, 5 ml of molybdate has been sufficient and has avoided excess acidity with extremely acid samples.
6. Wash down neck of flask with a small amount of water and dilute to about 22 ml.
7. Pipet 1 ml of the dilute SnCl₂ solution into the flask, dilute to volume (25 ml) with distilled water, and mix contents immediately.
8. After 10 minutes but less than 20 minutes after adding the dilute SnCl₂ to the flask and mixing, measure the adsorbance (A) of the blue solution, using the colorimeter or spectrophotometer at 660 nm. Read and understand instructions for operating the instrument correctly before using.

TABLE 2. STANDARDS FOR SODIUM BICARBONATE EXTRACTABLE PHOSPHORUS

P concentration (ppm P)	Volume of dilute (2 ppm) P Standard (ml)	Volume of H ₂ O (ml)	Volume of NaHCO ₃ (ml)
Blank	0	13	5
0.08	1	12	5
0.16	2	11	5
0.24	3	10	5
0.32	4	9	5
0.40	5	8	5
0.48	6	7	5
0.56	7	6	5
0.64	8	5	5
0.72	9	4	5
0.80	10	3	5
0.88	11	2	5
0.96	12	1	5
1.04	13	0	5

9. Prepare standard curve of P concentration as follows:

- a. Using 25 ml volumetric flasks, prepare phosphorus standards from Table 2.
- b. Develop the color as in steps 5 and 7.
- c. Make a standard curve by plotting absorbance (A) vs. P concentration (ppm) on linear graph paper.
- d. Find ppm in sample extract by finding absorbance (A) of the extract on the standard curve and reading ppm of P directly from the curve.

3.2.5.6 Calculations--

1. ppm P in the rock or soil = ppm (read from the curve) X 50.

NOTE: The 50 is obtained from the following equation:

$50 = (25 \text{ ml extracting solution} / 1.25 \text{ g sample}) \times (25 \text{ ml final volume} / 10 \text{ ml extract})$.

2. pp2m P in the soil = (ppm P in soil) X 2.

3.2.6 HCl-Extractable, HNO₃-Extractable and Non-Extractable Total Sulfur

3.2.6.1 Principle--

In doubtful cases, as stated in 3.2.4.1, this method should be used to rule out HCl-extractable and non-extractable forms of sulfur which are not considered to be acid formers. The HNO₃-extractable sulfur is determined by calculations. This form of sulfur will react with oxygen to produce acid.

3.2.6.2 Comments--

It is necessary to remove chlorides and nitrates by water leachings after the hydrochloric and nitric acid (respectively) extractions before running total sulfur.

Care should be taken that no sample is lost by run over, splashing or breaking through the filter paper during all leachings.

3.2.6.3 Chemicals--

1. Hydrochloric acid (HCl), 2 parts acid to 3 parts water: Mix 400 ml of concentrated HCl with 600 ml of distilled water.
2. Nitric acid (HNO₃), 1 part acid to 7 parts water: Mix 125 ml of concentrated HNO₃ with 875 ml of distilled water.
3. Silver Nitrate (AgNO₃), 10%: Dissolve 10.0 g of AgNO₃ in 90 ml of distilled water. Store in amber bottle away from light.
4. Nessler's Solution (Fisher Scientific Co. No. So-N-24 or equivalent).

3.2.6.4 Materials--

1. Leco Induction Furnace and Automatic Sulfur Titrator as in 3.2.4.4.
2. Funnels, 28 mm I.D. polyethylene.
3. Filter paper, 5.5 cm glass fiber.
4. Flasks, Erlenmeyer, 250 ml.

5. Beakers, 100 ml.
6. Syringe.
7. Balance, can be read to 0.001 g.

3.2.6.5 Procedure (Revised and updated from Smith et al., 1974)--

1. Take three 0.500 g subsamples of less than 60 mesh material.
2. Take one subsample and analyze for total sulfur (see 3.2.4).
3. Taking care not to sharply crease the glass fibers, fold filter paper to fit a polyethylene funnel.
4. Place second subsample in filter. NOTE: Make sure all material is placed in the filter.
5. Place subsample and filter onto funnel holder in sink or other suitable pan which can receive outflow from funnel.
6. Using a syringe, pipette, or other graduated dispenser, add 2:3 HCl to almost the top of the filter paper. Caution: During this step and all other leaching steps, be careful not to lose any sample by runover, splashing, or breaking through the filter paper.
7. Repeat step 6 until a total of 50 ml of acid has been added.
8. Place funnel holder, containing funnel and subsample, over a 100 ml beaker.
9. Leach subsample with 50 ml of distilled and deionized water. Discard leachate. NOTE: Stop here if procedure cannot be completed in one day. CAUTION: Samples must be kept moist.
10. Leach subsample with another 50 ml of distilled and deionized water.
11. Test leachate for chlorides by adding 3 drops of 10% AgNO₃ with a dropper. NOTE: The presence of chlorides will be detected by a white precipitate.
12. Discard leachate and repeat steps 10 and 11 until no precipitate forms.
13. Discard leachate.
14. Air dry subsample and filter overnight.
15. Carefully fold glass fiber filter around the sample and transfer to a ceramic crucible for total sulfur analysis (see 3.2.4).
16. Place third subsample in a 250 ml Erlenmeyer flask. NOTE: Make sure all of the subsample is placed in the flask.

17. Add 50 ml of HNO_3 (1:7).
18. Let stand overnight at room temperature.
19. Taking care not to sharply crease the glass fibers, fold a filter to fit a polyethylene funnel.
20. Place a funnel holder over a sink or other suitable pan which can receive outflow from funnel.
21. Carefully pour subsample and acid from the Erlenmeyer flask into the funnel. NOTE: Do not get material above top of filter paper.
22. Repeat step 21 using distilled and deionized water to wash all materials remaining in the Erlenmeyer flask into the funnel.
23. Place funnel holder containing funnel and subsample over a 100 ml beaker. NOTE: Stop here if procedure cannot be completed in one day. CAUTION: Sample must be kept moist.
24. Leach subsample with 50 ml of distilled and deionized water. Discard leachate.
25. Leach subsample with another 50 ml of distilled and deionized water.
26. Test leachate for presence of nitrates by adding 3 drops of Nessler's Solution with a dropper. NOTE: If nitrates are present, the leachate will turn yellow within 30 seconds as seen against a white background.
27. Discard leachate and repeat steps 25 and 26 until no nitrates are detected.
28. Discard leachate.
29. Air dry subsample and filter overnight.
30. Carefully fold glass fiber filter around the sample and transfer to a ceramic crucible for total sulfur analysis (see 3.2.4).

3.2.6.6 Calculations--

1. HCl -extractable sulfur (mostly sulfates) = (Total sulfur of untreated sample) minus (Total sulfur after HCl treatment).
2. HNO_3 -extractable sulfur (mostly pyritic sulfur) = (Total sulfur after HCl treatment) minus (Total sulfur after HNO_3 treatment).
3. Non-extractable sulfur (mostly organic sulfur) = Total sulfur after HNO_3 treatment.

3.2.7 Lime Requirement By Ca (OH)₂ Titration

3.2.7.1 Principle--

When calcium hydroxide is added to the soil, it initially reacts with and neutralizes any acidity in solution. The calcium hydroxide further reacts with the acidity contained on the soil particles. A time period of four days is required for the reaction to go to equilibrium. Because 5 ml of 0.04 N calcium hydroxide is equivalent to 1 ton of pulverized limestone per 1000 tons of material, various amounts can be added to the sample making this treatment similar to liming the soil. After the 4 day incubation period, pH determinations are made. A titration curve is drawn comparing pH to the amount of pulverized limestone per 1000 tons of material. From this curve the amount of pulverized limestone per 1000 tons of material can be determined to bring the soil to a pH of 6.5 (Dunn, 1943).

3.2.7.2 Comments--

The calcium hydroxide must be protected from carbon dioxide in the air by using soda lime or ascarite in a guard tube. The method is time consuming due to a 4 day incubation period; however, it is a reliable and accurate method for determining the lime requirement.

3.2.7.3 Chemicals--

1. Calcium hydroxide (Ca(OH)₂), 0.04 N, saturated solution: Dissolve 1.5 g Ca(OH)₂ (use some excess) and dilute to 1 liter with carbon dioxide-free water (see 3.2.3.3. No. 1). Filter to remove calcium carbonate (CaCO₃) and protect filtrate from CO₂ in the air with soda lime or ascarite in a guard tube.
2. Standard buffer solutions, pH - 4.00 and pH - 7.00.
3. Chloroform (CHCl₃).

3.2.7.4 Materials--

1. Flasks, Erlenmeyer, 250 ml with rubber stoppers.
2. Balance, can be read to 0.1 g.
3. pH meter (Corning model 12 or equivalent) with combination electrode.

3.2.7.5 Procedure--

1. Place 10 g samples of less than 60 mesh air-dry soil in 7 flasks.
2. Add Ca(OH)₂ at the rates of 1/2, 1, 2, 3, 4, 5, 6 tons of pulverized limestone per 1000 tons of material using 5 ml of 0.04 N Ca(OH)₂ as the equivalent of 1 ton of pulverized limestone per 1000 tons of material.
3. Dilute to 100 ml with distilled water.

4. Add three drops of chloroform to prevent microbial activity.
5. Allow suspensions to stand in stoppered flasks for 4 days with thorough shaking twice a day.
6. After 4 day incubation period, calibrate pH meter using pH 4.00 and 7.00 standard buffer solutions (see 3.2.2) and determine suspension pH.
NOTE: Gently swirl the suspension to insure good electrode-suspension contact.

3.2.7.6 Calculations--

1. Construct a titration curve by plotting pH on the horizontal axis and tons of pulverized limestone per 1000 tons of material on the vertical axis.
2. Plot points and construct a best-fit curve through the points.
3. Draw a line vertically from pH 6.5 to the curve and put an (X) on the curve.
4. Draw a line horizontally from the (X) to the vertical axis.
5. Determine tons of pulverized limestone per 1000 tons of material needed to bring the soil to pH 6.5.

3.2.8 Lime Requirement By the Five Minute Boiling Method

3.2.8.1 Principle--

Calcium hydroxide neutralizes the acidity in solution first and then reacts with and neutralizes the acidity contained on the soil particles. This reaction time is greatly reduced by boiling the sample and calcium hydroxide mixture for 5 minutes and allowing it to cool before a measurement is taken. The procedure is similar to liming the samples, since 5 ml of 0.04 N calcium hydroxide is equivalent to 1 ton of pulverized limestone per 1000 tons of material. A titration curve is drawn comparing pH to tons of pulverized limestone per 1000 tons of material. The amount of pulverized limestone needed to bring the soil to a pH of 6.5 can be read directly from the curve. (Abruna and Vicente, 1955).

3.2.8.2 Comments--

Because of the 5 minute boiling period, the time element is reduced from 4 days (Ca(OH)₂ method) to about 1 hour.

The calcium hydroxide must be protected from carbon dioxide in the air by using soda lime or ascarite in a guard tube.

3.2.8.3 Chemicals--

1. Calcium hydroxide (Ca(OH)₂), 0.04 N, saturated solution: Dissolve 1.5 g Ca(OH)₂ (use some excess) and dilute to 1 liter with carbon dioxide-

free water (See 3.2.3.3 No. 1). Filter off calcium carbonate (CaCO_3) and protect from CO_2 in the air with soda lime or ascarite in a guard tube.

2. Standard buffer solutions, pH = 4.00 and pH = 7.00

3.2.8.4 Materials--

1. Flasks, Erlenmeyer, 250 ml.
2. Hot plate.
3. Thermometer, 0 - 100°C.
4. Water Tray.
5. Balance, can be read to 0.1 g.
6. pH meter, (Corning model 12 or equivalent) with combination electrode.

3.2.8.5 Procedure--

1. Place 10 g samples of less than 60 mesh air-dry soil in 7 flasks.
2. Add $\text{Ca}(\text{OH})_2$ at the rates of 1/2, 1, 2, 3, 4, 5, 6 tons of pulverized limestone per 1000 tons of material using 5 ml of 0.04 N $\text{Ca}(\text{OH})_2$ as the equivalent of 1 ton of pulverized limestone per 1000 tons of material.
3. Dilute with 50 ml of distilled water.
4. Boil on a hot plate for 5 minutes. NOTE: Intermittent stirring of the samples may be necessary to avoid excessive foaming.
5. Cool in water tray to 25°C.
6. Calibrate pH meter using pH 4.00 and 7.00 buffer solutions.
7. Immediately after cooling, determine pH of soil + water + 0.04 N $\text{Ca}(\text{OH})_2$ suspension using a glass electrode. NOTE: Gently swirl the beaker to insure good electrode-suspension contact.
8. Record pH.

3.2.8.6 Calculations--

See 3.2.7.6.

3.2.9 Lime Requirement by the Woodruff Buffer Method

3.2.9.1 Principle--

The solution used is calcium acetate buffered by p-nitrophenol. An excess of the buffered solution (at pH 7.0) is added to the sample and allowed to

equilibrate for an hour. The pH of the solution is read and the lime requirement is based on the drop in pH of the buffered solution. By allowing the buffer solution to stand in contact with the sample, calcium ions from the solution saturate the exchange complex and hydrogen ions go into solution, thus lowering the pH (Woodruff, 1948).

3.2.9.2 Comments--

The method is quick, reliable, and adaptable to use on soils of different exchange capacities. The Woodruff buffer solution is strongly buffered and may not accurately detect the lime requirement for weakly acid samples. On strongly acid minesoil samples, the Woodruff buffer method correlated with the $\text{Ca}(\text{OH})_2$ titration procedure of determining lime requirement (West Virginia University, 1971).

3.2.9.3 Chemicals--

1. p-Nitrophenol ($\text{NO}_2\text{C}_6\text{H}_4\text{OH}$).
2. Calcium acetate ($\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$).
3. Magnesium oxide (MgO), heavy, powder, laboratory grade (Fisher M-50 or equivalent).
4. Standard buffer solutions, pH = 4.00 and pH = 7.00.
5. Woodruff buffer, stock solution: In a 10 liter glass bottle mix 80.0 g of $\text{NO}_2\text{C}_6\text{H}_4\text{OH}$, 400.0 g $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$, 6.2 g MgO , and 4 liters of distilled water. Make to 10 liters with distilled water. Put on reciprocating shaker at low speed overnight. Filter solution. Adjust to pH 7.00 with HCl or MgO .
6. Woodruff buffer, dilute solution: Mix 20 ml of Woodruff buffer stock solution with 10 ml of distilled water.

3.2.9.4 Materials--

1. Glass bottle, 10 liter.
2. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 120 strokes per minute.
3. Paper cup or beaker
4. Automatic pipet, 5 ml.
5. Stirrer.
6. pH meter (Corning model 12 or equivalent) with combination electrode.
7. Balance, can be read to 0.01g.

3.2.9.5 Procedure--

1. Place 5.0 g of less than 60 mesh sample in a paper cup or beaker.
2. Add 5 ml of distilled water and mix with the soil. Let stand and mix occasionally for 1 hour.
3. Calibrate pH meter using pH buffer solutions of 4.00 and 7.00 (See 3.2.1).
4. Using pH meter, record pH of soil + water mixture by placing an electrode into the sample while shaking the cup. This insures good electrode contact with the mixture. NOTE: Soil + water mixture equalling or exceeding pH 6.5 have a lime requirement of zero tons of pulverized limestone per 1000 tons of material.
5. Add 5 ml of the Woodruff buffer stock solution.
6. Stir or shake for at least 30 minutes.
7. Using the dilute Woodruff buffer solution, adjust meter to a reading of exactly pH = 7.0.
8. Read and record pH of soil + water + Woodruff buffer stock solution mixture while shaking the cup to insure a good electrode contact. Record as buffered pH reading.

3.2.9.6 Calculations--

1. pH depression = $(7.0) - (\text{buffered pH reading})$.
2. Lime Requirement (L.R.) in tons pulverized limestone/1000 tons of material = $0.5 \times (\text{pH depression})$.

3.2.10 Lime Requirement by S.M.P. Buffer

3.2.10.1 Principle--

By measuring a change in pH of a buffer caused by the acids in a soil, Shoemaker, McLean, and Pratt (1962) determined the lime requirement of a soil. The lime requirement is read directly from a table based on pH of a soil after the S.M.P. buffer has been added.

3.2.10.2 Comments--

The S.M.P. buffer is very reliable for soils with a 2 ton per 1000 ton of material lime requirement. It adapts well for acid soils with a pH below 5.8 containing less than 10% organic matter and having appreciable quantities of soluble aluminum.

A sensitivity of 0.1 pH unit is needed for the interpretation of this method. A difference of 0.1 pH unit will result in a lime requirement difference of 0.5 to 0.9 tons of lime per 1000 tons of material for mineral soils.

Increased exposure time causes greater acidity thus causing a greater lime requirement. Increases in organic matter and/or clay content increases absorption of acidic cations. Buffer modifications may be necessary to prevent interference from hydroxy-iron and hydroxy-aluminum polymers. Air-dry soils may be stored several months in closed containers without affecting the SMP pH measurement.

3.2.10.3 Chemicals--

1. Standard buffer solutions, pH = 4.00 and pH = 7.00
2. SMP buffer solution : Dissolve 1.8 g p-nitrophenol ($\text{NO}_2\text{C}_6\text{H}_4\text{OH}$), 2.5 ml triethanolamine ($\text{C}_6\text{H}_{15}\text{NO}_3$), 3.0 g potassium chromate (K_2CrO_4), 2.0 g calcium acetate ($\text{Ca}(\text{CO}_2\text{CH}_3)_2$), and 53.1 g calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) with distilled water and dilute to 1 liter. Filter through a fiberglass sheet if suspended material is present. Connect an air inlet with a 2.54 X 30.5 cm (1 x 12 in) cylinder of drierite, a 2.54 X 30.5 cm cylinder of ascarite, and a 2.54 X 30.5 cm cylinder of drierite in series.

3.2.10.3 Materials--

1. Cup, 50 ml. glass, plastic, or waxed paper of similar size.
2. Pipet, 10 ml capacity.
3. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 250 strokes per minute.
4. pH meter (Corning model 12 or equivalent) with combination electrode.
5. Balance, can be read to 0.1 g.

3.2.10.5 Procedure--

1. Weigh 5 g of less than 60 mesh sample into a 50 ml cup.
2. Add 5 ml of distilled water. Mix for 5 seconds.
3. Wait for 10 minutes and read the soil pH (see 3.2.2).
4. Add 10 ml SMP buffer solution to the cup for mineral soils with a pH of 6.5 or less.
5. Shake for 10 minutes on reciprocating shaker at 250 strokes per minute or stir.
6. Let stand for 30 minutes.
7. Read pH of the soil-buffer solution to the nearest 0.1 pH unit (see 3.2.2).

TABLE 3. SOIL-SMP BUFFER pH AND CORRESPONDING LIME REQUIREMENT (L.R.) TO BRING MATERIAL TO pH 6.5*

pH	L.R. (Tons/1000 Tons)**	pH	L.R. (Tons/1000 Tons)**
6.9	0.3	5.8	8.1
6.8	1.0	5.7	8.9
6.7	1.8	5.6	9.6
6.6	2.4	5.5	10.4
6.5	3.1	5.4	11.1
6.4	3.9	5.3	11.7
6.3	4.6	5.2	12.5
6.2	5.3	5.1	13.2
6.1	6.1	5.0	14.0
6.0	6.0	4.9	14.7
5.9	5.9	4.8	15.5

*Adapted from Shoemaker, McLean, and Pratt, 1962.

**Agricultural ground limestone TNP at least 90%.

3.2.10.6 Calculations--

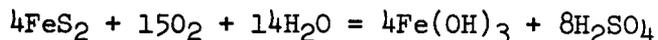
Determine lime requirement from Table 3.

3.2.11 Total Sulfur Estimation By Peroxide Oxidation

3.2.11.1 Principles--

Pyritic minerals begin to change into two new products when exposed to the atmosphere. The change may proceed slowly over a long period of time before the final products (yellowboy and sulfuric acid) are formed. The end product, "yellowboy," actually may form only when the sulfate is partially or completely neutralized by a basic substance. The chemical equation for this complete change in pyrite follows:

Pyrite + Oxygen + Water equals Yellowboy + Sulfuric Acid



Hydrogen peroxide greatly reduced the time needed for pyrite to oxidize to sulfuric acid and yellowboy.

3.2.11.2 Comments--

Alkaline materials interfere with the efficiency of hydrogen peroxide in oxidizing pyrite; therefore, overburden rock and minesoil samples containing carbonates need to be leached with acid and water as prescribed in steps 2 through 5 of the procedure.

When samples contain readily oxidizable organic matter, step 7 in the procedure may have to be repeated until the reaction stops.

The hydrogen peroxide used in this method must be 30% hydrogen peroxide. It must not contain stabilizers.

An important thing to remember is that this procedure works with fresh overburden and not with complex mixtures of minesoil material.

3.2.11.3 Chemicals--

1. Silver nitrate (AgNO_3), 10%: Dissolve 10.0 g of AgNO_3 with distilled water and make to a volume of 100 ml. Store in brown bottle away from light.
2. Hydrochloric acid (HCl), 2 parts acid to 3 parts water: Mix 400 ml of concentrated HCl with 600 ml of distilled water.
3. Hydrogen peroxide (H_2O_2), 30% (Fisher certified No. H-325 or equivalent).
4. Sodium hydroxide (NaOH), 1.0 N: Dissolve 40.0 g of NaOH pellets in carbon dioxide-free water (see 3.2.3.3. No. 1) and make to a volume of 1 liter. Protect from CO_2 in air with ascarite tube.
5. Sodium hydroxide (NaOH), 0.1 N: Dilute 10 ml of 1.0 N NaOH to a volume of 1 liter with carbon dioxide-free water (see 3.2.3.3. No. 1). Standardize solution (see 3.2.3.3. No. 4). Protect from CO_2 in air with ascarite tube.

3.2.11.4 Materials--

1. Sample, ground to pass a 60 mesh sieve.
2. Funnels.
3. Hotplate. NOTE: Bunsen burner may be substituted.
4. Thermometer, °C.

5. Beakers, 300 ml tall form.
6. Graduated cylinder, 25 ml.
7. Glass fiber filter (Reeve Angel 934AH or equivalent).
8. Burets, 50 ml capacity.
9. Balance, can be read to 0.01 g.
10. pH Meter (Corning Model 12 or equivalent) with combination electrode.

3.2.11.5 Procedure (modified and updated from Smith et al., 1974)--

1. Weigh 2.00 grams of less than 60 mesh sample.

NOTE: If the sample contains no carbonates and no sulfates, and the paste pH is less than 5.5, then steps 2 through 5 can be eliminated and procedure can be continued at step 6.

2. Place sample into a funnel fitted with filter paper and leach with 200 ml of 2:3 HCl in funnel-full increments.
3. Leach sample with distilled water (in funnel-full increments) until effluent is free from chloride as detected by 10% silver nitrate. Note: Add three drops of silver nitrate. If a white precipitate forms, chlorides are present.
4. Air dry filter and sample overnight, or place in 50°C forced air oven until dry.
5. Carefully scrape dried sample from filter surface and mix sample.
6. Place sample in a 300 ml tall form beaker.
7. Add 24 ml of 30% H₂O₂ and heat beaker on hotplate until solution is approximately 40°C. Remove beaker from hotplate and allow reaction to go to completion as shown when bubbling ceases. NOTE: Three blanks for each batch of samples should be handled in the same manner. CAUTION: Initial reaction may be quite turbulent when samples contain more than 0.1% sulfur.
8. Add an additional 12 ml of H₂O₂ (30%) to beaker and allow reaction to go to completion as shown when bubbling ceases.
9. Place beaker on hotplate and heat to approximately 90 to 95°C, solution temperature, until any unreacted H₂O₂ left in beaker is destroyed as shown when bubbling ceases. Do not allow to go to dryness.
10. Wash down the sides of the beaker with distilled water and make the volume of the solution to approximately 100 ml.

11. Place beaker on the hotplate and heat the solution to boiling to drive off any dissolved CO_2 , then cool the solution to room temperature.
12. Titrate the solution with 0.0100 N NaOH, that is free to CO_2 and protected from the atmosphere, to pH 7.0 using a pH meter.

3.2.11.6 Calculations--

1. $\text{meq H}^+/\text{100 g} = (\text{ml of NaOH}) \times (\text{N of NaOH}) \times (\text{100g/weight of sample})$.
2. $\% \text{ S} = 0.0185 (\text{meq H}^+/\text{100g}) - 0.0806$. (Grube, et al., 1973).
3. To convert percent sulfur (% S) to maximum CaCO_3 equivalents: Multiply %S by 31.25 to get tons CaCO_3 equivalent/1000 tons of material.

3.2.12 Double Acid Extractable Phosphorus, Potassium, Calcium, and Magnesium

3.2.12.1 Principle--

The method is a modified North Carolina Double Acid Method first published by Mehlich (1953) and then by Nelson, Mehlich and Winters (1953). Phosphorus, potassium, calcium, and magnesium are extracted from the sample using a solution containing dilute hydrochloric and sulfuric acid. Phosphorus concentration in the extract is determined using a colorimeter and calibration curve. The concentrations of potassium, calcium, and magnesium in the extract are determined using an atomic absorption spectrophotometer and calibration curve. The concentrations of each element can then be converted into pounds/1000 tons by calculations.

3.2.12.2 Comments--

With some soils a light to dark yellow color may develop in the extract. Decolorization is accomplished by the addition of activated charcoal in the extraction procedure. Lanthanum is added as a compensating element to remove phosphate and sulfate interference in the atomic absorption spectrophotometer methods for calcium and magnesium.

After the initial extraction, individual elements can be determined if data for all four elements are not required. Samples with elements higher in concentration than given in the calibration curves must be diluted and the resulting reading multiplied by the dilution factor.

3.2.12.3 Chemicals--

1. Hydrochloric acid (HCl), concentrated.
2. Sulfuric acid (H_2SO_4), concentrated.
3. Extracting solution: To make 0.05 N HCl and 0.025 N H_2SO_4 , measure about 10 liters deionized water into an 18 liter pyrex bottle. Add 12 ml H_2SO_4 (96%) and 73 ml HCl (37%). Make to 18 liters with distilled water and mix thoroughly by shaking. Allow 12 hours to come to equilibrium.

4. Ammonium molybdate $((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O})$.
5. Ammonium vanadate (NH_4VO_3) .
6. Nitric acid (HNO_3) , 1 N: Dilute 64 ml of concentrated HNO_3 (69.5%) to 1 liter with distilled water.
7. Molybdate - Vanadate solution: Dissolve 25 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ in 500 ml of distilled water. Dissolve 1.25 g of NH_4VO_3 in 500 ml of 1 N HNO_3 . Store in separate bottles. Mix equal volumes of these solutions (1 ml required per sample). Prepare fresh mixture each week.
8. Monobasic potassium phosphate (KH_2PO_4) .
9. Phosphorus standard solution: Dissolve 0.1098 g of KH_2PO_4 in 500 ml of extracting solution. Dilute to 1 liter with extracting solution.
10. Potassium atomic absorption standard (1000 ppm).
11. Calcium atomic absorption standard (1000 ppm).
12. Magnesium atomic absorption standard (1000 ppm).
13. Potassium (K) standard stock solution (100 ppm): Place 10 ml of potassium atomic absorption standard (1000 ppm) in a 100 ml volumetric flask. Bring to volume with deionized water. Make fresh daily.
14. Calcium (Ca) standard stock solution (200 ppm): Place 20 ml of calcium atomic absorption standard (1000 ppm) in a 100 ml volumetric flask. Bring to a volume with deionized water. Make fresh daily.
15. Magnesium (Mg) standard stock solution (100 ppm): Place 10 ml of magnesium atomic absorption standard (1000 ppm) in a 100 ml volumetric flask and dilute to volume with deionized water. Make fresh daily.
16. Lanthanum chloride $(\text{LaCl}_3\cdot 6\text{H}_2\text{O})$, 5%: Dissolve 127 g of $\text{LaCl}_3\cdot 6\text{H}_2\text{O}$ with deionized water and bring to a volume of 1 liter.
17. Activated charcoal (Darco G-60 or equivalent).

3.2.12.4 Materials—

1. Atomic absorption spectrophotometer (Perkin-Elmer Model 403 or equivalent).
2. Colorimeter (Bausch and Lomb Spectronic 20 or equivalent).
3. Flasks, Erlenmeyer, 50 ml.
4. Flasks, volumetric, 100 ml.
5. Flasks, volumetric, 200 ml.

6. Pipet, 1 ml.
7. Pipet, 2 ml.
8. Shaker, horizontal reciprocating type, 6.35 cm (2.5 in) stroke with 120 strokes per minute.
9. Filter paper (Whatman 40 or equivalent).
10. Pyrex bottle, 18 liters.
11. Pyrex bottle, 8 liters.
12. Balance, can be read to 0.1 g.

3.2.12.5 Procedure--

1. Place 5.0 g of less than 60 mesh sample in a 50 ml Erlenmeyer flask. Add 0.2 g of activated charcoal. Prepare two blanks using only 0.2 g of activated charcoal.
2. Add 25 ml of extracting solution and shake for 5 minutes on the reciprocating shaker at 120 strokes per minute.
3. Filter using filter paper and save filtrate for P, K, Ca, and Mg determinations. NOTE: If filtrate is cloudy, refilter.
4. Subdivisions 3.2.12.5.1 through 3.2.12.5.3 include the determination of individual elements.

3.2.12.5.1 Phosphorus (P)--These steps are used for the determination of phosphorus.

1. Turn on colorimeter 15 minutes before use and adjust according to instruction manual.
2. Pipet 4 ml of filtered extract into a colorimeter tube.
3. Add 1 ml of molybdate-vanadate solution and allow to stand 10 minutes.
4. Mix by inverting tube and shaking by hand for a few seconds.
5. Place tube in instrument and read percent transmission (% T).
6. Using % T, determine the ppm available P from a calibration curve prepared as follows: (A) To separate colorimeter tubes, add the amounts of chemicals given in Table 4; (B) Treat as outlined in 3.2.12.5.1 steps 2-5; (C) Plot ppm on the horizontal axis and % T on the vertical axis. NOTE: If sample does not fall on calibration curve, samples must be diluted and results multiplied by the dilution factor. The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.

TABLE 4. PHOSPHORUS (P) STANDARDS

Phosphorus Standard Solution (ml)	Extracting Solution (ml)	Molybdate-Vanadate Solution (ml)	Phosphorus in Standard (ppm)
0.0	4.0	1.0	0.0
0.5	3.5	1.0	2.5
1.0	3.0	1.0	5.0
1.5	2.5	1.0	7.5
2.0	2.0	1.0	10.0
2.5	1.5	1.0	12.5
3.0	1.0	1.0	15.0
3.5	0.5	1.0	17.5
4.0	0.0	1.0	20.0

3.2.12.5.2 Potassium (K)--These steps are used for the determination of potassium.

1. Set the atomic absorption spectrophotometer unit on emission mode following the instrument's instruction manual.
2. Use the extractant for zero setting.
3. Put the extracted sample solution under the aspirating tube and record readings.
4. Determine ppm of K in the sample from the calibration curve prepared as follows: (A) Into separate 100 ml volumetric flasks, dilute the K standard stock solution with extracting solution for a range of 0 to 80 ppm increments; (B) Take reading with the atomic absorption spectrophotometer; (C) Plot available K (ppm) on the horizontal axis and instrument reading on the vertical axis; (D) Plot a curve through the points. NOTE: If samples do not fall on the calibration curve, dilute samples with extracting solution and multiply results by dilution factor. The dilution factor is obtained by dividing the final volume by the initial aliquot.

3.2.12.5.3 Calcium (Ca) and magnesium (Mg)--These steps are used for the determination of calcium and magnesium.

1. Adjust the atomic absorption spectrophotometer following the instrument instruction manual.
2. Pipet 1.0 ml of sample extract and blank into separate 100 ml volumetric flasks. Add 1.0 ml of 5% $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ to each flask.
3. Bring to volume with extracting solution and mix by hand shaking.
4. In separate 100 ml volumetric flasks, prepare the calcium standards as shown in Table 5. Aspirate each standard into the instrument until a steady reading is obtained. Record reading.
5. Make a calibration curve plotting Ca (ppm) on the horizontal axis and instrument reading on the vertical axis. Plot a curve through the points.
6. Into separate 200 ml volumetric flasks, prepare the magnesium standards as shown in Table 6. Aspirate each standard into the instrument until a steady reading is obtained. Record reading.
7. Make a calibration curve plotting extractable Mg (ppm) on the horizontal axis and instrument reading on the vertical axis. Plot a curve through the points.
8. Aspirate sample extracts into the atomic absorption spectrophotometer and record readings.
9. Determine ppm of calcium and magnesium from calibration curves. If samples do not fall within the range of the calibration curve, dilute sample with extracting solution and add 5% $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$, but not to exceed 1% La in the final dilution. Multiply results by dilution factor. The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.

3.2.12.6 Calculations--

1. Dilution factor (DF) equals 1 unless the samples have to be diluted to fall within the range of the standard curve. The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.
2. ppm P in the soil = ppm (read from the curve) X 6.25 X DF. NOTE: The 6.25 is obtained from the following equation: $6.25 = 25 \text{ ml extracting solution} / 5 \text{ g sample} \times (5 \text{ ml final volume} / 4 \text{ ml extract})$.
3. ppm K in the soil = ppm (read from the curve) X 5 X DF. NOTE: The 5 is obtained from the following equation: $5 = (25 \text{ ml extracting solution}) / (5 \text{ g sample})$.

TABLE 5. CALCIUM (Ca) STANDARDS

Stock Ca Solution 200 ppm (ml)	$\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ (ml)	Extracting Solution (ml)	Calcium in Standard (ppm)
0.0	2.0	98.0	0.0
1.0	2.0	97.0	2.0
2.0	2.0	96.0	4.0
3.0	2.0	95.0	6.0
4.0	2.0	94.0	8.0
5.0	2.0	93.0	10.0

TABLE 6. MAGNESIUM (Mg) STANDARDS

Stock Mg Solution 100 ppm (ml)	$\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ (ml)	Extracting Solution (ml)	Magnesium in Standard (ppm)
0.0	4.0	196.0	0.00
0.5	4.0	195.5	0.25
1.0	4.0	195.0	0.50
1.5	4.0	194.5	0.75
2.0	4.0	194.0	1.00
2.5	4.0	193.5	1.25
3.0	4.0	193.0	1.50

4. ppm Ca in the soil = ppm (read from the curve) X 500 X DF. NOTE: The 500 is obtained from the following equation: $500 = (25 \text{ ml extracting solution} / 5 \text{ g sample}) \times (100 \text{ ml final volume} / 1 \text{ ml extract})$.

5. ppm Mg in the soil = ppm (read from the curve) X 500 X DF. NOTE: The 500 is obtained from the following equation: $500 = (25 \text{ ml extracting solution} / 5 \text{ g sample}) \times (100 \text{ ml final volume} / 1 \text{ ml extract})$.

6. pp2m of element in the soil = (ppm of element in the soil) X 2.

3.2.13 Organic Carbon by Walkley-Black Method

3.2.13.1 Principle--

The method involves the oxidation of organic carbon by an oxidizing agent, potassium dichromate. The reaction is aided by the addition of sulfuric acid which generates heat. After the reaction is complete, the remaining dichromate is determined by titration with standard ferrous sulfate solution. From the amount of dichromate reduced, the amount of oxidized organic carbon can be calculated (Allison, 1965; Jackson, 1958).

3.2.13.2 Comments--

Some interference can result from chlorides, higher oxides of manganese and reduced iron. With the use of proper precautions, this interference can be eliminated or greatly reduced (Walkley, 1947; Jackson, 1958). Ferrous iron and chlorides tend to give positive or high organic carbon values, whereas, oxides of manganese tend to give negative or low values (Allison, 1965).

All samples should be ground in a porcelain or agate mortar. Iron or steel mortar is avoided because of the introduction of reducing material in the form of metallic iron.

3.2.13.3 Chemicals--

1. Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), 1 N: Dissolve 49.04 g $\text{K}_2\text{Cr}_2\text{O}_7$ (dried at 105°C) in distilled water and dilute to a volume of 1 liter.
2. Sulfuric acid (H_2SO_4), concentrated.
3. Ferrous solution, 0.025 M (available from Fisher Scientific Company)
4. Ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 0.5 N: Dissolve 140.0 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water. Add 15 ml of concentrated H_2SO_4 and allow to cool. Dilute to 1 liter with distilled water. Standardize reagent daily by titrating it against 10 ml of 1 N $\text{K}_2\text{Cr}_2\text{O}_7$.

3.2.13.4 Materials--

1. Porcelain or agate mortar and pestle.

2. Flasks, 500 ml, Erlenmeyer, wide-mouth.
3. Pipet, 10 ml.
4. Pipet, 20 ml.
5. Buret, 50 ml
6. Balance, can be read to 0.001 g.
7. Sieve, 0.25 mm (60 mesh) openings, nonferrous.
8. Buchner funnel.
9. Filter paper, (Whatman 40 or equivalent).
10. Weighing pans.

3.2.13.5 Procedure--

1. Grind air-dry samples to pass 60 mesh sieve with a porcelain or agate mortar.
2. Weigh and record tare weights of two clean and dry weighing pans.
3. In previously tared weighing pans, weigh 2.00 g (0.50 g of Horizon 1 and carbolith material) air-dry soil samples. NOTE: One sample is used for the procedure. The second sample is placed in an oven at 105°C for 16 hours, allowed to cool in a desiccator, and its oven-dry weight recorded. (See 3.2.13.6. No. 3).
4. Place weighted air-dry sample in a 500 ml Erlenmeyer flask.
5. Pipet exactly 10 ml of 1 N $K_2Cr_2O_7$ solution into the soil. Swirl flask gently until mixed.
6. Rapidly pipet 20 ml of concentrated H_2SO_4 , directing the stream into the suspension. Mix by gentle rotation for 1 minute to insure complete contact of reagent with sample. NOTE: Avoid throwing soil up onto the sides of the flask and out of contact with the reagent.
7. Allow mixture to stand on an asbestos sheet for 30 minutes.
8. Dilute to 200 ml with distilled and deionized water.
9. Add 4 drops to 0.025 M Ferroin indicator.
10. Back titrate with 0.5 N ferrous sulfate solution from a buret. As the endpoint is approached, the solution has a greenish cast which changes to dark green. At this point, add ferrous sulfate drop by drop until the color changes sharply from blue to red (maroon color in reflected light against a white background). CAUTION: Discard and rerun with less soil

if 8 ml or more of the dichromate is reduced. If the endpoint cannot be clearly distinguished as described above, rerun sample and filter suspension using a buchner funnel before doing steps 9 and 10.

3.2.13.6 Calculations--

1. meq $K_2Cr_2O_7$ = (ml $K_2Cr_2O_7$ used) X (\underline{N} $K_2Cr_2O_7$).
2. meq $FeSO_4$ = (ml $FeSO_4$ used) X (\underline{N} $FeSO_4$).
3. Oven-dry weight of sample = (wt. oven-dry sample and tared pan) - (wt. of tared pan).
4. % organic carbon = [(meq $K_2Cr_2O_7$ - meq $FeSO_4$) X (0.003 X 100 X 1.33)] / Oven dry sample wt.

3.2.14 Organic Carbon Determination By Low Temperature Ignition

3.2.14.1 Principle--

Water and hydroxides are driven off the sample by heating to 105°C. Organic matter is oxidized by heating at 400°C for 7 hours. The percent organic matter can be determined by weight loss.

3.2.14.2 Comments--

Mineral matter is assumed to be unchanged at the 400°C temperature range. For soils containing amorphous materials, the discrimination between organic and mineral matter is far from complete (Jackson, 1958).

3.2.14.3 Chemicals--

None required.

3.2.14.4 Materials--

1. Muffle furnace.
2. Drying oven.
3. Desiccator with drierite desiccant.
4. Balance, can be read to 0.01 g.
5. Crucibles or evaporating dishes.

3.2.14.5 Procedure (Modified from Jackson, 1958)--

1. Weigh a clean and dry crucible. Record tare weight (A).
2. Weigh 10.00 g of less than 60 mesh sample in tared crucible.

3. Place in oven and heat for 4 hours at 105°C.
4. Remove sample and allow to cool in desiccator.
5. Weigh sample. Record weight (B).
6. Place sample in oven and heat for 7 hours at 400°C.
7. Remove sample and allow to cool in desiccator.
8. Weigh sample. Record weight (C).

3.2.14.6 Calculations

1. Legend:

A = Tare weight of crucible.

B = Weight of sample and crucible after heating 4 hours at 105°C.

C = Weight of sample and crucible after heating 7 hours at 400°C.

D = Weight of sample after heating 4 hours at 105°C.

E = Weight of sample after heating 7 hours at 400°C.

2. $D = B - A$.

3. $E = C - A$.

4. Organic matter oxidized by heating = $D - E$.

5. % organic matter in sample = $(\text{Organic matter oxidized by heating}/D) \times 100$.

3.2.15 Total Nitrogen by Kjeldahl Method

3.2.15.1 Principle--

In the Kjeldahl procedure, nitrogen is converted to ammonium ion by oxidation with concentrated sulfuric acid. With the addition of a catalyst such as copper, selenium, or mercury, this oxidation, which normally progresses very slowly, can be accelerated. Raising the boiling point by the addition of such salts as sodium sulfate or potassium sulfate also accelerates the reaction.

The ammonium ion produced by this oxidation is determined by making the solution strongly alkaline with sodium hydroxide, the liberated ammonia is distilled into a boric acid solution. The resulting ammonium borate is back titrated to boric acid with a standard acid (Bremner, 1965; Winkler, 1913).

3.2.15.2 Comments

Continuous boiling of the concentrated sulfuric acid and Kel-pak mixture for several hours requires insulation and venting of the system so the sulfuric acid condenses about one-third of the way up the digestion flask neck.

Materials adhering to the walls must be dislodged and brought into contact with the acid by rotation of the flask. Clay soils are particularly troublesome because clay promotes splattering. With the addition of glass beads, bumping during digestion can usually be eliminated. Optimum digestion temperature is between 360 and 400°C. Loss of nitrogen may occur if heated above 410°C.

3.2.15.3 Chemicals--

1. Kel-pak powder No. 3 ($\text{HgO} + \text{K}_2\text{SO}_4$) (available from Matheson Scientific Co.).
2. Sulfuric acid (H_2SO_4), concentrated.
3. Sulfuric acid (H_2SO_4), dilute (approximately 0.1 N): Dilute 44.8 ml of concentrate H_2SO_4 to 16 liters with distilled water.
4. Sodium hydroxide (NaOH), 45% with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$): Under a fume hood in a rubber bucket mix 4545.9 g of NaOH flakes (for nitrogen determination) with 438.0 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$. Dissolve and dilute to 11.355 liters (3 gal) with carbon dioxide-free water (See 3.2.2.2 No. 1). Cool overnight and siphon into dispensing apparatus. Protect from CO_2 in the air with soda lime or ascarite in a guard tube.
5. Boric acid (H_3BO_3), 4%: Dissolve 720.0 g of H_3BO_3 in distilled and deionized water on a hot plate. Dilute to 18 liters with distilled and deionized water. Add 60 ml of Bromocresol green-methyl red indicator (see below).
6. Bromocresol green-methyl red indicator: Mix 0.5 g of bromocresol green and 0.2 g methyl red with 100 ml of ethyl alcohol (90%). Adjust to medium color (brown) with a few drops of weak NaOH.
7. Zinc (Zn), granular.

3.2.15.4 Materials--

1. Kjeldahl electric digestion manifold
2. Kjeldahl electric distillation rack.
3. Room equipped with exhaust fan.
4. Flasks, Kjeldahl, 800 ml.

5. Flasks, Erlenmeyer, widemouth, 500 ml, marked at 230 ml.
6. Sieve, 20 mesh.
7. Balance, can be read to 0.1 g.
8. Asbestos gloves.

3.2.15.5 Procedure--

1. Place 10 g unground sample (sieved to 20 mesh) wrapped in filter paper in Kjeldahl flask. Also prepare two blanks without soil, but containing filter paper.
2. Add 2 packets of No. 3 Kel-pak.
3. Turn on exhaust fan.
4. Add 40 ml concentrated H_2SO_4 . NOTE: While rotating flask, run acid down side to carry down sample.
5. Mix contents by gentle swirling and place flask carefully on Kjeldahl rack.
6. When all flasks are in place, set all knobs so that a moderate boiling and digestion of the sample can be seen.
7. After 30 minutes increase heat to a rapid boil for 30 minutes so that sulfur dioxide can be released and to insure complete digestion of the sample.
8. Rotate flasks 180° and continue heating until all the black organic matter is digested (usually about 1 hour).
9. Allow sample to cool on digestion rack and stopper. CAUTION. Do not place stopper in hot flask as it may implode upon cooling.
10. Let stand until solution reaches room temperature and cautiously add 300 ml distilled water to each flask. NOTE: Rotate flasks while pouring to wash neck.
11. Swirl flasks gently to dissolve crystals.
12. Add $1/4$ teaspoon granular zinc to each flask.
13. Pour 30 ml H_3BO_3 (4% containing indicator) into 500 ml wide mouth Erlenmeyer flasks. NOTE: One required for each sample and blank and numbered to correspond to each Kjeldahl flask.
14. Place Erlenmeyer flasks on Kjeldahl distillation rack. NOTE: Top of glass delivery tube must be below surface of H_3BO_3 .

15. Turn condenser water switch to manual. After 30 minutes turn water switch to automatic if unit is so equipped.
16. Add 133 ml NaOH (45%) slowly to each Kjeldahl flask. NOTE: Allow NaOH to run down side of flask so that it lies on the bottom.
17. Place each flask on Kjeldahl distillation rack as NaOH is added, using steps 18-21.
18. Wet hands with distilled water and apply water to rubber stoppers.
19. Place stopper securely in flask. Set flask on burner.
20. As soon as flask is in position, turn burner switch to make a moderate boil but not enough to cause solution to boil into flask neck.
21. Swirl flask to mix NaOH layer with the rest of the sample solution and set back in position making sure stopper is tight.
22. When 200 ml has distilled into receiving flask, set receiving flask (Erlenmeyer) down and turn off heat. CAUTION: Be sure to set flask down before turning off heat or distillate may suck back through condensers. NOTE: Distillate color should be green or dark blue.
23. Wash delivery tube with a small stream of distilled water from a wash bottle before removing receiving flask.
24. When cool, titrate distillate with 0.1 N H₂SO₄ until solution becomes clear and then turns pink.
25. Record reading.

3.2.15.6 Calculations--

1. Average of sample blanks = [reading (blank 1) + reading (blank 2)]/2.
2. Corrected sample reading = (sample reading) - (average of sample blanks).
3. Constant = (N acid) X (meq. wt. of N) X (100) X (1/wt. of sample); where N acid = 0.1, meq. wt. of nitrogen = 0.014, and 100 changes constant to percent.

The equation can then be written:

Constant = (0.1) X (0.014) X (100) X (1/wt. of sample), which can be simplified to:

Constant = (0.14) X (1/wt. of sample).

4. % nitrogen = (corrected sample reading) X constant.

3.2.16 Calcium Saturation Cation Exchange Capacity

3.2.16.1 Principle--

Cation exchange capacity (CEC) is defined as the sum of the exchangeable cations in a soil. Several methods are used for determining the CEC of a soil.

In this method, a solution of calcium chloride is used to saturate the soil exchange complex and remove all other exchangeable cations from the exchange sites. Calcium is then removed from the exchange complex by saturating the soil with magnesium acetate. By determining the amount of calcium in the magnesium acetate extract, the CEC of the soil can be measured.

3.2.16.2 Comments--

Soils with a pH greater than 5.5 or surface soils less than pH 5.5 which have been treated with lime must be pretreated with 1.0 N sodium acetate (pH 5.0) to move free carbonates (Jackson, 1958 pp. 62-63). To avoid this pretreatment, sodium saturated CEC (see 3.2.17) can be used.

Since calcium chloride is used to saturate the soil instead of a buffered acetate, the pH of the soil is not affected and the CEC is determined at the actual pH of the soil. This is important because it is well known that as pH rises the CEC increases (Coleman and Thomas, 1967).

3.2.16.3 Chemicals--

1. Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 1 N: Dissolve 147.03 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and dilute to 1 liter with distilled water.
2. Methanol (CH_3OH), 95%: Dilute 950 ml of methanol with 50 ml distilled water.
3. Magnesium acetate ($\text{Mg}(\text{OAc})_2$), 1 N: Dissolve 107.25 g of $\text{Mg}(\text{OAc})_2$ and dilute to 1 liter with distilled and deionized water.
4. Calcium atomic absorption standard (1000 ppm).
5. Calcium (Ca) standard stock solution (100 ppm): Pipet 10 ml of calcium atomic absorption standard (1000 ppm) in a 100 ml volumetric flask. Bring to volume with deionized water. Make fresh daily.
6. Silver nitrate (AgNO_3), 0.1%: Dissolve 0.10 g of AgNO_3 and dilute to 100 ml with distilled water. Store in brown bottle.
7. Lanthanum chloride ($\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$), 5%: Dissolve 127 g of $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ with deionized water and make to a volume of 1 liter.

3.2.16.4 Materials--

1. Balance, can be read to 0.0001 g.

2. Centrifuge tubes, 100 ml.
3. Rubber stoppers (to fit centrifuge tubes).
4. Shaker, horizontal reciprocating type, 6.35 cm (2.5 in) stroke, 120 strokes per minute.
5. Centrifuge (International Equipment Company Model K with No. 279 head or equivalent centrifuge and 12-place head).
6. Graduated cylinder, 100 ml.
7. Beaker, 100 ml.
8. Dropper bottle.
9. Bottle, polyethylene, 100 ml (one needed per sample).
10. Atomic Absorption unit (Perkin-Elmer Model 403 or equivalent).
11. Flasks, volumetric, 100 ml (7 required for standards).
12. Desiccator with drierite drying agent.

3.2.16.5 Procedure (Modified from Rich, 1961)--

1. Weigh 5 g of less than 60 mesh soil into a 100 ml centrifuge tube.
2. Add 50 ml of 1 N CaCl₂.
3. Stopper centrifuge tube and shake horizontally for 45 minutes on a reciprocating shaker insuring that the solid material in the bottom of the tube is completely dispersed.
4. Remove stopper and centrifuge suspension until clear (at least 5 minutes at 2000 RPM).
5. Pour off clear solution.
6. Repeat steps 2 through 5 two more times.
7. Add 50 ml of distilled water to the soil in the centrifuge tube.
8. Stopper and shake horizontally for 15 minutes on a reciprocating shaker insuring that the solid material in the bottom of the tube is completely dispersed.
9. Remove stopper and centrifuge for at least 5 minutes at 2000 RPM. Pour off clear solution.
10. Repeat steps 7 through 9 one more time.

11. Add 50 ml of 95% methanol to the soil in the centrifuge tube.
12. Stopper and shake horizontally for 15 minutes on a reciprocating shaker insuring that the solid material in the bottom of the tube is completely dispersed.
13. Remove stopper and centrifuge for at least 5 minutes at 2000 RPM.
14. Repeat steps 11 through 13 one more time.
15. Repeat steps 11 through 14, but pour clear solution into a 100 ml beaker.
16. Add a few drops of 0.1% AgNO_3 to the solution in the beaker. NOTE: If no precipitations occur, no further washing with methanol is required. If precipitation occurs, repeat steps 15 through 16 until no precipitation occurs.
17. Dry soil in the centrifuge tube in a drierite desiccator.
18. Weigh 0.5000 g dry, Ca-saturated soil into a 100 ml centrifuge tube.
19. Add 50 ml of 1 N $\text{Mg}(\text{OAc})_2$.
20. Stopper and shake horizontally for 16 hours on a reciprocating shaker.
21. Remove stopper and centrifuge suspension until clear (for at least 5 minutes at 2000 RPM).
22. Pour solution into a 100 ml polyethylene bottle. Add 1.0 ml of 5% $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ and cap bottle. NOTE: This solution will be used for Ca determination by atomic absorption.
23. Prepare CEC determination standards from Table 7.
24. Aspirate the standards on the atomic absorption unit following the instruction manual of instrument.
25. Make a standard curve plotting ppm of calcium on the horizontal axis and instrument reading on vertical axis.
26. Analyze samples for calcium and determine ppm of calcium from the prepared curve. NOTE: If unknown does not fall within the range of the standard curve, dilute sample with 1 N $\text{Mg}(\text{OAc})_2$ and add 5% $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$, but not exceeding 1% La in the final dilution. The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.

3.2.16.6 Calculations--

1. Legend:

A = ppm of calcium as read from standard curve.

TABLE 7. STANDARDS FOR CALCIUM CEC DETERMINATION

Flask No. (100 ml)	ml of Ca stock solution (100 ppm)	ml of Mg(OAc) ₂ (1 N)	ml of LaCl ₃ ·6H ₂ O (5%)	Represented Ca (ppm)
1	0.0	98.0	2.0	0
2	1.0	97.0	2.0	1
3	2.0	96.0	2.0	2
4	4.0	94.0	2.0	4
5	6.0	92.0	2.0	6
6	8.0	90.0	2.0	8
7	10.0	88.0	2.0	10

DF = dilution factor, which is 1 if no dilution was necessary to read within the range of the standard curve. The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.

2. CEC (meq/100 g) = (A) X (DF) X (0.51), where the 0.51 is derived from the equation: (ppm/1,000,000) X (volume extracting solution/sample wt.) X (1000 meq per eq/eq. wt of Ca) X 100 g basis. NOTE: The volume of the extracting solution = 50 ml extracting solution + 1 ml LaCl₃·6H₂O = 51 ml.

3.2.17 Sodium Saturation Cation Exchange Capacity

3.2.17.1 Principle--

In this method, the soil is saturated with a solution of sodium acetate to replace all other exchangeable cations on the exchange sites with sodium. Sodium is then removed from the exchange complex by saturating the soil with an ammonium acetate solution. CEC is measured by determining the amount of sodium in the ammonium acetate extract.

3.2.17.2 Comments--

This method is used for both calcareous and noncalcareous soils. In mine-soils, it is recommended that the sodium acetate method for determining CEC be used. Minesoils with a pH as low as 5.5 can contain free carbonates which interfere with the CEC determination by calcium saturation.

Cation exchange capacity may also be determined using ammonium acetate as a saturating solution; however, because of variable amounts of calcium

carbonate and gypsum present in minesoils and their solubility in ammonium acetate, it is recommended that either sodium acetate or calcium chloride saturation be used for determining CEC. Solubility of calcium carbonate in 1 N sodium acetate at pH 8.2 is much lower than it is in neutral 1 N ammonium acetate.

Interferences occur in the sodium determination with some atomic absorption units. This interference can usually be corrected by the addition of 2,000 ppm of potassium to both the standards and the unknowns.

3.2.17.3 Chemicals--

1. Sodium acetate (NaOAc), 1.0 N: Dissolve 136 g of NaOAc in distilled water and dilute to 1 liter. NOTE: The pH of this solution should be 8.2. If needed, add a few drops of acetic acid or NaOH solution to adjust the pH to 8.2.
2. Ammonium acetate (NH₄OAc), 1.0 N: Dilute 114 ml of glacial acetic acid (99.5%) with distilled water to a volume of approximately 1 liter. Then carefully add 138 ml of concentrated ammonium hydroxide (NH₄OH) and slowly add distilled water to obtain a volume of approximately 1980 ml. Check the pH of the solution and add more NH₄OH as needed to obtain a pH of 7.0. Dilute the solution to a volume of 2 liters with distilled water.
3. Isopropyl alcohol, 99%.
4. Potassium stock solution, 10,000 ppm: Dissolve 19.07 g of potassium chloride (KCl) in 1 liter of deionized water.
5. Standard sodium solution, 1000 ppm, atomic absorption spectroscopy grade.

3.2.17.4 Materials--

1. Centrifuge tubes, 50 ml, round bottom polypropylene.
2. Rubber stoppers (to fit centrifuge tubes).
3. Shaker, horizontal reciprocating type, 6.35 cm (2.5 in.) stroke, 120 strokes per minute.
4. Centrifuge (International Equipment Company Model K with No. 279 head or equivalent centrifuge and 12-place head).
5. Volumetric flasks, 100 ml.
6. Atomic absorption spectrophotometer (Perkin-Elmer model 403 or equivalent).
7. Balance, can be read to 0.01 g.

3.2.17.5 Procedure--

1. Weigh 4.0 g of less than 60 mesh material and transfer to 50 ml centrifuge tube. NOTE: If the material is very coarse textured (loamy sand or sand), a 6.0 g sample is used.
2. Record weight of sample (A).
3. Add 33 ml of 1.0 N NaOAc solution to the centrifuge tube.
4. Stopper the tube and shake in a reciprocating shaker at 120 strokes per minute for 5 minutes insuring that the solid material in the bottom of the tube is completely dispersed.
5. Unstopper the tube and centrifuge until the supernatant liquid is clear (at least 5 minutes at 2000 RPM). Decant and discard the liquid.
6. Repeat steps 3 through 5 three more times.
7. Add 33 ml of 99% isopropyl alcohol to centrifuge tube.
8. Stopper tube and shake on reciprocating shaker for 5 minutes insuring that the solid material in the bottom of the tube is completely dispersed.
9. Unstopper centrifuge tube and centrifuge it until the supernatant liquid is clear (at least 5 minutes at 2000 RPM). Then decant and discard the liquid.
10. Repeat steps 7 through 9 two more times.
11. Add 33 ml of 1 N NH₄OAc to centrifuge tube, stopper tube and shake for 5 minutes insuring that the solid material in the bottom of the tube is completely dispersed.
12. Unstopper tube and centrifuge until supernatant liquid is clear (at least 5 minutes at 2000 RPM).
13. Decant liquid into a 100 ml volumetric flask.
14. Repeat steps 11 through 13 two more times.
15. Fill the volumetric flask to the 100 ml mark using the 1N NH₄OAc solution.
16. Take 10 clean 100 ml volumetric falsks and label them 0, 5, 10, 20, 30, 40, 50, 60, 70, and 80 ppm sodium.
17. Pipet 0.5 ml of the 100 ppm sodium standard into the flask labeled 5 ppm sodium. Into the flasks labeled 10 through 80 ppm, pipet 1 ml through 8 ml, respectively, of the 1000 ppm sodium standard solution.

18. Dilute all flasks to volume with 1 N NH_4OA solution. NOTE: The flask labeled 0 ppm will contain only the 1 N NH_4OAc extracting solution.

19. Turn on the atomic absorption unit and wet it for emission mode. Read instruction manual carefully and set all operating parameters according to the instrument instruction manual.

20. After the atomic absorption unit is ready, zero the instrument using the 1 N ammonium acetate extracting solution, not distilled water. Aspirate standards and record readings.

21. Plot a standard curve using ppm sodium on the horizontal axis and the instrument readings on the vertical axis.

22. Record the instrument readings for all unknowns and read the concentration (B) of sodium from the standard curve. NOTE: If the unknown does not fall within the range of the standard curve which you have plotted, dilute the unknown with NH_4OAc and potassium stock solution using 2 ml of the potassium stock solution for every 10 ml of NH_4OAc . Then measure the amount of sodium present.

3.2.17.6 Calculations--

1. Legend:

A = Sample weight.

B = ppm of sodium as read from the standard curve.

DF = dilution factor, which is 1 or unity if no dilution of the unknown had to be made to get it to read within the range of the standard curve.

2. CEC (meq/100g) =

$(B/1,000,000) \times (DF) \times (\text{Vol. extracting solution/sample wt.}) \times (1000 \text{ meq/eq. wt Na}) \times 100\text{g}$,

Where:

Vol. extracting solution = 100 ml

eq. wt of Na = 23.

The above equation can be reduced to:

$\text{CEC (meq/100g)} = (B \times \text{DF} \times 10) / (23 \times A)$.

3.2.18 Electrical Conductance of Soil Extract

3.2.18.1 Principle--

Pure water (water which contains no dissolved substances) is not a good

conductor of an electrical current. Water becomes a better electric current conductor with the addition of dissolved salts. The amount of electric current conducted through this water is approximately proportional to the amount of salts dissolved in the water. Based on this fact, a measurement of the amount of electric current that is conducted by a soil extract will provide information as to the amount of salts present in the soil. This simple measurement provides an accurate indication of the concentration of ionized constituents in the soil extract. The electrical conductivity of a soil extract is closely related to the sum of cations (or anions) as determined chemically. This measurement usually correlates closely with the total dissolved solids.

3.2.18.2 Comments--

Extracts to be used for electrical conductivity measurements should be taken from a saturated soil paste. Measuring the salt concentration of an extract obtained at the field moisture state would be an ideal method; however, it is much easier to obtain a soil extract from a saturated paste. This is extremely important when doing electrical conductivity measurements on a routine basis.

When making a saturated soil paste, some practice is necessary to obtain consistent results. Dried peat or muck usually require an overnight wetting period to obtain a satisfactory saturated paste. Add water to fine textured soils without stirring and allow the sample to wet slowly. This will enable the fine textured material to reach saturation without puddling occurring. Care must be taken not to overwet coarse textured soils. If water stands on the surface, the soil has been over saturated and a small additional amount of soil must be added.

The soil material used for electrical conductivity measurements should not be oven dried. Material should be air dried and ground to pass a 60 mesh sieve (see 3.1.2).

3.2.18.3 Chemicals--

1. Distilled water.
2. Potassium chloride (KCl), 0.01 N: Dissolve 0.7456 g of KCl in distilled water, and dilute with distilled water to 1 liter. This is the standard reference solution and at 25°C it has an electrical conductivity to 0.00141 mho/cm.
3. Sodium metaphosphate ((NaPO₃)₆), 0.1%: Dissolve 0.1 g of (NaPO₃)₆ (Fisher Scientific #S-333) in distilled water and dilute to 100 ml.

3.2.18.4 Materials--

1. Wheatstone bridge, alternating-current type, suitable for conductivity measurements. (Industrial Instruments Incorporated Model RC-16B2 or equivalent).

2. Conductivity cell, pipette-type, with platinized platinum electrodes. The cell constant should be approximately 1.0 reciprocal centimeter.
3. Flask, volumetric, 1000 ml.
4. Balance, can be read to 0.01 g.
5. Aluminum can with lid (large enough to contain sample).
6. Spatula.
7. Aluminum weighing pan.
8. Drying oven.
9. Dessicator, with silica gel dessicant.
10. Buchner type filtering funnel, 11 cm inside diameter.
11. Filter flask.
12. Filter paper (Whatman 42 or equivalent).
13. Vacuum source.
14. Graduated cylinder, 100 ml volume.
15. Pipette, measuring, 10 ml capacity.

3.2.18.5 Procedure (modified from U.S. Salinity Laboratory Staff, 1954)--

1. Weigh 400 g of air-dried soil. Transfer the soil to an aluminum can (with lid).
2. Add water to the sample in small increments by pouring the water down the side of the can. Water is added to the sample in this fashion until the saturation point of the soil is almost reached.

NOTE: Do not stir soil sample while adding water. Since water movement through puddled soil is very slow, the soil is allowed to wet by capillarity and then mixed to ensure against puddling.

3. Stir the wetted soil with a spatula until a condition of saturation is reached. Small amounts of water may be added while mixing to insure that the saturation point has been reached. NOTE: At saturation the soil paste glistens as it reflects light and the mixture slides off of the spatula easily.
4. After the mixing has been completed, place the lid on the aluminum can and let sample stand for 1 hour or more.

5. After sample has set for the required amount of time, check sample for saturation. NOTE: If the paste has stiffened or lost its glisten, add more water and mix it again. On the other hand, if free water has collected on the surface of the paste, add additional air-dry soil to absorb free water and remix the sample.
6. After a saturated paste has been obtained, remove a teaspoon-full of the saturated paste for oven-drying and replace lid. Allow the saturated soil paste to stand at least 4 hours.
7. Weigh an oven-dry aluminum weighing pan to the nearest 0.01 g. Record weight (A).
8. Place subsample of the saturated soil paste (from step 6) in aluminum weighing pan. Weigh pan and sample to the nearest 0.01 g. Record weight (B).
9. Place weighing pan and sample in an oven at 105°C for 16 hours (or overnight). Remove from oven and cool in a desiccator.
10. Weigh oven-dry sample and pan. Record weight (C).
11. After the saturated soil paste has stood for at least 4 hours (from step 6), transfer it to a Buchner funnel fitted with one sheet of Whatman #42 (or equivalent) filter paper.
12. Attach filter flask to vacuum source, apply vacuum, and collect filtrate. Terminate filtration when air begins to pass through the filter. NOTE: Refilter if filtrate is turbid.
13. Add one drop of 0.1% sodium hexametaphosphate solution for each 25 ml of extract.
14. Allow the standard 0.01 N KCl solution and the sample of the soil-water extract to adjust to room temperature. NOTE: As long as the temperature of the room is within the range of 20-30°C, the absolute temperature of the solutions are not important. However, it is extremely important that the standard solution and the extract be at the same temperature. If greater precision is required bring the standard solution and soil-water extracts to a temperature of 25°C in a constant temperature bath.
15. Turn on Wheatstone bridge and allow instrument to warm up.
16. When instrument is ready, rinse and fill the conductivity cell with the standard 0.01 N KCl solution.
17. Balance the wheatstone bridge according to the instruction manual provided by the manufacturer. Record the cell resistance (D) in ohms.
18. Rinse and fill the cell with the soil-water extract. NOTE: If the volume of the extract is limited, rinse the cell with distilled water followed by acetone. Dry the cell by drawing air through it until the acetone has evaporated. Allow the cell to come to room temperature.

19. Balance the bridge and record the cell resistance (E) in ohms.

3.2.18.6 Calculations--

1. Legend:

A = Weight of oven-dry weighing pan.

B = Weight of saturated soil and weighing pan.

C = Weight of oven-dry soil and weighing pan.

D = Initial cell resistance.

F = Final cell resistance.

2. % Moisture of sample at saturation = $[(B-C)/(C-A)] \times 100$.

3. Electrical conductivity (EC) mmhos/cm, at 25°C = $[(0.0014118 \times D)/F]$.

4. Total cation concentration, meq/liter = $10 \times (EC)$.

3.2.19 Sodium-Absorption-Ratio

3.2.19.1 Principle--

Plants growing in saline soils are affected by the salt concentrated in the soil solution. The principle cations present are calcium, magnesium, and sodium with small amounts of potassium. If the proportion of sodium is high, the alkali hazard is high. By making a soil-water extract and measuring the salt concentration of the extract, the salinity hazard of the soil can be determined.

3.2.19.2 Comments--

Lanthanum chloride must be added to both the standards and the extract to eliminate interferences in determining calcium and magnesium by atomic absorption. Interferences may also occur in the sodium determination and should be corrected by the addition of an excess (1000-2000 ppm) of potassium or lithium to both the standards and samples (see manuals supplied with atomic absorption unit).

3.2.19.3 Chemicals--

1. Calcium atomic absorption standard (1000 ppm).
2. Magnesium atomic absorption standard (1000 ppm).
3. Sodium atomic absorption standard (1000 ppm).
4. Lanthanum chloride ($\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$), 5%: Dissolve 127 g of $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ with deionized water and bring to a volume of 1 liter.

5. Sodium metaphosphate ($(\text{NaPO}_3)_6$), 0.1%: Dissolve 0.1 g of $(\text{NaPO}_3)_6$ (Fisher Scientific No. S-333) in distilled water and dilute to 100 ml.

3.2.19.4 Materials--

1. Atomic absorption spectrophotometer (Perkin-Elmer Model 403 or equivalent).
2. Flasks, volumetric, 100 ml.
3. Pipet, 1 ml.
4. Balance, can be read to 0.01 g.
5. Aluminum can with lid (large enough to contain sample).
6. Spatula.
7. Weighing pan.
8. Drying oven.
9. Desiccator.
10. Buchner filter funnel.
11. Filter paper (Whatman 42 or equivalent)
12. Vacuum source pulling a constant vacuum.
13. Bottle (to collect filtrate).

3.2.19.5 Procedure (modified from Bower and Wilcox, 1965; U.S. Salinity Laboratory Staff, 1954)--

1. Weight 400 g of air-dry soil. Transfer soil to an aluminum can (with lid).
2. Add water to the sample in small increments by pouring the water down the side of the can. Water is added to the sample in this fashion until the saturation point of the soil is almost reached. NOTE: Do not stir sample while adding water. Since water movement through puddled soil is very slow, the soil is allowed to wet by capillarity and then mixed to insure against puddling.
3. Stir the wetted soil with a spatula until a condition of saturation is reached. Small amounts of water may be added while mixing to insure that the saturation point has been reached. NOTE: At saturation the soil paste glistens as it reflects light and the mixture slides off of the spatula easily.

4. After the mixing has been completed, place the lid on the aluminum can and let stand for at least 1 hour.
5. After sample has set for the required amount of time, check sample for saturation. NOTE: If the paste has stiffened or lost its glisten, add more water and remix. If free water has collected on the surface, add additional air-dry soil to absorb the free water and remix.
6. After a saturation paste has been obtained, remove a teaspoonful of the saturated paste for oven-drying and replace lid. Allow the saturated soil paste to stand at least 4 hours.
7. Weigh an oven-dry aluminum weighing pan to the nearest 0.01 g. Record weight (A).
8. Place subsample of saturated soil paste (from step 6) in aluminum weighing pan. Weigh pan and sample to the nearest 0.01 g. Record weight (B).
9. Place weighing pan and sample in an oven at 105°C for 16 hours. Remove from oven and cool in dessicator.
10. Weigh oven-dry sample and pan. Record weight (C).
11. After the saturated soil paste has stood for at least 4 hours (from step 6), transfer it to a Buchner funnel fitted with one sheet of Whatman No. 42 (or equivalent) filter paper.
12. Attach filter flask to vacuum source, apply vacuum, and collect filtrate. Terminate filtration when air begins to pass through the filter. NOTE: Refilter if filtrate is turbid.
13. Add one drop of 0.1% sodium hexametaphosphate solution for each 25 ml of extract.
14. Take 10 clean 100 ml volumetric flasks and label them 0, 5, 10, 20, 30, 40, 50, 60, 70, and 80 ppm sodium.
15. Pipet 0.5 ml of 1000 ppm sodium standard into the flask labeled 5 ppm sodium. Into the flasks labeled 10 through 80 ppm, pipet 1 through 8 ml, respectively, of the 1000 ppm sodium standard solution.
16. Dilute all flasks to volume with deionized water. NOTE: The flasked labeled 0 ppm will contain only deionized water.
17. Turn on the atomic absorption unit and set it for emission mode. Read instrument instructions manual carefully and do all settings accordingly.
18. After the atomic absorption unit is ready, zero the instrument using the 0 ppm standard. Record the reading for each of the other standards.

19. Plot standard curve using ppm sodium on the horizontal axis and instrument reading on the vertical axis.
20. Measure the amount of sodium present in the unknowns. NOTE: If the unknown does not fall within the range of the standard curve, dilute with deionized water and remeasure the amount of sodium present. Record the dilution factor (DF). The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.
21. For each 100 ml of extract, or part thereof, of the volume found in step 13, add 2 ml of 5% $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$.
22. Prepare calcium and magnesium standards as shown in Table 8 using 100 ml volumetric flasks.
23. Set atomic absorption unit to absorption setting according to the instruments instruction manual.
24. After the atomic absorption unit is ready, zero the instrument using the 0 ppm standard (flask no. 1). Record the reading for each of the other standards.
25. Plot standard curves using ppm of element on the horizontal axis and instrument reading on the vertical axis.
26. Measure the amount of calcium and magnesium present in the unknowns. NOTE: If the unknown does not fall within the range of the standard curve, dilute with deionized water and add 5% $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$, but not to exceed 1% La in the final dilution. Remeasure the amount of calcium and magnesium present and record the dilution factor. The dilution factor is obtained by taking the final volume and dividing it by the initial aliquot.

3.2.19.6 Calculations--

1. Legend:

A = Weight of oven-dry weighing pan.

B = Weight of saturated soil and paste and weighing pan.

C = Weight of oven-dry soil paste and weighing pan.

2. $\text{Meq/l of Na} = \text{ppm of Na (read from curve)}/23.00$, where 23.00 is the equivalent weight of sodium.

3. $\text{Meq/l of Ca} = \text{ppm of Ca (read from curve)}/20.04$, where 20.04 is the equivalent weight of calcium.

4. $\text{Meq/l of Mg} = \text{ppm of Mg (read from curve)}/12.16$, where 12.16 is the equivalent weight of magnesium.

TABLE 8. CALCIUM AND MAGNESIUM STANDARDS FOR SODIUM-ADSORPTION RATIO

Flask No.	Calcium stock solution (100 ppm) (ml)	Magnesium stock solution (10 ppm) (ml)	LaCl ₃ ·6H ₂ O (5%) (ml)	Deionized water (ml)	Represents ppm	
					Ca	Mg
1	0.0	0.0	2.0	98.0	0.0	0.0
2	1.0	2.0	2.0	95.0	1.0	0.2
3	2.0	4.0	2.0	92.0	2.0	0.4
4	4.0	6.0	2.0	88.0	4.0	0.6
5	6.0	8.0	2.0	84.0	6.0	0.8
6	8.0	10.0	2.0	80.0	8.0	1.0
7	10.0	15.0	2.0	73.0	10.0	1.5

5. Sodium-adsorption-ratio = $Na^+ / \sqrt{(Ca^{++} + Mg^{++})/2}$, where Na^+ , Ca^{++} , and Mg^{++} refer to the concentrations of designated cations expressed in millequivalents per liter as found in calculations no. 2 through 4.

6. Saturated water percentage = $[(B-C)/(C-A)] \times 100$.

3.3 MINERALOGICAL METHODS

3.3.1 Summary

Minerals occurring in overburden materials can be identified using a petrographic microscope or x-ray diffraction unit. Individual soil or rock grains are identified by placing the grains in an oil with a known index of refraction and examining them with the aid of a petrographic microscope. Individual grains and their relationships to surrounding grains are identified and examined in thin section using the petrographic microscope. Using x-ray diffraction, the types of clay minerals present in a sample can be determined.

All of the procedures require some technical knowledge for the mineral identification. A person experienced in the use of a petrographic microscope and/or x-ray diffraction instrument should make the identifications.

3.3.2 Identification of Grains by Immersion Method

3.3.2.1 Principle--

Many minerals may be identified by measuring their indices of refraction and then referring to determinative tables. The index may be measured by using the immersion method. Liquids of known index of refraction ranging from about 1.43 to 1.71 in steps of 0.01 should be available. The mineral grains to be identified are placed on a glass slide, covered with liquid of known index, and a small cover glass placed on top of the liquid. The grains are then observed under a petrographic microscope using a medium power objective. If the mineral grains have the same index as the liquid, they will be practically invisible. If the grains do not "match" the liquid, one can determine whether the grains have a higher or lower index than the liquid by the Becke Line Test. When a mineral grain is slightly out of focus, a narrow line of light known as the Becke line forms near the edge of the grain. The line is usually more conspicuous if light is reduced by partially closing the diaphragm in the substage. If the tube of the microscope is raised (or microscope stage lowered), the Becke line will move into the medium of higher index. In this way, it is possible to determine whether the grain has an index higher or lower than the liquid. As an example, if the grain is lower than the liquid, a new immersion is prepared using a liquid of lower index of refraction. If the grain still does not have the same index as the liquid, different liquids are used until a match is attained. In using a white light source, two Becke lines form when the grain and liquid are nearly matched--one line is yellowish and the other line bluish. When the microscope tube is raised, the brighter of these two lines moves toward the medium of higher index. The grain and liquid have the same index of refraction when the intensities of these two lines are the same.

3.3.2.2 Comments--

Amorphous material (no crystal structure) and isometric (cubic) crystals are said to be optically isotropic, having only one index of refraction which can be measured at any position of the microscope stage. All other minerals have two or more indices of refraction and are said to be anisotropic. Isotropic substances remain dark as the microscope stage is rotated with crossed nicols (upper polarizing element inserted). Anisotropic minerals, on the other hand, will generally be illuminated under crossed nicols, becoming dark every 90 degree turn of the microscope stage. These dark settings are called extinction positions. At these extinction positions the indices of refraction of anisotropic minerals are measured.

Hexagonal and tetragonal minerals have two indices of refraction called n_o and n_e . A mineral is said to be positive when n_o is less than n_e and negative when n_o is larger than n_e . The index n_o can be measured on any grain by turning the stage to the low index extinction position in a positive mineral or to the high index position for a negative mineral. At the other extinction position an index called n_e' lying between n_o and the true n_e is obtained.

In positive minerals the highest value of n_F' , as determined on several grains of the same mineral, is closest to true n_E . In a negative mineral, the lowest value obtained would be closest to n_E . Precise measurements of n_E require use of interference figures which are explained in standard optical mineralogy textbooks.

Orthorhombic, monoclinic, and triclinic minerals have three indices of refraction with the lowest index called n_x , the intermediate index n_y , and the highest index n_z . Exact determination of these indices involves somewhat complicated techniques which are explained in standard optical mineralogy textbooks. However, if several grains of the same mineral are examined, the lowest index obtainable on any of these grains will be fairly close to the n_x index. The highest index obtainable on the grains will be close to the n_z index. The difference between the highest and lowest index in a given mineral is called birefringence.

Most minerals under crossed nicols will show spectral colors called interference colors. Interference colors result from the double refraction of light in the crystal. As the two rays emerge from the grain, they undergo interference as they combine in passing through the upper nicol. The color sequence is the same as in Newton Colors. The actual color observed depends on the thickness of the grain, its orientation, and difference between its highest and lowest index (birefringence). A mineral in randomly oriented grains of the same thickness will show all of the colors up to a certain maximum on the Newton Scale. This maximum color is very useful in identification. Although the general properties of two minerals may be quite similar, their interference colors may be clearly different.

Most of the 50-100 mesh constituents in a soil can be readily identified with the petrographic microscope by the immersion method using liquid 1.544. The most common minerals and rock particles with their distinguishing features are as follows:

1. Quartz can be distinguished from most other minerals by the fact that its low index (n_o) is always 1.544. The birefringence of quartz is weak and is similar to that of feldspar. However, unlike feldspar, quartz has no cleavage and is free of alteration or weathering to argillaceous or clayey material.
2. Chert, which is an aggregate of fine-grained quartz, may occur as grains in soil. Chert has the same optical properties as coarse quartz but under crossed nicols shows a mosaic or salt and pepper effect because of the diverse orientation of constituent quartz domains. Chert is distinguished from aggregates of clay which sometimes have a mosaic appearance by its lower index of refraction.
3. Orthoclase can be distinguished from most other minerals because its indices of refraction are noticeably lower than 1.544. The grain edges are commonly straight and parallel because of cleavage. Orthoclase is usually not as clear as quartz due to alteration. The birefringence of orthoclase is weak.

4. Microcline is similar to orthoclase but under crossed nicols shows spindle-shaped twin plates. Plates meet at right angles forming a grid-iron-like pattern.
5. Plagioclase commonly shows parallel bands or stripes under crossed nicols because of twinning. The indices of refraction of plagioclase vary with its composition. The more sodium-rich plagioclases have indices below 1.544 but not as low as potassium-rich feldspars. Plagioclases with a small amount of calcium have indices close to 1.544. The calcium-rich plagioclases have indices well above 1.544.
6. Muscovite occurs in colorless flakes with indices of refraction considerably higher than that of quartz or feldspar. In immersions, these flakes have a gray color under crossed nicols.
7. Biotite also occurs in flakes and in immersions under plain light is dark brown or less commonly green. Under crossed nicols hardly any light passes through the flakes.
8. Carbonate in the form of calcite or dolomite has a very high index in one extinction position and a low index near or below 1.544 in the other extinction position. This change in index as the stage is rotated is ordinarily very conspicuous and distinguishes carbonate from most other minerals. Under crossed nicols, carbonates have a unique pinkish tan color. Small carbonate particles mixed with clay may be recognized by introducing the substage condensing lens and crossing the nicols. Under these conditions, the carbonate will normally appear as bright specks.
9. Pyrite is opaque even with strong transmitted light obtained with the substage condensing lens. In reflected light pyrite has a brass yellow color and the crystal faces or polished surfaces look like metallic mirrors.
10. Limonite (goethite) is yellow or brown on thin edges under strong transmitted light and opaque in thicker masses. Under reflected light, limonite is yellowish brown to brown.
11. Hematite is opaque and black in reflected light where massive but commonly translucent and red in reflected light at thin edges of the material.
12. Sandstone fragments are recognized by the constituent grains of quartz which have the characteristic low index of 1.544 and gray to white interference colors. The grains may be bound together by cement of quartz, carbonate, clay or iron oxide.
13. Shale and mudstone usually show a fine layering due to parallel alignment of flakes of clay minerals. The index of refraction is moderately high (distinctly higher than 1.544) and interference colors are white to yellowish white. The fragments go to extinction when the layering is parallel to the polarizing elements because of the parallel alignment of the constituent clay minerals.

14. Limestone fragments have a high index of refraction which causes the fragments to appear somewhat dark. Under crossed nicols the fragments have a slight pinkish tan color. If the constituent grains are sufficiently coarse, a change from high to low index can be observed on a given grain as the microscope stage is rotated.

15. Glass may develop in the partial fusion of shale (red dog). Glass is amorphous (no crystal structure) and remains dark as the stage is turned under crossed nicols. Glass also has a lower index than most minerals (lower than 1.544) and commonly contains small air bubbles.

3.3.2.3 Chemicals--

1. Acetone (CH_3COCH_3), reagent grade.
2. Dispersing agent: Dissolve 35.7 g sodium metaphosphate (Fisher S-333 or equivalent) and 7.94 g sodium carbonate and dilute to 1 liter with distilled water.
3. 1.544 Index oil (available from R. P. Cargille Laboratories, Inc., Cedar Grove, N.J. 07009 or other suppliers).

3.3.2.4. Materials--

1. Polarized petographic microscope with micrometer stage with 10 X eyepiece and a range in objectives from 3.5 to 50 X.
2. Variable intensity white light source.
3. 7.62 X 2.54 cm (3 x 1 in) glass microscope slide.
4. Slide cover glasses.
5. Thermometer 0-100°C in 1°C divisions.
6. Sieve, 0.25 mm openings (60 mesh).
7. Sieve, 0.177 mm openings (80 mesh).
8. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 120 strokes per minute.
9. Beakers, 250 ml, low-form.
10. Bottles, 950 ml (32 oz dry square).
11. Two beakers, 400 ml, low-form.
12. Two polyethylene bottles, 250 ml.
13. Lens paper.

14. Balance, can be read to 0.1 g.

3.3.2.5 Procedure--

1. Mix bulk field sample thoroughly. CAUTION: Do not use steel utensils to mix sample as some magnetic minerals may be attracted to the iron in the steel.
2. Weigh approximately 100 g of fine-textured (50 g of coarse textured) minesoil.
3. Place weighed sample in a 950 ml (32 oz) dry square bottle.
4. Add 20 ml of dispersing agent.
5. Place bottle on reciprocating shaker. Shake sample overnight.
6. Make a nested series with the 60 mesh and 80 mesh sieves placing the 60 mesh sieve on top.
7. Wet sieve entire sample, being sure to thoroughly wash sample from the bottle.
8. Place sample retained on 80 mesh in a 250 ml beaker. Oven dry sample.
9. Thoroughly mix dried sample. NOTE: See caution step 1.
10. Fill two 250 ml polyethylene bottles with acetone. Label one bottle "Acetone Wash" and the second bottle "Acetone Rinse." Similarly label two 400 ml beakers.
11. Pour acetone from bottle marked "Acetone Wash" into corresponding 400 ml beaker. Repeat for bottle marked "Acetone Rinse."
12. Thoroughly wash and then rinse glass microscope slide and glass slide cover.
13. Air dry glass microscope slide and glass slide cover.
14. Return acetone to appropriate polyethylene bottles. NOTE: The acetone can be used for several wash and rinse cycles. Throw out acetone in "Acetone Wash" bottle when it becomes too contaminated to thoroughly wash slides. Replace discarded acetone from "Acetone Wash" bottle with acetone in "Acetone Rinse" bottle. Put fresh acetone in "Acetone Rinse" bottle.
15. Thoroughly clean glass microscope slide and cover glass with lens paper.
16. Thoroughly clean microscope lens and mirror with lens paper.
17. Place a few grains of the thoroughly mixed oven-dry sample on a glass microscope slide.

18. Add 1.544 index oil by drops until all grains are covered.
19. Place one edge of the cover glass on the microscope slide. Gently lower the opposite edge being careful not to trap air bubbles under the cover glass.
20. Place slide on microscope stage.
21. Adjust white light source, microscope mirror, and microscope diaphragm for best light refraction without being strongly bright.
22. Move stage micrometer to one corner of the cover glass. Note both micrometer readings.
23. While moving stage micrometer in increments of one and doing one row at a time, count various types of grains (see 3.3.2.2) as they appear under the cross hairs until the area under cover glass has been completely covered.
24. Record results for each slide.
25. Thoroughly wash and rinse slide and cover glass (see steps 11-14). Discard grains from bottom of beaker after washing.

3.3.3 Petrographic Analysis of Thin Sections

3.3.3.1 Principle--

Overburdens and minesoils can be studied in slices called thin sections which are 30 microns thick. Thin sections are examined under the petrographic microscope and are useful in observing how individual constituents are arranged and in determining the size and shape of pores. The technique of studying morphological features under the microscope is basically an extension of methods used in studying samples with a hand lens or the unaided eye.

3.3.3.2 Comments--

Coherent samples can be thin sectioned directly but friable samples have to be impregnated usually with a polyester resin (Buol and Fadness, 1961). Normally thin sections are prepared by professionals who advertise in several geological and mineralogical journals and magazines.

Thin sections should first be examined with low magnification to study larger scale features and familiarize the observer with the minerals and fabrics within the thin section. Magnification is increased to observe finer details. Observation with transmitted light is conducted in plain light, crossed polarized light with and without substage condensing lens, and with light stopped down to different degrees. Some features may be more visible in reflected light. The determination of approximate percentage

of constituents can be ascertained by visual estimation. More accurate determinations can be made by counting the different kinds of grains, voids and special features.

The mineral composition of the larger grains (skeletal grains) observed in thin section can be determined by procedures similar to those used in the immersion method (see 3.3.2.2). The larger grains are generally embedded in a matrix of very fine-grained material composed largely of fine silt and clay. Some of the matrix material may be identified (see 3.3.2.2) but complete identifications require x-ray diffraction and differential thermal analysis.

More information on identification of minerals in thin sections may be obtained from Cady (1965) and Kerr (1959); however, the following special features may be seen in thin sections:

1. Cutan is a general term coined by Brewer (1964) to designate accumulations on soil particle surfaces or textural changes along a surface of movement. Accumulations may be composed of various materials such as clay, organic matter, silica, iron oxides or hydroxides, or manganese oxides or hydroxides.
2. Argillans or clay skins are cutans composed of clay minerals and occur on the natural surfaces of soil particles. They have a smooth or ropy surface with a waxy luster in reflected light. In thin sections they have the same index as the clay matrix but a higher index than that of quartz or feldspar grains. Under crossed nicols argillans commonly appear as white borders on grains or soil units. The borders go to extinction at the points where they are parallel to the polarizing elements ("north-south and east-west").
3. Ferrans are iron oxides or hydroxides occurring on soil surfaces. In thin sections they are translucent or opaque. Under reflected light they are yellow, brown, or red.
4. Mangans are manganese oxides or hydroxides which are usually opaque and very dark brown or black in reflected light.
5. Concretions differ in composition from the material which surrounds them. Concretions may be composed of any mineral matter, but they are more commonly made up of carbonate, chert, sulfate, or oxides and hydroxides of iron or manganese. Normal techniques of mineral identification are used to distinguish between the different types of concretions.
6. Earthworm casts appear as tubes of material commonly containing rounded aggregates representing excreta. The aggregates have dark outer borders of humus and may occur in clusters.
7. Root channels are voids left from decayed roots. These voids resemble worm burrows but may contain remnants of roots with characteristic cellular structure. The channel system may also show more of a tree-like pattern than do the worm burrows.

8. Pores (voids) in thin sections are colorless in plain light and remain black when the stage is rotated under crossed nicols. The percentage of observed pores can be estimated by visual inspection or determined more accurately by the point count method as described by Anderson and Binnie (1961).

The shape of the pore may be spherical, tubular, planar, or irregular. Spherical voids are commonly referred to as vesicles, some of which result from gas bubbles or solution of spherical grains. The apparent shape of the other pores depends on how they are cut during thin section preparation. Tubular pores will appear round in sections cut at right angles to the tubes. The shape will be elliptical and more elongate the more nearly the section parallels the length of the tubes. Tubular pores commonly result from burrowing by earthworms and insects or from the decay of plant roots. The cut of the section is not as critical in recognizing planar voids, although the true width of the opening can be determined only on sections at right angles to the plane of the voids. The planar voids commonly originate as a result of shrinkage of soil material as it dries. Our data show that soil fabrics, as described by Brewer (1964), do not normally occur in young mine soils. Remnant soil fabrics may be seen in mine soils that have been "top soiled" or mixed with a natural soil.

More details on soil fabrics can be found in Brewer (1964). Sampling procedures and number of grains to be counted are given by Cady (1965), Kerr (1959), and Winchell (1937). Accurate percentage determinations by point counting methods are given by Hutchison (1974) and Anderson and Binnie (1961).

3.3.3.3 Chemicals--

None required.

3.3.3.4 Materials--

1. Polarized petrographic microscope with micrometer stage with 10 X eyepiece and a range of objectives from 3.5 to 50 X.
2. Variable intensity white light source.

3.3.3.5 Procedure--

1. Place thin section on the stage of the petrographic microscope.
2. Adjust light intensity so grains can be easily seen.
3. Examine thin section under low magnification. Use higher magnification to clarify details.
4. Determine kinds of voids and percent porosity.
5. Examine for oriented clay bodies and determine position and percentage in sample.

6. Determine mineralogy and percentage of the skeletal grains.
7. Examine for special features (see 3.3.2.2) and record percentage of each.

3.3.4 Identification of Clay Minerals by X-Ray Diffraction

3.3.4.1 Principle--

The clay minerals of greatest interest (e.g. kaolinite, illite (mica), vermiculite, chlorite, and montmorillonite) are mostly flaky or platy in shape. They are readily identified and distinguished from one another by observing the effect of different chemical and heat treatments on the inter-layer spacings along the axis perpendicular to the platy surfaces with the use of x-ray diffraction.

The pretreatment used to distinguish montmorillonite from vermiculite and chlorite and to identify illites is saturation of the exchange complex of the clay with magnesium and treatment with glycerol. Vermiculite is distinguished from chlorite and kaolinite by saturating the clays with potassium and heating on a glass slide at 500°C. Intermediate heat treatments, 110°C and 250°C, can be used to study interlayering in the collapsing minerals or other special problems. Stronger x-ray diffraction peak intensities are obtained due to preferred orientation of the clays on the glass slide. This preferred orientation results since the clay plates settle parallel or nearly parallel upon drying from the suspension.

3.3.4.2 Comments--

Due to the length of time involved in sample preparation, several samples should be prepared at the same time. The commonly used radiation sources are copper and cobalt. If copper radiation is used, free iron oxides will have to be removed (see Jackson, 1958 p. 168) to eliminate interference. Interpretation of data should be performed by a person qualified in x-ray analysis and clay mineralogy.

3.3.4.3 Chemicals--

1. Sodium hydroxide (NaOH), 1 N: Dissolve 40.0 g of NaOH pellets with carbon dioxide-free water (See 3.2.3.3 No. 1) and dilute to a volume of 1 liter. Protect from CO₂ in air with ascarite tube.
2. Sodium carbonate (Na₂CO₃), 1 N: Dissolve 53 g of Na₂CO₃ with carbon dioxide-free water (See 3.2.3.3 No. 1) and dilute to a volume of 1 liter. Protect from CO₂ in air with ascarite in a guard tube.
3. pH 10 water: Dilute 10 ml of 1.0 N Na₂CO₃ to 10 liters with distilled and deionized water. Check pH with a pH meter and adjust to pH 10 by the addition of 0.1 N HCl or 1.0 N Na₂CO₃.
4. Acetone (CH₃COCH₃).

5. Hydrochloric acid (HCl), 1.0 N: Dilute 83 ml of concentrated HCl to a volume of 1 liter with distilled water.
6. Hydrochloric acid (HCl), 0.1 N: Dilute 100 ml of 1 N HCl to a volume of 1 liter with distilled water.
7. Bromophenol Blue.
8. Hydrogen peroxide (H₂O₂), 30%, ACS certified (without added preservative).
9. Sodium acetate (NaC₂H₃O₂), 1 N: Dissolve 82 g of NaC₂H₃O₂ with distilled water and dilute to a volume of 1 liter. Buffer to pH 5.0 with acetic acid or sodium hydroxide.
10. Magnesium chloride (MgCl₂·6H₂O), 1 N: Dissolve 102 g of MgCl₂·6H₂O with distilled water and dilute to a volume of 1 liter.
11. Magnesium chloride (MgCl₂·6H₂O), 10 N: Dissolve 1020 g of MgCl₂·6H₂O with distilled water and dilute to a volume of 1 liter.
12. Potassium chloride (KCl), 1 N: Dissolve 74.5 g of KCl with distilled water and dilute to a volume of 1 liter.
13. Potassium chloride (KCl), 10 N: Dissolve 745 g of KCl with distilled water and dilute to a volume of 1 liter.
14. Magnesium acetate (Mg(C₂H₃O₂)·4H₂O), 1 N: Dissolve 107 g of Mg(C₂H₃O₂)·4H₂O with distilled water and dilute to a volume of 1 liter.
15. Potassium acetate (CH₃COOK), 1 N: Dissolve 98 g of CH₃COOK with distilled water and dilute to a volume of 1 liter.
16. Methanol (CH₃OH).
17. Silver nitrate AgNO₃, 10% : Dissolve 10 g of AgNO₃ with distilled water and dilute to a volume of 100 ml.
18. Glycerol solution, 20% : Dilute and mix 20 ml of glycerine (CH₂OHCHOHCH₂OH) to 100 ml with distilled water.

3.3.4.4 Materials--

1. Balance, can be read to 0.1 g.
2. Soil dispersion mixer with baffled cup.
3. Sieve, 300 mesh.
4. Rubber policeman.
5. Funnel, large powder, polyethylene.

6. Beakers, 1000 ml, 600 ml, and 400 ml.
7. Drying oven.
8. Spatula.
9. Spot plate.
10. Hot plate.
11. Centrifuge bottles, 250 ml with screw caps and centrifuge tubes, 50 ml.
12. Centrifuge; equipped with tachometer and timer (IEC Model K with No. 277 and 279 heads or equivalent centrifuge with 4-place and 12-place heads).
13. Bottle, French square, 1 liter (32 oz) capacity.
14. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 120 strokes per minute.
15. Flask, Florence, 2000 ml with rubber stopper.
16. Vacuum desiccator.
17. X-ray diffraction instrument.
18. Glass x-ray slides.
19. Vortex mixer.

3.3.4.5 Procedure--

NOTE: If soil samples are analyzed instead of rock samples, omit 3.3.4.5.1.

3.3.4.5.1 Separation of clay from rock samples -- The following steps are for the separation of clay from rock samples only.

1. Weigh 100 g of ground (less than 60 mesh) rock material in a 600 ml beaker.
2. Add 300 ml distilled water.
3. Adjust the pH to 8.5 with 1.0 N NaOH.
4. Transfer suspension to metal container used with the soil dispersion mixer.
5. Fill to two-thirds of the container's volume with pH 10 water.
6. Stir suspension vigorously (using soil dispersion mixer) for 30 to 60 minutes.

7. Pour most of the suspension (all except heavy soup near bottom of container) into a 300 mesh sieve, aiding passage of the suspension through the sieve with a gentle jet of pH 10 water. NOTE: Sieve should be mounted in a large polyethylene funnel leading into a 1000 ml beaker.
8. Wash residue remaining on sieve with a gentle jet of pH 10 water, gently breaking up any clay or silt lumps with a rubber policeman.
9. Add pH 10 water to residue in the metal container and repeat steps 5 through 8.
10. Repeat step 9 until most of the silt and clay has been washed from the container. Transfer the sand in the container to the sieve and wash several times with a jet of pH 10 water, being certain to break up the lumps of silt and clay with a rubber policeman.
11. Wash sand with acetone to remove most of the water.
12. Dry the sand. After drying, shake sieve for about 10 minutes either by hand or mechanical shaker. Add material that passes sieve to the 1000 ml beaker containing silt and clay fractions.
13. Carefully pour contents of the sieve onto a black glazed paper, turning the sieve over and tapping its rim with the handle of a spatula.
14. Weigh sand and store in vial if analysis of the sand is desired. If sand is not needed for analysis, discard after weighing.
15. Go to 3.3.4.5.2 step 18.

3.3.4.5.2 Separation of soil fractions -- The following steps are for separation of soil fractions only.

1. Weigh 50 g of soil and transfer to a 1000 ml beaker.
2. Add 150 ml deionized water.
3. Adjust to pH 3.5 with 1.0 N HCl using bromophenol blue indicator and a spot plate. So as not to lose any soil, wash the drops of suspension on the spot plate back into the original beaker.
4. Add 25 ml of 30% H₂O₂ and cover with a watch glass. Allow to stand overnight without heating.
5. The following day, add an additional 25 ml of 30% H₂O₂ and heat gently to 90°C on a hot plate. Continue heating for at least one hour maintaining the sample at 90°C. NOTE: If the soil contains a large amount of organic matter, add an additional 25 ml of H₂O₂ after 1 to 2 hours and continue to repeat the additions until most of the organic "scum" is destroyed (this may take all day with 3 or 4 applications of H₂O₂).

6. Wash soil into a 250 ml centrifuge bottle, adjust to pH 3.5 if necessary, and centrifuge at 1,500 RPM until the supernatant liquid is clear (about 5 minutes).
7. Discard the clear liquid, transfer soil to a 400 ml beaker and adjust to pH 10 using a pH meter and 1.0 N Na₂CO₃ solution.
8. Pour suspension into a 1 liter bottle and fill to two-thirds of volume with pH 10 water.
9. Place horizontally on a reciprocating shaker and shake for 16 hours at 120 strokes per minute.
10. Place a 300 mesh sieve in a large polyethylene funnel and place funnel over a 1000 ml beaker.
11. Pour most of the suspension from the bottle into the sieve, washing silt and clay through sieve with a gentle jet of pH 10 water.
12. Wash remaining material from the bottle onto the sieve with a gentle jet of pH 10 water.
13. Wash silt and clay through sieve with a jet of pH 10 water, breaking silt and clay lumps with a rubber policeman.
14. Wash remaining sand with acetone to remove most of the water.
15. Dry sand. Add a sieve cover and receiving pan to the sieve and vigorously shake either by hand or mechanical shaker for 10 minutes. Material passing sieve is added to the 1000 ml beaker containing silt and clay fractions.
16. Carefully pour the contents of the sieve onto black glazed paper, turning the sieve over and tapping its rim with the handle of a spatula.
17. Weigh sand and transfer to storage vial. If sand is not needed for analysis, discard after weighing.
18. Pour contents of the 1000 ml beaker into a 250 ml centrifuge bottle, using one bottle per sample. Balance bottles. Centrifuge at 2000 RPM for 5 minutes. Pour supernatant suspension into a 2000 ml Florence flask labeled less than 2 micron fraction. NOTE: Since all of the suspension in the 1000 ml beakers will not fit into the 250 ml centrifuge bottle, centrifuging must be repeated by making additions to the bottle after each decantation until all of the suspension in the 1000 ml beaker is centrifuged. Do not stir material in the bottom of the centrifuge bottle between these additions.
19. After all of the suspension in the 1000 ml beaker has been added and centrifuged, add pH 10 water to the bottles, stir, and centrifuge at 1500 RPM for 10 minutes. Decant supernatant liquid into the Florence flask.

20. Add pH 10 water, stir, and this time centrifuge at 1600 RPM for exactly 2 minutes. Decant into the Florence flask.

21. Repeat step 20 until the supernatant liquid is about clear. NOTE: If the Florence flask becomes filled, an auxiliary container such as a 2000 ml beaker can be used. Both the Florence flask and the auxiliary container must be kept covered with a stopper or watch glass.

22. Add 20 ml of 1 N $\text{NaC}_2\text{H}_3\text{O}_2$ to flocculate the clay. After clay flocculates, siphon off as much of the liquid as possible without removing any of the clay.

23. Stir remaining liquid and clay by hand and pour mixture into a beaker.

24. Wash remaining clay from the Florence flask into the beaker with deionized water.

25. Place beaker in a vacuum desiccator, attach vacuum line, and evacuate until clay is air-dry.

3.3.4.5.3 Mg (or K) saturation of clay fraction -- The following steps include procedures for either Mg or K saturation of clays depending on analyses required.

1. Weigh 0.10 g of air-dry clay and suspend in 100 ml of 1 N $\text{NaC}_2\text{H}_3\text{O}_2$ (buffered to pH 5.0) in a 250 ml centrifuge bottle. Boil gently for 5 minutes.

2. Add 20 ml of 1 N $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (or KCl for samples to be K saturated) to the suspension.

3. Mix the suspension thoroughly and centrifuge at 2000 RPM for 5 minutes.

4. If the supernatant liquid is clear, discard the liquid; if not, add 10 ml of 10 N $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (or KCl) to insure flocculation. Centrifuge and discard clear supernatant liquid.

5. Wash clay once with 1 N $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2) \cdot 4\text{H}_2\text{O}$ (or CH_3COOK) and twice with 1 N $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (or KCl) to remove acetates centrifuging and discarding clear supernatant liquid between washings.

6. Wash and centrifuge clay twice with 20 ml deionized water and then with methanol until free from chlorides (Cl^- is present if a precipitate occurs with the addition of a few drops of 10% AgNO_3 to a few ml. of the supernatant).

7. Pipet about half the suspension. Spread suspension on a glass slide and allow to dry at room temperature (25°C) (see 3.3.4.5.5, step 2).

8. Dry remaining clay over drierite in a desiccator at room temperature (25°C) and proceed directly to glycerol solvation (3.3.4.5.4) if glycerol treatment is needed; if not, proceed to 3.3.4.5.5.

3.3.4.5.4 Glycerol solvation -- The following steps are necessary if glycerol saturation is required.

1. Weigh 0.050 g air-dry clay (Mg saturated) and place in a 50 ml centrifuge tube.
2. Add 0.5 ml of 20% glycerol solution.
3. Let stand for 30 minutes, centrifuge, and let drain for at least 30 minutes.
4. Add about 1 ml of water to the centrifuge tube to make a free flowing slurry and mix using a Vortex mixer.

3.3.4.5.5 Slide preparation and treatment selection -- From the following steps, x-ray slides can be prepared and proper treatments chosen.

1. Select appropriate treatments and temperatures from Tables 9 and 10.
NOTE: All heat treatments are heated at the given temperatures for 2 hours.
2. Carefully pipet 1 or 2 ml suspension onto glass slide. Do not allow suspension to run off the glass slide, but cover an area large enough to cover the entire x-ray beam.
3. Dry at room temperature (25°C).
4. Run x-ray analysis following manual supplied with the x-ray diffraction equipment.
5. Perform appropriate treatments as determined in 3.3.4.5.5, step 1. Rerun slides on x-ray diffraction unit.
6. Determine basal spacing in angstroms (Å) depending on type of radiation used.
7. Determine type of clay minerals using data from Tables 9 and 10.

3.3.4.5.6 Explanatory notes -- The following notes are for Tables 9 and 10.

1. Peaks sharp and higher orders distinct; beidellite closes easier on K saturation than montmorillonite from bentonite.
2. Peaks broad; higher orders very weak.
3. Peaks broader than mica; higher orders not quite as distinct and may not be exactly integral; may be interstratified with montmorillonite; 10 Å peak sharpened on heating clay.
4. First order peak sharper than montmorillonite but not as sharp as mica; higher orders are weak.
5. Strong 2nd order reflection; peaks sharp; spacing may vary $\pm 0.2 \text{ Å}$.

TABLE 9. BASAL SPACINGS OF CLAY MINERALS AS INFLUENCED BY
Mg-SATURATION AND GLYCEROL TREATMENTS

Mineral Name	Basal Spacings (Å units)		Notes (see 3.3.4.5.6)
	H ₂ O 25°C	glyc 25°C	
Mica	10	10	1
Montmorillonite (mont.)	12-15	18	2, 10
Illite	10	10-11	3, 10
Vermiculite (verm.)	14.5	14.7	4, 10
Chlorite	14.4	14.4	5, 10
Interstratified mont. and illite	11-14	15-17	6, 10
Interstratified verm. and chlorite	14.5	14.6	6, 10
Interstratified verm. and illite	11-14	11-14	6, 10
Montmorillonite with "interlayer islands"	14-15	14-15	7, 10
Vermiculite with "interlayer islands"	14-15	14-15	7, 10
Interstratified mont. with "islands" and illite	11-14	11-14	7, 10
Interstratified verm. with "islands" and illite	11-14	11-14	7, 10
Kaolinite	7.0-7.2	7.0-7.2	8, 10
Hydrated halloysite	10	10	8, 9
Dehydrated halloysite	7.2	7.2	9

TABLE 10. BASAL SPACINGS OF CLAY MINERALS AS INFLUENCED
BY K-SATURATION AND HEAT TREATMENTS

Mineral Name	Basal Spacing (\AA units)				Notes (see 3.3.4.5.6)
	25°C	100°C	300°C	525°C	
Mica	10	10	10	10	1
Montmorillonite (mont.)	10-12	10	10	10	2, 10
Illite	10	10	10	10	3, 10
Vermiculite (verm.)	10.5	10.2	10.1	10.1	4, 10
Chlorite	14.4	14.4	14.4	14.4	5, 10
Interstratified mont. and illite	10-11	10	10	10	6, 10
Interstratified verm. and chlorite	11-13	11-13	11-13	11-13	6, 10
Interstratified verm. and illite	10-11	10-11	10-11	10	6, 10
Montmorillonite with "interlayer islands"	14	13-14	11-12	10-11	7, 10
Vermiculite with "interlayer islands"	11-14	11-14	11-12	10-11	7, 10
Interstratified mont. with "islands" and illite	11-14	11-14	11-13	10	7, 10
Interstratified verm. with "islands" and illite	11-14	11-13	11-13	10	7, 10
Kaolinite	7.0-7.2	7.0-7.2	7.0-7.2	None	8, 10
Hydrated halloysite	10	7.2	7.2	None	8, 9
Dehydrated halloysite	7.2	7.2	7.2	None	9

6. Higher orders not integral.
7. Peaks may not sharpen on heating but shift to smaller "d" values.
8. Well crystallized kaolinite has a sharp peak at 7.0 Å and higher orders are present; poorly crystallized kaolinite has a broader peak at 7.2 and may be confused with halloysite.
9. If hydrated halloysite is once dried in the absence of some excess salts it does not reexpand to 10 Å. If dried in a slurry of HCl or NH₄Cl the spacing remains at 10 Å.
10. All 2:1 minerals are probably interstratified or interlayered with "islands" of nonexchangeable groups to some degree. When it is slight there is some shifting of the spacing indicated. The greater the proportion of a particular phase, the more the spacing will be like that of the pure mineral.

3.4 PHYSICAL METHODS

3.4.1 Summary--

The methods listed in this section are primarily for minesoil and soil materials. These methods measure parameters that dictate the long-term use of the soil. Where chemical properties are of extreme importance in the short term, physical properties of minesoils are extremely important to long-term management and use. Chemical properties can be more easily modified and changed than physical properties.

The size distribution of particles can be measured by either of the two methods, pipette or hydrometer. The pipette (3.4.2) is the more exact while the hydrometer method (3.4.3) is less time consuming. Bulk density can be measured by methods 3.4.4 through 3.4.7. The type of materials found in minesoils with the large variety of particle sizes dictated that more than one method be presented to the user for measuring bulk density. The other methods are self-explanatory and need no further clarification; however, material used in each of the physical methods is only sieved when taken from the field and not subjected to grinding.

3.4.2 Particle Size Analysis (Pipette Method)

3.4.2.1 Principle --

The pipette method depends on differential settling rates of silt- and clay-size soil particles from a water suspension. Since large particles settle faster than small particles of similar density (as stated by Stokes' Law), sampling a suspension at constant depth over increasing longer periods of time will yield increasingly smaller sizes of the suspended solids. By sampling the suspension with a pipette at a 10 cm depth at the time calculated from Stokes' Law, a sample of specific equivalent particle sizes will have already settled past the 10 cm depth at each sampling time.

3.4.2.2 Comments --

Normally soil samples are pretreated with hydrogen peroxide to remove organic matter. Materials which contain concentrations of soluble salts and gypsum must be leached with enough water to remove them before good dispersion of the sample can be accomplished. If the need should arise, procedures for the removal of organic matter, soluble salts, and gypsum are available (Day, 1956; Kilmer and Alexander, 1949).

3.4.2.3 Chemicals --

Dispersing agent: Dissolve 34.7 g sodium metaphosphate ($\text{Na}(\text{PO}_3)_6$) (Fisher Scientific Co. No. S-333 or equivalent) and 7.94 g sodium carbonate (Na_2CO_3) in distilled water and dilute to one liter. The Na_2CO_3 is used as an alkaline buffer to prevent the hydrolysis of the metaphosphate back to the orthophosphate which occurs in acidic solutions. NOTE: Instant Calgon available from Calgon Corp., Pittsburgh, Pa. can be used.

3.4.2.4 Materials--

1. Sieve, 2 mm (10 mesh) openings.
2. Sieve, approximately .05 mm (300 mesh) openings.
3. Bottles, pyrex nursing, 237 ml (8 oz) with rubber stoppers.
4. Pipette, Lowry automatic, 25 ml (available from Arthur H. Thomas, Co., Philadelphia, Pa.).
5. Shaw pipette rack. NOTE: If not available, a substitute pipette rack can be made using a cathetometer or a support stand with a sliding clamp. The pipette rack is required for lowering and positioning the tip of the pipette at a controlled depth below the upper mark of the 1000 ml graduated cylinder.
6. Balance, can be read to 0.001 g.
7. Drying oven.
8. Hot plate
9. Wooden rolling pin.
10. Beakers, 250 ml.
11. Watch glass.
12. Desiccator with drierite desiccant.
13. Funnel.
14. Rubber policeman.

15. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 120 strokes per minute.
16. Cylinders with a 1000 ml graduation (KIMAX brand (20023) or equivalent).
17. Aluminum pan.
18. Plunger (see 3.4.3.4 No. 6).
19. Vacuum assembly, vacuum source and suction hose with valves and trap to control rate (Kilmer and Mullins 1954, Figure 6, p. 440).
20. Weighing bottles, 60 ml capacity or 100 ml beakers.

3.4.2.5 Procedure (Adapted from Kilmer and Alexander, 1949)--

1. Mix and quarter air-dry soil.
2. Roll one quarter with a wooden rolling pin to break up clods.
3. Pass sample through 2 mm sieve. NOTE: Rolling and sieving are repeated until only rock fragments and pebbles are retained on the sieve. CAUTION: Care must be taken to avoid breaking the rock fragments.
4. Material not passing the 2 mm sieve are weighed and reported as a percentage of the air-dry weight of the whole sample.
5. Two 10.000 g samples of air-dry material passing the 2 mm sieve are weighed.
6. One sample is placed in a weighing bottle and dried at 105°C overnight. Then it is cooled in a dessicator and weighed to the nearest milligram. This weight is recorded as organic-free, oven-dry weight.
7. The other 10.000 g subsample is placed in a 237 ml (8 oz) nurse bottle with 10 ml of the dispersing agent.
8. Add distilled water to bring volume to about 177 ml (6 oz.). Stopper bottle and shake overnight in a horizontal position on a reciprocating shaker at 120 strokes per minute.
9. After shaking, bring bottle to room temperature by allowing it to stand for a few minutes if necessary.
10. Place 300 mesh sieve in a funnel and then place funnel in a 1000 ml graduated cylinder.
11. Wash the dispersed sample on the 300 mesh sieve. Wash all sample from the bottle using a jet of distilled water. CAUTION: Jets of water should be avoided in washing the sample through the sieve. The funnel should be gently tapped with the side of the hand to facilitate the washing procedure. Care should be taken not to spill any material over the top of the sieve.

12. Continue washing until the volume in the cylinder totals about 500 ml.
NOTE: Sands remain on the sieve. It is necessary that all particles of less than about 50 microns diameter be washed through the sieve.
13. Remove sieve, place in a tarred aluminum pan and dry in the oven. Cool sieve and transfer sands to the pan using a brush. Dry the pan and contents for about 2 hours at 105°C. The pan is then placed in a desiccator to cool and the contents weighed to the nearest 0.01 g.
14. Wash materials retained in the funnel into the cylinder. Bring volume to 1000 ml graduation mark with distilled water.
15. Cover cylinder with a watch glass and set in sedimentation cabinet.
16. Place the Lowry pipette on the pipette rack.
17. Make adjustments required to immerse the pipette 10 cm in the suspension when proper sampling time has arrived.
18. Attach vacuum line to pipette and adjust vacuum assembly to fill pipette in 12 sec using distilled water.
19. Stir the material in the sedimentation cylinder for 6 minutes (8 minutes if the suspension has stood for more than 16 hours) with a motor driven stirrer. CAUTION: Do not let any of the suspension spill over the top of the cylinder.
20. After mechanical stirring, stir the sample using an up and down motion for 2 minutes with the plunger (see 3.4.3.4 No. 6). Record time at completion of stirring and suspension temperature. NOTE: The temperature should remain constant during the settling process by using a constant temperature room or placing cylinders in a constant temperature bath. Samples should be placed where they are free of vibrations.
21. Using Table 11, determine the settling time for the less than 20 micron fraction.
22. About one minute before the determined settling time, the tip of the pipette is lowered slowly into the suspension to a depth of 10 cm by means of the pipette rack.
23. At the appropriate time, fill the pipette by controlled suction calibrated to require 12 seconds to fill. Remove pipette. Drain freely into a pre-weighed weighing bottle or beaker.
24. Add one rinse from the pipette to the weighing bottle or beaker using distilled water.
25. Repeat steps 22 through 24 until all cylinders have been sampled for the less than 20 micron fraction.
26. Restir samples for 2 minutes using an up and down motion with the plunger.

TABLE 11. TIMES FOR PARTICLE SIZE ANALYSIS (PIPETTE METHOD)
BASED ON TEMPERATURE

°C	Less Than 20 Micron	Less Than 2 Micron
20°	4 min. 40 sec.	466 min. = 7 hr. 46 min.
21°	4 min. 33 sec.	455 min. = 7 hr. 35 min.
22°	4 min. 27 sec.	444 min. = 7 hr. 24 min.
23°	4 min. 20 sec.	434 min. = 7 hr. 14 min.
24°	4 min. 14 sec.	424 min. = 7 hr. 4 min.
25°	4 min. 9 sec.	415 min. = 6 hr. 55 min.
26°	4 min. 3 sec.	405 min. = 6 hr. 45 min.
27°	3 min. 58 sec.	396 min. = 6 hr. 36 min.
28°	3 min. 53 sec.	388 min. = 6 hr. 28 min.
29°	3 min. 48 sec.	379 min. = 6 hr. 19 min.
30°	3 min. 43 sec.	372 min. = 6 hr. 12 min.

27. Record time and temperature.

28. Using Table 11, determine the settling time for the less than 2 micron fraction.

29. Repeat steps 22 through 24 until all cylinders are sampled.

30. Dry weighing bottles or beakers in an oven at 90°C until the volume has been reduced by one-half. Then dry for 12 hours at 105°C.

31. Cool in desiccator and record weights of the individual fractions.

32. Prepare a blank by placing 10 ml of the dispersing agent in a 1000 ml graduated cylinder. Bring volume to 1000 ml with distilled water. Pipette 25 ml and place in preweighed weighing bottle or beaker along with one rinse of the pipette. Dry at 105°C, cool in desiccator, and record weight as weight correction factor for dispersing agent.

3.4.2.6 Calculations--

1. % sand = (Weight of sand fraction/Weight of oven-dry, organic-free total sample) X 100.

2. Constant (K) = 1000/Volume of pipette.

3. D = 100/Weight oven-dry, organic-free total sample.

4. % Clay = (A - B) KD, where:

A = Weight in grams of the less than 2 micron fraction plus dispersing agent.

B = Weight in grams of dispersing agent correction.

5. % (20 to 2 micron) = [(A - B)KD] - (% clay), where:

A = Weight in grams of the less than 20 micron fraction plus dispersing agent.

B = Weight in grams of dispersing agent correction.

6. % (50 to 20 micron) = 100 - [% sand + % clay + % (20 to 2 micron)].

7. % silt = (% 20 to 2 micron) + (% 50 to 20 micron).

3.4.3 Particle Size Analysis (Hydrometer Method)

3.4.3.1 Principle--

This method depends on the rate at which soil particles settle from a water suspension. The soil particles are put into suspension by mechanical stirring with the aid of a dispersing agent. Sodium metaphosphate solution is used to disperse the soil and avoid flocculation of the clays. Sodium replaces exchangeable calcium and the precipitation of the calcium, in the form of calcium phosphate, prevents its recombination with the clays. The net negative charge on the clay particles increases due to the addition of sodium ions, causing the particles to repel each other and disperse. Since large particles settle faster than the same kind of small particles as stated by Stokes' Law, the concentration of soil particles in suspension at a given time is dependent upon the size of the particles.

3.4.3.2 Comments--

Temperature is important in the sedimentation procedure since the density and viscosity of water change with temperature. As the temperature increases, the time required for particles to settle out of suspension decreases. The hydrometer is usually calibrated for 19.4 or 20°C (67 or 68°F). For each °F above the hydrometer calibration temperature, add 0.2 g to the reading. Subtract 0.2 g from the hydrometer reading for each °F below the calibration temperature. Although the correction factor for

temperature can be used, it is best to carry out the procedure in a constant temperature room or maintain the sedimentation cylinders in a constant temperature bath.

The material is mixed using a reciprocating shaker; however, a soil dispersion mixer with baffled cup (similar to a drink mixer) can be used as an alternate method if a reciprocating shaker is not available. With this apparatus, a weighed sample is placed in the baffled cup with distilled water and dispersing agent. The cup is placed on the mixer and stirred for a maximum of 5 minutes. Many minesoil samples can usually be mixed in 3 minutes.

3.4.3.3 Chemicals--

1. Dispersing agent: Dissolve 35.7 g sodium metaphosphate ($\text{Na}(\text{PO}_3)_6$) (Fisher Scientific Co. No. S-333 or equivalent) and 7.94 g sodium carbonate (Na_2CO_3) in distilled water and dilute to a volume of 1 liter. The Na_2CO_3 is used as an alkaline buffer to prevent the hydrolysis of the metaphosphate back to orthophosphate which occurs in acidic solutions. NOTE: Instant Calgon available from Calgon Corp., Pittsburgh, Pa. can be substituted.

2. Distilled water.

3.4.3.4 Materials--

1. Bottles, French square, 1 liter (32 oz) with caps.

2. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 120 strokes per minute.

3. Glass sedimentation cylinder with markings at the 1130 ml and 1205 ml levels (Bouyoucos cylinder).

4. Standard hydrometer (ASTM 152 H, with Bouyoucos scale in grams per liter).

5. Balance, can be read to 0.1 g.

6. Plunger. NOTE: This can be made using 3 mm (0.125 in) diameter wire. At one end make a circle 5.5 cm (2.125 in) in diameter. The wire should be manipulated so the handle extends at a right angle from the center of the circle for 56 cm (22 in). Stretched rubber bands bisecting the wire circle are spaced around the circumference until it is largely covered by rubber bands overlapping at the center.

7. Thermometer, 0-100°F.

3.4.3.5 Procedure (Modified from Bouyoucos, 1951)--

1. Weigh 50 g (oven-dried at 105°C overnight) of a fine textured or 100 g of coarse textured (90-100% sand) soil and place in a shaker bottle.

2. Add 125 ml of dispersing agent and 400 ml of distilled water to shaker bottle.
3. Cap bottle snugly and place horizontally on a reciprocating shaker for 16 hours at 120 strokes per minute.
4. Remove bottle and bring suspension to room temperature.
5. Wash all contents of shaker bottle into a sedimentation cylinder.
6. Set cylinder in a place away from vibrations.
7. Place hydrometer in suspension.
8. Fill to lower mark (1130 ml) with distilled water for a 50 g sample. Fill to upper mark (1205 ml) for a 100 g sample.
9. Remove hydrometer. Take plunger in one hand holding the cylinder with the other. Strongly move plunger up and down being careful not to spill contents of cylinder.
10. After all sediment is off cylinder bottom, carefully remove plunger and record time immediately. NOTE: Add a drop of amyl alcohol if the surface is covered with foam and restir the suspension if necessary.
11. Record hydrometer reading at meniscus top at the end of 40 seconds. NOTE: About 10 seconds before taking reading, carefully insert hydrometer and steady by hand.
12. Remove hydrometer from suspension. CAUTION: Do not leave hydrometer in suspension longer than 20 seconds as particles will settle out on its shoulders.
13. Measure and record suspension temperature. For each °F above the calibrated temperature of the hydrometer add 0.2 g to the reading. For each °F below the calibrated temperature subtract 0.2 g.
14. Record corrected hydrometer reading.
15. With the plunger, restir suspension. Take a reading at the end of two hours. Correct hydrometer reading (see step 13) and record corrected hydrometer reading.
16. Make 3 blanks by placing 125 ml of dispersing agent in 3 sedimentation cylinders. NOTE: Blanks should be run for each new batch of dispersing agent.
17. Fill cylinders two-thirds full with distilled water. Insert hydrometer and fill cylinder to the lower mark (1130 ml) with distilled water.
18. Take hydrometer reading and temperature of suspension. Correct hydrometer reading using step 13.

3.4.3.6 Calculations--

1. Dispersing agent correction factor = Sum total of temperature corrected hydrometer readings of blanks/3.
2. Weight corrected 2 hour reading = (Temperature corrected 2 hour hydrometer reading) - (Dispersing agent correction factor).
3. Weight corrected 40 second reading = (Temp. corrected 40 second hydrometer reading) - (Dispersing agent correction factor).
4. % Clay = (Weight corrected 2 hour reading/oven-dry weight of total sample) X 100.
5. % Silt = [(Weight corrected 40 second reading - Weight corrected 2 hour reading)/oven-dry weight of total sample] X 100.
6. % Sand = 100 - (% clay + % silt).

3.4.4 Bulk Density (Core Method)

3.4.4.1 Principles--

The soil bulk density determination is based on two measurements, a mass measurement and a volume measurement. The mass is measured by oven drying the sample at 105°C until a constant weight is obtained. The bulk volume measurement includes the space between the soil particles as well as the space occupied by the soil particles. Bulk density, the ratio of sample mass to sample volume, is expressed as grams per cubic centimeter (Blake, 1965).

3.4.4.2 Comments--

This method may be difficult or impractical in soil containing many rock fragments.

A flat soil surface is prepared at the desired depth and the core sampler is driven into the soil. If driven with a heavy hammer, the head of the tool must be protected with a tough wooden plank or block. Care must be taken to see that no compaction takes place so that a known volume of soil is obtained. The sample is transferred to the laboratory and weighed while still moist. The sample is then dried in an oven and weighed again. This sample must be immediately placed in a desiccator after removing from the oven as the dry sample will absorb moisture from the atmosphere (Baver, 1956, p. 180-182).

3.4.4.3 Chemicals--

None required.

3.4.4.4 Materials--

1. Double-cylinder core sampler with steel cutting edge, driving head,

and removable brass or aluminum sleeves.

2. Core cylinder, 7.6 cm (3 in) in diameter and 7.6 cm (3 in) in height with 3.2 mm (0.125 in) thick walls.
3. Balance, can be read to 0.1 g.
4. Drying oven.
5. One-pint containers.
6. Air tight plastic bags.
7. Aluminum weighing pans.
8. Cloth diapers
9. Desiccator containing drierite.

3.4.4.5 Procedure--

1. Assemble double-cylinder core sampler according to the instruction manual.
2. Prepare a flat soil surface at depth in profile to be sampled.
3. Drive core sampler into the soil with the driving head until the soil fills the brass or aluminum sleeve and extends slightly above it.
4. Remove driving head and twist double-cylinder core sampler.
5. Excavate soil on one side of the core sampler until the bottom of the cutting edge can be clearly seen.
6. To insure that the contact of the core with the main soil body is broken, run a knife across the bottom of the cutting edge. NOTE: Do this step taking care not to disrupt the soil core.
7. Pack a cloth diaper into the top of the double-cylinder core sampler until it rests on the top of the soil core and hold in place with one hand.
8. Gently tilt the top of the sampler towards the excavated side until the cutting edge of the sampler is exposed. Put the other hand across the bottom of the cutting edge to hold soil core in place. Remove core sampler from excavation.
9. Remove the core and sleeve from sampler by raising the cutting edge and applying gentle pressure to bottom of soil core while using the cloth diaper to insure that the soil core does not slide or fall from the sleeve.
10. Trim any excess soil off both ends of the soil core so a flat surface exists flush with the edges of the sleeve.

11. Remove the soil from the sleeve ring and place in a pint container lined with a plastic bag. Take care that no soil is lost in transfer.
12. Label the sample as to location, depth sampled and any other pertinent information.
13. Transfer the samples to the laboratory.
14. Weigh a labeled aluminum pan and record the weight (A).
15. Transfer the moist soil sample to the pan and record the weight (B).
16. Place the pan with sample in an oven and allow to dry for 24 hours at 105°C.
17. Remove the pan with sample from the oven and cool in a desiccator. Weigh pan and contents. Record weight (C).

3.4.4.6 Calculations--

1. Bulk Density = $(C - A)/347.5$ cc, where 347.5 cc is the volume of the cylinder.
2. Percent Field Moisture = $((B - C)/(C - A)) \times 100$.

3.4.5 Bulk Density (Saran Method)

3.4.5.1 Principle--

See 3.4.4.1

3.4.5.2 Comments--

Care should be exercised when handling methyl ethyl ketone. This chemical is toxic and flammable. An exhaust hood should be used during the mixing of the plastic solution. Containers used for storing the solvent and the plastic solution must have lids which provide a tight seal.

One sampling pit can be used to collect samples from several different depths. Start at the surface and work downwards. Take a sample at the surface and then remove all material until the horizontal layer at the desired depth is exposed. Then take sample and repeat process until all samples needed are collected.

When trimming a clod to the desired size, be careful not to compact or otherwise destroy it. Careful handling of the clod is necessary until final coatings of plastic have been applied.

3.4.5.3 Chemicals--

1. Water.

2. Methyl ethyl ketone ($\text{CH}_3\text{COC}_2\text{H}_5$).

3. Dow Saran F310 solution in methyl ethyl ketone. NOTE: This solution consists of 1 part Saran and 7 parts of methyl ethyl ketone. It is prepared as follows: Under an exhaust hood, add 2310 ml of methyl ethyl ketone to a 3.785 liter (1 gallon) paint can. Add 330 g of Dow Saran Resin F310 to the solvent. The plastic is mixed with an air-powered or nonsparking electric stirrer until the resin dissolves. If a high-speed stirrer is used, the resin should dissolve in about one hour. Seal the container tightly with lid to prevent evaporation of solvent. Care should be exercised when using methyl ethyl ketone since the solvent is flammable and its vapors mix with air to form explosive mixtures. Always work with this solvent under an exhaust hood.

3.4.5.4 Materials--

1. Tile spade and shovel.
2. Sharp knife.
3. Scissors.
4. Thread or fine wire.
5. Plastic bags (large enough to contain sample) with ties.
6. Boxes, heavy, cardboard (large enough to contain samples).
7. Cloth diapers or other suitable packing material.
8. Exhaust hood.
9. Beaker, 600 ml.
10. Balance, can be read to 0.1 g.
11. Weighing pan, aluminum or other metal.
12. Support stand with ring clamp.
13. Drying oven.
14. Wooden rolling pin.
15. Paper (to crush clods on).
16. Sieve with 2 mm openings (10 mesh).

3.4.5.5 Procedure--

1. Dig a pit from the surface of the soil downward until a vertical cross-section of the soil is exposed.

2. Starting at the surface, work downward and remove a section of soil larger than the clod to be studied from the face of the pit with a tile spade.
3. Take a soil clod, about 5 cm in diameter, weighing from 30 to 150 g from a larger piece of soil using a sharp knife to carefully cut away excess material.
4. Carefully break or cut off all protruding points, cut off all roots with scissors, and brush all loose materials from clod.
5. Loop thread or fine wire around clod and tie securely. Be sure to leave a loose end of at least 50 cm (20 in) of thread or fine wire.
6. Open can containing the plastic solution. Holding the clod by the loose thread or fine wire, immerse it in the plastic solution for 5-10 seconds.
7. Remove clod from plastic solution and suspend from a previously prepared line (like a clothes line) for 30 minutes to allow coating to dry. NOTE: Seal container containing plastic solution tightly to prevent evaporation of solvent.
8. When dry, place coated sample in airtight plastic bag. Label the sample. Record location, depth sampled, and other pertinent information in data book.
9. Put the bag in a rigid cardboard container to prevent breaking or crushing of clod. NOTE: To insure that sample bag will be immobilized, use cloth diapers for packing material around the plastic bag.
10. Transport sample to the laboratory.
11. Under an exhaust hood, open can containing plastic solution. Remove sample from plastic bag holding it by the loose thread or fine wire and immerse it in the plastic solution for 30 seconds.
12. Remove clod from plastic solution, reseal container of plastic solution, and hang clod on a line under the exhaust hood for 30 minutes.
13. Repeat steps 11 and 12 four more times.
14. Fill a 600 ml beaker with approximately 350 ml of water.
15. Place beaker, with water, on a balance and weigh it to the nearest 0.1 g. Record weight (A).
16. Attach a ring clamp to the top of a support stand and position stand so that the ring clamp extends over the beaker of water on the balance.
17. After the final coating has dried, take loose end of thread or fine wire and lower clod into beaker of water until clod is resting on the

bottom of the beaker. Record weight (B). NOTE: Do not allow loose end of thread to fall into the beaker.

18. Loop loose end of thread or fine wire over ring clamp and slowly raise clod off the bottom of beaker.

19. When clod is completely surrounded by water, record weight (C).
NOTE: It is extremely important that the clod is not touching any part of the beaker and is entirely surrounded by water.

20. Remove clod from beaker and place on tray in an oven at 105°C for 48 hours.

21. Remove clod from oven, cool in a desiccator and weigh to the nearest 0.1 g. Record weight (D).

22. Take a knife and carefully cut plastic coating and thread or fine wire from clod.

23. Put all clod material on a sheet of paper and crush with a wooden rolling pin. NOTE: Be careful not to crush soft coarse fragments, but be sure to remove all fines from coarse fragments.

24. Pass crushed material through a 2 mm sieve.

25. Transfer all material caught on 2 mm sieve to a weighing pan and dry in an oven at 105°C for 4 hours.

26. Cool weighing pan and sample in a desiccator. Weigh to nearest 0.1 g and record weight (E).

27. Discard material and weigh weighing pan. Record weight (F).

3.4.5.6 Calculations--

1. Legend:

A = Weight of beaker and water.

B = Weight of beaker, water, and moist clod.

C = Weight of beaker, water, and moist clod suspended in water.

D = Weight of oven-dry clod.

E = Weight of weighing pan and clod material greater than 2 mm in effective diameter.

F = Weight of weighing pan empty.

V = Volume of moist clod.

X = Volume of coarse fragments in clod.

2. Bulk density of clod = D/V ,

Where $V = (C-A)/(1.00 \text{ g/ml})$, the density of water is assumed to be 1.00 g/ml.

3. Bulk density of the less than 2 mm material of the clod = $[D - (E - F)] / (V - X)$, where: $X = (E - F)/(2.65 \text{ g/ml})$ NOTE: This calculation assumes that all material greater than 2 mm in effective diameter has no porosity and has a particle density fo 2.65 g/ml.

4. Percent moisture of field sample on an oven-dry weight basis = $[(B - A) - D]/D \times 100$.

3.4.6 Bulk Density (Varsol Method)

3.4.6.1 Principle--

(See 3.4.4.1)

3.4.6.2 Comments--

The nonpolar liquid, Varsol, is used because of its availability, cheapness and absence of an offensive odor. Because of its nonpolar nature, it can replace air trapped in pores without causing the clod to slake like a polar liquid (water).

Clods used must hold together without breaking during routine field and laboratory work. When samples are packed for transportation to the laboratory, cushioning agents (i.e. diapers, styrofoam chips, crumpled paper) should be used to reduce the chances of clod breakage. Corrections can be made for soils containing coarse fragments using steps 28 through 31 in the procedure.

The density of each new container of Varsol should be determined by using a clean and dry 50 ml volumetric pipet and pipetting the Varsol into a clean and dry preweighed beaker. The weight of the Varsol is recorded to 0.01 g. The pipetting and weighing is repeated a total of three times. An average weight of the three readings is divided by 50 (ml of Varsol used to determine the density).

3.4.6.3 Chemicals--

Varsol - Trade name of EXXON cleaning fluid (but can usually be purchased from other suppliers). We have found Varsol to have a rather consistent density of 0.77 g/cc.

3.4.6.4 Materials--

1. Digging implements (spade and shovel).
2. Knife.

3. Plastic bags (large enough to contain sample) with ties.
4. Containers, rigid cardboard (large enough to contain samples).
5. Drying oven.
6. Thread (or similar light weight, thin cord).
7. Balance, can be read to 0.1 g.
8. Weighing pan (preferably aluminum, but glass or other metal can be substituted).
9. Blotting paper.
10. Desiccator apparatus, vacuum type, with hole in center of lid for a rubber stopper (Corning 3100 or equivalent). Supported above the bottom of the desiccator is a perforated porcelain desiccator plate having a large center hole. A two-hole rubber stopper is placed in the desiccator lid. In one hole is placed a 8 mm o.d., T-shaped tubing connector. From one end of the T-connector, a short piece of tubing with a hosecock clamp is applied to allow air back into the desiccator after evacuation. From the other end of the T-connector, attach a length of vacuum hose with a hosecock clamp to the vacuum source equipped with vacuum gauge. A short piece of 8 mm o.d. glass tubing (bent at 90°) is inserted into the second hole of the rubber stopper with the 90° bend being outside the desiccator. A length of tygon tubing is attached to the inside end of the glass tubing so that when the desiccator is closed, the tubing extends below and through the center hole of the porcelain plate. Another piece of tygon tubing with hosecock is applied to the other end of the glass tubing and cut to extend to near the bottom of the Varsol container.
11. Support stand with ring clamp (aluminum rod can be substituted for the ring clamp).
12. Beaker, 600 ml.
13. Wooden rolling pin (optional).
14. Brown paper (optional).

3.4.6.5 Procedure--

1. Dig a pit from the surface of the soil downward until a vertical cross section of the soil is exposed through the depths to be sampled.
2. Remove a large layer of soil with a spade from the face of the sampling pit. Take a soil clod about 5 cm in diameter and weighing from 30 to 150 g from the layer of soil. NOTE: Use a knife to cut the clod from the soil. More than one clod can be taken for testing.

3. Put the sample in an airtight plastic bag. Label the sample. Record location, depth sampled, and other pertinent information in data book.
4. Put the bag in a rigid cardboard container to prevent breaking or crushing the clod. NOTE: To insure that sample bag will be immobilized, use cloth diapers for packing material around the plastic bag.
5. Transport the sample to the laboratory.
6. Weigh an oven-dry weighing pan and record the weight (A).
7. Carefully break off all protruding points and brush all loose material from the clod.
8. Loop thread around clod and tie leaving about 50 cm (20 in) of thread loose.
9. Place moist clod in weighing pan and weigh it to the nearest 0.1 g. Record weight (B).
10. Place moist clod on a small square of heavy blotting paper in the vacuum desiccator.
11. Apply grease to the ground glass surfaces of the lid and the bowl of the desiccator.
12. Place the lid on the bowl and make a tight seal. NOTE: Make sure the tubing extends below and through the center hole of the porcelain base plate in the bottom of the desiccator.
13. Clamp off the hoses that lead to the supply of Varsol and air inlet.
14. Evacuate to a pressure of less than 0.1 bar.
15. Clamp off hose leading to vacuum source.
16. Open clamp to hose leading to Varsol and admit fluid slowly until it completely covers sample.
17. After sample is completely covered with Varsol, allow sample to soak for one hour.
18. Fill a 600 ml beaker with enough Varsol to cover sample completely (approximately 350 ml) and weigh on balance to nearest 0.1 g. Record weight (C).
19. Take a support stand and attach a ring clamp at the top of stand. Position stand in such a manner that the ring clamp extends over the beaker of Varsol on the balance.

20. After soaking, open clamp on air inlet to allow the inside of the desiccator to return to atmospheric pressure.
21. Remove lid of desiccator carefully. Remove clod on its base of blotting paper from the fluid.
22. Separate blotting paper and clod. Carefully place clod into beaker of fluid on the balance pan and allow clod to rest on beaker bottom. Do not let the loose end of thread fall into the beaker. Record weight (D).
NOTE: Separation of blotting paper and clod after removal of both from Varsol will eliminate a few drops of surplus fluid from the sample, but the drainage tension will be slight.
23. Take loose end of thread attached to clod and loop thread over the ring clamp (or straight rod) and slowly raise the clod off the bottom of beaker.
24. When clod is completely surrounded by fluid, record weight (E).
NOTE: It is essential that clod is not touching the sides or bottom of the beaker and is entirely surrounded by the fluid.
25. Remove clod from beaker and place in weighing pan (pre-weighed in step 6). Allow samples to air dry overnight under a hood.
26. Dry clods in an oven at 105°C for 24 hours.
27. Remove samples from oven. Cool in desiccator and weigh to nearest 0.1 g. Record weight (F). NOTE: Steps 28 thru 31 are necessary if bulk density and porosity of the textural particles without coarse fragments are required.
28. Put clod on sheet of brown paper and crush with a wooden rolling pin.
NOTE: Be careful not to crush small, soft coarse fragments, but be sure to remove all fines from coarse fragments.
29. Pass sample through a 2 mm sieve.
30. All material caught on 2 mm sieve is transferred to a weighing pan (pre-weighed in step 6) and dried in an oven at 105°C for 4 hours.
31. Cool weighing pan and sample in deiccator. Then weigh sample plus weighing pan and record weight (G).

3.4.6.6 Calculations—

1. Legend:

A = Oven-dry weight of weighing pan.

B = Weight of moist clod and weighing pan.

C = Weight of beaker and Varsol.

D = Weight of beaker, Varsol, and clod.

E = Weight of beaker, Varsol, and clod suspended in Varsol.

F = Oven-dry weight of clod and weighing pan.

G = Oven-dry weight of coarse fragments contained in clod and weighing pan.

X = Volume of water in clod (equals the volume of pore space filled with water).

Y = Volume of Varsol in clod (equals the volume of pore space filled with Varsol).

Z = Volume of clod.

T = Volume of coarse fragments.

Density of water = 1.00 g/cc.

Density of Varsol = 0.77 g/cc (see 3.4.6.2).

2. Bulk density of clod = $(F - A)/Z$, where:

$Z = (E - C)/\text{Density of Varsol}$.

3. Total pore space = $X + Y$, where:

$X = (B - F)/\text{Density of water}$; and

$Y = [(D - C) - (B - A) - (F - A)]/\text{Density of Varsol}$.

4. Total porosity = $[(X + Y)/Z] \times 100$.

5. Bulk density of the less than 2 mm material in the clod.

Bulk density = $(F - G)/(Z - T)$, where: $T = (G - A)/2.65$

NOTE: The coarse fragments are assumed to have no porosity; therefore, a particle density of 2.65 g/cc is used to find the volume of the coarse fragments. When coarse fragments have porosity the calculated bulk density and porosity of the fines (less than 2 mm material) will be incorrect, but bulk density and porosity of the whole clod, including coarse fragments, will be correct.

3.4.7 Bulk Density (Sand Method)

3.4.7.1 Principle--

See 3.4.4.1

3.4.7.2 Comments--

The calculated volume of the jar and attachment remain constant as long as

both maintain the same relative position to each other. If the two are to be separated, match marks should be made to permit reassembly to this position. The individual measured volumes of water (Q_1 , Q_2 , and Q_3) require filling the jar and attachment repeatedly (see 3.4.7.6, no. 1). Replicates should not differ more than 3 ml between the highest and lowest volume determined. Vibration of the sand during any of the weighings or density determinations may cause an increase in the sand bulk density and a decrease in accuracy. Sand bulk density (T) may change over time due to changes in moisture content or effective graduation. Field measurements should be run as soon as possible after the sand density (T) has been determined. Each new bag of sand must have its sand density determined (ASTM, 1974).

Care should be taken in excavating to minimize compaction of the soil surrounding the hole. Any material falling from the sides of the hole must be removed and placed with the material to be weighed. In this method, discrimination of very thin horizons is lost; however, due to the relatively large sample size, small errors in measuring the sand weight results in insignificant errors (Blake, 1965).

This method is especially suited to minesoils where coarse fragments prevent using a core sampler. The procedure also works well in coarse textured or unconsolidated materials that cannot be tested with either the Varsol or Saran techniques.

3.4.7.3 Chemicals--

Acetone (CH_3COCH_3) (optional).

3.4.7.4 Materials--

1. Template consisting of a thin, flat, metal plate 30.5 cm (12 in) square, with a 16.5 cm (6.5 in) diameter hole in its center.
2. Sand-funnel apparatus consisting of a lower cone flanged to 16.5 cm (6.5 in) to fit the above template and a top cone section that is threaded to receive the sand jug. A valve is located between the two cones to control the sand flow into the density hole (specifications in ASTM, 1974 p. 211).
3. A standard sand that is clean, dry, and free-flowing. Particle size should be uniform passing a sieve with 0.841 mm openings (20 mesh) and retained on a sieve with a 0.250 mm openings (60 mesh). (Ottawa sand or equivalent).
4. Balance, 20 kg (44.10 lb) capacity which can be read to 1.0 g (Model L-500 available from Soiltest, Inc., Evanston, IL or equivalent).
5. Large spoon.
6. Sand scoop.
7. Brown paper (optional).

8. Wooden rolling pin (optional).
9. Sieve, 2 mm openings (10 mesh).

3.4.7.5 Procedure (modified from ASTM, 1974)—

NOTE: Steps 1-16 and 3.4.7.6 no. 1-3 should be completed in the laboratory prior to going to the field. Steps 1-8 and 3.4.7.6 no. 1 must be completed when either the jar or funnel apparatus is replaced. Steps 9-12 and 3.4.7.6 no. 2 must be repeated for each new bag of sand. Steps 13-16 and 3.4.7.6 no. 3 must be repeated if the funnel apparatus is replaced.

1. Assemble apparatus and place match marks on both the jar and funnel apparatus to permit accurate realignment in case of separation.
2. Weigh assembled apparatus empty and record weight (A).
3. Place apparatus upright. Open valve and fill with water until the water appears over the valve.
4. Close valve and pour off excess water. Remove any water remaining in the funnel by sponging and then wiping dry.
5. Weigh apparatus filled with water. Record weight (B). Determine temperature of the water and record temperature (C).
6. Discard water in apparatus.
7. Repeat steps 3-6 two more times and determine the volume of the apparatus from an average of the three weighings.
8. Thoroughly dry apparatus by the addition of acetone to absorb water, followed by drying with a jet of moisture-free air or drying on a drying rack.
9. Place dry density apparatus upright on a firm, level surface. Close valve and fill funnel with sand.
10. Open valve and fill apparatus. NOTE: Keep funnel at least half full of sand during the filling procedures.
11. Close valve sharply and remove sand remaining in funnel.
12. Weigh apparatus filled with sand and record weight (D).
13. Invert apparatus and seat in template on a clean, level, planar surface.
14. Open valve and keep open until sand stops running.
15. Close valve sharply. Weigh apparatus and remaining sand. Record weight (E).

16. Replace sand following steps 9-11.
17. In the field, prepare the surface of the location to be tested so that it is a level plane.
18. Place template on surface.
19. Using a large spoon, dig the test hole inside the template hole, being careful to avoid disturbing the soil bounding the hole. NOTE: The excavated hole should have a diameter equal to the diameter of the template hole. The excavated walls of the finished hole should be as close to vertical as possible. The hole depth should be at least 7.6 cm (3 in) but not exceeding 16.5 cm (6.5 in) deep.
20. Place all loosened soil in a container, being careful not to lose any material.
21. Seat the density apparatus on the template and open the valve. After the sand has stopped flowing, close the valve sharply.
22. Weigh apparatus and remaining sand. Record weight (F).
23. Replace as much sand as possible from the hole back into the jar, being careful not to get contaminants in the sand from the hole.
24. Refill apparatus with sand using steps 9-11.
25. Prewrite a weighing pan and record weight (G).
26. Place moist material removed from the test hole on the preweighed pan. Record weight (H).
27. Place material in an oven at 105°C for 16 hours.
28. Cool in desiccator and reweigh. Record weight (I).
NOTE: Steps 29-32 are optional and are used when bulk density without coarse fragments is required.
29. Put excavated material on a sheet of brown paper and crush with a wooden rolling pin. NOTE: Be careful not to crush small soft coarse fragments, but be sure to remove all fines from coarse fragments.
30. Pass sample through a 2 mm sieve.
31. All material caught on a 2 mm sieve is transferred to weighing pan and dried in an oven at 105°C for 4 hours.
32. Cool weighing pan and sample in desiccator. Weigh sample plus weighing pan and record weight (J).

3.4.7.6 Calculations--

1. Legend:

A = Weight of empty apparatus.

B_1 = Weight of apparatus filled with water from first weighing
(see 3.4.7.5, steps 2 through 7).

B_2 = Weight of apparatus filled with water from second weighing
(see 3.4.7.5, steps 2 through 7).

B_3 = Weight of apparatus filled with water from third weighing
(see 3.4.7.5, steps 2 through 7).

C = Temperature of water.

D = Weight of apparatus filled with sand.

E = Weight of apparatus and sand excluding sand in funnel.

F = Weight of apparatus and sand excluding sand in excavated hole and
sand in funnel.

G = Weight of weighing pan.

H = Weight of moist sample and weighing pan.

I = Weight of oven-dry sample and weighing pan.

J = Weight of coarse fragments and weighing pan.

K = Volume of coarse fragments.

N_1 = Weight of water required to fill apparatus on first weighing
(see 3.4.7.5, steps 2 through 7).

N_2 = Weight of water required to fill apparatus on second weighing
(see 3.4.7.5, steps 2 through 7).

N_3 = Weight of water required to fill apparatus on third weighing
(see 3.4.7.5, steps 2 through 7).

P_1 = Volume-temperature correction factor from Table 12 for first weighing
(see 3.4.7.5, steps 2 through 7).

P_2 = Volume-temperature correction factor from Table 12 for second weighing
(see 3.4.7.5, steps 2 through 7).

P_3 = Volume-temperature correction factor from Table 12 for third weighing
(see 3.4.7.5, steps 2 through 7).

TABLE 12. VOLUME OF WATER PER GRAM BASED ON TEMPERATURE

Temperature (C) °C	Volume of Water (P) ml/g	Temperature (C) °C	Volume of Water (P) ml/g
12	1.00048	22	1.00221
14	1.00073	24	1.00268
16	1.00103	26	1.00320
18	1.00138	28	1.00375
20	1.00177	30	1.00435
		32	1.00497

Q_1 = Volume of water required to fill apparatus from first weighing (see 3.4.7.5, steps 2 through 7).

Q_2 = Volume of water required to fill apparatus from second weighing (see 3.4.7.5, steps 2 through 7).

Q_3 = Volume of water required to fill apparatus from third weighing (see 3.4.7.5, steps 2 through 7).

R = Average volume of density apparatus.

S = Weight of sand required to fill apparatus.

T = Bulk density of sand.

U = Weight of sand required to fill funnel.

V = Weight of sand required to fill excavated hole and funnel.

W = Volume of excavated hole.

Y = Weight of oven-dry sample

Z = Weight of moist sample.

2. $R = (Q_1 + Q_2 + Q_3)/3$, where:

$Q_1 = N_1 \times P_1$.

$$Q_2 = N_2 \times P_2.$$

$$Q_3 = N_3 \times P_3, \text{ and}$$

$$N_1 = B_1 - A.$$

$$N_2 = B_2 - A.$$

$$N_3 = B_3 - A.$$

3. $T = S/R$, where:

$$S = D - A.$$

4. $U = D - E.$

5. $V = D - F.$

6. $W = (V - U)/T.$

7. Bulk density of soil = Y/W , where $Y = I - G.$

8. Percent moisture = $[(Z - Y)/Y] \times (100)$, where $Z = H - G.$

9. Bulk density of the less than 2 mm material in sample.

$$\text{Bulk density} = [Y - (J - G)] / (W - K), \text{ where } K = (J - G) / 2.65.$$

NOTE: The coarse fragments are assumed to have no porosity; therefore, a particle density of 2.65 g/cc (density of quartz) is used to find the volume of the coarse fragments. See note under 3.4.6.6.

3.4.8 Particle Density

3.4.8.1 Principle--

The relationship of the solid soil particles to their total volume excluding the pore spaces between particles is called the particle density. It is normally expressed as grams per cubic centimeter. The mass of the solid particles is found by weighing and their total volume is determined by the displacement of a liquid whose mass and density are known (Blake, 1965).

3.4.8.2 Comments--

If measurements of volumes and weights are done carefully, this method is precise. A lack of precision in either measurement may result in serious error.

A non-polar liquid, Varsol, is used in the procedure instead of water because of the higher density values water gives for finely divided, active powders. Other polar liquids (e.g. toluene, xylene, or carbon tetrachloride) can be used, but they need special care in handling.

This measurement is used to mathematically determine porosity, airspace, and sedimentation rates for particle-size analysis. Minesoil samples are screened through a 2 mm sieve after rolling with a rolling pin. Sample is not ground with mortar and pestle.

3.4.8.3 Chemicals --

Varsol - Trade name of EXXON cleaning fluid (but can usually be purchased from other suppliers). We have found Varsol to have a rather consistent density of 0.77 g/cc.

3.4.8.4 Materials--

1. Pycnometer flask with ground glass lid (modified Hubbard-Carmick, Pyrex brand 1620 or equivalent).
2. Balance, can be read to 0.0001 g.
3. Vacuum desiccator.

3.4.8.5 Procedure (modified from Blake, 1965 and Gradwell, 1955)--

NOTE: All weights are recorded to ± 0.0001 .

1. Oven dry the less than 2 mm sample at 60°C overnight.
2. Weigh a clean, dry pycnometer flask and lid. Record weight (W_a).
3. Add about 10 g of oven-dry sample to pycnometer. Clean outside and neck of pycnometer of any soil that may have spilled during transfer.
4. Weigh the pycnometer, including lid, and its contents. Record weight (W_s).
5. Fill pycnometer about one-half full with Varsol, washing any soil adhering to the neck into the pycnometer.
6. Place pycnometer into the vacuum desiccator, apply vacuum, and remove any entrapped air. Entrapped air will be removed when all bubbling ceases.
7. Remove the pycnometer and shake gently. NOTE: Repeat steps 6 and 7 until all bubbling ceases.
8. Fill the pycnometer with enough Varsol so that when the lid is put in place, the hole in the lid will be completely filled with Varsol.
9. Insert the lid and seat it carefully.
10. Thoroughly dry and clean the outside of the pycnometer with a dry cloth.
11. Weigh the pycnometer and its contents. Record weight (W_{sv}).

12. Remove sample and Varsol from the pycnometer. NOTE: Thoroughly wash pycnometer and lid with Varsol to insure removal of sample.
13. Fill pycnometer with enough Varsol so that the hole in the lid will be filled with Varsol when the lid is seated.
14. Insert and seat lid. Thoroughly dry the outside with a dry cloth.
15. Weigh pycnometer filled with Varsol. Record weight (W_V).

3.4.8.6 Calculations--

Particle density (D_p) = $dv (W_s - W_a) / [(W_s - W_a) - (W_{sv} - W_v)]$, where:

dv = Density of Varsol in g/cc (see note below).

W_s = Weight pycnometer plus sample.

W_a = Weight of pycnometer filled with air.

W_{sv} = Weight of pycnometer filled with sample and Varsol.

W_v = Weight of pycnometer filled with Varsol.

NOTE: The density of Varsol must be determined for each new supply of Varsol. Using a pipette, add exactly 50 cc to a previously tared beaker. Record weight of the Varsol.

dv = weight (g) of Varsol/50 cc.

3.4.9 Total Porosity

3.4.9.1 Principle--

The bulk volume of a field moist soil sample contains soil particles, moisture, and air. The portion of the bulk volume filled with moisture and air is called pore space. Bulk density measurements (3.4.4-3.4.7) are calculated by dividing the oven-dry weight of the mass (in grams) by the bulk volume. This value is considerably lower than the average particle density (3.4.8). This means that part of the bulk volume is pores filled with air. Calculation of total porosity is done by converting data from densities into volumes. The volume (VB) of the bulk sample is derived from a bulk density measurement. The volume (VP) is the collective volume occupied by solid particles and is derived from the particle density measurement. Therefore, VP/VB is the fraction of the volume occupied by solid particles. In this manner, total porosity can be calculated using the equation in 3.4.9.6 (Vomocil, 1965).

3.4.9.2 Comments--

Total porosity can be measured directly if the "Varsol" method is used to find the bulk density. The procedure and calculations are given in 3.4.6.

Do not use an assumed particle density of 2.65 g/cm^3 if carbolithic materials are present in the sample in appreciable amounts. Measure the particle density of this material using 3.4.8.

3.4.9.3 Chemicals--

None required.

3.4.9.4 Materials--

None required.

3.4.9.5 Procedure--

1. Determine the bulk density using one of the following methods:
(a) 3.4.4; (b) 3.4.5; (c) 3.4.6; or (d) 3.4.7.
2. Determine particle density using method 3.4.8. NOTE: In cases where great accuracy is not required, use the assumed value of 2.65 g/cm^3 for the particle density of mineral soils.

3.4.9.6 Calculations--

1. TP = Total porosity: percentage of the bulk volume not occupied by solids.
2. BD = Bulk density of soil.
3. PD = Particle density of soil.
4. $TP = [(PD - BD)/PD] \times 100$.

3.4.10 Free Swelling (Settling Volume)

3.4.10.1 Principle--

Swelling is an innate property of the clays. Swelling may arise in two different ways: (1) water molecules becoming positioned between the particles of clay; (2) water molecules becoming positioned within the molecular structure of the clay mineral. Kaolinite and mica-like clays will only exhibit swelling due to the former process; therefore, these clays will have limited volume change, especially kaolinite. Clays of the montmorillonite type exhibit extensive swelling mainly because of the latter process. Free swelling is an important property of this type of clay mineral (Marshall, 1949).

3.4.10.2 Comments--

Step number 4 of 3.4.10.5 (procedure) should be performed very carefully and slowly so that no sample is lost. Also, step number 10 should be performed exactly as described.

This simple method can be used effectively to evaluate the stability of materials. Materials exhibiting extensive swelling would be unstable on steep slope, haul-road, etc. Also, future land use would be affected by such materials.

3.4.10.3 Chemicals--

Distilled water (H₂O).

3.4.10.4 Materials--

1. Graduated cylinders, 100 ml capacity with 1 ml graduations.
2. Powder funnels.
3. Sieve, 0.25 mm openings (60 mesh).
4. Polypropylene wash bottle.
5. Balance, can be read to 0.001 g.
6. Pencil, yellow or any color that can be seen easily through turbid water.
7. Standard liquid limit device (Sowers, 1965, Fig. 1-1, p. 395) adjusted to drop a distance of 1 cm.

3.4.10.5 Procedure--

1. Weigh a 10.00 g air-dry sample of earthy material ground to pass a 60 mesh sieve.
2. Fill a 100 ml graduated cylinder to the 85 ml mark with distilled water.
3. Put a powder funnel in the neck of the graduated cylinder.
4. Slowly add the 10.00 g of earthy material to the graduated cylinder in several small increments. NOTE: This step must be done slowly so that all earthy material is transferred into the cylinder without unnecessary entrapment of air.
5. Add distilled water to the cylinder until the liquid level reaches the 100 ml mark, washing off any particles adhering to the sides of the cylinder.
6. Set cylinder aside and let stand undisturbed for 6 hours.
7. At the end of 6 hours, place cylinder on cup of liquid limit device and turn crank 30 times at a rate of one revolution per second. NOTE: After every five revolutions straighten cylinder without changing the rate if necessary to keep cylinder upright.
8. Set cylinder aside and let stand undisturbed for an additional 18 hours.

9. At the end of the prescribed time, take a yellow pencil and place it behind the cylinder so that the pencil can only be seen by looking through the cylinder and the material in the cylinder.
10. Starting at the top of the cylinder, lower pencil down the back of the cylinder until the pencil can no longer be seen.
11. Record the volume at the point where the pencil cannot be seen.

3.4.10.6 Calculations--

Free swelling (settling volume) is expressed on a volume per mass basis (cc/g).

Free swelling = volume/10.00 g air-dry sample.

3.4.11 Moisture Retention (Pressure Plate Method)

3.4.11.1 Principle--

The amount of work needed to remove water from soil is measured by the pressure plate apparatus. This work equals the energy with which the soil sample holds the water. In this procedure a saturated soil sample rests on a semipermeable membrane and is subjected to controlled pressures in excess of atmospheric pressure. A water continuum, which is at atmospheric pressure outside the apparatus, exists from the surface of the soil sample to the open-air side of the semipermeable membrane; therefore, the compressed gas forces water out of the pores of the sample through the membrane by way of the water continuum. Water outflow from the chamber ceases when equilibrium has been reached (i.e., when the pressure exerted by the gas is counteracted by the tension (negative pressure) with which the soil particles hold onto the water). It is possible to determine directly the moisture content of the soil at that particular tension. Normally a curve called the moisture characteristic curve is developed by equilibrating soils at pressures from 0 through 15 bars or higher (Richards, 1965).

3.4.11.2 Comments--

Errors in these measurements can come from many sources. Some of the principle errors come from nonrepresentative subsamples, losses due to evaporation during the approach to equilibrium due to a leak in the air pressure chamber of the semipermeable membrane, pressure to temperature effects in excess of 1°C causing hysteresis effect, failure to obtain outflow equilibrium, and inadequate pre-wetting of samples. Additional errors can also come from evaporation losses when the samples are being removed from the chamber and loss of sample during removal from the chamber; however, these errors can be overcome as the operator becomes more proficient.

The semipermeable membrane, which may be a ceramic plate or cellulose disc, has a definite bubbling pressure. Below bubbling pressure of these membranes, the membrane will allow free movement of moisture from one side to the other; however, soil particles and air are not transmitted. The membrane contains

pores which are full of water and form the continuum from the soil sample through the membrane to the atmosphere on the outside. When the bubbling pressure of a membrane has been exceeded, some pores contain gas instead of water and the gas moves freely through the membrane and pressure is lost. After the system and the apparatus have been checked, determine which pressure range will be measured. The USDA - SCS commonly measures moisture retention at 1/3 and 15 bar tensions; however, the range of tensions of prime interest for plant growth may be from 0 to 2 bars or other ranges. For highly disturbed soils, coarse fragments sometimes constitute a major part of the soil volume. Therefore, the particle sizes used to get a moisture characteristic curve are not necessarily the same as for soils with few coarse fragments. Soils sieved to contain only particle sizes of less than 6.35 mm effective diameter are used at West Virginia University to determine a moisture characteristic curve. Also, moisture characteristic curves can be determined for the particle size range of 6.35 mm to 2 mm in effective diameter, as well as for the less than 2 mm particles (Richards, 1965).

3.4.11.3 Chemicals--

1. Distilled water.
2. Compressed nitrogen gas.

3.4.11.4 Materials--

1. Five bar pressure plate extractor (Soil Moisture Equipment Company Catalog No. 1600 or equivalent).
2. Pressure control manifold, accuracy of control within 1/100 psi in the 0.50 psi range (Soil Moisture Equipment Company Catalog No. 700-3 or equivalent).
3. One bar pressure plate cells (Soil Moisture Equipment Company Catalog No. 1290 or equivalent).
4. Three bar pressure plate cells (Soil Moisture Equipment Company Catalog No. 1690 or equivalent).
5. Soil sample retaining rings (Soil Moisture Equipment Company Catalog No. 1093 or equivalent).
6. Connecting hose (Soil Moisture Equipment Company Catalog No. 1293 or equivalent).
7. Nitrogen gas tank gauges - 1 for tank pressure and 1 for outflow pressure.
8. Large spatula or small pancake turner.
9. Wax paper.

10. Plastic teaspoon.
11. Balance, can be read to 0.01 g.
12. Drying oven.
13. Aluminum pans, for weighing samples.
14. Laboratory notebook.
15. Desiccator, with silica gel desiccant.

3.4.11.5 Procedure--

NOTE: This apparatus and procedure are used for negative pressures of 0 to -3 bar. Read instrument's instruction manual before starting procedure.

1. Check pressure in the nitrogen tank.
2. Check all fittings by pressurizing system. NOTE: Take a toothbrush and a bar of soap and mix up a soapy foam. Brush foam over each fitting to see if there are any leaks in the system when pressurized.
3. Check ceramic plates by forcing compressed air into outlet valve. Seal off valve and submerge ceramic plate in pan of water. If any bubbles appear, there is a hole in the rubber gasket sealed to the plate. Repair the leak or do not use the plate.
4. Place the ceramic plate to be used in a pan of distilled water and soak overnight (12-16 hrs). This is done when the ceramic plates have been dried over a period of time. If the ceramic plate has been used for a previous determination, this prolonged soaking is not necessary.
5. Take the aluminum pans and place a soil sample retaining ring inside the pan. Draw a line around the top of the ring so that the approximate height of the ring is outlined on the inside of the aluminum pan. The desired volume of subsample that would be put into the aluminum pan would be slightly less than needed to fill the soil sample retaining ring.
6. Use a thin plastic teaspoon and lift the soil from the container and fill the aluminum pan to the volume mark. Do two replicates in the same manner. NOTE: Be sure that all the pans are marked with the soil sample number.
7. After the ceramic plate has been soaked overnight, place the soil sample retaining rings on the ceramic plate in such a fashion that a diagram can be easily made of the set up showing the sample number for each particular ring.
8. Take the aluminum pan containing the approximate volume of soil sample needed and carefully dump it into the proper soil sampling retaining ring on the ceramic plate. Take the spatula or the spoon and carefully flatten

the sample until it is level with the top edge of the soil sample retaining ring. NOTE: Do not compact this material. Just carefully flatten by spreading.

9. After all the soil samples have been placed on the soaked ceramic plates, add an excess of water to the surface of the ceramic plate and allow the samples to soak for 16 hours. NOTE: Be sure there is enough water on the ceramic plate to allow samples to wet without removing water from the pores of the plates.
10. Cover samples and ceramic plate with wax paper to prevent evaporation.
11. After the samples have soaked overnight (16 hours), remove the excess water from the surface of the ceramic plate by means of a pipette.
12. Remove the wax paper from the soil samples. Connect the outflow tube on the ceramic plate to the outflow tube on the wall of the extractor.
13. Cover the extractor with the metal top. NOTE: Be sure that the "O" ring seal is in place.
14. Clamp the lid to the bottom of the extractor with clamping bolts. Tighten the wing nuts on the clamping bolts by hand.
15. With the needle valve, the "Nullmatic" type regulator, and the coarse adjustment regulator on the manifold all closed, pressurize the system by means of the controls on the nitrogen tank. Turn the "Nullmatic" type regulator valve to wide open and use the coarse adjustment valve on the manifold to get a reading on the pressure gauge of very slightly in excess of the desired pressure.
16. Use the "Nullmatic" type regulator to get the desired pressure reading on the manifold's pressure gauge.
17. Slowly open the needle valve at the end of the manifold and pressurize the pressure plate extractor. NOTE: Two hours after system is pressurized, check pressure gauge on manifold for any final adjustment.
18. Samples that are 1 cm high can be removed any time after 48 hours from initiation of the extraction. Some soils approach equilibrium in 18 to 20 hours; therefore, after 20 hours the outflow tube is tested periodically with blotter paper. If no moisture accumulates on the blotter paper after it has been held against the outflow tube for approximately 1 minute, equilibrium has been reached and the extraction can be stopped.
19. Clean aluminum pan previously used. Oven dry, cool in desiccator, and weigh to nearest 0.01 g. Record weight (A).
20. Put a piece of tubing over the outflow tube and clamp the tubing off with a pinch clamp. Shut the pressure source off, then drain the system of compressed gas slowly by using the coarse adjustment valve on the manifold.

21. After the system has been drained of compressed gas, disconnect the hose leading to the extractor. NOTE: This will insure that the extractor is no longer pressurized.
22. Remove the clamping bolts and extractor lid.
23. Remove the samples one at a time and place in weighed aluminum pans.
24. Quickly weigh the aluminum weighing pan and the sample. Record weight (B).
25. Place samples in the drying oven at 105°C. Allow samples to dry overnight.
26. Remove samples from drying oven and place in a desiccator filled with silica gel desiccant. Allow samples to cool.
27. Weigh samples and weighing pan. Record weight (C).
28. Discard sample.
29. Make sure that the pressure at which the extraction was carried out is recorded in the laboratory notebook.

3.4.11.6 Calculations--

1. Legend:

A = Weight of aluminum weighing pan.

B = Weight of moist sample and aluminum weighing pan.

C = Weight of aluminum weighing pan and oven-dry sample.

2. Percent moisture = $[(B - C)/(C - A)] \times 100$.

3.4.12 Moisture Retention (Pressure Membrane Method)

3.4.12.1 Principle--

See 3.4.11.1

3.4.12.2 Comments--

See 3.4.11.2

3.4.12.3 Chemicals--

1. Distilled water.
2. Compressed nitrogen gas.

3.4.12.4 Materials--

1. Pressure membrane extractor (Soil Moisture Equipment Company Catalog No. 1000 or equivalent).
2. Pressure control manifold, 0-225 psi range with Mercury Differential Regulator (Soil Moisture Equipment Company Catalog No. 700-1 or equivalent).
3. Torque wrench and socket (Soil Moisture Equipment Company Catalog No. 1090 or equivalent).
4. Two connecting hoses (Soil Moisture Equipment Company Catalog No. 1091 or equivalent).
5. Soil sample retaining rings (Soil Moisture Equipment Company Catalog No. 1093 or equivalent).
6. Cut cellulose membrane discs (Soil Moisture Equipment Company Catalog No. 1096 or equivalent).
7. Two nitrogen gas tank gauges - one for tank pressure (0-4,000 psi) and one for outflow pressure (0-500 psi).
8. Large spatula or small pancake turner.
9. Wax paper.
10. Plastic teaspoon.
11. Balance, can be read to 0.01 g.
12. Drying oven.
13. Aluminum pans, for weighing samples.
14. Laboratory notebook.
15. Desiccator, with silica gel desiccant.

3.4.12.5 Procedure--

NOTE: Read instrument's instruction manual before starting procedure.

1. Place a cut cellulose membrane disc in a pan of distilled water and allow disc to soak for at least 30 minutes.
2. Check pressure in the nitrogen tank.
3. Check all fittings by pressurizing system. NOTE: Take a toothbrush and a bar of soap and mix up a soapy foam. Brush foam over each fitting to see if there are any leaks in the system when pressurized.

4. Take the aluminum pans and place a soil sample retaining ring inside the pan. Draw a line around the top of the ring so that the approximate height of the ring is outlined on the inside of the aluminum pan. The desired volume of subsample that would be put into the aluminum pan would be slightly less than needed to fill the soil sample retaining ring.
5. Use a thin plastic teaspoon and lift the soil from the container and fill the aluminum pan to the volume mark. Do two replicates in the same manner. NOTE: Be sure that all the pans are marked with the soil sample number.
6. Remove the screen drain plate from the base of the pressure membrane extractor. Clean the screen to remove all soil grains that might puncture the membrane. Wet screen drain plate with distilled water and be sure that the drain hole is open.
7. Place the screen drain plate in its proper position. Remove the cellulose membrane disc from the water and place it on the screen drain plate. NOTE: The membrane should completely cover the screen drain plate. Arrange the membrane so there is a minimum of wrinkling. The membrane cannot be handled in this manner when it is dry because cracking will occur.
8. Place an "O" RING on the cellulose membrane. Put the standard cylinder (16 mm high) on top of the "O" RING. NOTE: Be sure that the "O" RING is in the lower groove of the standard cylinder and that the air-entry is pointing to the back of the pressure membrane extractor where the PM Hinge is mounted.
9. Latch the turn buttons (eccentric clamping screw assembly) into the grooves on the outside of the standard cylinder and tighten wing nuts. NOTE: The turn buttons hold everything in place when the samples are left to soak overnight.
10. Place soil sample retaining rings on the membrane. Draw a diagram of the arrangement of the rings on the membrane using the air-entry port as a guide. NOTE: A sample number is shown on the diagram for each soil sample retaining ring.
11. Attach a short piece of rubber tubing to the outflow tube on the bottom of the screen drain plate. Close off the outflow tube by attaching a pinch clamp to the rubber tubing.
12. Take the aluminum pan containing the approximate volume of soil sample needed and carefully dump it into the proper soil sampling retaining ring on the membrane. Take the spatula or the spoon and carefully flatten the sample until it is level with the top edge of the soil sample retaining ring. NOTE: Do not compact this material. Just carefully flatten by spreading.
13. Add an excess of water to the surface of the membrane and allow the samples to soak for 16 hours. NOTE: Be sure there is enough water on the membrane to allow samples to wet without removing water from the pores of the membrane.

14. Cover samples and membrane with wax paper to prevent evaporation.
15. After the samples have soaked overnight (16 hours) remove the excess water from the surface of the membrane by means of a pipette.
16. Place an "O" RING in the groove on the top of the standard cylinder.
17. Depress the PM hinge, put the lid in place and close the cell. NOTE: Be sure that the rubber diaphragm is between the lid and the top "O" RING before closing the cell.
18. Bolt the pressure membrane extractor shut using a torque wrench to tighten the bolts uniformly. A torque of 25 foot-pounds is usually adequate for air pressure up to 15.5 bars (225 psi).
19. The connecting hose coming from the mercury differential regulator is attached to the air-entry port on the side of the standard cylinder.
20. The other connecting hose is attached to the air-entry port on the top of the lid.
21. Remove pinch clamp from rubber tubing on outflow tube on bottom of pressure membrane extractor. Put 100 ml beaker under outflow tube and catch excess water.
22. Pressurize the system up to the first regulator on the manifold by turning the tank regulator on. Set the gas pressure in the line 2 bars (29 psi) higher than the desired cell pressure.
23. Open the bypass valve on the mercury differential regulator.
24. Admit gas into the cell slowly using the regulator on the manifold until the desired pressure is attained.
25. After about 2 hours, or when the outflow rate has decreased appreciably, close the bypass valve at the top of the "U" tube and open the exhaust valve on the air pressure test gauge side of the manifold. When gas is heard bubbling past the mercury in the "U" tube, close the exhaust valve and readjust the gas pressure using the first regulator on the manifold. NOTE: The membrane should be tested for leaks by submerging the rubber tubing connected to the outflow tube in a beaker of water. If there is rapid bubbling and/or a hissing of gas can be heard, then there is a leak in the membrane. The gas should be shut off and the procedure started again using a new membrane.
26. Check the pressure gauge reading after a few hours and readjust the gas pressure if needed.
27. Samples that are 1 cm high can be removed any time after 48 hours from initiation of the extraction. Some soils approach equilibrium in 18 to 20 hours; therefore, after 20 hours the outflow tube is tested periodically with blotter paper. If no moisture accumulates on the blotter paper after

it has been held against the outflow tube for approximately 1 minute, equilibrium has been reached and the extraction can be stopped.

28. Clean aluminum pan previously used. Oven dry, cool in desiccator, and weigh to nearest 0.01 g. Record weight (A).

29. Attach piece of tubing to the outflow tube and clamp with a pinch clamp. Open the bypass valve and shut the pressure source off. Drain the system of compressed gas slowly using the first regulator on the manifold.

30. After the system has been drained of compressed gas, disconnect the hoses leading to the top and side of the extractor. NOTE: This will insure that the extractor is no longer pressurized.

31. Remove the clamping bolts, extractor lid, and rubber diaphragm.

32. Remove the samples one at a time and place in weighed aluminum pans.

33. Quickly weigh the aluminum weighing pan and the sample. Record weight (B).

34. Place samples in the drying oven at 105°C. Allow samples to dry overnight.

35. Remove samples from drying oven and place in a desiccator filled with silica gel desiccant. Allow samples to cool.

36. Weigh samples and weighing pan. Record weight (C).

37. Discard sample.

38. Make sure that the pressure at which the extraction was carried out is recorded in your laboratory notebook.

3.4.12.6 Calculations--

1. Legend:

A = Weight of aluminum weighing pan.

B = Weight of moist sample and aluminum weighing pan.

C = Weight of aluminum weighing pan and oven-dry sample.

2. Percent moisture = $[(B - C)/(C - A)] \times 100$.

3.5 MICROBIOLOGICAL METHODS

3.5.1 Summary

Early soil microbiologists developed and published original versions of the

procedures described in this publication. These procedures were used in soil investigations by their contemporaries and later soil microbiologists the world over. The data obtained have played an important role in our continual quest to unravel the mysteries of the soil.

These procedures, on the whole, are simple, easy to use, and require a minimum of equipment. Because they were developed years ago, none of the so-called "modern sophisticated" laboratory apparatus is involved.

These procedures were used in minesoil or strip mine spoil investigations over a 15-year period. Some were chosen because no better method was available, or because of a lack of equipment. These generally simple methods used in studying minesoils have again played an important role as they did earlier on conventional soils.

Though mainly simple procedures, careful planning, careful work, and a conscientious worker are basic requirements. A technician can be trained to perform the routine laboratory work described. Complex biological interpretations of these laboratory measurements in relation to field problems should include a person knowledgeable in soil microbiology.

3.5.2 Buried Slide Technique

3.5.2.1 Principle--

This technique was developed independently by both Cholodony (1930) and Rossi et al. (1936). It is a simple procedure and provides useful information concerning the microbes, particularly to their spatial relationships to each other, plant roots, debris, and soil particles. If the organisms remain intact, observations may be made of colony characteristics, feeding of organisms on materials, and response of organisms to environmental factors, such as water films (Frederick, 1965).

3.5.2.2 Comments--

The method is not quantitative but can be used to show microbial differences among various treatments of a native soil or minesoil. Burying two or more slides in each minesoil and/or treatment and removing one from each at weekly intervals will yield information on relative abundance and associations of the microbes.

Often the actual microorganism is no longer attached to the slide, but after staining, the size, shape, and location of the missing entity is revealed by stain deposition. This often reveals locations where organic debris and sometimes soil aggregates have been in contact with the slide.

Some determination of individual organisms can be made by placing the slide flat on the surface of an agar medium plate. The plate is incubated 2-3 hours, the slide aseptically removed, then incubation continued for at least 24 hours. This procedure will require duplicate slides, as a slide used in this manner is no longer useful for staining and microscopic observation and most organisms on a stained slide are dead.

One familiar with bacteria, fungi, actinomyces and diatoms, for example, will find the interpretation of the microscopic examination of a stained contact slide much easier than one without such familiarity. The technique is more useful when used in conjunction with other microbial methods for soil microbial studies than when used alone.

3.5.2.3 Chemicals--

NOTE: All chemicals must be ACS Certified pure grade.

1. Phenol (C_6H_5OH), 5% aqueous: Dissolve 5.0 g of phenol in distilled water and dilute to a volume of 100 ml.
2. Phenolic rose bengal stain: Weigh 1.0 g of rose bengal. Add 100.0 ml of 5% aqueous phenol. Add 0.05 g certified grade calcium chloride ($CaCl_2$).

3.5.2.4 Materials--

1. Straw, autoclave-sterilized, ground to 40 mesh, or any other material under study.
2. Alfalfa, autoclave-sterilized, ground to 40 mesh, or any other material under study.
3. Samples from the top 13 cm (5 in) of a soil (or any constant depth under study).
4. Sieve, 2 mm openings (10 mesh).
5. Sterile straight-sided water tumblers, or similar glass containers such as beakers.
6. 7.62 X 2.54 cm (3 X 1 in) sterile glass microscope slides.
7. Microscope with 10X or 15X eyepiece and 97X oil immersion objective.
8. Autoclave, steam, capable of holding 15 psi and 121°C.
9. Hilgard soil cups. NOTE: A sieve with 1 mm openings can be used.
10. Spatula.
11. Filter paper
12. Humid chamber. NOTE: A container large enough for the pan that will retain moisture can be used.
13. Balance, can be read to 0.01 g.
14. Drying oven.
15. Glass rods.

16. Pan. NOTE: Any pan that will hold the cups can be used.

3.5.2.5 Procedure (modified from Allen, 1949; Wilson and Hedrick, 1957a; Frederick, 1965)--

1. Place a circle of filter paper, cut to fit exactly, on the brass perforated bottom of a Hilgard cup, and moisten the filter paper.
2. Weigh the complete unit. Record weight (A).
3. Fill the cup with air-dried minesoil.
4. Compact the minesoil by dropping the cup 10 times through a distance of approximately 3 cm (1 in).
5. Level the soil surface with a spatula.
6. Weigh cup and minesoil. Record weight (B).
7. Lay two glass rods on the bottom of the pan.
8. Place the cup of minesoil on glass rods.
9. Add water to the pan to reach about half cup height.
10. Allow the soil to become saturated and remain in pan and water for 24 hours.
11. Remove cup, carefully wipe outside cup surfaces and underneath bottom to remove adhering water.
12. Weigh the cup with the soil in a saturated condition. Record weight (C).
13. Place cup in drying oven for 24 hours at 105°C.
14. Cool in desiccator and weigh immediately. Record weight (D). Remove soil, brush cup and filter paper free of soil and weigh immediately. Record weight (E).
15. Calculate soil moisture of the air-dried soil as well as the water holding capacity (see 3.5.2.6).
16. Prepare glass microscope slides by cleaning them thoroughly. If desired, flame slides just before use to insure sterility. NOTE: It is desirable to use new slides.
17. Pass samples through 2 mm hardware cloth sieve to remove the rocks and pieces of coal.
18. To 3 tumblers containing 150 g (oven-dried at 105°C for 16 hours) or some constant weight of soil, add the following: (First tumbler) no treatment -

- control; (Second tumbler) soil thoroughly mixed with 0.5% autoclave-sterilized ground straw; (Third tumbler) soil thoroughly mixed with 0.5% autoclave-sterilized ground alfalfa.
19. Bring the soil moisture to 50% of the sample's water-holding capacity in the glass containers. Add slightly more so the treatment material will be moistened without diminishing the 50% water-holding capacity.
 20. Insert carefully the prepared glass slides (2 per tumbler) vertically into the soil leaving about 13 mm (0.5 in) of each slide above the surface.
 21. Press soil gently against slide.
 22. Weigh tumbler, sample, and slides.
 23. Cover tumblers with paper caps to prevent excessive evaporation, but not to exclude aeration.
 24. Incubate soil tumblers at room temperature for one week.
 25. Add water during incubation (about twice a week) to replace that lost by evaporation. NOTE: Add water until weight of sample and tumbler is same as weight found in step 22.
 26. After incubation remove soil from only one side of one slide using a spatula. NOTE: Gently break the slide away from the soil without sliding the slide.
 27. Remove large clumps of sand and soil from the slide surface to be observed by means of a dissecting needle or some small sharp pointed instrument.
 28. Air dry slide.
 29. With the aid of a small gentle stream of water, remove excess soil from the undisturbed side until only a thin film remains.
 30. Clean disturbed side with a damp cloth. NOTE: This is the side that will not be stained.
 31. Air dry slide.
 32. Fix slide by passing it over a bunsen burner at low flame four or five times. Do not cook. This "fixes" the material on the slide reducing the likelihood of loss during the staining procedure.
 33. Place the fixed slide over a steam bath (or beaker of boiling water). Flood slide for 6 to 10 minutes with phenolic rose bengal. NOTE: Avoid drying slide by adding stain as needed.
 34. Remove excess stain by washing the slide gently with water until no more stain is removed.

35. Air dry slide.

36. Examine the slide microscopically using a 10X or 15X eyepiece with a 97X oil immersion objective. CAUTION: Avoid scratching oil immersion objective by making sure that all soil particles have been removed from the slide.

37. Continue incubation of second slide for another week. Examine slide in identical manner.

38. Examine at least 5 fields per slide.

39. Make drawings of representative fields. Arrange the drawings in two rows so that a comparison of the slides per treatment can be readily observed.

3.5.2.6 Calculations--

1. Legend:

A = Weight of cup and moist paper.

B = Weight of cup, moist paper, and air-dried soil.

C = Weight of cup, moist paper, and saturated soil.

D = Oven-dry weight of cup, paper, and soil.

E = Oven-dry weight of cup and paper.

S = Weight air-dry soil.

T = Weight saturated soil.

U = Weight oven-dry soil.

V = Weight water in air-dry soil.

W = Weight water in saturated soil (water loss).

X = Percent moisture in air-dry soil.

Y = Percent water-holding capacity of soil.

Z = Grams of water per 100 g oven-dry soil needed to make 50% water-holding capacity.

2. $S = B - A.$

3. $T = C - A.$

4. $U = D - E.$

5. $V = S - U.$
6. $W = T - U.$
7. $X = (V/U) \times 100.$
8. $Y = (W/U) \times 100.$
9. $Z = Y/2.$

3.5.3 Total Microbial Count (Agar-Plate Method)

3.5.3.1 Principle--

When a soil dilution is dispersed in appropriate agar medium and incubated under favorable conditions, discrete and macroscopically visible colonies of microorganisms will develop. Calculations using the number of colonies developing on the agar will give the "total count." The total count obtained, however, is only a fraction of the total number of microbes present. If the conditions are uniform throughout, relative if not absolute, microbial populations can be counted successfully (Clark, 1965).

3.5.3.2 Comments--

The agar-plate method is highly empirical. Care must be taken of details in the technique if individual workers are to obtain comparable results.

Soil samples should be processed the same day they are collected in their natural, undried condition. Drying of the soil reduces the total count, whereas storing moist samples at room temperature more than one day increases the total count.

Primary soil sample dilutions should be withdrawn within ten minutes after shaking. Rapid multiplication of organisms may result if counting is delayed. All samples should be withdrawn from the middle of the suspension immediately after vigorous hand shaking, since soil particle settling tends to move microorganisms to the bottom of the suspension. Care should be taken not to count soil particles that have settled from the solution as colonies.

The melted medium must be cooled to a temperature of 42° to 45°C before mixing with the soil, as some of the organisms are killed at higher temperatures. If the flask containing the melted medium is too hot when touched to the cheek, it's too hot for microorganisms.

Although the procedure uses soil-extract agar, egg-albumen, or yeast-extract agar can be used (Clark, 1965).

3.5.3.3 Chemicals--

NOTE: All chemicals must be ACS Certified pure grade.

1. Soil-extract (Lockhead, 1940): Mix the following: 1000.0 g of fertile soil and 1500.0 ml of distilled water. Autoclave mixture for 30 minutes at 15 psi. After partial cooling and settling, filter suspension using a Buchner funnel, filter-aid, and medium-grade filter paper. If extract cannot be filtered easily in this way, pour turbid soil:water suspension into a 2 liter graduated cylinder and let stand in a refrigerator at 4°C overnight. Settling and clearing will usually result.
2. Soil-extract agar (Lockhead, 1940): Mix 20.0 g agar, 0.5 g dipotassium phosphate (K_2HPO_4), and 0.1 g of dextrose with 1000.0 ml of soil-extract. Adjust pH to between 6.8 and 7.0 with 3 N HCl or 3 N NaOH. Sterilize medium by autoclaving at 15 psi for 15 minutes.
3. Egg-albumen agar (Waksman and Fred, 1922): Dissolve 0.25 g of egg albumen in 10 ml of 0.1 N NaOH. Add 15.0 g agar, 1.0 g dextrose, 0.5 g dipotassium phosphate (K_2HPO_4), 0.2 magnesium sulfate ($MgSO_4 \cdot 7H_2O$), a trace amount of ferric sulfate ($Fe_2(SO_4)_3$), and 1000.0 ml of distilled water. After a preliminary heating of the medium, adjust pH to 6.8 with 3 N HCl or 3 N NaOH. Sterilize medium by autoclaving for 30 minutes at 15 psi.
4. Yeast-extract agar (Stevenson and Rovatt, 1953): Mix 15.0 g agar, 1.0 g dextrose, 1.0 g sodium chloride (NaCl), 0.01 g ferric chloride ($FeCl_3$), 1.0 g yeast extract, and 1000.0 ml distilled water. Adjust pH to 6.8 with 3 N HCl or 3 N NaOH. Sterilize medium by autoclaving for 30 minutes at 15 psi.

3.5.3.4 Materials--

1. Bottle, French square, 237 ml (8 oz) with caps. NOTE: 8 required per sample.
2. Three dozen spherical glass beads of 2 mm (0.079 in) diameter.
3. Autoclave, steam, capable of holding 15 psi and 121°C.
4. Sieve, 2 mm (10 mesh) openings.
5. Shaker, horizontal reciprocating type, 6.3 cm (2.5 in) stroke, 120 strokes per minute.
6. 10 ml pipette, sterile
7. 1 ml pipette, sterile.
8. Petri dishes, sterile. NOTE: 15 required per sample.
9. Balance, can be read to 0.01 g.
10. Humidified incubator. NOTE: A glass container with moistened paper towels in the bottom can be used. Place container in an oven.

11. Quebec colony counter or a wide-field, low-power microscope.
12. Sample bags.

3.5.3.5 Procedure (Adapted from Clark, 1965)--

1. From a thoroughly mixed bulk sample, transfer about 900 g of soil to a polyethylene bag for transport to the laboratory. NOTE: Containers must be clean and at least sanitized so as not to harbor other microorganisms not in the bulk sample. Avoid exposing the sample to heat or drying. If the sample is not used that day, it may be stored in a closed container (pinholed for aeration) at 4°C for 1 or 2 weeks without serious detriment.
2. Pass entire sample through a 2 mm sieve.
3. Mix sample thoroughly.
4. Withdraw a 10 g subsample and weigh. Record weight (A). Oven dry subsample in weighing container, cool in desiccator, and reweigh. Record weight (B). Determine soil moisture.
5. Put approximately 3 dozen spherical glass beads and 95 ml of water in a 237 ml screw cap bottle. NOTE: The purpose of the beads is to facilitate disintegration of soil aggregates.
6. To seven 237 ml screw cap bottles add 90 ml of water and no beads. NOTE: More than 7 bottles will be required for dilution series if sample is high in microorganisms. Less than 7 bottles required if low in microorganisms.
7. Cap all bottles.
8. Sterilize bottles by autoclaving at 15 pounds pressure for 15 minutes and cool to room temperature prior to use. Make sure caps are loose during autoclaving.
9. Transfer 10 g of moist soil into the bottle containing 95 ml of water and glass beads.
10. Tightly cap bottle.
11. Shake bottle containing sample for 3 minutes in a horizontal position in a reciprocating shaker or for an equal time by hand.
12. No longer than 10 minutes after removing the bottle from the shaker, shake bottle vigorously by hand for a few seconds and immediately transfer 10 ml from the center of the suspension to a bottle containing 90 ml water and no beads, using a sterile 10 ml pipette. NOTE: This establishes a 10^{-2} dilution.
13. Continue this dilution process by similarly transferring 10 ml quantities to successive bottles of 90 ml and no beads to provide a dilution

series through 10^{-7} . NOTE: Experience with different soils will provide a basis for estimating whether the highest dilution will need to be no more than 10^{-6} or 10^{-7} , or whether it will need to be as high as 10^{-8} or 10^{-9} .

14. From the highest dilution prepared, transfer a 1 ml portion of the freshly agitated suspension to each of 5 sterile petri dishes by means of a sterile, 1 ml pipette. Shake a few times before withdrawing the 1 ml portion.

15. Make similar transfers from the two next lower dilutions into other dishes. Shake as above.

16. Into each petri dish, pour about 15 ml of soil-extract agar, which previously has been steamed sufficiently to insure complete melting, and then cooled to 42°C . NOTE: Agar media that are starting to solidify, at about 40°C , are not suitable for pouring into plates.

17. Immediately after adding agar, cover dish and carefully rotate by hand to swirl the agar and to insure its thorough mixing with the inoculant. CAUTION: Do not splash medium-sample mixture on petri dish cover. If this should occur, discard and replace.

18. Permit poured plates to stand upright until the agar has solidified.

19. Invert plates in a humidified incubator at 28°C . NOTE: Some workers prefer 25°C ; others 30°C . Do not use 37°C as is commonly the practice in medical bacteriology laboratories.

20. Leave the plates undisturbed for 4 days for fast growing bacteria. NOTE: Incubation time would depend upon type of bacteria being determined. If slow growing bacteria are being determined, 7 days are required and preferably 10 to 14 days. Actinomycetes require 10 days. Fungi can cover a medium if left more than 5 days. However, once a time period is established all samples must be counted at the established period of time.

21. Remove plates from the incubator.

22. Inspect all plates prepared from a single sample to see whether a proper dilution range has been plated and whether a proper dilution effect is apparent. NOTE: The proper dilution effect means that a plate prepared from a given dilution should have only approximately one-tenth as many colonies as the plate prepared from the next lower dilution. If there are numerous colonies on the plates or a dilution effect is not apparent, contamination has occurred. Discard all plates and rerun.

23. If incubation plates appear satisfactory, select the plates from the dilution at which 30 to 300 colonies have developed per plate. NOTE: (1) If the plate from the highest dilution shows greater than 300 colonies, the dilution has been too low. (2) If the lowest dilution shows less than 30 colonies, the dilution has been too high. In either event, discard all the plates. (3) If one or two plates within the 30 to 300 colony range have one or more large bacterial or fungal colonies (greater than 2 cm in diameter),

discard such plates without counting.

24. With the aid of a Quebec colony counter or a wide-field, low-power microscope, count the total number of colonies on each of the three or more remaining suitable plates.

3.5.3.6 Calculations--

1. Legend:

A = Weight moist soil.

B = Weight oven-dry soil.

2. Water loss = A - B.

3. Percent soil moisture = (Water loss/B) X 100.

4. Total viable count per gram of the initial moist soil sample = (average number of colonies per plate for a given dilution) X (dilution factor).

5. Grams of dry matter per gram of moist soil = B/A.

6. Total count per gram of dry soil = (count per gram of moist soil)/(grams of dry matter per gram of moist soil).

3.5.4 MPN of Aerobic Cellulose-Decomposing Bacteria

3.5.4.1 Principle--

The most-probable-number (MPN) method permits estimation of aerobic cellulose-decomposing bacteria without actually counting single cells or colonies. The method is based on the presence or absence of cellulose-decomposing bacteria on strips of paper. A strip of paper is needed for each dilution of a minesoil. A positive (or presence) reading indicates that at least one (it could be several) cellulose-decomposing bacterium was present (Alexander, 1965).

3.5.4.2 Comments--

Cellulose-decomposing bacteria must meet one of the following conditions: (1) Bacteria must bring about a change in the medium that is easily recognizable or (2) after the bacteria have multiplied, they must be recognizable on the strip of paper on which they are growing.

Single cellulose-decomposing bacterial cells must be capable of growth in the medium or the method is not reliable. That is, no growth in the medium without cellulose source, but growth with cellulose source added.

Some quantitative changes in the original number of bacteria can occur over a period of time, even with refrigeration. The samples should be passed

through a 2.0 mm sieve to remove rocks and coal. Samples must be prepared the same day as collected (Alexander, 1965).

3.5.4.3 Chemicals--

NOTE: All chemicals must be ACS Certified pure grade.

1. Ammonium sulfate-cellulose solution (Fred and Waksman, 1928): Mix 1.0 g ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 1.0 g dipotassium phosphate (K_2HPO_4), 0.5 g magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 2.0 g calcium carbonate (CaCO_3), trace amount of sodium chloride (NaCl), and 1000.0 ml of distilled water. NOTE: The CaCO_3 can be left out and a trace of FeSO_4 introduced.

3.5.4.4 Materials--

1. Samples sieved through 2 mm sieve from depth of 0-13 cm (0-5 in) or any other depth range of interest.
2. Medium-sized test tubes, 150 X 18 mm (6 X 0.7 in).
3. Strips of filter paper (see 3.5.4.5, No. 1).
4. Pipette, 1 ml, sterilized.
5. Microscope slides, glass, 7.62 X 2.54 cm (3 X 1 in), sterile.
6. Microscope with 10X or 15X eyepiece and 97X oil immersion objective.
7. Autoclave, steam, capable of holding 15 psi and 121°C.
8. Rubber stoppers (to fit test tubes).

3.5.4.5 Procedure (Fred and Waksman, 1928)--

1. Prepare a series of medium-sized test tubes containing 5 ml of the medium and a strip of filter paper. Part of the paper should protrude above the surface of the medium.
2. Plug test tubes with rubber stoppers. Sterilize by autoclaving for 15 minutes at 15 psi. NOTE: Make certain stoppers are loose or they will blow out. Fold a bit of paper and insert between tube and stopper before autoclaving and remove after autoclaving.
3. Prepare a 10-fold soil:water dilution series, stopping at 10^{-9} . (See 3.5.3.5 Steps 5 through 13).
4. Withdraw by sterile 1 ml pipette, 5 aliquots from the 10^{-9} soil suspension. Discharge 1 ml into each of 5 test tubes containing the medium.
5. Repeat step 4 for the next four lower dilutions, 10^{-8} through 10^{-5} . NOTE: Use lower dilutions if the number of organisms is expected to be small.

6. Incubate tubes at 25°C or 30°C.
7. Examine tubes daily.
8. Presence of cellulose-decomposing bacteria will be shown by the decomposition of the paper just at the surface of the liquid.
9. After 4 weeks storage, make final observations.
10. Record the number of tubes at each dilution in which growth has occurred as positive tubes.
11. Calculate the most-probable-number (MPN) of bacteria.

3.5.4.6 Calculations (Alexander, 1965)--

1. Select as P(1) the number of positive tubes of the least concentrated dilution in which all tubes are positive or in which the greatest number of tubes are positive.
2. P(2) and P(3) are the next two higher dilutions.
3. Using Table 13, find the row of numbers in which P(1) and P(2) correspond to the experimentally observed values.
4. Follow the row of numbers across the table to the column headed by the observed value of P(3). NOTE: This number is the MPN of organisms in the quantity of the original sample represented in the inoculum added in the second dilution, P(2) dilution factor.
5. Multiply the number found in step 4 by the dilution factor of P(2) to obtain the MPN for the original sample.

Example A -

Using a 10-fold dilution and 5 tubes per dilution, the following numbers of positive tubes were observed: 5 at 10^{-5} ; 5 at 10^{-6} ; 4 at 10^{-7} ; 2 at 10^{-8} ; 1 at 10^{-9} . In this series, P(1) = 5, P(2) = 4, and P(3) = 2. Table 13 gives a value of 2.2 for a dilution series of 10^{-7} , the dilution of P(2). Multiplying 2.2 times 10^7 gives a MPN for the original sample of 22 million bacteria, $2.2 \times 10^7 = 22,000,000$.

6. The 95% confidence limits for MPN values can be determined from Table 14. Upper confidence limit at 95% level = (MPN value) X (factor from Table 14).

Lower confidence limit at 95% level = (MPN value)/(factor from Table 14).

TABLE 13. MOST-PROBABLE-NUMBERS FOR USE WITH 10-FOLD
DILUTIONS AND 5 TUBES PER DILUTION
(FROM COCHRAN, 1950)

P ₁	P ₂	Most probable number for indicated values of P ₃					
		0	1	2	3	4	5
0	0	-	0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.020	0.040	0.060	0.080	0.10	0.12
1	1	0.040	0.061	0.081	0.10	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.15	0.17
1	3	0.083	0.10	0.13	0.15	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.045	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.20	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.3
5	5	2.4	3.5	5.4	9.2	16	-

Example B -

The factor for example A using five tubes with a dilution of 10-fold and a MPN equaling 2.2 obtained from Table 14 is 3.30.

Upper confidence limit at 95% level = (2.2) X (3.30).

Upper confidence limit at 95% level = 7.26.

Lower confidence limit at 95% level = (2.2)/(3.3).

Lower confidence limit at 95% level = 0.67.

NOTE: New tables must be used if these particular number of tubes and dilutions are not used. MPN has a low order of precision. Large numbers of tubes must be inoculated for each dilution for precise estimates. Increasing the number of tubes inoculated at each dilution or narrowing the dilution ratio, reduces the confidence limit intervals at the 95% level.

3.5.5 Carbon Dioxide Production

3.5.5.1 Principle--

This method determines the amount of carbon dioxide produced, under laboratory conditions, by microbial decomposition of finely ground (40 mesh) straw (or any other additive). The quantity of carbon dioxide produced is an index of intensity for microbial activity. Minesoils, like other soils, have a microbial population. Vegetated minesoils are expected to contain larger numbers and a wider variety of microorganisms than nonvegetated minesoils (Hedrick and Wilson, 1956; Wilson and Hedrick, 1957b).

3.5.5.2 Comments--

The simplicity of this method is the ready accessibility of the materials. Care must be taken in preparation and standardization of the barium hydroxide, $Ba(OH)_2$, since exact concentration (Normality) is important (Hedrick and Wilson, 1956; Wilson and Hedrick, 1957b).

3.5.5.3 Chemicals--

NOTE: All chemicals must be ACS Certified pure grade.

1. Calcium hydroxide ($Ca(OH)_2$), 0.04 N, saturated solution: Dissolve 1.5 g (use some excess) of $Ca(OH)_2$ in carbon dioxide-free water (See 3.2.3.3 No. 1) and dilute to 1 liter. Filter off $CaCO_3$ and protect from CO_2 of the air with soda lime or ascarite in a guard tube.
2. Sodium nitrate ($NaNO_3$).
3. Calcium phosphate ($CaHPO_4$).

TABLE 14. FACTORS FOR CALCULATING THE CONFIDENCE LIMITS FOR THE MOST-PROBABLE-NUMBER COUNT (FROM COCHRAN, 1950)

No. of tubes per dilution (n)	Factor for 95% confidence limits with indicated dilution ratios			
	2	4	5	10
1	4.00	7.14	8.32	14.45
2	2.67	4.00	4.47	6.61
3	2.23	3.10	3.39	4.68
4	2.00	2.68	2.88	3.80
5	1.86	2.41	2.58	3.30
6	1.76	2.23	2.38	2.98
7	1.69	2.10	2.23	2.74
8	1.64	2.00	2.12	2.57
9	1.58	1.92	2.02	2.43
10	1.55	1.86	1.95	2.32

4. Monopotassium phosphate (KH_2PO_4).

5. Barium hydroxide ($\text{Ba}(\text{OH})_2$), 0.1 N: Dissolve 15.75 g of $\text{Ba}(\text{OH})_2$ in carbon dioxide-free water (See 3.2.3.3 No. 1) and dilute to 1 liter. Filter off BaCO_3 and protect from CO_2 of the air with soda lime or ascarite in a guard tube.

6. Hydrochloric acid (HCl), 0.1 N.

7. Phenolphthalein indicator.

3.5.5.4 Materials--

1. Sieve, 2 mm openings (10 mesh).

2. pH meter (Corning model 12 or equivalent) with combination electrode.

3. Refrigeration unit.

4. Straw, ground, 40 mesh, sterilized, or other biodegradable material.
5. Griffin beaker, 50 ml.
6. Griffin beaker, 600 ml.
7. Mason jars, 473.2 ml (1 pt).
8. Plastic cylinder, 3.81 cm (1.5 in) length.
9. Rubber stoppers.
10. Fiber board (pokerchip).
11. Metal lids, 2 piece to fit Mason jar.
12. Flasks, Erlenmeyer, 250 ml.
13. Balance, can be read to 0.1 g.

3.5.5.5 Procedure (Adapted from Hedrick and Wilson, 1956; Wilson and Hedrick, 1957b)--

1. Collect bulk samples representing the minesoil and depth in question, usually 0-8 cm (0-3 in).
2. Save material that will crush easily with fingers and pass through a 2 mm sieve.
3. Determine water-holding capacity (See 3.5.2.5, Steps 3 through 15).
4. Place 10.0 g of minesoil sample into a series of 250 ml Erlenmeyer flasks.
5. Add different amounts of 0.04 N Ca(OH)₂ to the flasks. NOTE: 5 ml of 0.04 N Ca(OH)₂ is equivalent of 1 ton of pulverized limestone per acre.
6. Dilute to 100 ml with distilled water.
7. Add 3 drops of chloroform. NOTE: The chloroform is added to prevent microbial activity.
8. Stopper flasks.
9. Thoroughly shake flasks twice a day.
10. Repeat step 9 for 4 days.
11. Determine pH values of the suspension.
12. Note the amount of 0.04 N Ca(OH)₂ required for 10.0 g of minesoil to have a pH of about 7.0.

13. Place 100 g of minesoil into each of two 600 ml beakers.
NOTE: One beaker will have the untreated sample and the other beaker will have the treated sample.
14. Adjust minesoil in both beakers to a pH of about 7.0 with $\text{Ca}(\text{OH})_2$ using the data acquired from step 12.
15. To one beaker, add 1.0 g of ground straw, or other additive, and thoroughly mix.
16. To the same beaker add nitrogen (as NaNO_3), phosphorus (as CaHPO_4), and potassium (as KH_2PO_4) at an equivalent rate of 1000 lbs per acre of 4-12-4 fertilizer and thoroughly mix.
17. Close both ends of two 3.81 cm (1.5 in) plastic cylinders with rubber stoppers.
18. On one end cement a small disc of fiber board (pokerchip) to each cylinder.
19. Place a cylinder inside each of the Mason jars (incubation chambers) with the pokerchip end up.
20. While holding the cylinder firmly against the bottom, transfer the 100 g sample from the 600 ml beakers.
21. Bring minesoil to 50 percent water-holding capacity by the addition of distilled and/or deionized water.
22. Shake the jar gently to level the material and then gently tap it on a table to settle the material around the cylinder.
23. Place a 50 ml beaker containing 20 ml of 0.1 N $\text{Ba}(\text{OH})_2$ and 7 drops of phenolphthalein on top of the pokerchip. NOTE: $\text{Ba}(\text{OH})_2$ is used to absorb the CO_2 and the phenolphthalein is used as an indicator to show if the $\text{Ba}(\text{OH})_2$ was converted to BaCO_3 before the one day incubation period was completed. If this occurs, quickly open the incubation chamber and replace with a new beaker of $\text{Ba}(\text{OH})_2$ and note for the calculations.
24. Close the jars with two-piece metal lids.
25. After 24 hours, remove the 50 ml beakers from the Mason jars.
26. Titrate the $\text{Ba}(\text{OH})_2$ with 0.1 N HCl until it clears.
27. Make a blank for each titration. NOTE: This is necessary to determine the amount of CO_2 in the stock $\text{Ba}(\text{OH})_2$ solution.
28. After each titration, thoroughly aerate the incubation chamber by rapidly drawing carbon dioxide-free air into the jar for about 3 minutes.
29. Repeat steps 23 through 28 for 10 days.

3.5.5.6 Calculations--

1. $\text{ml BaCO}_3 = (\text{ml Ba(OH)}_2 \text{ used}) - (\text{ml of HCl}/2)$.
2. $\text{mg CO}_2 = (\text{ml BaCO}_3) \times (\underline{N} \text{ of Ba(OH)}_2 \times 44)$.
3. Total mg CO₂/10 days = Sum total of mg CO₂ for each of the ten days.

3.5.6 MPN of Sulfur-Oxidizing Bacteria

3.5.6.1 Principle--

When sulfur is added to a minesoil, the sulfur at first oxidizes slowly. As the soil becomes acid, sulfur begins to oxidize rapidly.

Inoculation is made in a medium free of any organic compounds and carbonates. Sulfur is added as the only energy source. The bacteria convert sulfur into sulfuric acid thus lowering the pH.

3.5.6.2 Comments--

Since many organisms will not live in acid conditions, the medium has a reaction at about pH 4.0. The sulfur-oxidizing bacteria can develop at this low pH. The high acidity and high dilutions of the culture results in a pure culture.

Sterilization of the medium must be by flowing steam. The sterilization must be on 3 CONSECUTIVE days at 30 minutes each. This process is called intermittent sterilization. The first day kills vegetated cells; the second day kills spores that have germinated; and the third day kills any remaining vegetated cells. NOTE: Passing steam around the medium is the best procedure; however, an autoclave can be used if: (1) there is NO PRESSURE BUILDUP, and (2) temperature REMAINS at about 100°C.

The medium becomes turbid as bacterial growth develops and sulfur crystals can be seen in the medium. The medium also allows for pH determination.

3.5.6.3 Chemicals--

NOTE: All chemicals must be ACS Certified pure grade.

1. Sulfur-phosphate medium (Fred and Waksman, 1928): Mix 0.2 g ammonium sulfate ((NH₄)₂SO₄), 3.0 g monopotassium phosphate (KH₂PO₄), 0.25 g magnesium sulfate (MgSO₄·7H₂O), a trace amount of ferrous sulfate (FeSO₄·7H₂O), 10.0 g of powdered sulfur, and 1000.0 ml of distilled water. Weigh 1.0 g of sulfur into individual 250 ml Erlenmeyer flasks. Add 100 ml of the liquid medium to each flask. Reaction of the medium is about pH 4.0. Sterilize flasks in flowing steam for 30 minutes on 3 CONSECUTIVE days (See 3.5.6.2).

3.5.5.4 Materials--

1. Flasks, Erlenmeyer, 250 ml.

2. pH meter (Corning model 12 or equivalent) with combination electrode.
3. Microscope with 10X or 15X eyepiece.
4. Sieve, 2 mm (10 mesh) openings.

3.6.6.5 Procedure (Fred and Waksman, 1928)--

1. Sieve sample with 2 mm sieve.
2. Prepare 20 flasks with 100 ml of the medium.
3. Prepare a 10-fold soil:water dilution series, stopping at 10^{-9} (See 3.5.3.5 steps 5 through 13).
4. Withdraw by sterile 1 ml pipet, 5 aliquots from the 10^{-9} soil suspension. Discharge into flasks containing medium.
5. Repeat step 4 for the next four lower dilutions, 10^{-8} through 10^{-5} .
NOTE: Use lower dilutions if the number of organisms is expected to be small.
6. Incubate flasks at 25° to 30°C .
7. After 7, 14, and 30 days, determine pH of flasks and note if medium has become turbid. NOTE: It is a good practice to check the turbid medium microscopically to determine if the turbidity is due to the presence of sulfur-oxidizing bacteria.
8. Record the number of flasks at each dilution in which turbidity has been observed. Record these tubes as positive tubes.
9. Determine the most-probable-number (MPN) of bacteria (See 3.5.4.6).