Investigations in Biology and Chemistry I – Lab work Point Value: 35

Name: ___________________________________ Date Due: _____________

Points for Completion: Name, Date, Title (3), Purpose (3), Safety Precautions (3), Data/Results (24) (total possible pts for completion: 35)

Lab #26: Profiling a Soil Samples – How Does Agriculturalization Affect Nutrient Levels in Soil?

Pre-Lab Activities

1. Survey the headings and subheadings to familiarize yourself with this lab.

2. Read the **Introduction** from start to finish. Now go back and re-read it, this time highlighting main ideas and annotating the text.

3. Re-read the **Introduction** a third time**,** completing the given tasks along the way. In addition, pick 3 new terms, underline them, and define them in your own words in the margins on this sheet. These terms do not need to be "science" words.

4. Read through the **Procedure** and circle any new or unfamiliar materials.

5. Access the MSDS for appropriate circled materials at the LaMotte Homepage. This link will take you to the MSDS page: http://www.lamotte.com/pages/common/msds/msdslook.cfm : type in the "code number" provided for each item in **Materials**; then click on the MSDS file that appears. Record any important information, such as health hazards or emergency procedures in the space below.

6. Flip to the ***Nutrients*** section of the **Introduction**. Take a few moments and as a group build the molecules and ions listed in the discussion of soil nitrogen.

7. Draw the Lewis Structures for ammonia and nitrogen gas.

8. Set up your lab notebook for Lab #26. Be sure to transfer any important safety information and/or safety symbols into the "Safety Precautions" section in your lab notebook.

9. Interactive Lecture: How can we develop a chemical profile for an ecosystem?

10. Familiarize yourself with the new lab techniques during your Lab Technique Discussion. Record any notes to yourself.

11. Read the entire lab sheet. Revisit any tasks in the **Introduction** that you may not have completed. In addition, circle concepts and and/or activities that relate directly to Lab Rubric Items. This will help you "Achieve" or "Exceed Standard" on your lab write-up.

12. As a group, write a hypothesis about the nutrient levels you expect to find in each of the soil samples.

13. As a group, decide which data you will be gathering during this lab, and construct appropriate data tables in your lab notebooks.

14. Determine which partners will be responsible for testing three samples of the six soil samples provided and which partners will test the other three samples.

15. In lab teams complete Procedure Steps #1-4.

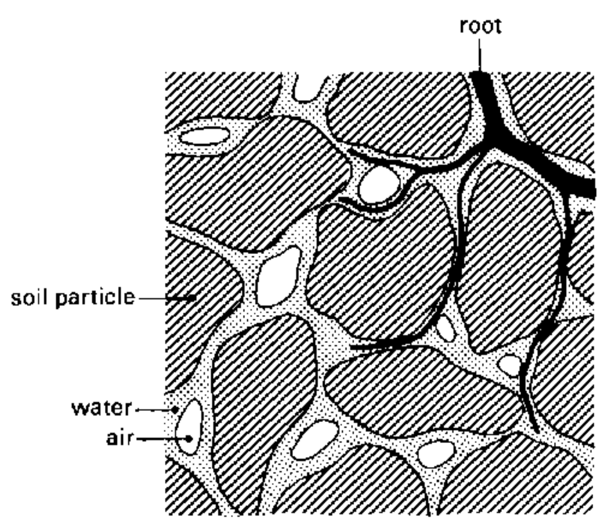
Introduction

In studying the environment, scientists often collect data to create what is known as a *profile*. A profile is a survey of major characteristics, done so that we can see all parts of a sample in an organized fashion. On TV, you may have seen an entertainment talk show give a "celebrity profile," where the reporter documents various characteristics of that celebrity's life. In the environment, there are many celebrities worth profiling—and they matter a lot more to our survival than the latest movie star. In Lab #26 we will be profiling a soil sample. Soils are diverse around the world, and soil profiles can tell us a good deal about what can grow where, and thus what food webs can be sustained in a given ecosystem.

Soil

Soil is made up of solids, liquids and gases (**Figure 1**). For growing plants, each phase is essential for their life and growth. The solid part is mixture of the mineral/inorganic material and the organic matter. Water and air make up the liquid and gas parts of the soil. The amounts of each type of matter in the soil determine the type of soil, as classified by scientists. An "average" mineral soil sample, which comprises most of the soil in northwest Canada, consists of about 45% mineral matter, 25% air, 25% water and 1% - 5% organic matter. Organic soils, found in bogs and

Figure 1: Close-up of Soil Composition



wetlands of northern Canada, are composed of at least 20% organic matter. Why would the soil of northwest Canada be interesting to us? Take a moment and in the circle provided create a pie graph that represents the distribution of matter in the soil of northwest Canada. Be sure to indicate solid, gas and liquid phases and provide a key.

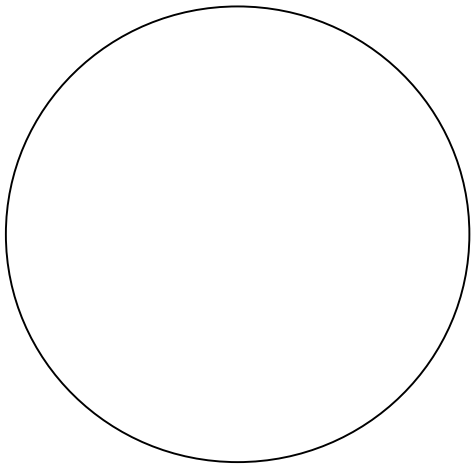


Figure 2: Distribution of Materials in Northwest Canada Soil

Key:

The source matter from which a soil is derived is called the *parent material*. It may be underlying rock or material transported and deposited by water, ice, wind, or gravity. Soils can develop from underlying rock; from water-deposited sediments in lake bottoms, stream flood plains, or oceans; from material deposited from receding glaciers; and may also be derived from sediments transported and deposited by wind. Kodiak Island in Alaska is a relatively undeveloped patch of land whereas St. Petersburg, Russia has been agriculturalized and industrialized. As part of that process, the bogs and wetlands once indigenous to St. Petersburg were dredged to create drainage canals, and the dredged materials were used to fill in low places. This is a process very similar to what happened in the urbanization of Manhattan. What impact do you think this human intervention has on the natural processes of soil formation in those areas?

Soil formation, a process called *weathering*, occurs from the breakdown of the parent materials and is a component of the rock cycle (Think back to Lab #4). Climatic factors such as air and water continue to change the composition and mineralogy of rock fragments and sediments through physical and chemical processes. Plants and animals contribute additional nutrients and chemicals from their metabolic pathways, and these influence the rate of the weathering process and its byproducts.

As soils weather, they develop distinct physical and chemical properties that determine the type and amount of plant species that inhabit a specific location. *Physical properties* of a soil include texture, which is determined by the percent composition of mineral sand, silt and clay within a given soil, and structure, which is determined by the physical distribution and conglomeration of these mineral particles. *Chemical properties* of a soil include pH and nutrient levels such as nitrogen, phosphorous and potassium. All of these characteristics influence the interaction of plant roots, soil organisms, air, water and nutrients in solution with each other, in turn favoring certain types of plant growth in each soil and as we already know the classification of world ecosystem is based in part on the distinctive plant life found in a given location. Take a moment and write down some the distinctive plants found in the taiga or the tundra.

Taiga	Tundra

By studying both the physical and the chemical properties of soil we can predict what types of plant life would be best suited to a given ecosystem. In Lab #26 we will be doing exactly that. We will be determining the texture, pH and nitrogen, phosphorus and potassium content of a soil sample and then comparing it to what we might expect to find in a soil sample from Kodiak Island or St. Petersburg, Russia.

pH and Soil

pH provides an indication of acidity or alkalinity in soil. How have we defined pH in the past? (look back in notes, a textbook, or an internet source)

It really describes the acidity of the water within the soil. Pure water has a pH of 7.0 and is considered neutral. Most soils range from pH 4.5 to 8.0. Take a moment and look back at Lab #11 Figure 2. Write down the substances which have pH values correlating to that of the range for soil:

Soil pH has a direct influence on the availability of nutrients for plants and the functioning of soil micro-organisms. Based on your work in Lab #13 or other experiences that you have had, why do you think that soil pH may have such a big impact on plants?

pH 6.5 is considered the ideal garden soil because the greatest number of basic nutrients are available to plants, but most plants will thrive between pH 6.0 and 7.5. Very acidic soils below 5.0 often have high concentrations of metals, such as aluminum, which are toxic to some plants, but needed by those who require acidic soil. Ericaceous plants, such as heathers, rhododendrons, azaleas and blueberries, demand acidic soils. In highly alkaline soils above 7.5, trace elements such as iron, are not available to plants needing these elements. There are few plants that absolutely require very alkaline soils. There are some though, such as carnations and pinks, which grow best in a more alkaline soil

In a non-agriculturalized plot of soil, natural processes will help to establish and maintain desired pH levels. Many conifers (e.g., pine trees), as a defense mechanism, will release acidic matter into the soil surrounding their root system. This creates an overall acidic soil plot which is not desirable to many other types of plants—especially competing deciduous trees. The pine seedlings grow very well in this soil. The result of this process is a *stand*, or grouping, of pine trees with more acidic than average soil. The ongoing presence of conifers will maintain the soil pH until such a time as there is a disturbance. This is one way that plants mark their territory. In the case of St. Petersburg, Russia, pine stands have been removed to make room for agricultural fields. Of course, the soil was acidic so farmers needed to treat the soil to get a pH suitable for crops. This was done by adding materials such as lime, which is calcium carbonate, to the soil. The process of liming is still very common today on both large scale and small scale farms.

In Lab #26 we will be using a pH indicator solution which is a combination of Bromocresol Green, Bromocresol Purple and Cresol Red. Refer back to Lab #11 to review how pH indicators enable us to determine the relative pH of a solution. All of these indicators are in the same family and will therefore not react with each other. Use **Reference Table M** to state the pH range for Bromocresol Green:

Bromocresol Purple has a range of 5.2 – 7 and Cresol Red has a range of 7.1 – 8.9. Why do you think these three indicators may have been selected for testing the soil samples?

Nutrients: Nitrogen, Phosphorus and Potassium

An ecosystem, just like a living organism, has needs. Just as we need things such as carbon, nitrogen, oxygen, calcium and sodium to keep our bodies running effectively, ecosystems require nutrients to maintain themselves. In ED I we looked at the carbon and oxygen cycles. These are both macronutrients essential to an ecosystem. Sketch out (oval) and write a caption (box) for a simple carbon/oxygen cycle on Kodiak Island, AK.

Figure 4: Carbon/Oxygen Cycle

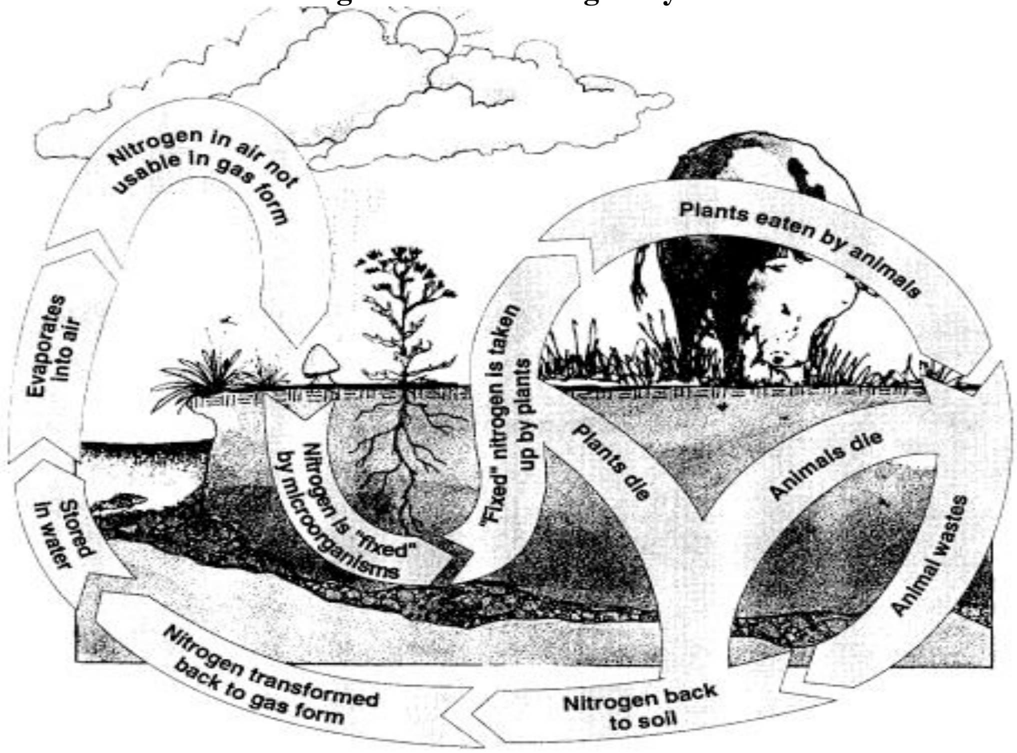
There are other nutrients that are essential for the proper functioning of an ecosystem. These include but are not limited to: Nitrogen, Phosphorus, Calcium, Potassium, Magnesium and Iron. In Lab #26 we will be focusing on three of these elements, Nitrogen, Phosphorus and Potassium.

Nitrogen is essential for the formation of nucleotides and proteins in all living things. Nitrogen is also one of the most abundant elements in the atmosphere but nitrogen in the air can't be used directly by plants and animals; it must be captured and transformed, or *fixed*, by mutualistic bacteria or algae into a usable form. The Nitrogen-fixing organisms that live on the roots of certain plants called *legumes*, can change nitrogen gas and/or ammonias into forms that can be used by plants. These forms of *organic nitrogen*, are part of the organic matter of the soil where and can be readily accessed by plants. This biological transformation involves the following:

- Uptake of ammonia (NH_3) and nitrate (NO_3^-) by plants and micro-organisms to form organic nitrogen.
- Fixation of nitrogen gas (N_2) by plants and bacteria to produce organic nitrogen.
- Ammonification of organic nitrogen to produce ammonia (NH_3) during decomposition of organic matter.
- Oxidation of ammonia to nitrite (NO_2^-) and nitrate (NO_3^-) under aerobic conditions.
- Denitrification of nitrate by bacteria to nitrous oxide (N_2O) and molecular nitrogen (N_2) under anaerobic (low oxygen) conditions.

When plants and animals die, their bodies are decomposed by other microorganisms such as fungi or bacteria, and nitrogen is returned to the air. This is a critical link in the nutrient cycling process. The whole process of nitrogen moving through the atmosphere, soil and water is known as the *nitrogen cycle* and is diagramed below in **Figure 5**. Take a moment and label the three spheres in the diagram. Refer back to your notes from ED I to help you.

Figure 5: The Nitrogen Cycle

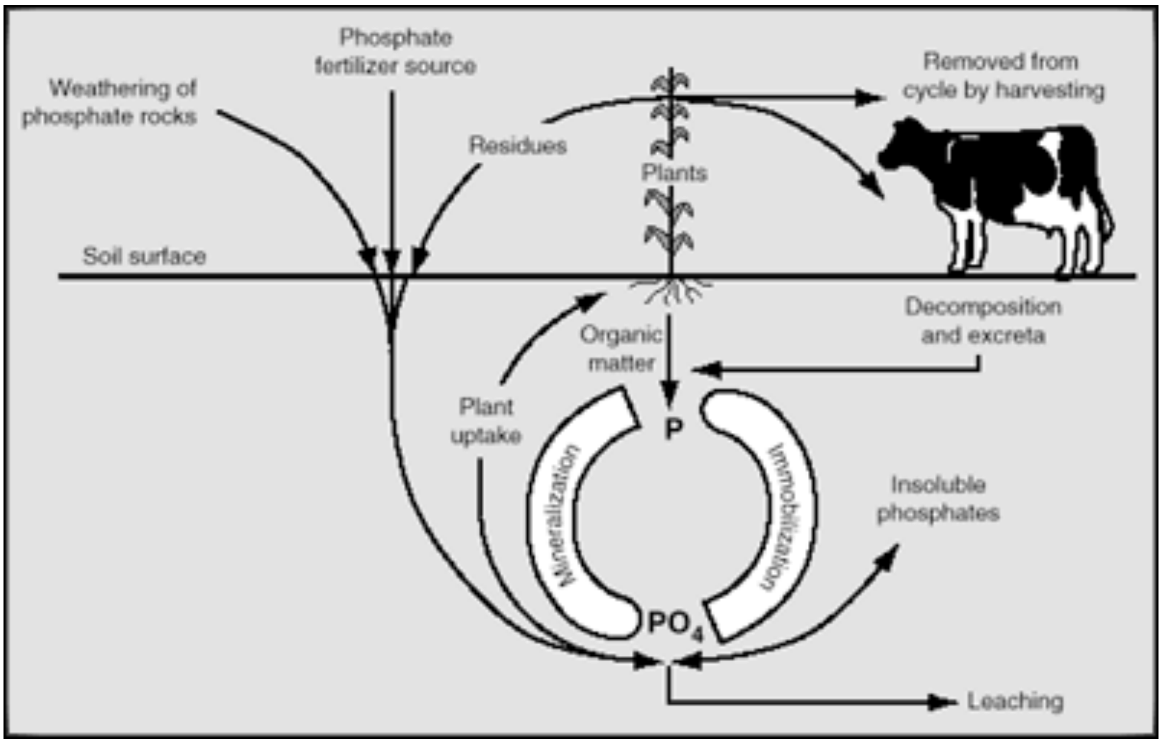


If the above natural cycle is left to regulate itself there is generally enough nitrogen available to support the local food webs. However, natural or human disruptions can upset the balance in a given area. A massive landslide, for example, may cause the top layer of soil, where much of the available nitrogen is located, to be removed from a given ecosystem. Short-sighted agricultural practices can strip an area of nitrogen by keeping a single crop in a field for too long. If that entire particular crop takes out nitrogen, and then the crop is harvested so there is no decaying plant matter, eventually that portion of land will become nitrogen-depleted and most things will not be able to grow there.

Phosphorus is a key element in the structure of nucleic acids. Think back to GUD I: each nucleotide of a nucleic acid is composed of a nitrogenous base, a sugar and a phosphate group. Take a moment and use **Reference Table E** to 1) write the molecular formula and 2)draw the Lewis Structure for a phosphate group:

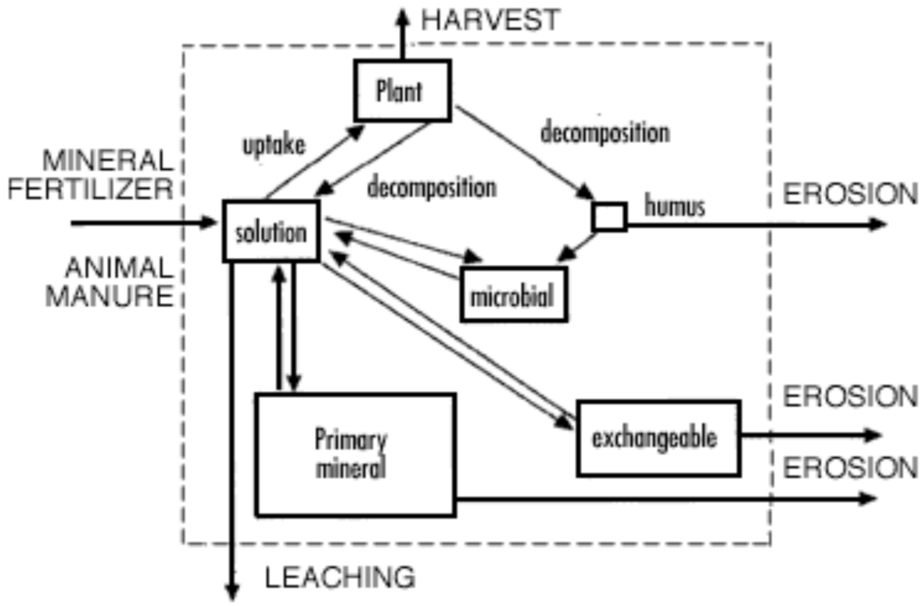
Natural sources of phosphorus are mainly derived from the weathering of phosphorus-bearing rocks and the decomposition of phosphorus containing organic matter. Wastewater containing detergents, industrial fluids, and fertilizer runoff contribute to elevated phosphorus levels in surface waters which can lead to algal blooms. Unlike nitrogen, phosphorus is not particularly mobile in soils, and phosphate ions do not leach readily. In general most phosphorus is removed from soils either by crop uptake or by soil erosion. Therefore, the basic pathway of phosphorus includes plant uptake, fertilization, and residue decomposition (See **Figure 6**).

Figure 6: The Phosphorus Cycle in an Agricultural Area



<u>*Potassium*</u> is absorbed by plants in the ionic form K^+. It is important in the formation of carbohydrates and proteins; also it helps regulate the water level within plant cells. In animals, potassium is essential for osmotic balance in a cell and the proper functioning of the muscle and nervous systems. The potassium in unfertilized soils originally came from certain mineral compounds, such as mica, that form when hot solutions solidify below or on the Earth's surface. Although such compounds constitute the single largest potassium reservoir in soils, they give up element very slowly. Other sources of potassium include clay minerals and potassium ions in solution, located in the water found in soil pores. Potassium that is bound up in a compound is unavailable to plants, so only ionic potassium is able to be used by plants. As plants die and decay, they give up some of their cellular potassium to the soil solution, and potassium again becomes available to plants and microorganisms. Microorganisms also can use some of the potassium that remains in the organic matter as it breaks down (See **Figure 7**).

Figure 7: The Potassium Cycle in an Agricultural Area



In order to determine the presence and relative amount of each of the nutrients, we will need to perform some simple extractions to remove the nutrients from the soil sample and then test for it. We have done several extractions in prior labs. <u>Take a moment and write down 2 different labs which required extractions, and what technique was used to perform each extraction</u>.

In each test in Lab #26, a solvent will be used to loosen the element in question from the soil sample. In the extraction of nitrogen, hydrochloric acid (HCl) will added to the soil sample and shaken. The nitrogen compounds will dissolve into the HCl. When the soil layer settles, the liquid known at *supernatant*, can be removed and tested. The same situation exists for the extraction of potassium and phosphorous although in both of those tests acetic acid (CH_3COOH) will be used.

These compounds will dissolve into these solvents because both the substances being tested for <u>and</u> the solvents are comprised of charged particles. As we have seen previously, "like dissolves like". If both the solute and the solvent are charged, then the process of dissolving can occur. Each of the acids can be broken down into positive and negative ions. The soil nitrogen, phosphorus, and the valuable potassium are all ionic. The positive and negative ions will be attracted to one another more than to the soil. Thus, the substances being extracted will move into the solvents and as the soil particles settle down to the bottom the liquid layer can be removed.

Then through the use of indicators, the liquid layer can be tested for the presence of the element in question. Just as in Lab #10 and Lab #11, color changes will indicate the presence of specific materials. Remember: *color change* is one of four signs that a chemical reaction has occurred.

The kit company has already standardized the materials we are using, so by using their color charts we will be able to determine presence of and relative quantity of the elements being investigated.

Materials per Team of 2
6 Soil Samples
Test Tubes
Stopper or square of Parafilm®
Distilled Water
Test Tube Rack
LaMotte® Garden Guide Soil Test Kit for pH, Nitrogen, Phosphorus, Potassium

Quantity in Kit	Item	LaMotte Code Number
120 mL	pH indicator	5701
120 mL	Nitrogen Extracting Solution	5702
10 g	Nitrogen Indicator Powder	5703
120 mL	Phosphorus Extracting Solution	5704
15 mL	Phosphorus Indicator Reagent	5705
25	Phosphorus Test Tablets	5706
120 mL	Potassium Extracting Solution	5707
25	Potassium Indicator Tablet	5708
15 mL	Potassium Test Solution	5709
7 – 1-8 mL	Test Tubes with caps	
1	Test Tube Brush	
3	Pipets, Transfer	
1- 0.25 g	Spoon, plastic	
1- 0.50 g	Spoon, plastic	
1	Nitrogen Color Chart	
1	Phosphorus Color Chart	
1	Potassium End Point Color Chart	
1	pH Wide Range Color Chart	

Procedure

LaMotte Kit Testing—Note* each lab team will perform only one of the tests on six samples of soil. Lab groups will then share data.

pH Test

1. Fill kit test tube to Line 4 with pH indicator.
2. Use 0.5 g spoon to add 3 measures of soil sample.
3. Cap and mix gently for one minute
4. Allow tube to stand for 10 minutes to allow soil to settle
5. Correlate color of liquid portion of sample to pH Color Chart provided with the kit. Record pH.

Phosphorus Test

6. Fill kit test tube to Line 6 with phosphorus extracting solution
7. Use 0.5 g spoon to add 3 measures of soil sample.
8. Cap and gently shake for 1 minutes
9. Remove cap and all to stand until soil has settled and liquid is clear.
10. Use pipet to remove clear liquid from the test. Be sure to squeeze bulb before inserting into liquid so as to not disturb the sediment.
11. Transfer liquid to a second test tube. Discard sediment in first test tube.
12. Add 6 drops of Phosphorus Indicator Reagent to liquid extract in 2nd test tube.
13. Cap tube and mix
14. Add 1 phosphorus Test Tablet to the test tube
15. Cap and mix until tablet dissolves.
16. Correlate color of liquid to Phosphorus Color Chart provided with the kit. Record results.

With this kit:	Low =	0-50 lb/acre
	Medium =	5-100 lb/acre
	High =	+100 lb/acre

Nitrogen Test

17. Fill a test tube to Line 7 with Nitrogen Extracting Solution
18. Use 0.5g spoon to add 2 measures of soil sample
19. Cap and mix gently for 1 minute.
20. Remove cap and allow sample to settle.
21. Use pipet to remove clear liquid from the test. Be sure to squeeze bulb before inserting into liquid so as to not disturb the sediment.
22. Fill second test tube to Line 3 with liquid from first test tube. Discard sediment from first test tube.
23. Use 0.25 g spoon to add 2 measures of Nitrogen Indicator Powder to liquid extract in second test tube.
24. Cap and gently mix
25. Wait about 5 minutes for pink color change to occur above the powder.
26. Correlate color of liquid with Nitrogen Color Chart provided with the kit. Record results.

With this kit:	Low =	0-30 lb/acre
	Medium =	30-60 lb/acre
	High =	+60 lb/acre

Potassium Test

27. Fill test tube to line 7 with Potassium Extracting Solution
28. Use 0.5 g spoon to add 4 measures of soil sample to test tube
29. Cap and shake vigorously for one minute
30. Remove cap and allow soil to settle.
31. Use pipet to remove clear liquid from the test. Be sure to squeeze bulb before inserting into liquid so as to not disturb the sediment.
32. Fill second test tube to Line 5 with liquid from first test tube. If additional extract is needed to fill to Line 5 repeat Steps 37-40. Discard sediment from first test tube.
33. Add one Potassium Indicator Tablet to the liquid extract in the second tube.
34. Cap and mix until tablet dissolves.
35. Add Potassium Test Solution 2 drops a time be sure to keep track of the number of drops. Swirl test tube after each addition to mix contents. Stop adding drops when color goes

from purplish to blue. Use the Potassium Endpoint Color Chart as a guide to help see the end of the reaction.

36. Record the number the total number of drops. Use the table below to help interpret results:

Number of Drops Added	**Potassium Level**
0-8	Very High
10	High
12	Medium High
14	Medium
16	Medium Low
18	Low
20 or more	Very Low

With this kit:	Low =	0-120 lbs/acre
	Medium =	120-200 lbs/acre
	High =	+200 lbs/acre

Post-Lab Activities

1. Share LaMotte Kit® test data with lab group members and class.

2. Organize all of the results from the nutrient tests into graphs.

3. Does your data support your hypothesis? If so, how? If not, why? Support your answer with data.

4. What other types of information do think would be helpful to have regarding collect, in order to learn more about the soil samples?

5. Class debrief.

Sources

Figure 1: http://www.fao.org/docrep/R4082E/2.1.1%20soil%20composition
Figure 3: http://homepages.which.net/~fred.moor/soil/formed/f0107.htm
Figure 5: http://www.co.missoula.mt.us/measures/soils.htm
Figure 6: http://www.epa.gov/owow/watershed/wacademy/wam/watqual.html
Figure 7: http://www.acsh.org/publications/priorities/0904/soils.html
Figure 8: http://www.suite101.com/article.cfm/222/93184