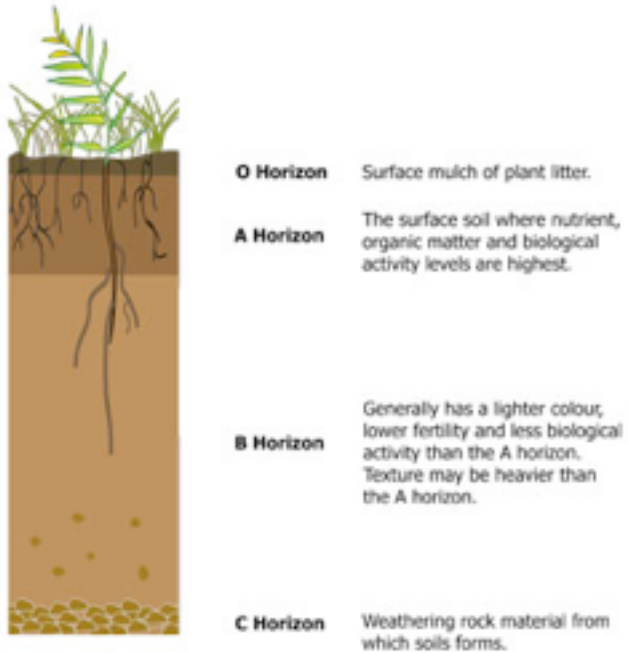
ENVS2001/2014 LABORATORY #4
SOIL ANALYSIS

INTRODUCTION

The chemical and physical properties of soil are vital characteristics which influence the plant and animal communities that grow within it. Many human factors alter the abundance and chemistry of soil, threatening future agriculture and terrestrial biodiversity. From a plant's perspective, soils contain several factors vital for healthy growth, including nitrogen (N), phosphorus (P), and organic carbon (C). We will measure carbon and phosphorus this time, nitrogen later.

Sample	% moisture	pH	$[PO_4^{2-}]$ (ug L^{-1})	% sand	% silt	% clay	% organic	notes
Shek O								
Compost A				x	x	x		
Compost B				x	x	x		



NAME:_____ **DATE:**_____

STATION #1: SAMPLING AND SOIL EXTRACTION

<u>Field Sampling</u> for soils is varied and depends on the soil composition and the purpose for sampling. Pit cores can be sampled simply by careful digging with a shovel or a motorized post-hole digger. More precise sampling (for analyzing soil layers) can be taken using a variety of soil augers and probes. Depending on the application, care must be taken when removing the core and preparing the sample for transport and storage.

1) measure soil temperature as a function of depth using the **digital temperature probe**
2) practice taking a small soil core using the **soil probe**.
3) observe how a large soil core can be taken using the **auger**.
4) homogenize a subsample of soil from each of the samples provided (see page 1) using a **mortar & pestle**
5) sieve soil samples using the **sieve shaker** in the laboratory.




mortar & pestle (left), soil push probe (left middle), a “russian” auger (right middle), and a soil sieve shaker (right).

Soil Extraction **is a fundamental procedure for the study of soil chemistry. Briefly, soil particles are generally charged and attract positive and negative ions (cations and anions). Clays tend to be negatively charged and thus strongly bind cations (like Ca, Mg, K) while organic matter tends to have mixed charges and thus bind all ions depending on it's composition. Plants can secrete ions (like H^+) to "push" other ions off of soil particles. If the pH is slightly acidic, this process is easier for plants. We can analyze ions by forcing ions off using strong ionic solutions. Today we will DEMO the KCl extraction a common technique primarily used to assess dissolved inorganic nitrogen. However, we will see if we can analyze phosphate instead (note that phosphate extractions are usually conducted using a bicarbonate extraction, which is not practical for our lab).**

Soil Moisture

1) Dry one 10g subsample (field moist) in oven for 48 hours @ 105°C
2) return to re-weigh the oven-dry sample.
3) calculate % moisture using:

% moisture = (field moist mass - dry mass)/field moist mass

Extracting Soil

4) Weigh out a 20g subsample of field moist soil in a specimen cup. With a graduated cylinder measure 40 ml 2M KCl and add to soil. Cap specimen cups.
5) Make several "blank" cups of KCl.
6) Shake for 1 hour at 200 rpm.
7) Allow samples to settle for 1 hour after shaking.
8) Set up vacuum filter with 9cm filter. Pretreat filters by leaching them 2x with 2M KCl.
9) Vacuum filter the supernatant through filter paper and collect extract into pre-labeled vials or sample bottles.
10) Freeze extracts to store.

A filtration system for preparing multiple extracts simultaneously.

STATION #2: SOIL PHOSPHATE DETERMINATION (SPECTROPHOTOMETRY)

Phosphate (PO_4^{2-}) limits terrestrial plant productivity and is vital for essential plant functions including ATP cycling (energy), photosynthesis (reductants like NADP), sugar cycling, and DNA synthesis. Lack of P reduces leaf and root growth and extension. Again, we will measure soluble reactive phosphate (SRP or orthophosphate = dissolved inorganic phosphate), which is directly taken up by plant cells.

1) create a combined reagent <u>in the following order</u> with mixing after each addition:
- **i.** 5mL H_2SO_4 (5N)
- **ii.** 0.5mL potassium antimonyl tartrate
- **iii.** 1.5mL ammonium molybdate
- **iv.** 3mL ascorbic acid

2) all solutions should be at room temperature before proceeding.

3) pipette 5mL of each standard and soil extract** into a 15mL "Falcon" tube (see table to the right)

4) add 0.8mL combined reagent to each tube. Screw on cap and vortex.

5) wait 10 minutes but no more than 30 minutes to measure

6) pour 5 mL of each standard and soil extract into the cuvette and record the absorbance at **880 nm**. Make sure the mark on the cuvette is lined up with the mark on the spectrophotometer. Rinse cuvette with DI water between each sample.

** Recall that the soil extract was made by diluting 100g soil in 200mL KCl solution**

9) calculate the equation of the line of best fit from the standard curve using the laptop. (Absorbance = slope * concentration + intercept.

10) using the absorbance values from your extracts, calculate their concentration based on the best fit line.

Concentration (ug L^{-1})	Absorbance (880)	Extract	Absorbance (880)	Concentration (ug L^{-1})
0		Shek O		
100		A		
200		B		
400				

QUESTION: HOW DO YOU CALCULATE THE FINAL SOIL EXTRACT CONCENTRATION?

STATION #3: Soil pH

Soil pH is critical for plant growth as the relative acidity/alkalinity of the soil affects the abundance of dissolved free ions in water surrounding soil particles. relatively acidic soils contain more free ions and thus more nutrients available for plant growth. Use of the pH probe is as before (instructions below). This time we will use a **magnetic stir plate** with the soil solution to improve the precision of our analysis. The probe is calibrated to pH 4 and 7.

1) add 5g of each soil sample to 5mL DI water in a small beaker. stir.
2) allow the solution to equilibrate for 15min. stir.
3) carefully remove the pH probe from the storage solution by first unscrewing the cap, removing the bottle, and then sliding the cap off the probe. GENTLY! **DO NOT YANK THE STORAGE BOTTLE OFF THE PROBE OR YOU MAY BREAK IT!**
4) open the blue vent on the side of the probe.
5) rinse the probe with DI water
6) Use the pH probe to measure the pH of two standards pH 4.0 and pH 7.0 **while stirring**. **rinse the probe between each measurement** with DI water.
7) if the standards do not match the pH reading, please inform your demonstrators.
8) if the readings match, Record pH for each unknown, while stirring and **rinse the probe between each measurement** with DI water.
9) rinse and store the pH probe in the provided storage solution bottle. First, slide on the cap and o-ring with the threads facing down. Then insert the probe into the bottle's solution. Bring the cap down and screw on gently.
10)replace the vent cap

Figure 1. Soil pH and Plant Growth

Soil Reaction	pH	Plant Growth
	>8.3	Too alkaline for most plants
	7.5	Iron availability becomes a problem on alkaline soils.
Alkaline soil	**7.2**	6.8 to 7.2 – "near neutral"
Neutral soil	**7.0**	6.0 to 7.5 – acceptable for most plants
Acid soil	**6.8**	
	6.0	
	5.5	Reduced soil microbial activity
	<4.6	Too acid for most plants

STATION #4: ORGANIC CONTENT

% Organic is calculated by mass difference. A mass of soil is weighed and then combusted in a **muffle furnace.** Unlike a drying oven which we've used for drying filters and incubating microbes, a muffle furnace is capable of much higher temperatures (>500 ºC). As such, the muffle furnace is a suitable tool for removing organic matter via burning. For example, the GF/F filters we used in the water quality laboratory were pre-combusted in the muffle furnace to remove organic matter contamination.

1) pre-weigh a ceramic crucible. to this add 1 g oven-dried soil of each type
2) label the crucible on the bottom with pencil (marker will burn off in the furnace).
3) carefully place in the muffle furnace.
4) combust at 500ºC for at least 6 hrs (this will be done for you).
5) move samples from the furnace to a low humidity dessicator to avoid water re-absorption.
6) in 24 hrs, re-weigh the crucible and subtract the initial mass from the final mass to determine the mass of material remaining. the "ashed" sample now contains only the inorganic fraction of the soil sample.
7) calculate % organic matter using the equation below.

A common muffle furnace. Note the thick insulating layer on the inside to prevent overheating of the exterior

% organic matter = (oven dry weight - ash free dry weight) / oven dry weight

STATION #5: SOIL TEXTURE

Soil texture is an important characteristic that is altered by the proportion of sand, silt, and clay. Clays are the smallest particle size, and although they have a high surface area : volume they can compact to the point that limits water, oxygen, and nutrient availability. On the other end of the spectrum, sand does a poor job of retaining water and is prone to low moisture. <u>You will conduct this exercise using soil from Shek O.</u>

1) sieve ~75g dried and crushed soil through a 2mm sieve
2) weigh and record the retained gravel in the sieve
3) add 50g of the sieved sample to a 1L beaker
4) add 2g of the detergent sodium hexametaphosphate* and mix
5) add 500mL of DI water to the beaker
6) mix for 5-10 minutes
7) rinse all the solution and particles into a 1000mL graduated cylinder. top up with DI water to 1000mL
8) cap the cylinder and invert several times to mix.
9) remove the cap and immediately add the soil hydrometer and record the hydrometer value (g soil L^{-1}) at exactly 40 seconds
10) remove and clean the hydrometer
11) record the water temperature using a digital thermometer
12) after 2hr measure again using the hydrometer, record temp.
13) pass the entire suspension through a 0.053-mm sieve, rinse
14) transfer the retained sand to a pre-weighed container and oven dry for 24hrs at 110ºC. weigh the sand after drying
15) calculate the % sand as:
% sand = (dry weight of sand * 100)/ (dry weight of soil)
16) correct the 2 hr hydrometer reading for temperature by adding (or subtracting) 0.36 g L^{-1} per degree above (or below) 20ºC.
17) calculate % clay as:
%clay = (corrected 2hr reading *100)/dry weight of soil
18) calculate the % silt as: **%silt = 100 - (%sand + %clay)**

*** this is a common detergent and acts to separate the soil particles and prevent clumping in solution.**

A soil texture triangle assists with classifying soil type.

Although we cannot demonstrate it effectively in Hong Kong, soil profiles are an important aspect of terrestrial ecology. The depth of each layer can influence the abundance and biodiversity of plant and animal species inhabiting surface and sub-surface environments. See below for some examples of how soil horizons vary across different biomes. **Which one do you think most closely represents Hong Kong? Why?**