SIREN

pH project protocol
version 1.0

Objective: The purpose of this experiment is to quantify the effect of the addition of elemental sulfur and lime or different pH solutions on soil pH and plant growth and nutrient uptake.

- Soil should be collected in 5-gallon pails from the soil surface (top six to eight inches) of a garden or agricultural field. Sufficient soil should be collected to prepare enough pots for the experiment. [Note: It takes approximately 3.5 pounds (1.6 kilograms) of soil to nearly fill a six-inch greenhouse pot. It takes about 4 pounds of sandy soil (1.9 kilograms)].
- Choice of the type plant(s) to grow is at the discretion of the instructor. More than one plant species may be grown if there is sufficient soil. It is recommended that plant choice be made from the list below. We do not recommend that students eat any of the plants that they grow. Changes in soil pH, up or down, can result in increased solubility and availability of one or more elements which could potentially be harmful if taken up into the plant and ingested.
- A minimum of three treatments are recommended: soil at the pH at which it was collected (control) and a pH at least 1.5 pH unit above and at least 1.5 pH unit below the control. More treatments can be added if there is sufficient soil for additional treatments (e.g. +/-1 unit, +/-1.5 unit). The EXCEL spreadsheet SIRENWorksheet is provided for calculating the lime/sulfur application rate. We will supply you with the necessary amount of Aluminum sulfate ($AlSO_4$) and calcium carbonate ($CaCO_3$).
  An alternative approach to using lime and sulfur to adjust soil pH is to use a strong acid, hydrochloric acid (HCl), and a strong base, potassium hydroxide (KOH), to adjust the pH of distilled water applied as treatments to the pots. Hydrochloric acid and KOH solutions can be prepared and the pH adjusted using a pH meter. A range of pH values can be prepared in this manner, for example pH 3, 5, 7, 9, 11. Treatment volumes of 400 mL would be added once at the beginning of the experiment.
- A minimum of four replications.
  (1 soil *3 pH levels *1 crop*4 reps) = 12 greenhouse pots.
- Fertilizer must be applied. Adequate nitrogen, phosphorus, and potassium should be applied in order to provide the necessary plant nutrition. The EXCEL spreadsheet SIRENWorksheet is provided for calculating the appropriate amounts of nitrogen-phosphorus-potassium (N-P-K) for your crop and should be based on University Extension recommended fertilizer rates.
- The duration of the experiment should be 42 days after emergence.
- Soil and Plant data collection and analysis:
  Soil samples should be collected four times during the experiment as shown in Table 2 and analyzed for pH. Plant samples are collected at the termination of the experiment, dried and sent to the University of Minnesota SWROC for nutrient uptake analysis.

  Plant height, stalk/stem diameter, plant sturdiness, leaf color, and leaf /plant deformity should be measured and tabulated once per week.

Experimental set up.
Measure the pH of your soil following the protocol below. Once you know the pH of your soil decide on the treatments. Use the spreadsheet provided to determine the amount of $AlSO_4$ and $CaCO_3$ required for each of your treatments. The spreadsheet tab labeled "pH" gives you a starting point for determining the expected response to pH adjustment for a soil that has a starting pH of about 6.0 and one with a starting pH of about 8.0.

A total of ~ 1.6 kg of dry soil plus the required amount of fertilizer and lime/sulfur treatment for each of the four replications should be thoroughly mixed in a 5 L plastic bag. Depending on the plant being grown, you will want to add appropriate amount of N-P-K at this time. Use the spreadsheet tabs labeled "nitrogen", "phosphorus" and "potassium" in the SIRENWorksheet to help you determine the amounts to be added to each pot. The spreadsheets are set up for urea and various other sources of nitrogen, diammonium phosphate (DAP) and various other sources of phosphorus, and potassium chloride (KCl) and various other sources of potassium. If you use a product other than a single element fertilizer, for example DAP, be sure to subtract the amount of N from the total needed from your chosen N source from you calculation.

After combining the soil and treatments, the entire mix will be placed in a 6-inch diameter ½ gallon plastic pot placed on a saucer. Plants can be from transplants or direct seeded. If direct seeding plant seed shallow, approximately ½ inch below the soil surface. Plant more seeds per pot than needed for the experiment (e.g. 3-4). Water will be provided as needed and daily if necessary to maintain soil moisture. Initially add 25% of the mass of soil as water, so for 1638 g soil add 410 mL of water. Leaching of the soluble nutrients will be contained by returning any water leached into the saucers back into the pots. One week after seedling emergence, pots will be thinned to one plant per pot.

Soil
A subsample of ~ **20 g** soil from each pot will occur at initiation (1 d) and termination (42 d) for pH. Soil samples will also be collected on day 14 and 28 after emergence.

Chemical Lab analysis (destructive sampling)
1. Initial
    a. Sample 20-g dry soil + amendment
    b. pH (2:1)
2. Intermediate 14 and 28 days
    a. Sample 20-g dry soil + amendment
    b. pH (2:1)
3. Final at 42 days
    a. Sample 20-g dry soil + amendment
    b. pH (2:1)

Protocol for soil pH measurement (2:1 ratio method)
Equipment
1. pH meter
2. One ounce paper or plastic cups

3. Standard buffers, pH 4, 7and 10
4. Plastic or glass rod
5. Distilled water
6. Rinse bottle filled with distilled water
7. Paper towels

Procedure

1. Weigh 20 grams of soil into a paper or plastic cup.
2. Add 40 mL of distilled water to each cup.
3. Mix thoroughly for 5 seconds.
4. Let stand for 10 minutes.
5. Insert the pH meter electrode into the container and stir the soil suspension by swirling the cup.
6. **Read** the pH immediately on the standardized pH meter. **Record** as soil pH in water or $pH_{w2:1}$.
7. Rinse the pH meter electrode with distilled water in the rinse bottle between successive samples and blot excess water off the electrode before immersing in the next sample.

Table 1.	
Crop	**Target pH**
Alfalfa	6.8
Barley	6.6
Bean, dry kidney	6.0
Bean, snap	6.8
Beet, table	6.0
Broccoli	6.0
Brussels sprouts	6.0
Buckwheat	5.6
Cabbage	6.0
Canola	5.8
Carrot	5.8
Cauliflower	6.0
Clover, red	6.3
Corn, grain	6.0
Cucumber	5.8
Flax	6.0
Lettuce	5.8
Oats	5.8
Onion	5.6
Pea	6.0
Pepper	6.0
Potato	6.0
Pumpkin	6.0
Rye, grain	5.6
Soybean, grain	6.3
Spinach	6.0
Squash	6.0
Sunflower	6.0
Tomato	6.0
Triticale	6.0
Wheat	6.0

Table 2.	
Date	Amount of sample to collect per plot
Day 0 (planting date)	20 grams
Day 14	20 grams
Day 28	20 grams
Day 42 (termination date)	20 grams

Calibration Experiment

Objective: The purpose of this experiment is to create pH calibration equations for an unknown soil by either raising or lowering soil pH.

- Sufficient soil should be collected to prepare enough beakers for the experiment. [Note: It will take approximately 200 grams of soil per 250 mL beaker for this experiment].
- A minimum of four treatments plus a control are recommended: soil at the pH at which it was collected (control) and at least four rates of liming material to raise and lower the pH, respectively. There are numerous commercially available products that can be purchased at a nursery or garden center to manipulate soil pH. Products include various liming materials to raise soil pH and various sulfur materials to lower soil pH. We used reagent grade Aluminum sulfate ($AlSO_4$) and calcium carbonate ($CaCO_3$) from Fisher Scientific. Various grades of limestone or sulfur can be used.
- The duration of the experiment is variable but should last at least 30 days but could go as long as 60 or 90 days depending on the frequency of soil sampling and objectives of the experiment.

Experimental set up.
Rates of material added to the soil can be based on agricultural rates, for example 0 (control), 2, 4, 6, and 8 tons material/acre. By setting up a simple ratio we can determine the number of grams of material to add in order to raise or lower pH. Remember that the goal is to raise and/or lower soil pH of a soil that we are not sure how it will respond to treatment. If we assume that an acre of soil, six inches thick weighs 2,000,000 pounds and that we will need 200 grams of soil to perform our experiment we can determine how many grams of material to add to our beakers. The amount of soil will depend on the amount of time the experiment is run and the objectives.

Amount of material (tons/acre)	Material required to change pH (grams/200 grams soil)	Example calculation:
0	0	4000 lb/2,000,000 lb = Xg/200 g 200 g (4000/2,000,000) = X g X = **0.4 g** of material to raise or lower soil pH.
2	0.4	
4	0.8	
6	1.2	
8	1.6	
1 ton = 2000 pounds.		

A mass of dry soil plus the required amount of material to adjust pH should be thoroughly mixed in a 1 L plastic bag. After combining the soil and treatments, the entire mix will be placed in a 250 mL or larger beaker. Initially add 20 to 25% of the mass of soil as water, so for 200 g soil add 40 to 50 mL of water. One day after the experiment is started a subsample of ~ **20 g** soil is collected from each beaker. Additional soil samples can be collected on a weekly or bi-weekly basis for pH analysis using the protocol below. Plot the data in a spreadsheet.

Protocol for soil pH measurement (2:1 ratio method)

Equipment

1. pH meter
2. One ounce paper or plastic cups
3. Standard buffers, pH 4, 7and 10
4. Plastic or glass rod
5. Distilled water
6. Rinse bottle filled with distilled water
7. Paper towels

Procedure

1. Weigh 20 grams of soil into a paper or plastic cup.
2. Add 40 mL of distilled water to each cup.
3. Mix thoroughly for 5 seconds.
4. Let stand for 10 minutes.
5. Insert the pH meter electrode into the container and stir the soil suspension by swirling the cup.
6. **Read** the pH immediately on the standardized pH meter. **Record** as soil pH in water or $pH_{w2:1}$.
7. Rinse the pH meter electrode with distilled water in the rinse bottle between successive samples and blot excess water off the electrode before immersing in the next sample.