SCEEC Environmental Outreach Program

Check out this insightful report by CCTV News!!!

https://www.youtube.com/watch?v=xUUk2ZBKDyI&ab_channel=CCTVNews

Soil Pollution Lab

*** Watch the video above and answer the questions below, including the background questions before starting the lab.*

1. What were the specific heavy metals accused of polluting the soil in Jiangsu province?

2. Why would scientists AND entrepreneurs want to spend their time with soil remediation according to the video?

3. How could the lack of government regulation hamper the environmental industry?

The mission of the Santa Clarita Environmental Education Consortium is to proactively provide educational resources to promote environmental literacy in SCV. For more information, or additional resources go to SCEEC.org. For questions and requests regarding environmental

science lab modules, please contact Patrick Allen at pwallen1415@gmail.com -

SCEEC lab module: Soil Quality

Objectives:

1. To introduce the variables that affect & represent soil health and how to measure them.
2. To introduce methods of soil remediation, including bioremediation.
3. Provide experience in collecting soil samples, as well as basic experimental design.

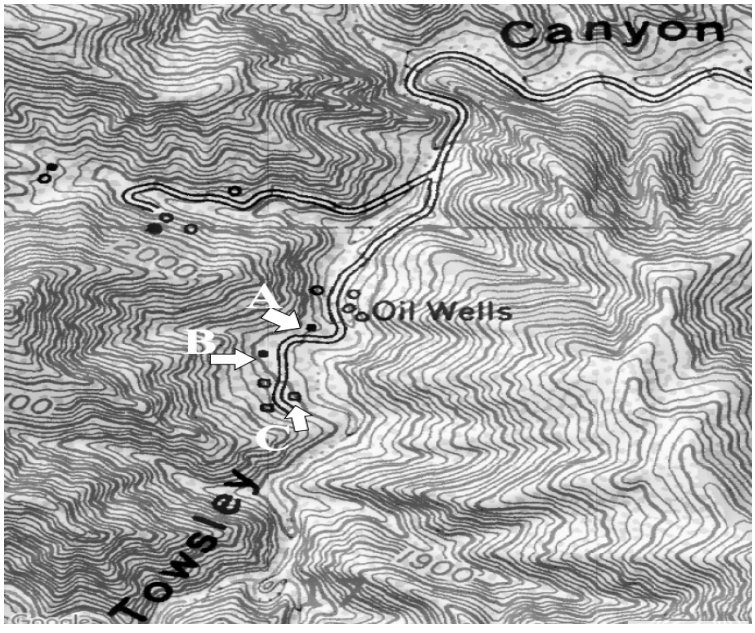
Soil Quality background

China isn't the only country to deal with soil pollution. Even countries with sufficient regulations against heavy metal contamination, such as the United States, still face other sources of man-made contaminants through oil spills. Regardless of where someone stands on the usage of fossil fuels, everyone can agree that technology and trained personnel are needed to clear any threats to soil health.

So what exactly is soil health? Soil health is an assessment of how well soil performs all of its functions now and how those functions are being preserved for future use **[8]**. Just as a nurse looks at multiple indicators in a patient (blood pressure, heartbeat, respiratory, etc) to treat them, so too must farmers and soil scientists measure multiple indicators of soil health.

There are three main indicator branches to assess soil health **[8]**; There are physical properties such as water holding capacity, water retention, and soil structure (known as tilth). There are biological indicators such as soil respiration, microbial catalytic potential of Carbon and Nitrogen, and overall microbial activity. Lastly the chemical indicators are pH levels, nutrient (N, K, & P levels, and electrical conductivity. There are almost as many indicators for soil health as there are for a human being's health! However this lab will focus on the chemical indicators for pH, Nitrogen (N), Potassium (K), and Phosphorous (P) within soil gathered from Newhall's Towsley Canyon hiking trails.

Figure 1. Topographical map of Towsley Canyon



4. Based on Figure 1, why do you think you will be testing the chemical properties of soil samples taken from sites A, B, and C? How would this activity be relevant to soil health?

5. Which soil horizon do you think will be the most useful in testing samples from these three sites? Why?

There are ranges for chemical properties where plant life will either not grow, or become stunted during growth. One of the most influential factors for plant growth is soil pH, and most plants thrive between a pH of 6-7 **[6]**. Acidic soil, perhaps caused by rainwater leaching Calcium and Mangesium ions, can reduce the availability of essential nutrients**[2]**. Even slight changes to soil pH can make macro-nutrients such as Nitrogen, Phosphorous, and Potassium (NPK) almost untouchable despite being in the surrounding soil.

Even though NPK are needed by plants to survive, plants cannot absorb their elemental forms. Pure Nitrogen (N_2) is a gas, and the triple bonds make it thermodynamically difficult to break. Essentially pure Nitrogen would be more trouble than it is worth. Many of the elemental forms of nutrients would take too much energy to process for what plants would get out of them, or would be unstable and dangerous (pure potassium would explode violently the instant it touches water).

The essential NPK nutrients come in milder forms, but their ionic charges are affected by changes in soil pH. Nitrogen for example is taken up by plants as NO_3^- ions or NH_4^+ (ammonium) ions**[4]**. Phosphorous is most available in its $H_2PO_4^-$ ion **[7]**, but plants' ability to absorb Phosphorous decreases when Phosphoric acid gains or loses another hydrogen (H_3PO_4 and HPO_4^{-2} respectively). Potassium can come in multiple forms, but all are generally referred to as potash.

6. Given the five macronutrient ions above and their availability to plants at neutral pH, which would be affected more in a low pH soil? Which ones would be more affected in high pH soil?

Anytime soil is contaminated, the concentrations of these nutrients and the microbe populations that can assist in making them, can drop. This will inevitably decrease the soil health, affecting plant growth or even the diversity of plant life in the region. As shown in the video on pg 1, there are methods that can reverse the effects of pollution. This practice is known as remediation, or if new bacteria/microbes are introduced to the soil it is called bioremediation.

Before any remediation can be applied, the soil health must be assessed to see exactly what indicators are deficient, and what contaminants are present. The tools needed to collect samples for testing are shown in Figure 2. This lab will only use the Soil Probe to collect control samples from an area guaranteed to have high concentrations of available NPK macronutrients and neutral pH.

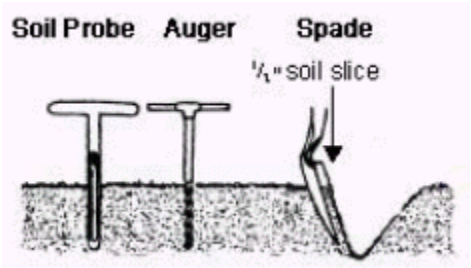


Figure 2. Three common tools used to collect soil samples.**[1]**

Each group will test three different sets of soil samples. The first two will come from Towsley Canyon, one sample polluted by crude oil and one unpolluted sample. The third will be collected by each group from a plot of healthy soil from the Valencia campus (the class must agree on one area from Figure 3 prior to starting the lab). The second and third samples are control sets, which will determine to what extent the crude oil affects the chemical properties in Towsley Canyon. Including controls in experiments is one technique environmental scientists use to control variables that could affect the results. Having another data set to compare against provides more meaningful conclusions.

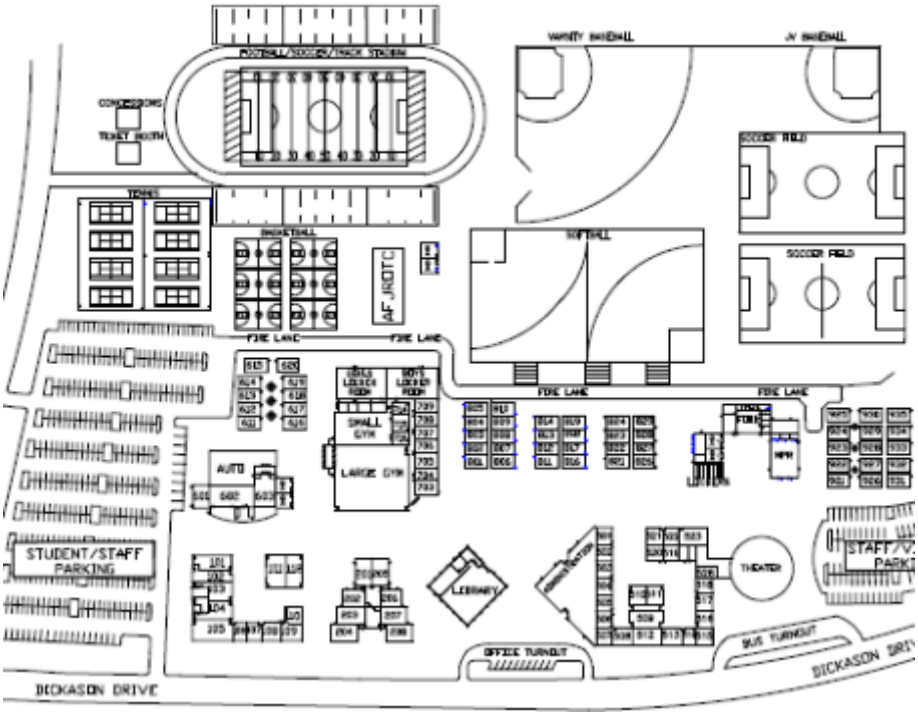


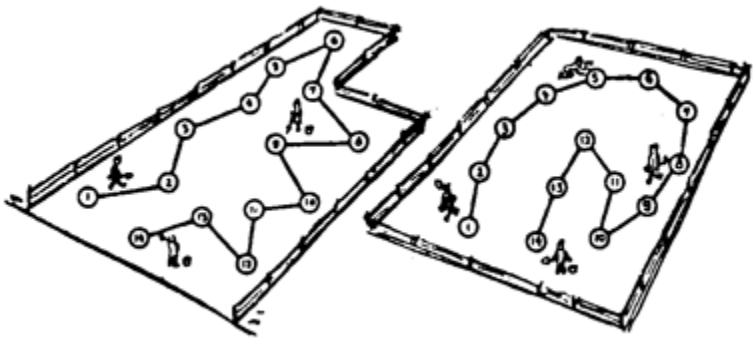
Figure 3. map of Valencia High School campus. *Circle or highlight where you think would provide the ideal set of soil sample controls.*

7. Why are there two control sets to compare against the polluted soil sample? What is the purpose of the unpolluted Towsley Canyon soil sample when the Valencia campus soil sample is supposed to be the ideal healthy soil at neutral pH and sufficient nutrient values?

In choosing a plot of land to collect samples, the samples must be representative. That is, each sample cannot be completely random for this lab and need to have consistency. Perhaps within a field of grass there is a section where the grass is much greener and taller or a section where no grass is growing. Regardless, these sections make up a small portion of the field and will not be representative of the entire field. Sometimes the eyes cannot qualitatively determine what is representative and what is not. The grass and plant life may appear to be consistent on the top, but one soil sample among many may not have consistent nutrient values with the other samples (even if the sample is representative!). This is why scientists never include just one sample in their data set.

8. Looking at figure 4, how would you solve the problem of single representative yet inconsistent soil sample introduced on page four? How does the technique introduced here help minimize the statistical influence of one bad data point?

Figure 4. A demonstration of how to take multiple, representative soil samples. **[3]**



Sources (Always cite your Sources!!)-

[1] A&L Eastern Laboratories.com

[2] Bickelhaupt, Donald. "Soil PH: What It Means." Soil PH: What It Means. State University of New York/ College of ESF, n.d.

[3] Bionutrient.org

[4] Crawford, Nigel M., and Anthony D.m Glass. "Molecular and Physiological Aspects of Nitrate Uptake in Plants." Trends in Plant Science 3.10 (1998): 389-95.

[5] Kisic, Ivica. "The Effect of Drilling Fluids and Crude Oil on Some Chemical Characteristics of Soil and Crops." Science Direct. Elsevier Geoderma, 2008.

[6] Rank, Isama Lawrence, International Journal Of Modern Engineering Research (Ijmer), Vol. 3, Issue. 6, Nov - Dec. 2013 Pp-3336-3342, and Issn: 2249-6645. "Effects of Crude Oil Spillage on Physico-Chemical Properties in Ugborodo Community." Internation Journal of Modern Engineering Research 3.6 (2013): n. pag.

[7] Shachtman, Daniel P. "Phosphorus Uptake by Plants: From Soil to Cell." Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiology/ Department of Botany and Soil Science/Australia, 1998.

[8] Various Authors. "Natural Resources Conservation Service." Soil Health Literature Matrix. NRCS, 8 May 2016.

SCEEC Soil Pollution Lab

Lab Supplies List per group

-12 colored containers for testing pH and NPK values. (three sets of the four colors) -

-Three of each colored testing pills (12 total). -

-Three paper cups (two for the Towsley samples, and one for school campus sample) -

-Two ziplock bags full of one polluted and one nonpolluted Towsley samples -

-One empty ziplock bag for school campus sample -

-Three 1 ml transfer pipette -

-Source of deionized (DI) water (no tap water!) -

-One soil probe -

-One plastic stirrer (could be a pen/capped marker) -

-One dry erase marker/sharpie -

SCEEC Soil Pollution Lab Procedure

(Record all Results on page 7 & 8)

-Each group will have 3-4 students per station. Pick a group number. -
-Check that the station has each and every one of the supplies listed on page 5. -
****Remember that only the green pH containers are filled with DI water. The remaining NPK containers are filled with the soil water from the beakers.****

1. While the class is discussing where they should take school campus control samples, open the ziplock bags containing the Towsley sample sets. Fill the two green pH containers to the marked line with each soil sample respectively. Then label them "polluted" and "clean" and your group number. ****pull apart the green pill over the pH container like you are cooking an egg. Add the powder inside and fill with DI water to the 2nd marked line. Let it wait undisturbed with the beakers.****

2. Place the remaining soil samples into two labeled paper cups. Fill but do not overflow with DI water. Mix with the plastic stirrer and leave them untouched for 20-30 minutes.

3. While waiting for the two Towsley beakers to settle, each group will go with the class to collect one soil sample from the agreed upon area on campus. Use the soil probes to fill the ziplock bag with the soil from one use of the soil probe. Organize the soil sample sites according to Figure 4.

4. Upon retrieving the school campus samples perform steps 1 & 2 for this sample.

5. While waiting for the school campus sample to settle for 20-30 min, use the transfer pipette to fill the colored NPK containers (6 total for the polluted and clean samples) up to the marked line. Label them, and then pull apart the colored pills over the matching colored containers and add the powders.

****use a different transfer pipette for each of the three sets. Always transfer DI water to the pH container FIRST before transferring the soil water from the beakers to the NPK containers.****

6. Thoroughly shake the six labeled NPK (*do not shake the pH container*). Wait for 10-15 minutes before recording results on page 7. Record any differences in the soil particles observed within the containers on page 8.

7. Perform steps 5 & 6 on the school campus sample. While waiting for the macronutrient and pH results, clean your station. Thoroughly wash the beakers, colored containers, and transfer pipettes. Only throw away the colored pills into the trash can. Dispose of the soil into designated waste baskets.

8. Record all results for the three sample sets, then share data using a classroom-wide excel sheet. The excel sheet will be e-mailed after the lab for analysis.

Results and Discussion

In order to record the results of the Soil Pollution lab, scores will be assigned to each readout on the colored NPK containers. Samples with the "Depleted" color will be given a score of 0, while samples in "Surplus" of N, P, or K will be given a score of 5. pH already has values and will be recorded as is with no score.

****Some samples may give darker colors that go beyond the 7.5 value listed. Ask the teacher before recording a pH higher than 7.5. Also, some colors for NPK may appear to be inbetween the 5 readouts listed on the container. It is OK to assign a +0.5 score to these such as 2.5 or 3.5. Don't assign values such as 1.43 or 4.28370451394887.****

Sample Set	pH	N	P	K
Towsley Polluted Sample				
Towsley Clean Sample				
School Campus Sample				

Additional observations (record any observations about the soil particles in the colored containers used during the experiments).

******Attach the 4 bar graphs made from the averaged values (pH & NPK) in Google spreadsheet to the lab and answer discussion questions #1-4******

1. Did your school campus soil sample have ideal soil health according to the pH/NPK indicators? If so, why? If not, where would be an ideal place, locally, to collect ideal samples? Explain why the control may not have been ideal.

2. How did the polluted Towsley values for the chemical indicators tested differ from the clean Towsley values? In what ways were they the same?

3. Were there any tests where the colors for specific indicators did not conform to the color chart provided? Which ones were they, and how do those values compare to values recorded by other groups? Explain.

4. What conclusions can be made about the effects of crude oil on soil health at Towsley Canyons? What indicators were not affected?

Post Lab Questions

-Use knowledge gained from the lab background, the lab results, and class to answer the following questions.

1. DI water stands for De-ionized water, whereby ions are removed from water. Why is it so important that the water is controlled for this experiment? How could tap water affect the results and conclusions of this experiment? (be specific about the ions in question).

2a. In life-science research there are two ways to assess data, qualitatively and quantitatively. Qualitative essentially means that the senses are being used, and this experiment used eyesight to detect color changes. Even though scores 0-4 were used to identify the NPK concentration within soil, it cannot be truly quantitative as no exact values were involved (mg/ml or mg/kg for example)

Below are the results of a spectrophotometer used in the same experiment as this lab. A spectrophotometer is a tool that gives quantitative values to liquid samples by firing a light beam through a sample. As seen in Figure 5, one the other side of the sample is a reader that will measure the difference in the laser's intensity after traveling through the sample.

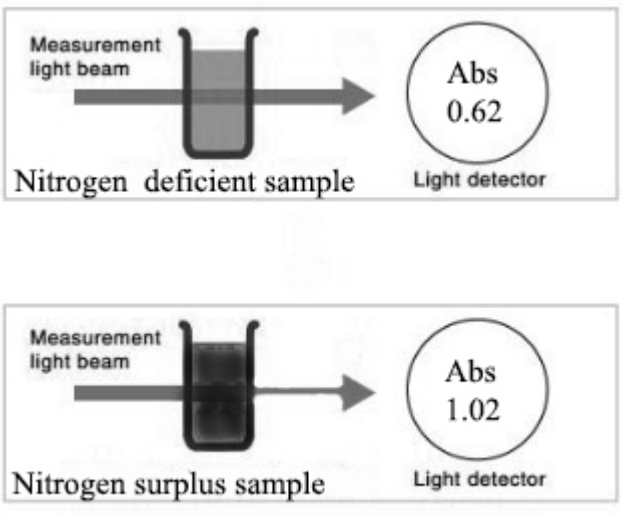


Figure 5. Two soil samples with different Nitrogen concentrations will absorb the light beam differently. Samples with higher concentrations will absorb MORE of the light beam. [Shimazdu.com]

Each sample will have an absorbance value (Abs) which for this experiment will correspond to how dark the color of each indicator is. So a polluted soil sample with a Nitrogen score of 2 (looking just slightly purple) will have a low Abs value. A soil sample with a score of 4 (dark purple) will have a higher Abs value.

Absorbance	**Nitrogen Concetration (mg/kg)**
00.6	0
0.17	1
0.33	2
0.52	4
0.74	8
1.4	10

Unknown Soil Samples Absobance Values: *a.* 0.47 *b.* 0.11 *c.* 1.33

Use google spreadsheet to create a scatter plot of the spectrophotometer data. Create a linear regression line and attach the graph with the equation to the lab. Use the resulting equation to determine the exact Nitrogen concentration of the three unknowns (a, b, & c).

2b. What benefit does the spectrophotometer provide over the color charts used in this experiment? How can this tool be helpful when soil health indicators do not have a convenient powder to change the color of the sample, and the sample is clear?

2c. Which method do you think is appropriate for experiments with clear samples, qualitative or quantitative? Why?

****Figure 6 below is one page from the International Letters of Natural Science, a scientific journal. It is the results section of a published paper regarding the remediation of soil affected by crude oil. Unlike this lab module, the published paper mixed their own soil and crude oil rather than take them from a site. These scientists wanted to measure the effects of two different remediation techniques on soil health and see if/to what extent the remediation worked. Use the picture and the knowledge you have gained to answer question 9 on experimental design.****

The electrical conductivity ranged from 35.30 – 83.54 µS/cm. Also there was an increase in the E.C of the soil in the plots even after [redacted] days of pollution and remediation when compared with plot A and B that was [redacted] and [redacted] respectively. The increase in the E.C could be attributed to introduction of the crude oil and subsequent addition of the [redacted] to the plots. A similar observation on the changes in the E.C. of crude oil contaminated soil has been reported [45].

Table 1: Physiochemical properties of the soil after [redacted] days of remediation

Plots	pH	E.C µS/cm	PO^{3-}_4, mg/kg	P mg/kg	Organic C %	Total N %	C/N
A	5.32	35.30	21.80	6.62	0.15	0.27	0.56
B	5.96	46.14	11.57	5.02	0.41	0.17	2.41
C	5.65	83.54	13.38	5.67	0.45	0.20	2.25
D	5.76	83.38	12.63	5.67	0.42	0.19	2.21
E	5.57	82.03	13.75	6.04	0.39	0.22	1.77

Table 2: Physiochemical properties of the soil after [redacted] days of remediation

Plots	pH	E.C µS/cm	PO^{3-}_4, mg/kg	P mg/kg	Organic C %	Total N %	C/N
A	5.37	35.70	21.82	6.54	0.14	0.28	0.50
B	5.86	48.31	11.53	5.25	0.40	0.14	2.22
C	7.03	56.24	14.76	5.86	0.38	0.18	2.11
D	6.74	73.68	13.57	5.43	0.34	0.16	2.13
E	6.98	48.05	15.05	6.23	0.32	0.20	1.60

The results indicate slight differences in the soil phosphate as shown on table 1 – 2 and fig.4, the values ranged from 11.53 – 21.83 mg/kg. The [redacted] and the [redacted] which were remediated had higher phosphate level than the [redacted] plot which was not remediated

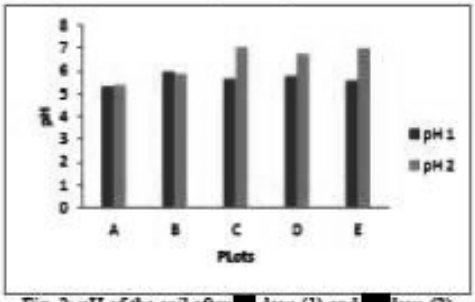


Fig. 2: pH of the soil after [redacted] days (1) and [redacted] days (2)

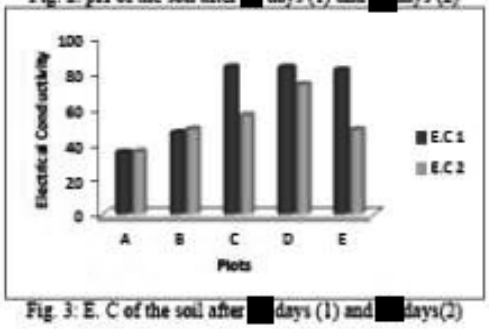


Fig. 3: E. C of the soil after [redacted] days (1) and [redacted] days(2)

Figure 6 (source)- Ibe, Francis Chizoruo, Bridget Onyekachi Ibe, Chinyere Benardette Chinaka Ikpa, and Magaret Chinyelue Eneldoh. "Remediation of Mild Crude Oil Polluted Fresh Water Wet Land with Organic and Inorganic Fertilizer." International Letters of Natural Sciences ILNS 54 (2016): 75-84.

9a. Research and choose two methods of soil remediation. Create an experiment based on how you think figure 6 was designed, and test the effects of soil remediation on soil polluted by crude oil. HINT: (How many plots did figure 6 use, and what sample sets would they represent? What would the controls be, and how many are needed? What indicators would you test? What equipment would you use to test them? How would you analyze the results? After how many days of remediation would you test for?). Defend each of your reasons within the experiment.

9b. Why are the indicators tested twice after ___ & ____ days? What is the benefit of testing them more than once?

9c. Why would a spectrophotometer be needed in determining whether soil remediation is working/how effective?