Stinking Mud: An Introductory Soil Science Laboratory Exercise Demonstrating Redox Reactions in Flooded Soils

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ABSTRACT

Essential for life, oxidation–reduction (redox) reactions can be considered the most important chemical reactions on the face of the Earth. Redox reactions are often difficult for students to understand and common demonstrations used to explain redox reactions, such as voltage cells with salt-bridges, often complicate the learning process. We propose that a laboratory exercise using flooded soil is a better tool to introduce redox reactions to introductory soil science students. In the laboratory, simulated flooded soil conditions were prepared in microcosms to give a time series of decreasing redox (20 min, 1 d, 1, 2, 3, and 4 wk). Students measure dissolved oxygen (DO), nitrate (NO_3^-), and ferrous iron (Fe^{2+}) concentrations, and the presence or absence of hydrogen sulfide [$H_2S(g)$] in the flooded soil sequence. In addition, students observe the changes in soil color and odor that accompany the changes in redox chemistry. As a result, students were able to associate the intense odor of H_2S and black sediment in the microcosms to soils with aquic conditions. Finally, the students prepared a laboratory report that consisted of a written interpretation of their data. This exercise improved students' understanding of redox reactions and potentials, the concept of electron donors and acceptors, and promoted student interest in soil microbiology and biogeochemistry. This lab exercise is easily expanded for use in upper division soil chemistry classes.

IN NATURE, redox reactions are significant in a number of areas, including biological energy production, soil formation, reclamation of metals from low-grade ores or toxic metallic wastes, acidification of mine waste water, and production of aquatic and atmospheric pollutants. Students are generally first exposed to oxidation–reduction (redox) reactions in general chemistry courses via a galvanic cell demonstration. Galvanic cell demonstrations made from a salt bridge and metal electrodes are hard to grasp conceptually and are far from any natural environmental situation. Within the soil science curriculum, most students are introduced to redox reactions by introductory soil science exercises involving the identification of redoximorphic features, such as mottles and low chroma colors (Vepraskas, 1996). These features are very important in the taxonomic classification of hydric soils (Vepraskas and Sprecher, 1997) or soils with aquic conditions (Soil Survey Staff, 1994). However, exercises emphasizing physical features are not effective in demonstrating the biogeochemical and redox processes involved in the formation of redoximorphic features.

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In soils, the cycling of chemical elements is largely a result of microbial respiration, whereby energy is obtained from the oxidation of reduced substrates and reduction of oxidized molecules. The most notable elements that are cycled are C, N, S, and P, and a variety of other trace elements such as Fe, Mn, and Se. The majority of biologically mediated redox reactions that occur in the natural environment lie between the reduction of O_2 and CO_2, many of which can easily be reproduced under laboratory conditions. Therefore, if we focus on redox reactions that can be found in the natural environment, such as a flooded soil, we can then find a vast array of interesting and relevant redox reactions that students can understand.

Turner and Patrick (1968), using a flooded soil apparatus, determined sequential changes in redox chemistry that occur in soils as a result of oxygen depletion. Tokunaga et al. (1996) used a specially designed laboratory column to monitor the reduction of selenate (SeO_4^{2-}) to elemental Se (Se^0) in sediments, by ponding seleniferous water over previously uncontaminated soil. Therefore, a laboratory exercise on flooded soils has practical *real world* applications, and we propose that it is a better technique to introduce students to redox reactions and microbial processes that occur in anaerobic soil systems.

MATERIALS AND METHODS

The exercise was designed for approximately 15 students in a 3-h laboratory period. The majority of the students enrolled in the course were junior and senior environmental science majors. All students in the course had previously completed an introductory chemistry course and were concurrently enrolled in a soil science lecture course. With the small number of students the instructor could observe and assist those who were having difficulties. Grades for the assignment were based on an individual laboratory report that included a table of results, graphical representation of the results, and written interpretation of the data.

Laboratory Preparation

The students prepared a series of six flasks that were sequentially flooded over 4 wk so that all measurements were made during one laboratory period. Equipment and supplies required for this laboratory exercise are listed in Table 1. In our case, students were divided into five groups of three students. A week before the laboratory, each of the soils were collected, sieved using a large mesh screen, and then stored under field-moist conditions at 5°C. The day before the laboratory, each of the soils were allowed to air-dry overnight at room temperature. Air drying of the soil is not a critical step and if desired, field-moist soil can be substituted for air-dried soil without creating any problems.

Abbreviations: DO, dissolved oxygen.

Table 1. Equipment and supplies needed to set up one flooded soil experiment for each group of three students.

Quantity	Description
>300 g	Soil amended with gypsum and alfalfa
6	250-mL Erlenmeyer flasks
6	Gas lock apparatus (20-cm narrow tubing, drilled no. 6 rubber stopper, 60-mL polyethylene bottle)
6	Nitrate (NO_3^-) quantitative test strips
6	Ferrous iron (Fe^{2+}) quantitative test strips
6	Lead acetate (PbOAc) strips
1	Dissolved oxygen detection probe
3 m^2	Aluminum foil

Almost any surface soil will work, provided it has an adequate Fe and Mn content.

Before the laboratory exercise, the soil had been treated with the following amendments (if time permits, the students should amend the soil themselves): (i) dried and ground plant material [20 g alfalfa (*Medicago sativa* L.) kg^{-1} soil], which serves as a C and energy source for the indigenous soil microbes and is the electron donor, and (ii) gypsum (10 g $CaSO_4 \cdot 2H_2O$ kg^{-1} soil), which provides sulfate as an alternative electron acceptor. Alternative C sources, such as sugars (e.g., glucose, fructose, and sucrose), should be avoided since the redox reactions will occur too fast. Although other plant material could be substituted for the alfalfa, the high N content of the alfalfa results in a measurable nitrification reaction and the appearance of NO_3^- a few days after flooding. To date, no other plant materials have been tested in this laboratory assignment. If alternative plant materials are to be used, it is recommended that plants high in N are chosen.

Each group was provided with 350 g of an air-dried agricultural soil. Four weeks before the final laboratory exercise, students weighed 50 g of their amended soil into each of six 250-mL Erlenmeyer flasks. During this laboratory period, 175 mL of water was added to one flask that would become the *oldest* or *longest flooded* soil (see water addition schedule, Table 2). The flasks were then capped with rubber stoppers fitted with a gas lock to prevent O_2 from diffusing back into the flasks while allowing CO_2 to escape (Fig. 1). A lead–acetate (PbOAc) strip, used to identify the presence of H_2S(g) in the headspace, was attached between the rubber stopper and the flask rim, hanging above the soil–water

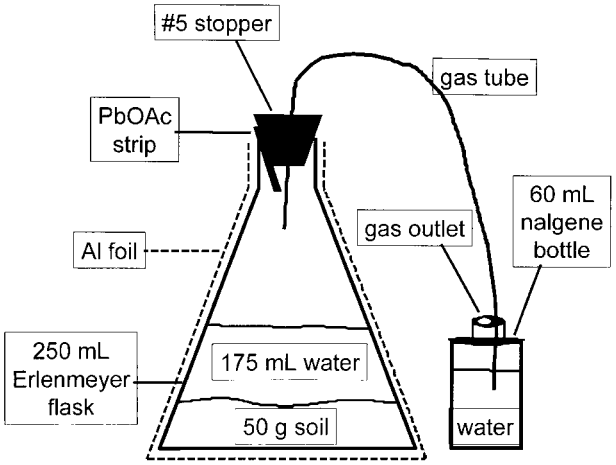

Fig. 1. Soil–water microcosm and gas trap apparatus.

Table 2. Time schedule for addition of 175 mL of distilled water to flasks to begin experiments and allow all measurements to be made during the fourth laboratory class.

Flask no.	Time submerged
1	4 wk
2	3 wk
3	2 wk
4	1 wk
5	1 d
6	20 min

suspension (Fig. 1). The PbOAc paper strips are available from Fisher Scientific (product no. 14-862). The gas trap is essential for maintaining anaerobic conditions and ensuring the reduction of the alternative electron acceptors. All sample flasks were incubated at room temperature under static conditions and covered with aluminum foil to prevent algal growth. Each week the students added water (175 mL) to the flasks according to the schedule in Table 2. On the day of the laboratory exercise, each group had a time series of a flooded soil to analyze.

Laboratory Lecture

On the day of the laboratory exercise a brief, 30-min discussion was given to acquaint the students to the concept of oxidation–reduction, nutrient cycling, and microbial energetics. The students were introduced to the terms *oxidation* and *reduction*, and were reminded that these reactions are always paired and are commonly called *redox reactions*. The following example was provided to the students to demonstrate what a typical balanced redox equation looks like:

$$NH_4^+ + 3H_2O \rightarrow NO_3^- + 10H^+ \quad \text{(oxidation)}$$
$$8e^- + 8H^+ + 2O_2 \rightarrow 4H_2O \quad \text{(reduction)}$$
$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad \text{(net equation)}$$

The students were exposed to a table of redox pairs, which listed common redox reactions that occur in soil and water environments, especially those relevant to the laboratory exercise. Excellent references that describe nutrient cycling and soil microbiology are Paul and Clark (1996) and Coyne (1999), while information on redox chemistry can be found in Sparks (1995) and Stumm and Morgan (1996). In an oxic soil, O_2 is the electron acceptor, while in an anoxic soil, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2, and organic intermediates can act as electron acceptors. The redox potential (often expressed as Eh and measured in mV) is a measure of the relative electron activity of the solution and determines which terminal electron acceptors the soil microorganisms will use. The redox pairs with the highest standard electrode potentials will be used before those with lower potentials. For example, O_2, the strongest oxidant, with a standard electrode potential of 820 mV, will be used before SO_4^{2-}, with a standard potential of –220 mV. In addition, understanding redox potentials is important in determining the solubility of metals in soils (Moore and Patrick, 1989; Amrhein et al., 1993).

Furthermore, a brief description of aerobic, facultative anaerobic, and anaerobic microbial respiration was provid-

ed to better help those students that had not previously taken a course in microbiology. Students were encouraged to view the above-mentioned references for additional information. In addition, a laboratory handout that contained all information necessary to understand and complete the lab was provided to the students.

Analysis of Samples

After the brief laboratory lecture, students recorded visual observations such as soil and solution color, and color of the PbOAc strips in each of the flasks. All observations and chemical data were recorded on a table provided by the instructor (Table 3). During the laboratory exercise, there was some student confusion over how to qualitatively assess the color of the PbOAc strips since some of the strips were discolored only around the edges. The students were instructed that the strips had to be fully discolored; darkening around the edges of the strip did not positively confirm the presence of H_2S. Each of the student groups were free to come up with a strip color scale of their own. Most students used color scales (e.g., white, light brown, and black), while other students used a series of plus signs (e.g., +, ++, and +++), with darker colors or more plus signs indicating a higher concentration of H_2S in the flask headspace.

The students measured the dissolved oxygen (DO) concentration with a DO electrode (model 820 dissolved oxygen meter, Orion Research, Boston, MA). The students were instructed not to open the flasks until they were ready for the DO reading. The DO electrode must be calibrated before use, which usually takes less than 5 min to perform. The calibration time will vary slightly depending on the type of DO probe being used. If fresh electrolyte solution is being put into the electrode, caution must be taken to prevent the trapping of air bubbles in the probe tip. If the DO reading fluctuates greatly without stabilizing, the user should check for air bubbles in the probe tip. If air bubbles are present, the probe tip should be removed and additional electrolyte solution should be added to flush out the air bubbles. The DO electrode should then be recalibrated.

Once the flasks had been opened, the Fe^{2+} and NO_3^- concentrations were determined using chemical test strips [product no. EM-1004-1 (Fe^{2+}) and EM-10020-1 (NO_3^-), VWR Scientific]. The concentration of NO_3^- and Fe^{2+} is determined by directly comparing the color of the test strip to a color scale. Nitrate and Fe^{2+} concentrations were estimated when the color of the chemical test strip fell between that of the color scale. Use of the chemical test strips is very straight forward and no problems were encountered by the students during their use.

Using the same time-series samples, additional analysis are easily adapted for use in a more advanced class. These analysis include: Eh measurement using a platinum electrode, quantification of sulfide with a specific ion electrode, soluble Mn by atomic absorption spectrophotometer, pH, alkalinity, and determination of the partial pressure of CO_2 by calculation using the pH and alkalinity. Additional analyses for redox-sensitive trace elements have been done on occasion with contaminated soils. At various times we have measured selenium (Se), arsenic (As), uranium (U), and vanadium (V) in solution in these flooded samples.

Table 3. Example of results obtained from a flooded soil.

Time	O_2	NO_3^-	Fe^{2+}	H_2S strip color
	mg L^{-1}			
20 min	7.2	10	0	White
1 d	1.4	50	0	White
1 wk	0	10	3	White
2 wk	0	0	7	Light brown
3 wk	0	0	15	Black
4 wk	0	0	7	Dark black

Laboratory Assignment

From the data collected, students were asked to plot the changes in solution chemistry of O_2, NO_3^-, and Fe^{2+} vs. time and interpret their results by writing a two-page laboratory report. In their report they were asked to include a brief introduction, results (data table and graph), and a discussion section. They were instructed to be specific about the order in which electron acceptors were used, based on their redox potentials. They were also asked to explain how their visual observations were related to the redox state of flooded soils.

RESULTS AND DISCUSSION

All 15 students were able to generate a graphical representation of their chemical data. Although the chemical concentrations varied among each of the soils, especially those of NO_3^- and Fe^{2+}, the trends produced were similar. A typical graph and data table produced by the students is shown in Fig. 2 and Table 3. The O_2 was rapidly depleted and none could be detected after Day 1; NO_3^- was initially low but rose after 1 d of flooding, due to nitrification reactions, and then declined through Week 2, due to denitrification. After 1 wk, the Fe^{2+} appeared in solution and peaked at Week 3, but then declined by Week 4. The decline in Fe^{2+} after 3 wk was linked to the formation of FeS. After 3 wk the PbOAc strips began to discolor indicating the presence of H_2S. Because of the graphical representation and PbOAc strip results, all of the students were able to positively identify the order in which the terminal electron acceptors were used (i.e., O_2 first, followed by NO_3^-, then Fe^{3+}, and finally SO_4^{2-}). Similar changes in redox chemistry of a flooded soil were observed by Turner and Patrick (1968).

Of additional importance to the chemical data are the visual observations and odors associated with each of

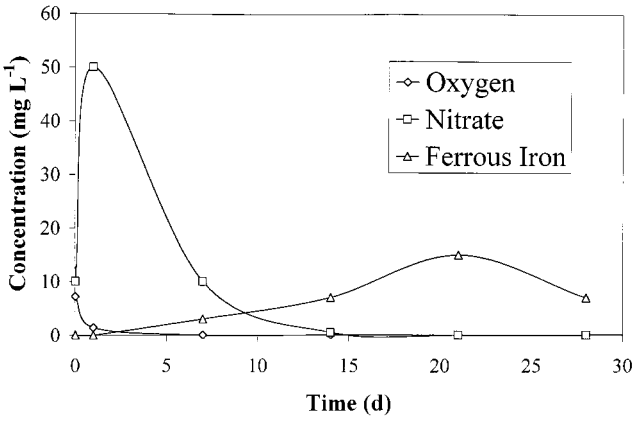

Fig. 2. Typical solution chemistry figure generated by students.

microcosms. The students were able to associate the intense odor of H_2S with the blackening of the PbOAc strips. Many students instantly recognized the odor and associated it with lake or pond sediment or wet, boggy soils. Visual evidence supporting redox reactions came from the black sediment that formed after 4 wk, confirming the production of FeS and other associated sulfide minerals.

CONCLUSIONS

This laboratory exercise provided a chemical, visual, and olfactory demonstration of redox reactions. Not only were the students able to relate to the environmentally relevant redox reactions, but they were able to observe how the reactions impacted the soil–water mixture. This created an ideal learning situation since most redox exercises are not effective in combining visual and olfactory stimulating demonstrations with chemical data in one exercise.

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