Utilizing Indicator of Reduction in Soils Tubes to Affirm a Serpentinitic Hydric Soil on the California Central Coast

A Senior Project Presented to

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Soil Science; Bachelor of Science

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Abstract

Wetlands are vital ecosystems that are crucial in maintaining the life of rare and unique soils, plants, and animals. These ecosystems are key players in water storage, water filtration, carbon storage, and harboring unique species. Since the intervention of human development on the Earth's surface, almost 50% of the Earth's original wetlands have either been damaged or destroyed. The identification and assessment of both new and old wetlands is crucial in the survival of these precious ecosystems and their conservation. A 3 month-long study was performed to confirm the hydric status of a soil derived from serpentinitic parent material. The serpentinite study area was confirmed to have wetland hydrology and vegetation, but due to the unique genesis of the soil, no hydric soil indicators were met. The incorporation of indicator of reduction in soils (IRIS) tubes was used to examine the potential reduction of iron in this problematic soil. All IRIS tubes were examined using Adobe Photoshop to obtain exact percentage amounts of reduction and oxidation. It was confirmed that the site is a wetland, with each extraction week providing more than 3 tubes with more than 30% iron reduction within 30cm of the soil surface. The study also incorporated a test of the human eye to the photoshop analysis to see the differences when analyzing IRIS tubes via those methods. It was found that there is a lack of study of serpentinitic wetlands and even more so, the study of sulfur reduction in these unique wetland ecosystems. Further studies could be performed on this site, or a similar site, to study the effect of serpentinite on wetland soils and sulfur reduction.

Keywords: Wetlands, USDA, Army Corps of Engineers, IRIS tubes, serpentinite

Literature Review

Wetland ecosystems are a unique and rare sight to any human no matter their interest. Wetlands consist of vegetation that can be seen in no other ecosystem, due to the adaptation of plants to hydric soil conditions. The soils in wetlands have been developed through years of fluctuating water inundation and unique microorganisms. Finally, wetlands also provide a home for many unique fauna that thrive in the strange hydrologic conditions brought on by wetlands. These special ecosystems are not only breeding grounds for unique soils and life forms, but they are also beneficial to humans (for recreation and aesthetic purposes) and other ecosystems surrounding wetlands. One of the largest contributing factors to the maintenance and importance of wetlands are the other hydrologic connections linked to the ecosystem; a wetland does not need to have standing water to become a wetland, therefore standing water does not constitute a wetland. Overall, wetlands are literal cradles of diverse life and one of the world's most productive ecosystems and they should not be overlooked as just more land to be built upon (Niu *et al.*, 2012).

The conservation and mitigation of wetlands is crucial to the survival of many plant species, macrofauna, and even humans. Certain states, like Ohio, have lost almost 90% of their wetlands; the earth on average has lost almost half of its wetlands since the intervention of humans in wetland ecosystems. Wetlands now only compose around seven percent of terrestrial lands (Mitsch, 2005). Since the approval of the 1985 Farm Bill, the Natural Resources Conservation Service (NRCS), and other agencies within the United States Department of Agriculture (USDA), have been at work trying to conserve wetlands within the lands of private landowners as well as wetlands that had been previously destroyed by the USDA. Michigan NRCS employees, for example, perform wetland determinations by cross-referencing 1985 aerial

slides with current determinations to see if the land was altered since the 1985 farm bill. All preliminary determinations are performed off site, typically using ESRi ArcGIS. If the client has any discretion, a Soil Scientist performs a field wetland determination.

Many scientists have been using different techniques to assess wetlands other than the Army Corps of Engineer field book (Rabenhorst, 2008). Instead of observing more of the soil and vegetation, certain researchers have used biological factors such as animals and bugs to assess wetland ecosystems. To conserve wetlands, the observation of adult damselflies and dragonflies (Insecta: Odonata) in freshwater disturbed and undisturbed wetlands has been applied to determine if an ecosystem is a wetland due to the insects' prevalence in freshwater wetlands (Kutcher and Bried, 2014).

Another common method in wetland determination is the observation of the soil itself. Wetlands can be constantly or seasonally under reducing chemical conditions due to the inundation of water, typically near the surface. Anaerobic soil conditions are required in wetlands, and in order for microbes to receive energy they must use elements aside from oxygen to fuel metabolic respiration (Rabenhorst, 2008). Microorganisms follow a typical succession of elements once all oxygen is used up in an aquatic system: nitrogen → manganese → iron → sulfur, with each following element being less efficient at delivery energy than the previous. Researchers may use this succession of energy retrieval to study if a wetland really is under reducing conditions by implementing Indicator of Reduction in Soils (IRIS) tubes. They are not intended to be the only indicator of a wetland, but when a soil is high in organic carbon the observation of redoxomorphic iron features in a soil may be masked by the presence of organic matter due to their high specific surface area and charge the true face of a soil ped would be masked without chemical observations (Rabenhorst, 2008).

Indicator of reduction in soils tubes are segments of 40 Polyvinyl Chloride (PVC) tubes painted with an approximate 70% Ferrihydrite - 30% Goethite mixture which are both oxidized forms of Iron (Fe(III)) and therefore orange in appearance. The tubes will typically be placed in the soil far enough for all of the paint to be covered (prior to a large water inducing event). Removal of the tubes will typically be after the depletion of all oxygen in the soil system and observation of Iron reduction can be observed by white spots along the tube. Another indicator of reducing conditions would be the presence of black sulfur concentrations along the tube (Jenkinson, 2002).

With the advancement of imaging technology wetland scientists can cross reference LIDAR data with topographic Geographic information system (GIS) data to observe changes in wetlands over long periods of time without the need to visit the site in a field, or to recognize wetlands otherwise unrecognizable due to primitive technology. In a country such as China, where technological advances have been thriving they were able to raster map land from 80 m resolution in 1978 to 20 m resolution in 2008. Their observations overall noted that over the 30 year period a total of 33% of the wetlands were lost just by observing changes in the maps. The technique of geospatially identifying wetlands is very useful in noticing large changes (whether removal or addition of) in wetlands. This method can also be used in mapping wetlands for public use to prevent the destruction of these lands (Niu *et al.*, 2012).

Wetlands are vital habitats that perform many natural feats such as water filtration, flood prevention, water table restoration, and carbon storage. They are also home to unique and rare vegetation, animal life, and hydric soils. It is estimated that almost half of the Earth's original wetlands are diminished due to human intervention (Mitsch, 2014). Finding different techniques, other than those provided by the United States Army Corps of Engineers (USACE), for the

assessment and delineation of wetlands can help lead humans to the discovery and conservation of these precious resources. Human intervention has led to the destruction of a substantial amount of the Earth’s wetlands, however, humans have the capabilities of conserving and protecting what is left of these ecosystems.

Introduction

Wetlands are unique ecosystems that have exclusive hydrology, vegetation, and soils that set them apart from any other natural ecosystem. Since the advent of wetland conservation, wetland scientists have adapted field techniques to recognize these exclusive traits to protect and conserve these ecosystems (Mitsch, 2005). One of the main wetland conservation agencies of the United States, the Army Corps of Engineers, has advised many of its field workers on how to assess these traits. Wetland hydrology is the result of long or short-term water inundation due to a variety of reasons. The presence of wetland hydrology leads to the development of hydric soils and hydrophytic vegetation in a wetland. Hydrology indicators are typically the most obvious when delineating a wetland, some of the most noticeable indicators being surface water or fully saturated soils (USACE, 2008).

Wetland vegetation, also known as hydrophytic vegetation, consists of plants that have special physical and chemical adaptations to the saturated and anaerobic conditions presented by wetlands. This indicator uses a quantitative approach to vegetation evaluation rather than qualitative. This situation means that rather than one certain plant being present in a wetland, the amount of total plants contributes to the status of hydrophytic vegetation. Therefore, when delineating wetlands, a dominance test is used by quantifying the amount of any vegetation (hydrophytic or non-hydrophytic) at the site in question (USACE, 2008). Water inundation is not the only contributing factor to hydrophytic vegetation; factors that could contribute to these rare plant species could range from (but not limited to): climate, local weather, topography, and the soils (even without wet status). As with hydrology contributing to the potential presence of hydrophytic vegetation, the presence of hydrophytic vegetation is key in the development of hydric soils and vice-versa (USACE, 2008).

The USACE has worked in conjunction with the National Technical Committee for Hydric Soils (NTCHS) for establishing the identification of hydric soils. Hydric soils are characterized by their unique morphological features as a result of prolonged water saturation and/or the slow decomposition of organic material (USACE, 2008). In a well-drained soil, oxygen acts as the primary electron acceptor used by microorganisms; microorganisms utilize oxygen as the reducing agent when decomposing organic material in an oxidation reaction. When oxygen becomes depleted, typically in a water-inundated soil, microorganisms turn to other ions as terminal electron acceptors in order to continue organic matter decomposition. Electron acceptors are typically reduced in the order of most reduction-oxidation potential to least: NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-}, and CO_2 (Vepraskas and Faulkner, 2001).

When oxygen is depleted because of prolonged water saturation, not only are alternate terminal electron sources required, but also the decomposition of organic matter is slowed. Craft explains that in an aerobic soil system, every mole of glucose decomposed provides 38 moles of ATP (energy) when using oxygen as a reducing agent; in an anaerobic condition, every mole of glucose decomposed provides only 2 moles of ATP when using alternate electron acceptors (2001). Because of the low amount of ATP produced in anaerobic decomposition, this causes a continual accumulation of organic matter in wetland soils (Craft, 2001). Mollisols are soils that are characterized by the accumulation of organic matter in the upper horizons of the profile. When organic matter accumulation occurs in conjunction with iron reduction, it can cause the masking of the redoximorphic features on the soil peds; because of the high reactivity of organic matter with the silicate minerals within a soil matrix, the coating of organic matter on soil peds can “cover-up” any iron or manganese redox features present on the peds (Thompson and Bell, 1996).

If an ecosystem meets the conditions for wetland vegetation and hydrology, but no conditions are found that meet the criteria for wetland soils the area is not eligible to be Wetland Waters of the United States (USACE, 2008). When a hydric soil is not identifiable, but has compelling evidence for wetland hydrology and vegetation the soil in question is termed a "problem hydric soil." The NTCHS, in conjunction with the USACE, has developed alternative hydric soil indicators for use when the problem conditions are present. These problem conditions can stem from a single condition or a mix of conditions: organic matter accumulation, parent material, soil mineralogy, or even soil order (USACE, 2008).

Serpentinite is a metamorphic rock derived from the low heat and pressure transformation of the ultramafic rock, peridotite. Alexander and DuShey explain that minerals within this rock are in the serpentine mineral group consisting of: chrysotile, lizardite, talc, and (but not limited to) antigorite; these minerals are rich in silica content and unusually high in magnesium (2011). This strange content matrix is caused because of the metamorphosis of the original minerals olivine and pyroxenes, which contribute to the green hue of the rock (Alexander and DuShey, 2011).

The study of serpentinitic derived soils is uncommon and therefore the study of wetlands formed upon serpentinite parent materials is even sparser. Typically, well developed serpentinite soils have a larger clay increase as the profile gets deeper (Lee *et al.*, 2004). This clay increase can be attributed to the extent of weathering on the serpentine minerals to produce smectitic minerals (Lee *et al.*, 2004). Serpentinite soils that are less developed still have a clay increase deeper in the profile, but tend to have a higher coarse fragment content in the soil. Soils that are poorly drained and have been derived from serpentinitic parent material also tend to show higher amounts of organic carbon content throughout the profile – 7-15% in the A and greater than 2%

as the profile gets deeper (Lee *et al.*, 2004). The presence of organic matter can inhibit the formation of secondary iron-oxide formation, which can lead to gley soil colors (Lee *et al.*, 2004). However, the presence of gley soil colors does not mean that a soil is automatically considered a hydric soil.

To counteract the masking of redox features or the interactions of parent material with iron, an alternative approach was created to delineating hydric soils. Created by Jenkinson, IRIS tubes are lab created alternatives to observing the interactions of iron-oxides with the soil matrix (2002). Castenson and Rabenhorst created the technical standard for IRIS tubes and it is accepted by the NTCHS (2006). The standard states that, with at least 3 of the tubes, there has to be at least 30% iron reduction in the top 30cm of the tubes (Castenson and Rabenhorst, 2006). This study employs the IRIS tubes to study the potential iron-oxide reduction in a serpentinite-derived soil. The objective was to observe if any reduction was present in a site with a problematic soil. A technical note is also incorporated into the study to compare the observations of the human eye and the observations of a computer to deduce the reduction of iron on the IRIS tubes. It was hypothesized that with the presence of wetland hydrology and hydrophytic vegetation that the IRIS tubes would confirm that the site is, in fact, a wetland.

Materials and Methods

1. Study Area

The problematic soil in question is located on the Serrano Ranch on the northern California Polytechnic State University, San Luis Obispo, campus (a portion of the Upper Stenner Creek Watershed). It is in the northeast quarter of section 12, township 30 south, range 12 east of the San Luis Obispo Quadrangle. The site is in close proximity to a non-county maintained portion of Stenner Creek Road, which is easily accessible through highway 1 (Fig.1, 2). Soils mapped at the site are mapped as unit 164, Los Osos-Diablo Complex, 15-30% slopes;

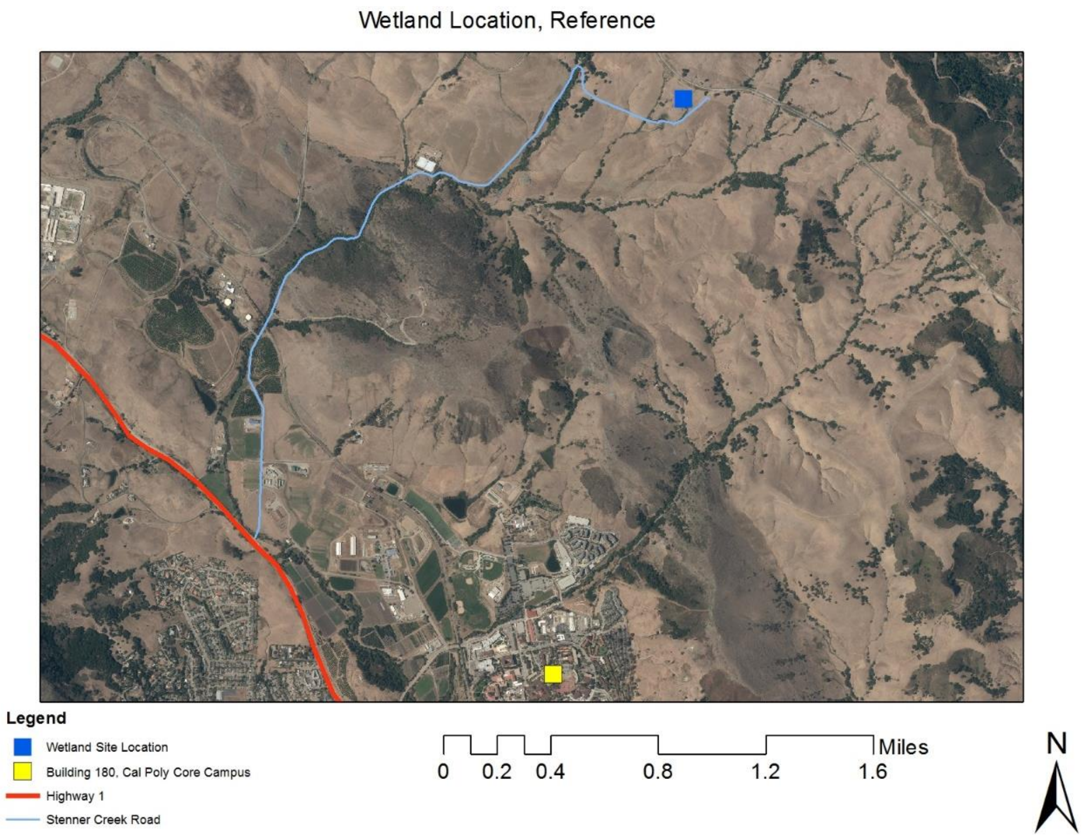

Figure 1. Location of study site. Using Highway 1 and the Warren J. Baker Center for Science and Mathematics as reference, the site is located on the northern Cal Poly campus.

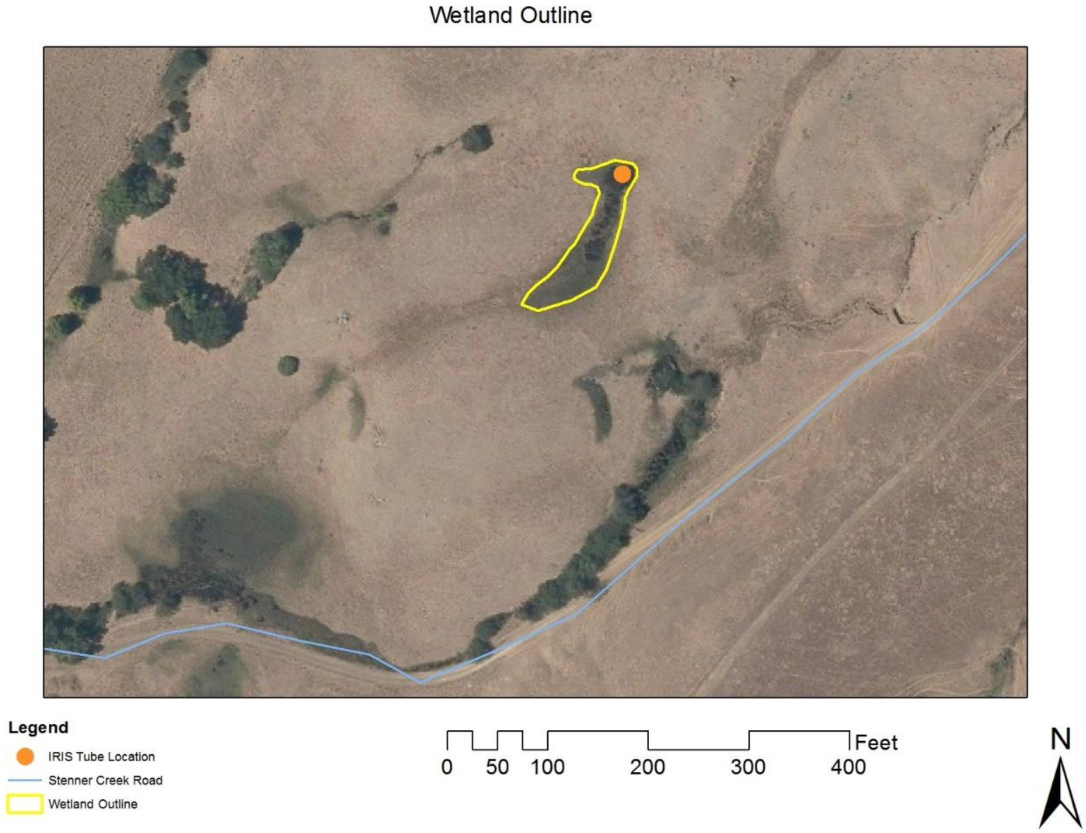

Figure 2. Outline of the study site with close proximity to the unmaintained portion of Stenner Creek Road.

the soil is mapped as a complex of the Los Osos Series (Typic Argixerolls) – a Mollisol with an argillic horizon – and the Diablo Series (Aridic Haploxerert) – a Vertisol with 5 mm thick cracks present for more than half of a normal year. The site is located on a backslope and has an approximate 13% slope (clinometer measurement) (Fig. 3).

Topographic Map

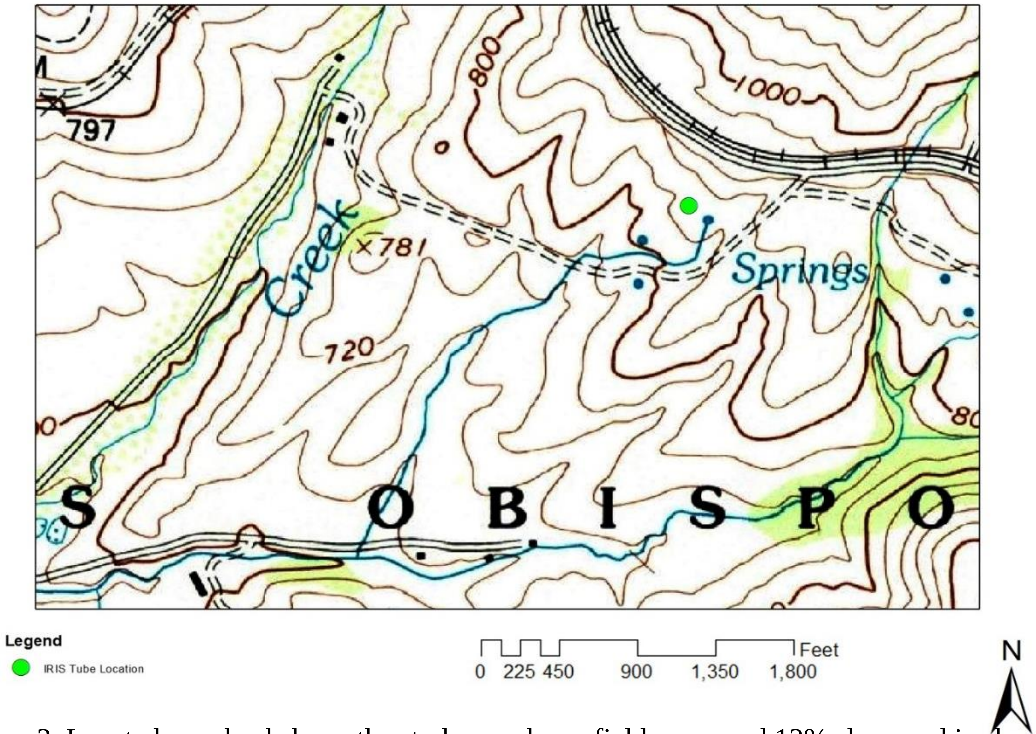

Figure 3. Located on a backslope, the study area has a field measured 13% slope and is closely located to a drainage path on the southeast. The green dot represents the placement of the IRIS tubes at the study site.

2. Laboratory Preparation

The main measurement method used in the study was the implementation of the IRIS tubes. The procedure was a slightly modified form of the original procedure of Jenkinson (2002). They are created from 0.5-inch schedule 40 PVC pipes, which were cut into lengths of 61 cm. The original procedure states to cut into 60 cm segments, but the original pipe lengths, when bought, were 305cm in length – easily divisible by 61cm when making cuts. Once the tubes were cut, they were cleaned using Klean Strip™ Acetone (paint thinner) in order to remove the original labeling on the PVC pipe.

Preparation of the IRIS paint began with the dissolution of 32 g of anhydrous $FeCl_3$ in 1000 mL of deionized water. Once the solid dissolved in the water, approximately 500 mL of KOH was titrated with the solution and brought to a pH of 8; this process allows for the partial precipitation of crystalline Goethite (30%) with amorphous Ferrihydrite (70%). As a result of the

titration, aqueous KCl remained in solution and had to be removed through centrifugation. The remainder of the excess salts was removed by the implementation of dialysis tubing in a deionized water bath. To assure no salts remained in the iron paint, the water bath was tested with $AgNO_3$: if no silver precipitated into the water then the paint was ready to be removed from the tubing. To achieve optimum consistency the paint was centrifuged multiple times to remove any excess water from the paint. All paint was kept in an enclosed glass container at 40°F (4°C) to slow water evaporation and therefore, paint solidification (Jenkinson, 2002) (Rabenhorst and Burch, 2006).

Painting of the tubes began with the sanding of the PVC pipes. A drill with a rubber stopper attached was used to spin the PVC pipe to allow a uniform concentric pattern of sanding and painting. The tubes were attached to the drill and sanded using very fine sand paper (~220 grit) to allow easy adherence of the paint to the pipe. A black line was made 10 cm from the end of the tube. The black line represented the 50 cm of paint to be applied onto the tube. Using a 2 in wide foam brush, the paint was applied in one coat to the pre-sanded PVC pipe; paint was applied above the black line to observe reactions that could potentially take place with any standing water (Jenkinson, 2002).

In order to analyze the effect of organic matter on visible redox features, carbon and nitrogen content was analyzed. After collecting soil samples from each horizon, 1000 mg of each horizon was incinerated in a Vario Max Carbon-Nitrogen analyzer.

3. Field Work

A preliminary site visit was performed in October of 2013 to assess the condition of the "wet" site. A small hole was dug to assess the physical characteristics of the soil in question. A field wetland determination was performed using the U.S. Army Corps of Engineers (USACE)

Wetland Determination Data Form (Version 2.0) – Arid West Region in conjunction with the USACE Regional Supplement to the Corps of Engineers Wetland Delineation Manual: Arid West Region (Version 2.0). The surface field determination was performed to assess vegetation at the site: the vegetation was delineated based on surface ground cover within a 10 ft radius of the IRIS tubes.

The field study began on January 23, 2014 and lasted until March 20, 2014. Fieldwork consisted of placing 5 IRIS tubes in a north-south orientation; using a T-push auger, small holes were made to allow easy entry for the IRIS tubes without scraping the paint and still allowing enough soil to tube contact. The tubes were placed in close proximity to one another – approximately 5 cm apart. Every two weeks (exactly 14 days) the tubes were removed and were replaced with a new set. On the first replacement date (February 6), the same holes were used for the IRIS tubes. All replacement dates after February 6, new holes were made for the IRIS tubes. A Decagon Devices Incorporated EM50 Data Logger was used in conjunction with 4 Decagon ECH_2O-EC Probes to measure soil electrical conductivity, temperature, and volumetric water content. Readings were taken twice a day. The probes were placed in the center of each morphological horizon throughout the soil profile. A bucket-auger hole was made at the beginning of the study to observe the water table level and any potential wetland hydrology. The hole was covered with wood to prevent any loss of water through evaporation or addition of water through precipitation (Fig. 4).

Figure 4. The pallet of wood covering the groundwater monitoring well, the Decagon Devices Inc. EM50 data logger, and the 5 IRIS tubes oriented south (top of photo) to north (bottom of photo). Photograph taken on March 6, 2014.

4. IRIS Tube Photoshop Analysis

After the IRIS tubes were removed at the end of each 2 week period they were gently rinsed, in a lab, with deionized water to allow for clean photographing of the tube. If the tubes were pulled and monosulfide reduction was present, the tubes were gently cleaned, immediately after pulling, and photographed in the field.

Three photographs were taken of each tube. Each “side” of the tube was photographed in order to analyze the reduction and oxidation in the form of one image. The tubes were turned 120° to allow equal coverage of every tube. Once all photographs were taken, the three images had their background removed and turned into one photograph using Microsoft Powerpoint (Fig. 5). After every respective tube had its own image they were all analyzed, pixel by pixel

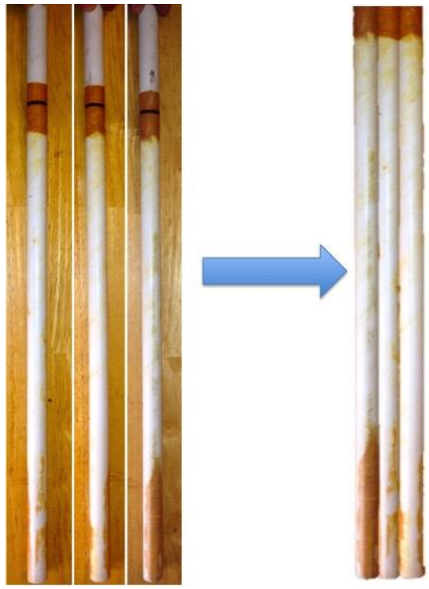

Figure 5. Before (left) and after (right) image of one tube, pulled on March 20, 2014. Made using Microsoft Powerpoint.

in Adobe Photoshop CS6. When all images were placed into Photoshop, they all subsequently had a remnant white background that could not be removed. By utilizing the “Quick Selection Tool” the number of pixels in the white background were counted and subtracted from the total picture pixels to give a “Tube Pixel Count.” The “Selection, Color Range” tool was used to select the oxidation pixel count (by selecting al pixels of the oxidation color) and turned into a percentage of the tube pixel count (Fig. 6). The reduction pixel amount was obtained by the difference in the tube pixel count and oxidation pixel count to obtain a similar percentage. This process reduced time because if the reduced pixels were chosen, the program selected the white background pixels by accident. If at any time the program accidentally chose reduced pixels for oxidized pixels, those could quickly be removed from selection by using the negative quick selection tool. If monosulfides were present on the tube, a similar process was used: oxidation

pixels were added to monosulfide reduction pixels and that difference was taken from the tube pixel count to obtain iron reduction pixels.

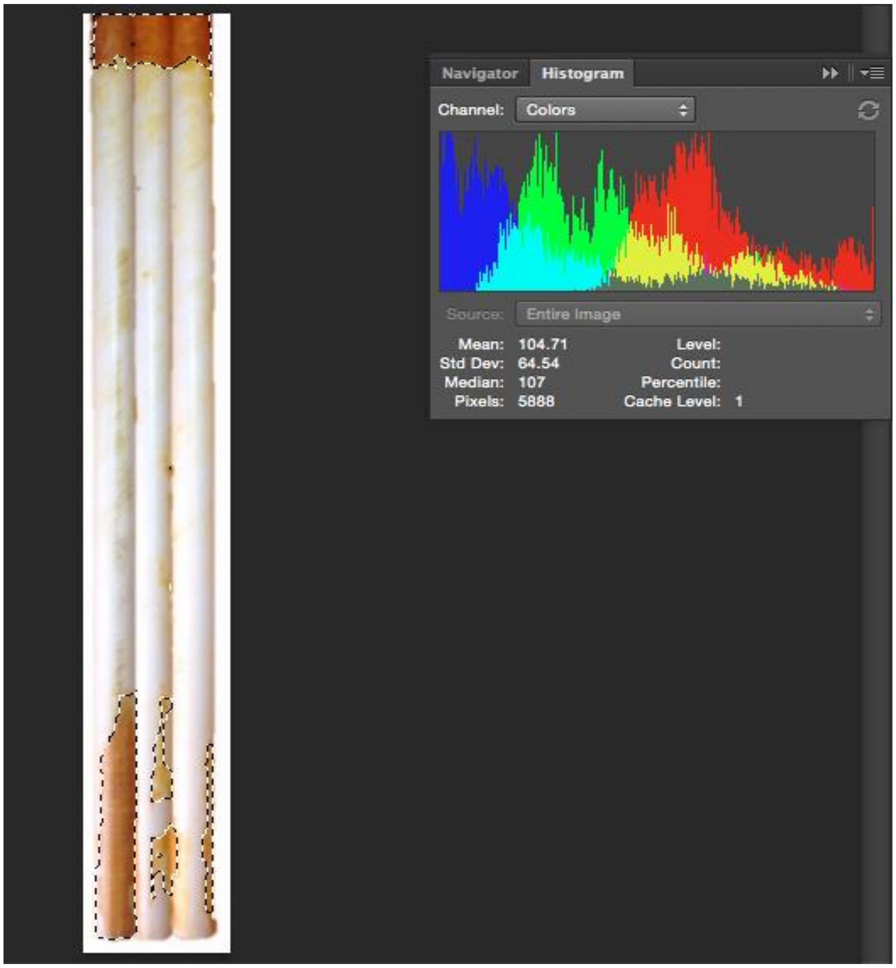

Figure 6. Using Adobe Photoshop CS6, this IRIS tube image from March 20, 2014 had its oxidized pixels counted using the selection – color range tool. The pixel count is given at the top right in the histogram feature.

5. Comparison of Photoshop Analysis to Human Eye Analysis

Ten students were sampled to use their analysis of IRIS tubes in comparison to the analysis performed by Photoshop. The students' educational background varied from Soil Science, Earth Science, Crop Science, and Fruit Science degrees. The process of IRIS tubes was explained to the students in order to comprehend the action asked of them.

Results and Discussion

1. Hydrology

Analysis of the well water during each site visit gave the result that water is present near the surface because each visit the well water was no more than 5cm away from the surface. The well water data was collected and analyzed in coordination with California Irrigation Management Information System (CIMIS) data (2014) (Table 1) (Fig. 8).

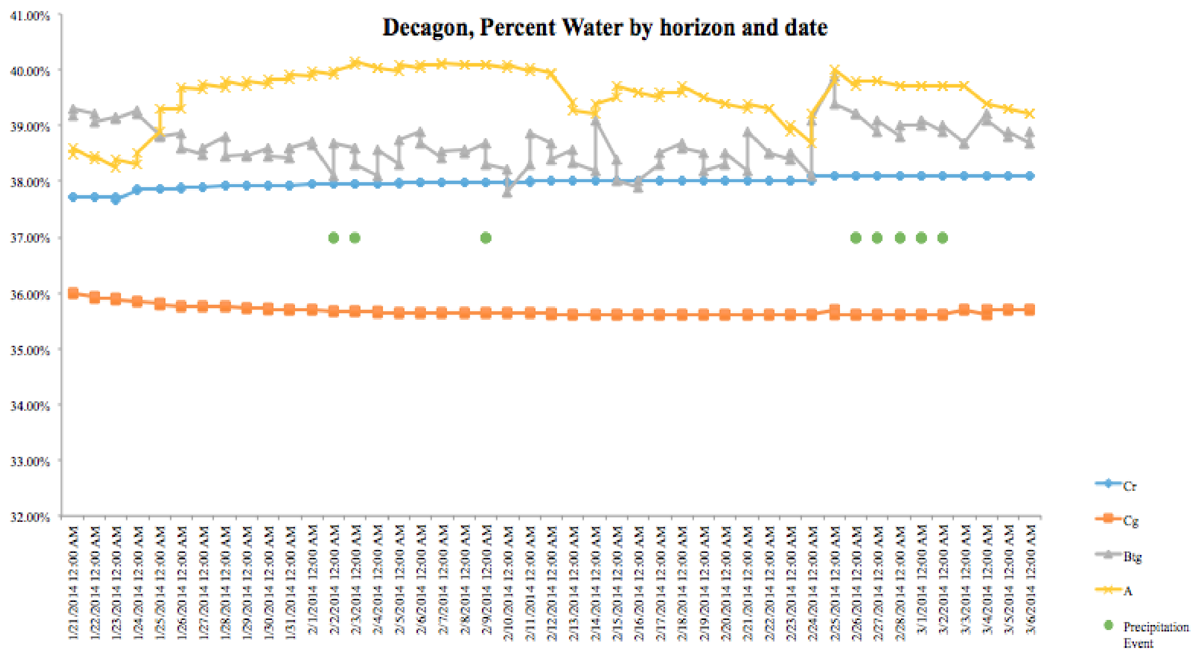

Figure 8. Comparison of precipitation events with volumetric water content in the soil. Data collected twice a day.

Table 1. California Irrigation Management Information System (CIMIS) data collected to analyze precipitation dates during the study (CIMIS, 2014).

Date	Precipitation (in)
2/2/14	0.45
2/3/14	0.13
2/9/14	0.39
2/26/14	0.86
2/27/14	0.31
2/28/14	1.8
3/1/14	0.62
3/2/14	0.19

Because the Decagon device took a few days to calibrate, the first 2 data collection days of the study were removed because of false data. Data could not be collected after March 6, 2014 because three probes had failed before my final data collection date on March 20. Analyses of the Cg and Cr horizon show it can be assumed that they were fully saturated throughout the course of the study, due to the lack of change in water content. Minor fluctuations can be seen in the A and Bt horizons throughout the duration of the study, but the fluctuation range is at a maximum of 2% volume water change, which is not a large amount. When comparing the soil hydrology data to precipitation event it is interesting to see that there was not a large interaction of the soil water with above surface water.

Throughout the majority of the study surface water was present at the wetland site. Combining visual observations with the Decagon water data allowed the deduction that the wetland site meets the criteria for Wetland Hydrology indicators A1 – Surface Water and A2 – High Water Table (USACE, 2008).

2. Vegetation

In accordance with the USACE manual for delineating wetlands, the vegetation was described to obtain a dominance test. All plant strata at the wet site were in one of the following wetland indicator categories: Obligate (OBL), Facultative-wet (FACW), Facultative (FAC), or Facultative-upland (FACU). These terms are used to generalize the probability of observing a certain strata at a site in question. Obligate is used to indicate that a certain strata almost always occurs in a wetland (<10% outside of a wetland). Facultative-wet is used to indicate that a certain strata usually occurs in wetlands, but can occur outside of a wetland (<33% chance outside of a wetland). Facultative is used to indicate that a plant can occur both in and out of wetlands (50/50). Facultative-upland is used to indicate that a plant occurs more often outside of wetlands

than in (<33% chance of being in a wetland) (USACE, 2008). All vegetation observed at the site were described scientifically (Table 2). Because 90% of the ground cover at the study site

Table 2. Scientific names of vegetation observed at the study site with percent ground cover and wetland indicator status.

Plant Species	Percent Ground Cover	Wetland Indicator Status
Juncus phaeocephalus	90%	FACW
Mimulus guttatus	4%	OBL
Rumex crispus	2%	FAC
Cirsium arvense	2%	FACU

consisted of *Juncus phaeocephalus* and it is FACW status, it meets the criteria for a wetland dominance test. The dominance test indicates that more than 50% of the vegetative cover has to be OBL, FACW, or FAC (Fig. 9).

Figure 9. Looking east, the wetland boundary can be seen by the absence of the dark green vegetation. All dark green vegetation is the species *Juncus phaeocephalus*.

3. Soil and IRIS Tubes

The soil present at this site was described using *The Keys to Soil Taxonomy, 11th ed.* in accordance with USDA-NRCS MLRA Soil Survey Staff requirements. The soil was found to be in the family Clayey-skeletal, smectitic, thermic Oxyaquic Haploxerolls (Table 3). The soil had a lack of redoxomorphic features and did not have the correct munsell colors to match the criteria for a hydric soil or a typical wetland Mollisol, hence the reason for the soil classification given

Table 3. Physical soil description for the soil found to be in the family, Clayey-Skeletal, smectitic, thermic Oxyaquic Haploxerolls.

Horizon	Depth	Description
A	0-19cm	black (2.5Y 2.5/1) loam; weak medium granular structure; friable, slightly plastic, slightly sticky; many medium roots; abrupt wavy boundary
Btg	19-36cm	black (N 2.5/0) gravelly sandy clay; moderate medium sub-angular blocky structure; firm, moderately plastic, moderately sticky; few fine roots; diffuse wavy boundary
Cg	36-49cm	very dark gray (N 3/0) very gravelly sandy clay; moderate medium sub-angular blocky structure; very firm, moderately platic, moderately sticky, moderately plastic; gradual wavy boundary
Cr	49-50+cm	Partially weathered serpentinite

and reason for this study; even though colors were found to be on the "Gley Page" of the *Munsell Soil Color Chart*, it still did not meet the requirements for a hydric soil.

The carbon content was gathered by the incineration of the soil in a VarioMax C:N Analyzer (Table 4). In the preliminary field visit, 0.1M Hydrochloric Acid was dropped onto

Table 4. Organic carbon and organic matter content for the soil present at the study site.

Horizon	Organic Carbon Content	Organic Matter Content
A	3.26%	6.52%
Btg	1.21%	2.42%
Cg	1.29%	2.58%
Cr	1.06%	2.12%

a soil ped to observe if there were any free carbonates present. No effervescence occurred, so it was assumed that all carbon present in the soil was organic. It is a common calculation in soil science that the organic matter content of the soil can be found by doubling the organic carbon content of the soil (Brady and Weil, 1999). This high of an accumulation of organic carbon is uncommon in Mollisols of the Central Coast. The high organic matter content could be directly related to an anaerobic system in the soil; because the microorganisms use alternate terminal

electron acceptors, the decomposition of organic matter is decelerated (Craft, 2001). The higher organic matter content can also result in the absence of redoximorphic features higher up in the soil profile (Thompson and Bell, 1996). The confusion of iron reduction with the mineral color can also be a result of the serpentinitic parent material; young serpentinitic soils naturally have a green or gley color, which makes the study of serpentinitic wetland soils that more complicated (Alexander and DuShey, 2011).

The technical standard for IRIS tubes states that at least 3 tubes need to have at least 30% reduction in the top 30cm (USDA-NRCS, 2007). All tubes were analyzed individually in Adobe Photoshop CS6 (Fig. 10). The results of the study show that each week, every tube has greater

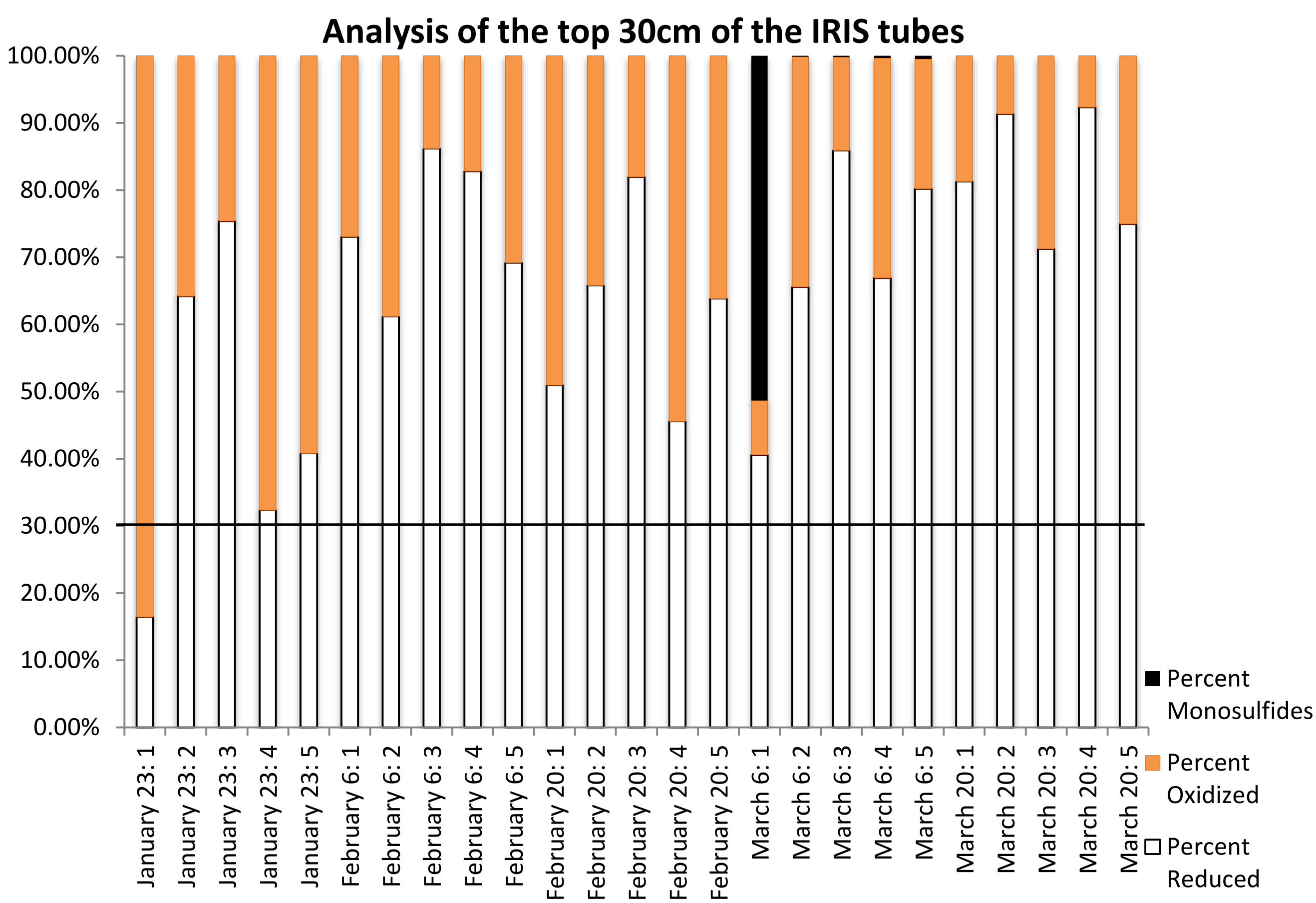

Figure 10. The image analysis results of the top 30cm of all IRIS tubes organized by tube number and date of extraction. The line at 30.00% represents the requirement of the technical standard.

than a 30% reduction, with the exception of the first tube on January 23. Based on these data, this soil meets the technical standard for a hydric soil. Analysis of the IRIS tube reduction data in conjunction with the well-water data show that higher reduction occurs after a precipitation event, most noticeable on March 6. The extraction date on March 6 occurred following a 5-day precipitation event (February 26-March 2). This large inundation of water could be a direct correlation to the presence of monosulfide reduction on March 6.

Sulfur reduction has to occur because it is the only form of a terminal electron acceptor available in the soil. Sulfur has one of the lowest redox potentials of all anaerobic terminal electron acceptors, which is why it is usually the last to be reduced; sulfur provides the lowest amount of energy to microorganisms (Vepraskas and Faulkner, 2001). For sulfur reduction and mineralization to occur, the following requirements must be present in the soil: sulfate (>10μM), oxidizable organic carbon, anaerobic conditions, sulfate reducing bacteria, and inorganic iron. Once free sulfide has been formed, typically in the form of hydrogen sulfide, the mineralization of sulfur can occur. The sulfur reacts with the iron paint on the tubes to form iron based monosulfides [1]. The environmental conditions for sulfur reduction are not stringent, because

$$Fe^{2+} + S^{2-} \rightarrow FeS^{-} \qquad [1]$$

sulfur reduction is typical of tidal zones. Tidal zones are the areas usually studied for sulfur reduction, therefore the study of serpentinitic wetlands and sulfur reduction is very sparse (Rabenhorst *et al.* 2002).

The presence of sulfur reduction at this wetland is an interesting result of the study. Sulfur is not a common component in soils outside of tidal zones, nor soils of the California Central Coast. The heightened sulfide reduction on only one tube, after a precipitation event, is a

larger anomaly (Fig. 11). As stated, the reduction of sulfur in serpentinitic wetlands is very sparse and monosulfide reduction is not considered a part of the IRIS tube technical standard.

Figure 11. Full IRIS tubes 1(left) and 2(right) from March 6. Black and gray portions of the tubes represent pyrite accumulation, note the large accumulation on tube 1 vs. tube 2.

Therefore, the presence of monosulfide accumulation on one tube could be the result of a heated zone in the wetland. Oxygen is more diffuse in warm and wet soils, and the connection of increased monosulfide accumulation on one single tube could be the result of a heat pocket.

Conclusions and Future Considerations

A study was performed on a serpentinite derived wetland with uncommon soil matrix colors that did not meet any requirements for a hydric soil. The IRIS tubes were utilized to attempt to confirm the hydric status of the serpentinitic soil. After a 3-month field study, it was confirmed that because of the pronounced reduction (>30%) on the IRIS tubes the soil is considered hydric. Because of the chemical observation of the IRIS tubes it can be concluded that the soil is actually in the family: Clayey-skeletal, smectitic, thermic Typic Endoaquolls.

The presence of a high organic matter content was found to have hidden true redoximorphic features in the surface horizons of the soil. The derivation of serpentinite colors into the soil matrix also lead to the confusion that there was a lack of iron reduction. Having a plant species considered FACW dominating the ground cover and the presence of surface water made the vegetation and hydrology wetland determinations time efficient. Analyzing both precipitation events and the IRIS tubes from March 6 gave the conclusion that sulfur is either present in larger amounts than normal of a serpentinite soil or that sulfur could have been added into the wetland, possibly even sulfuric acid based rain (Rabenhorst *et al.* 2002).

Further studies could be performed on a problematic serpentinitic soil. A field study could be performed to mimic natural field conditions, but controlling for precipitation. A lab study could also be performed to control for both the sulfur content in the serpentinite soil and the precipitation. Currently, no technical standard is recognized for the presence of monosulfides on IRIS tubes. The lack of pure iron reduction, but instead, monosulfide accumulation may have to cause a researcher to remove that tube from counting toward the confirmation of a hydric soil.

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Technical Note: Comparison of Eye Estimates to Photoshop Estimates

Ten students from multiple agriculture majors were chosen to examine the top 30cm of each IRIS tube. There is currently no standard for analyzing IRIS tube oxidation and reduction. This note is the start of potentially creating a standard for analyzing IRIS tubes. Separating extraction dates, the photoshop analysis used for this project was compared to the average of the ten analyses performed by the students (Figure 12a-12e).

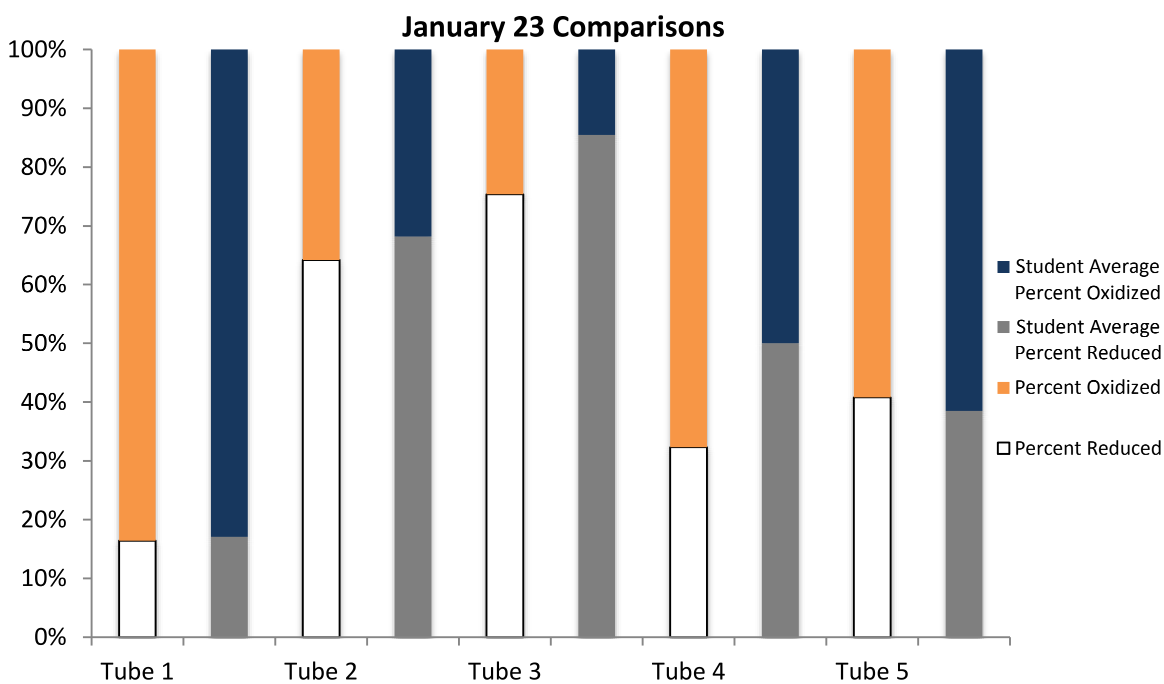

Figure 12a. The comparison of student average estimates to photoshop estimates for the extraction on January 23.

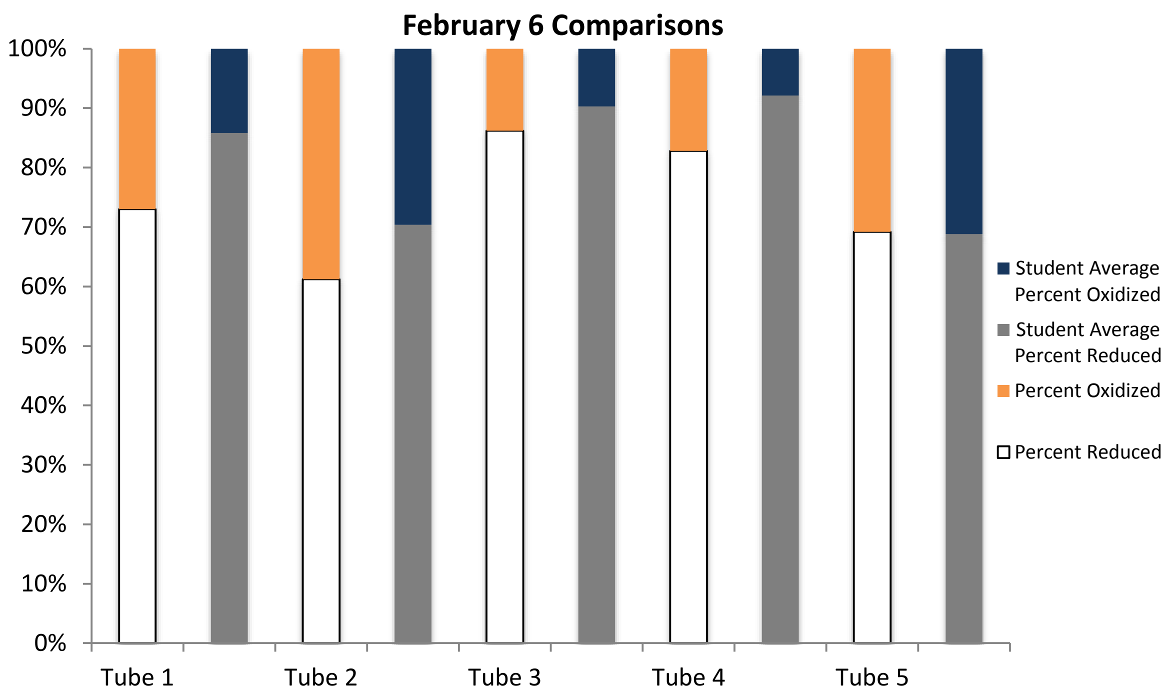

Figure 12b. The comparison of student average estimates to photoshop estimates for the extraction on February 6.

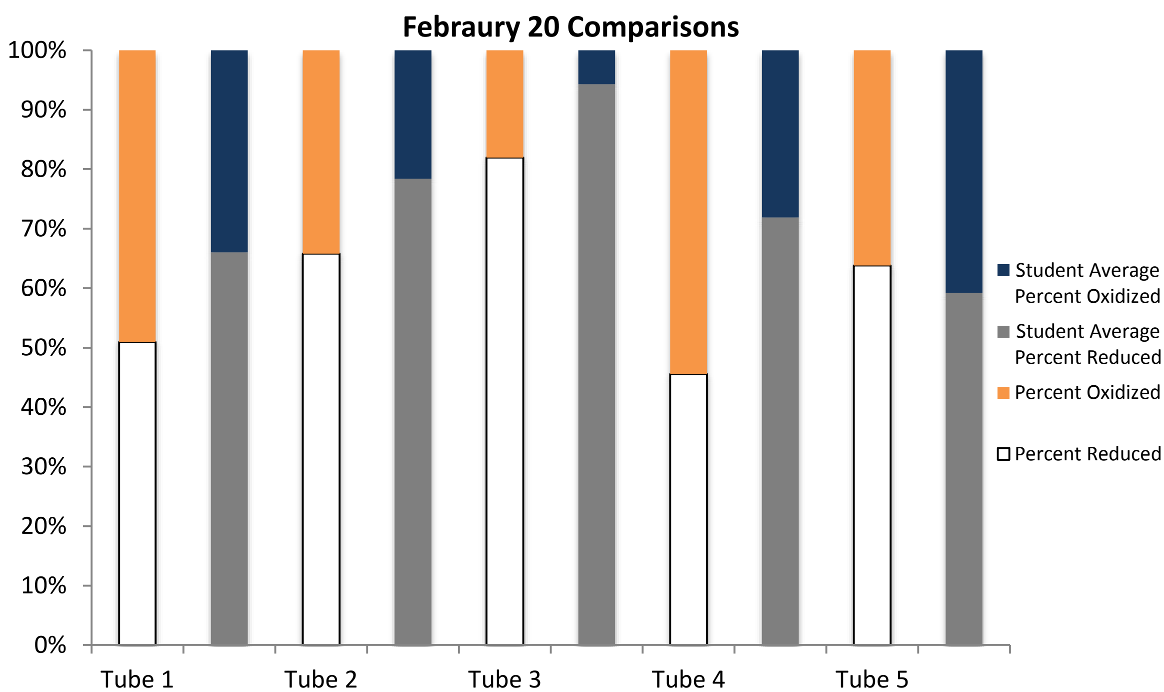

Figure 12c. The comparison of student average estimates to photoshop estimates for the extraction on February 20.

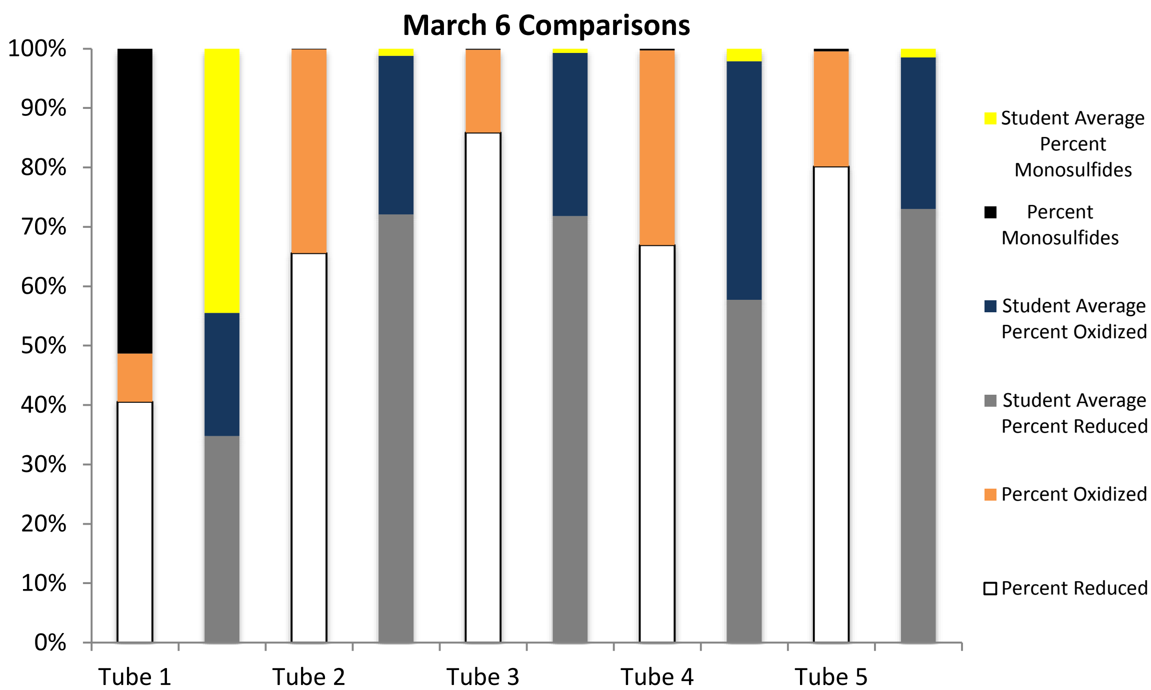

Figure 12d. The comparison of student average estimates to photoshop estimates for the extraction on March 6.

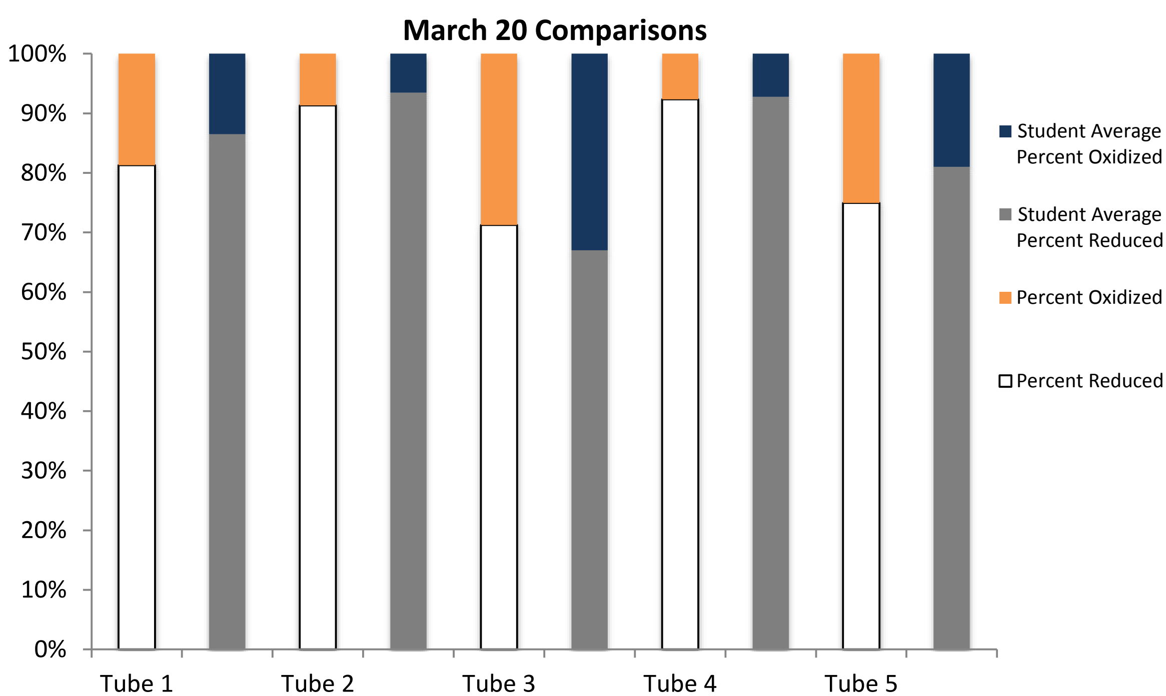

Figure 12e. The comparison of student average estimates to photoshop estimates for the extraction on March 20.

With the exclusion of March 6 (the date with sulfur reduction), on average, the students typically estimated the reduction higher than photoshop. When presented with monosulfides, the students tended to estimate the oxidized percentages higher. As stated, there is no current standard for analyzing IRIS tubes. It would be beneficial if all wetland scientists could use a standard method to avoid error when deducing IRIS tube analysis.

Appendix

Table 5. Data for photoshop and average student IRIS tube estimates.

Day - Tube number	Total Pixels	Pixels Reduced	Percent Reduced	Pixels Oxidized	Percent Oxidized	Pixels Monosulfides	Percent Monosulfides	Student % Reduced	Student % Oxidized	Student % Monosulfides
January 23: 1	22971	3775	16.43%	19196	83.57%			17%	83%	
January 23: 2	25163	16152	64.19%	9011	35.81%			68%	32%	
January 23: 3	27876	21008	75.36%	6868	24.64%			86%	15%	
January 23: 4	23465	7580	32.30%	15885	67.70%			50%	50%	
January 23: 5	24417	9958	40.78%	14459	59.22%			39%	62%	
February 6: 1	24094	17596	73.03%	6498	26.97%			86%	14%	
February 6: 2	24137	14773	61.20%	9364	38.80%			70%	30%	
February 6: 3	23844	20552	86.19%	3292	13.81%			90%	10%	
February 6: 4	25242	20902	82.81%	4340	17.19%			92%	8%	
February 6: 5	25001	17295	69.18%	7706	30.82%			69%	31%	
February 20: 1	24041	12249	50.95%	11792	49.05%			66%	34%	
February 20: 2	25674	16897	65.81%	8777	34.19%			78%	22%	
February 20: 3	24606	20168	81.96%	4438	18.04%			94%	6%	
February 20: 4	21453	9777	45.57%	11676	54.43%			72%	28%	
February 20: 5	23270	14854	63.83%	8416	36.17%			59%	41%	
March 6: 1	26862	10894	40.56%	2175	8.10%	13793	51.35%	35%	21%	45%
March 6: 2	29207	19158	65.59%	10008	34.27%	41	0.14%	72%	27%	1%
March 6: 3	28174	24199	85.89%	3929	13.95%	46	0.16%	72%	28%	1%
March 6: 4	27997	18730	66.90%	9178	32.78%	89	0.32%	58%	40%	2%
March 6: 5	28016	22464	80.18%	5422	19.35%	130	0.46%	73%	26%	2%
March 20: 1	23499	19107	81.31%	4392	18.69%			87%	14%	
March 20: 2	24323	22217	91.34%	2106	8.66%			94%	6%	
March 20: 3	98708	70338	71.26%	28370	28.74%			67%	33%	
March 20: 4	20126	18584	92.34%	1542	7.66%			93%	7%	
March 20: 5	22599	16934	74.93%	5665	25.07%			81%	19%	

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