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Developing an Inquiry-based Laboratory Project for CHEM 142L Course at BSU

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Submitted in Partial Completion of the Requirements for
Commonwealth Honors in Chemical Sciences

Bridgewater State University

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Abstract

In addition to content knowledge, critical and independent thinking, scientific reasoning, and problem-solving skills are essential in preparing next generation of successful workforce. Since one of the biggest advantages of STEM disciplines is a “must-have” hands-on laboratory experience, it is intuitive to exploit this learning space to reinforce afore-mentioned skills. In this context, project-based (PBL) or inquiry-based (IBL) laboratory experiences are rapidly becoming mainstream pedagogical choice for many STEM instructors across United States.^[1-4] PBL, and IBL are learning experiences that offer students an opportunity to experience realistic scientific process of discovery through carefully designed inquiry-driven and/or open-ended investigative laboratory experiences.^[1-4] PBL and IBL learning experiences also provide instructors with an opportunity to create projects that are related to real-world problems.^[1-4] In this context, the work described in this thesis sought to adapt and redesign a semester long project on phytoremediation of copper from soil, published in the *Journal of Chemical Education* ^[5], into a collection of mini-projects to be done by students over a period of 3 – 4 weeks. Specifically, work presented herein focuses on general chemistry (CHEM 142L) students exploring the effects of mass, pH and temperature of a commercially available garden soil on its adsorption capability of copper ions and understanding these effects in terms of equilibrium processes and thermodynamics of adsorption processes. The motivation for this work stems from the fact that the Chemistry Department at BSU is currently redesigning its second semester of general chemistry laboratory (CHEM 142L) curriculum with an environmental science focus.

1. Introduction

At Bridgewater State University (BSU), for a very long-time students have been working with “cook-book” style chemistry laboratory experiences. Research ^[1-5] shows that these types of experiments, by nature, do not fully challenge student’s intellect and many times fail to help students actively think about the work they are doing or the results they are observing. Among the student skills required by American Chemical Society ^[6], critical thinking skills are essential in order to produce chemistry graduates that are ready to tackle real world issues. An excellent way of developing these skills is by exposing students to project-based, inquiry-based, and problem-based laboratory projects that foster curiosity by challenging them to connect the course content to real world issues. ^[1-5] For this reason, this type of hands-on, undergraduate research focused pedagogy is gaining popularity across many other universities in the United States. Further, project-based laboratory experiences have the following additional advantages: ^[1-5]

- (a) Fostering high level of student engagement by providing an opportunity to design experiment protocols;
- (b) Encouraging independent and critical thinking and reasoning;
- (c) Providing an opportunity to gain research-like experiences;
- (d) Allowing them to perceive chemistry as a scientific process of discovery;
- (e) Providing an opportunity to learn and gain proficiency in common and advance scientific instrumentation;
- (f) Allowing students to gain proficiency in scientific writing skills and science communication skills.

Therefore, many universities across U.S., including BSU, are adapting inquiry/project-based learning (IBL/PBL) experiences into their undergraduate chemistry laboratory curricula.

In 2018, BSU’s Chemistry Department was one of the 24 departments (from 12 U.S. institutions) selected by the Council of Undergraduate Research (CUR) to participate in their ‘Transformations Project’ ^[7], which is funded by National Science Foundation. This project’s goal is to study various factors (e.g. student, faculty, and discipline), that influence the integration of research experiences throughout the undergraduate curriculum in a scaffolded fashion.^[7] As a result of this grant, BSU’s Chemistry Department is committed to incorporating research-like experiences via IBL/PBL exercises in all of its undergraduate courses, starting from its

introductory chemistry laboratory course sequence, namely CHEM 141L and CHEM 142L. Since the field of environmental science has been steadily gaining attention from researchers and general public alike ^[5,8-10], new CHEM 142L curriculum intends to incorporate three small IBL projects (3 – 4 weeks per project) in the area of environmental chemistry and the project described in this thesis is one of these small-scale projects.

The impetus for the current project stems from a recent article by Cessna et.al.^[5], in the *Journal of Chemical Education*, which describes a semester long project illustrating the equilibrium thermodynamics principles associated with copper phytoremediation from soil. Phytoremediation is a type of remediation process that uses plants to remove or break down toxic contaminants from soil, water, and other environments ^[5,8-10]. For example, while *Lemna minor* and *Elodea* are known to absorb copper from wastewater, and soil respectively ^[9], *Egeria densa* and *Brassica rapa* are known for their silver uptake ^[10]. Copper was chosen because:^[9]

- (a) It is one of the naturally occurring trace elements present in soil, sediment, air and water;
- (b) It is an essential micronutrient that plays an important role in making red blood cells as well as maintaining nerve cells and the immune system;
- (c) Relatable sources of contamination such as corrosion of household plumbing, manure, sewage sludge and fertilizers/pesticides;
- (d) Its salts are relatively inexpensive to use in large classroom setting and the colored nature of many of aqueous $\text{Cu}^{2+}_{(\text{aq})}$ complexes has an added advantage of visually engaging the students.

As mentioned earlier, the project described herein is one of the three mini-projects to be implemented into a one-semester CHEM 142L course and therefore, the semester long project described by Cessna et.al.^[5] cannot be adapted “as-is”. Hence, this project focused on developing experimental protocols students can use to study the influence of various factors on $\text{Cu}^{2+}_{(\text{aq})} \rightleftharpoons \text{Cu}^{2+}_{(\text{soil})}$ equilibrium, including its partition coefficient, and connect it to equilibrium and thermodynamics principles they would have learned in CHEM 142 lecture. Specifically, this thesis develops student protocols to evaluate the effect of soil mass, copper concentration, pH, and temperature on the adsorption of copper by soil.

This project's overarching goal is to deepen students' understanding of concepts learned in CHEM 142 course and showcase their connection to environmental pollution. Specifically, by completing the individual projects described in this thesis, students will:

- (a) Learn about principles behind chelation and redox chemistries and adsorption equilibrium processes;
- (b) Gain proficiency in dilution and aqueous solution preparation, including correct pipetting techniques;
- (c) Gain proficiency in analyzing equilibrium processes under various experimental conditions;
- (d) Learn about UV-Visible spectrophotometry and the Beer Lambert law and their application in measuring concentration of an analyte;
- (e) Develop a standard curve and use it to analytically determine concentration in aqueous solutions;
- (f) Experimentally determine Gibbs free energy, enthalpy and entropy changes associated with equilibrium processes;
- (g) Develop skills such as critical thinking, using Microsoft Excel for data plotting and linear fitting, scientific writing, and communication skills;
- (h) Learn to work in a team environment.

2. Experimental Details

Table 2.1 summarizes the optimal working conditions identified during the early stages of this project. These conditions should allow the students to complete each experiment within a three-hour laboratory period while yielding reproducible results.

Table 2.1. Optimal general experimental conditions identified through trial and error.

Parameter	Optimal value
Mass of Vigoro all-purpose garden soil	1.00 g
Volume & concentration of $\text{Cu}^{2+}_{(\text{aq})}$ solution added to soil	5 mL & 1000 ppm
Wait time for equilibration of soil and copper solution	60 min
After equilibration, volume of $\text{Cu}^{2+}_{(\text{aq})}$ tested	0.2 mL
Volume & concentration of glucose used for $[\text{Cu}^{2+}]_{\text{aq}}$ determination	2 mL & 100 g/L
Volume of commercially bought BCA used for $[\text{Cu}^{2+}]_{\text{aq}}$ determination	2 mL
Incubation time and temperature	15 min & $\sim 50\text{ }^{\circ}\text{C}$

2.1. Reagents and other materials

Reagent grade copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, CAS# 7758-99-8), D(+)-glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, CAS# 50-99-7), Bicinchoninic acid protein assay reagent A (BCA, CAS# 1245-13-2), 0.10 M aqueous hydrochloric acid (HCl, CAS# 7647-01-0) and 0.10 M aqueous sodium hydroxide (NaOH, CAS# 1310-73-2) were all purchased from Fisher Scientific and were used without further purification. Vigoro all-purpose garden soil (see Fig. 2.1) was purchased from local home improvement store and was sifted using a household kitchen swifter with fine mesh, to remove larger particles. In-house deionized (DI) water was used to prepare all the solutions.

2.2. Preparation of solutions

2.2.1. Copper(II) solution: Initial experiments tested 10, 100 and 1000 ppm aqueous Cu^{2+} solutions and based on the reproducibility of results and ease of preparation and use, 1000 ppm was chosen as the appropriate concentration for the entirety of this project. Desired volume of this



Figure 2.1. Image of the commercial garden soil used in this project.

concentration was prepared fresh for each experiment. For example, since 1000 ppm equals to 1 g in 1000 mL, to prepare 25 mL of 1000 ppm copper solution, 9.82×10^{-3} g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was added to a 25 mL volumetric flask and DI water was slowly added until the salt dissolved completely. Flask was filled up to the mark with DI water and was gently shaken to ensure homogeneity was achieved.

2.2.2. Glucose solution: As mentioned in the Introduction, purpose of glucose is to reduce Cu^{2+} to Cu^+ to facilitate its binding with BCA. Initial experiments tested 0.01 g/L, 0.1 g/L, 1 g/L and 100.0 g/L aqueous glucose solutions and based on the reproducibility of results and ease of preparation and use, 100 g/L was chosen as the appropriate concentration for the entirety of this project. The desired volume of this concentration was prepared fresh for each experiment. For example, to prepare 50 mL solution of this concentration, 5 g of glucose was added to a 50 mL volumetric flask and DI water was slowly added until glucose dissolved completely. After filling the flask up to the mark with DI water, it was gently shaken until the solution was homogeneously mixed.

2.2.3. Other solutions: As mentioned in Introduction section, BCA was chosen for detecting Cu^{2+} in aqueous solutions. The commercially purchased BCA assay reagent was stored in the refrigerator and a desired volume of this solution was retrieved as needed. It is worth pointing out that BCA assay reagent used in this project contains BCA, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.10 M NaOH (pH = 11.1 – 11.3). To test the pH effects on the partition

coefficient (K) of $\text{Cu}^{2+}_{(\text{aq})} \rightleftharpoons \text{Cu}^{2+}_{(\text{soil})}$ equilibrium, 0.10 M $\text{HCl}_{(\text{aq})}$ (pH = 1) and 0.1 M $\text{NaOH}_{(\text{aq})}$ (pH = 13) solutions were used as purchased. Solutions with pH values 3 and 11, respectively, were obtained by diluting these stock solutions to the desired volume.

2.3. Ultraviolet–visible (UV-Vis) spectroscopy

A table-top UV-Vis spectrophotometer (Perkin Elmer Lambda XLS+) capable of operating from 400 nm to 700 nm was utilized to monitor the absorption spectrum of $[\text{Cu}(\text{BCA})_2]^+$ complex. This instrument was chosen because this will be the one that students at BSU will use. All the analytes showed a broad peak with an absorption maximum (A_{max}) at 562 nm. DI water was used as the blank solution for all the experiments. Absorbance and wavelength values corresponding to the peak maximum were recorded for all the samples.

2.4. Analytical determination of copper concentration

2.4.1. General procedure to determine copper(II) ion concentration in aqueous phase,

$[\text{Cu}^{2+}]_{\text{aq}}$: Based on the procedure described by Cessna et.al.^[5], 0.2 mL of aqueous copper solution of desired concentration was added to a test tube containing a mixture of 2 mL of 100 g/L glucose solution and 2 mL of BCA reagent. The mixture slowly starts turning purple. The test tube was then placed in a ~ 60 °C water bath and the mixture was allowed to incubate for ~ 15 minutes, at the end of which it turns to a darker shade of purple. Mixture was then cooled to room temperature and ~ 3 mL of the purple-colored solution was transferred to a disposable plastic cuvette to collect its UV-vis spectrum in the 400 - 700 nm range. Since glucose can reduce Cu^{2+} to Cu^0 , Cessna et. al., suggested pre-mixing glucose and BCA to ensure that as soon as Cu^+ is formed, BCA reacts with it and thereby preventing any further reduction to Cu^0 . Further, the volumes of reagents were doubled from the original work^[5] in order to minimize experimental error in the measurements, because it was predicted that it would be difficult for the students to accurately measure the small volumes used in the reference paper.

2.4.2. Standard curve: To construct a standard calibration curve for copper concentration in aqueous phase, its concentration was varied between 0 – 10 ppm or 0 – 100 ppm or 0 – 1000 ppm in three different sets of experiments and A_{max} at $\lambda_{\text{max}} = 562$ nm for $[\text{Cu}(\text{BCA})_2]^+$ complex was obtained using the general procedure described earlier.

2.4.3. General procedure to determine $[\text{Cu}^{2+}]_{\text{soil}}$: Earlier experiments were carried out by altering the ratio of amount of soil to volume of aqueous copper solution and it was found that ~1:2 ratio yielded reliable results. Further, earlier experiments also evaluated the soaking times ranging from 15 minutes to 7 days and it was found that ~60 mins soaking time provides reliable results while allowing students to finish the experiment within a three-hour lab period.

For a given set of experimental conditions, desired amount of sifted soil was added to a 20 mL scintillation vial and soaked in an appropriate of volume of 1000 ppm aqueous copper solution for 60 minutes. After the waiting period, aqueous copper solution layer was carefully extracted using a syringe and then filtered using a 0.25 μm syringe filter. Analyte absorbance was then determined following the afore-mentioned procedure using BCA and glucose solutions. Finally, concentration of copper adsorbed by soil can be obtained as:

$$[\text{Cu}^{2+}]_{\text{soil}} \text{ (in ppm)} = [\text{Cu}^{2+}]_0 - [\text{Cu}^{2+}]_{\text{aq}} = 1000 \text{ ppm} - [\text{Cu}^{2+}]_{\text{aq}} \text{ (in ppm)} \quad (2.1)$$

2.5. Effect of soil mass on $[\text{Cu}^{2+}]_{\text{soil}}$

To study the relationship between the amount of soil and its capacity for copper adsorption, soil mass was varied from ~0.10 g to ~1.00 g, as shown in Table 2.2. Exactly 5.00 mL of 1000 ppm copper(II) solution was added to each sample and was left to equilibrate for 60 min. After 60 mins, each sample was analyzed for copper ion concentration in soil using the method mentioned in subsection 2.4.3.

2.6. Effect of soil pH on the amount of copper adsorbed

As a first attempt to test the effect of soil pH on the partition coefficient for the $\text{Cu}^{2+}_{(\text{aq})} \rightleftharpoons \text{Cu}^{2+}_{(\text{soil})}$ equilibrium, soil was equilibrated at desired pH for 60 minutes using water or $\text{HCl}_{(\text{aq})}$ or $\text{NaOH}_{(\text{aq})}$ solutions. However, due to a lot of scattering in the data (data not shown), experiment was repeated by soaking the soil in the pH altering reagent for 7 days. After the desired equilibration time has passed, the excess aqueous layer was carefully removed with a syringe without disturbing the soil and 5.00 mL of aqueous copper(II) solution was added to the soil. The mixture was left to equilibrate for another 60 minutes and the Cu^{2+} solution layer was extracted using a syringe and analyzed according to the procedure mentioned in subsection 2.4.1 and Cu^{2+} concentration in soil was estimated using equation 2.1.

Table 2.2. Summary of final experimental parameters used to study various effects on Cu²⁺ adsorption by soil.

Soil mass (g)	Vol. of DI water (mL); pH	Vol. of HCl _(aq) (mL); pH	Vol. of NaOH _(aq) (mL); pH	Soaking time with pH reagent	Vol. of 1000 ppm Cu ²⁺ (aq) (mL)	Soaking time with Cu ²⁺ (aq)	Temperature (°C)
<i>Effect of soil mass</i>							
0.102	N/A; 7	N/A	N/A	N/A	5	60 min	21
0.302							
0.700							
1.002							
<i>Effect of pH</i>							
1.000	0; N/A	5.00; 1.00	0; N/A	5 days	5	60 min	21
1.000	0; N/A	5.00; 3.00	0; N/A				
1.000	5.00; 7	0; N/A	0; N/A				
1.000	0; N/A	0; N/A	5.00; 11				
1.000	0; N/A	0; N/A	5.00; 13				
<i>Effect of temperature</i>							
1.000	N/A; 7	N/A	N/A	N/A	5	60 min	~0
1.000							22
1.000							40
1.000							60

2.7. Effect of soil temperature on the equilibrium constant (K)

Four soil samples, each containing ~1.00 g soil, were used to study the temperature effects on copper adsorption by the soil. To each of the soil samples, ~ 5 mL 1000 ppm aqueous copper(II) solution was added and allowed to equilibrate for 60 min at ~0 °C, ~22 °C (room temperature), 40 °C and 60 °C. The 40 °C and 60 °C temperatures were achieved with the help of hot water baths, while the 0 °C temperature was achieved by placing the soil sample in an ice bath. Removal and analysis of excess aqueous Cu^{2+} solution was carried out according to procedures described earlier in subsections 2.4.1 and 2.4.3.

3. Results and Discussion

3.1. Why BCA to detect $[\text{Cu}^{2+}]_{\text{aq}}$?

Although many analytical methods are available for the determination of copper concentration in a variety of samples, UV-visible spectroscopy remains popular for aqueous solutions because of its simplicity, low cost, instrument accessibility and familiarity to undergraduate students.^[11] Among the available spectrophotometric reagents^[11], a quinoline derivative 4,4'-Dicarboxy-2,2'-biquinoline acid (aka bicinchoninic acid, BCA, with $pK_{a_1} = 1.87$ and $pK_{a_2} = 2.85$) is a well-known complexing agent with a high sensitivity and selectivity for Cu(I) in aqueous media. Hence, it has been widely used to directly determine copper in soil and to indirectly determine proteins, sugars, ascorbic and uric acids.^[11,12] In the indirect method, species like proteins or sugars reduce copper(II) to copper(I) which then binds with aqueous BCA to produce an intensely purple colored complex with a formula $[\text{Cu}(\text{BCA})_2]^+$ and a molecular structure shown in Fig. 3.1. For example, reaction of Cu(II) with glucose, a reducing sugar, and BCA in a basic medium is shown below. The $[\text{Cu}(\text{BCA})_2]^+$ complex exhibits an absorption maximum at $\sim 560 \pm 2$ nm with a molar absorptivity coefficient in the range of $7.7 \times 10^3 - 8.7 \times 10^3$ L/mol·cm.^[11,12]

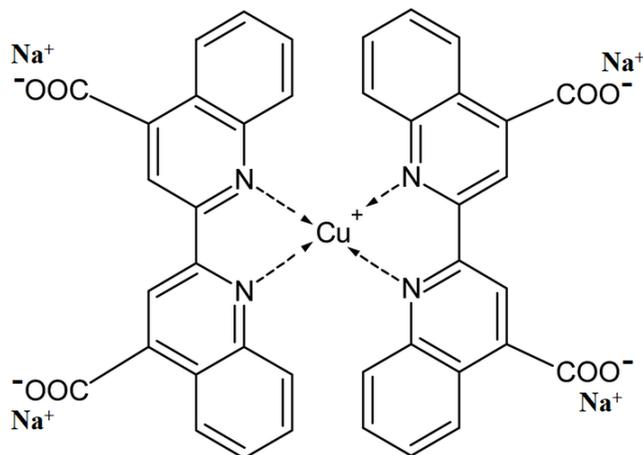
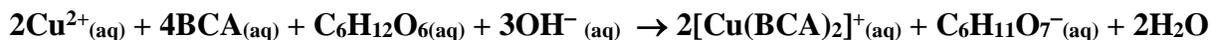


Figure 3.1. Chemical structure of purple $[\text{Cu}(\text{BCA})_2]^+$ chelate complex. Image is adapted from reference [13]. Sodium ions shown in the picture are from the commercially purchased BCA assay reagent solution used in this project. See subsection 2.2.3 for details.

In this project, glucose/BCA pair was chosen to detect Cu^{2+} ion concentration in aqueous media using UV-Vis spectroscopy because of the following reasons.

- (a) Glucose is one of the simplest reducing sugars that the students will be familiar with and it is a very inexpensive reagent;
- (b) $[\text{Cu}(\text{BCA})_2]^+$ complex shows a strong absorption maximum at ~ 560 nm which falls in the 400 – 700 nm range of the table top Perkin Elmer UV-Vis spectrometers widely available at BSU;
- (c) Absorbance of $[\text{Cu}(\text{BCA})_2]^+$ complex can be measured at any wavelength between 550 – 570 nm with less than 10% signal loss.^[12,13]
- (d) BCA chelation with Cu^+ in alkaline medium, allows one to introduce chelation and redox chemistries at the general chemistry level;
- (e) Dark purple color of $[\text{Cu}(\text{BCA})_2]^+$ complex provides an easy visualization of reaction progress;
- (f) Finally, as suggested by Cessna et. al.^[5], using BCA/glucose pair allows students to potentially estimate the ratio of $[\text{Cu}^{2+}]$ to $[\text{Cu}^+]$ in aqueous and soil media.

If the instructors of CHEM 142 find the detection method using glucose/BCA to be too tedious or overwhelming activity for general chemistry students, alternate methods such as chelation with NH_3 ($\lambda_{\text{max}} \sim 610$ nm), titration with EDTA or atomic absorption spectroscopy may be used to determine $[\text{Cu}^{2+}]_{\text{aq}}$ and $[\text{Cu}^{2+}]_{\text{soil}}$ concentrations.

3.2. Construction of standard curve for $[\text{Cu}^{2+}]_{\text{aq}}$ using BCA and glucose

To understand the partitioning of copper ions between soil and water i.e., the equilibrium $\text{Cu}_{\text{aq}}^{2+} \rightleftharpoons \text{Cu}_{\text{soil}}^{2+}$, students will need the values of Cu^{2+} ion concentration in water layer and in soil. Hence, they need to construct a standard curve with various concentrations of Cu^{2+} ions in water. Standard curves generated using UV-Vis spectrophotometric method rely on the Beer-Lambert Law (cf. Eq. 3.1). According to this law, absorbance (A) versus concentration (c) should yield a straight line with a slope equal to ϵl , where ϵ is the molar extinction coefficient (aka molar absorptivity) and l is the light path length or the width of a cuvette. Since the standard ~ 1 cm \times 1 cm \times 5 cm cuvettes are used, slope in the current case should be ϵ with $\text{ppm}^{-1}\text{cm}^{-1}$.

$$A = \epsilon cl \quad (3.1)$$

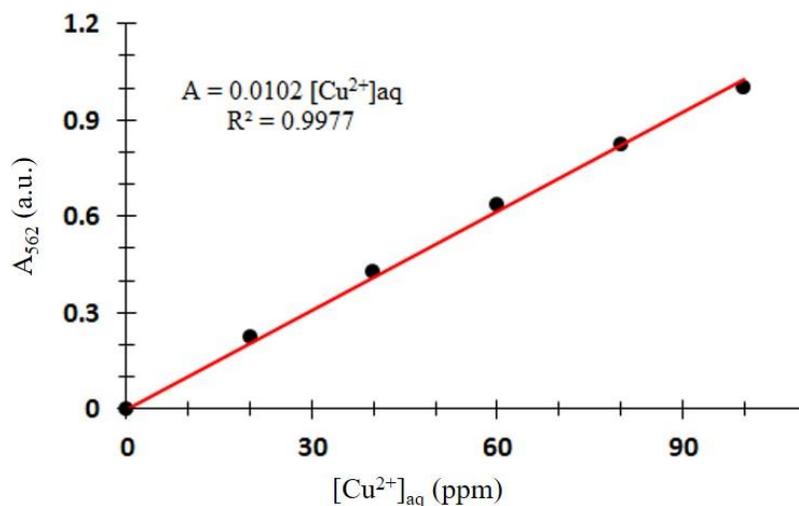


Figure 3.2. Calibration curve to determine copper concentration in aqueous solutions. Absorbance data (black circles) was collected for $[Cu(BCA)_2]^+$ complex's $\lambda_{max} = 562$ nm peak and the solid red line represents a fit to Beer Lamberts equation shown in Eq. 3.1.

As a first attempt to create a standard curve, $[Cu^{2+}]$ was varied from 100 – 1000 ppm. However, because of large molar absorptivity of $[Cu(BCA)_2]^+_{(aq)}$ complex,^[12,13] concentrations above 100 ppm turned out to be too concentrated for the table top UV-Vis spectrometer and had to be diluted by 10 times in order to collect the absorption spectra (data not shown). Hence, to simplify the number of steps for the students, copper concentration was changed from 0 – 100 ppm and a second standard curve, shown in Fig. 3.2, was obtained as $A = 0.0102 [Cu^{2+}]_{aq}$ with $R^2 = 0.9977$. Finally, our attempts to construct a standard curve for copper ion concentrations from 0 – 10 ppm (data not shown) yielded poorly fit data ($R^2 = 0.8893$), probably because of poor sensitivity of the tabletop UV-Vis spectrometer or instrumental deviation from Eq. 3.1 or experimental errors such as slightly warm BCA, not enough incubation etc. Hence, asking students to work with copper concentrations of 0 – 100 ppm, while keeping the number of required steps to a minimum, will provide students not only with a working standard curve they can use for all their analytes but also provide an opportunity to practice developing it.

3.3. Relation between soil mass and amount of copper adsorbed

Intuition suggests that increasing the amount of soil increases the copper adsorbed by soil. To confirm this relationship, an experiment was designed where the soil mass was varied while keeping the initial copper concentration constant at 1000 ppm. All samples were equilibrated for

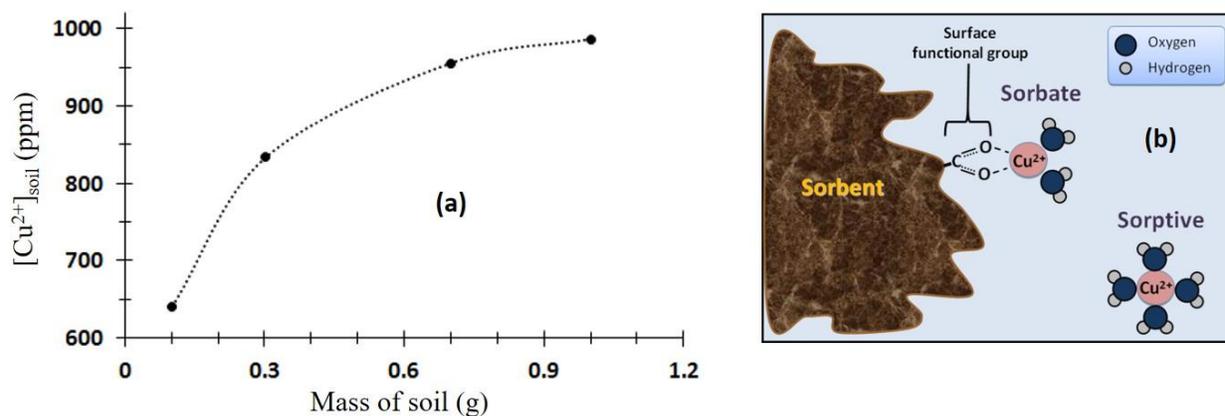


Figure 3.3. (a) Concentration of copper adsorbed as a function of soil mass at room temperature and atmospheric pressure. Data was obtained by monitoring the $\lambda_{\max} = 562$ nm peak for $[\text{Cu}(\text{BCA})_2]^+$ complex. Dotted line is added as a guide to the eye. (b) Cartoon diagram showing the interactions between Cu^{2+} ions and the soil surface. Image is adapted from reference [14].

60 minutes at room temperature and atmospheric pressure. While the proportionality between soil mass and copper sorbate is confirmed, it is interesting to note that the observed plateau behavior (see Fig. 3.3) resembles the adsorption isotherms for systems with a limited number of adsorption sites.^[14,15] This resemblance may be understood in terms of the increased number of pockets with surface functional groups (see Fig. 3.3b) as the soil mass increases, which in turn results in an increase of the amount of copper adsorbed by the soil. Based on Fig. 3.3, it may be concluded that ~1.00 g of Vigoro all-purpose garden soil is capable of adsorbing ~1000 ppm of copper ions. It may also be worth noting that this resemblance may be purely coincidental and may depend on the type of soil, constituents of the soil, and the size of the pocket spaces. For example, initial experiments done using the backyard soil from Dr. Nellutla's home were not successful. It is suspected that the poor porosity and/or absence of necessary functional groups might be the reason behind poor adsorption qualities of the backyard soil. Therefore, the idea of using backyard soils for this project is discarded. Also, further experimentation should be conducted for pre-existing amounts of copper in the soil samples used in order to ensure a greater accuracy of the results. Regardless of these variations, it is still expected that as the amount of soil is increased, the amount of copper adsorbed should also increase.

3.4. Effect of soil pH on the amount of copper adsorbed

To test the effect of soil pH on its adsorption capacity for copper, soil pH was adjusted by soaking it in aqueous HCl or NaOH solution of desired pH. Aqueous HCl and NaOH were chosen because they are the most common acid and base, respectively, and commercial solutions of varying concentrations are readily available in the Chemistry Department. Separate experiments to optimize parameters such as soil mass, volume and concentration of pH altering reagent, soaking times with or without Cu^{2+} ions were also carried out but not included in this thesis. These experiments indicate that soaking the soil in the desired pH solution for 5 days without the copper ions gave the best results. Detailed experimental parameters are listed in Table 2.2 but briefly, ~1.00 g of soil was soaked in either aqueous HCl or NaOH or plain water for 5 days before testing the effect of pH on the copper adsorption. Copper adsorption experiments were carried out as described in section 2.6 of this thesis, after the aqueous layer is carefully removed through pipetting. Although most of the aqueous layer was removed, soil was still wet, and the volume of this aqueous ‘solution’ was ignored in our $[\text{Cu}^{2+}]_{\text{soil}}$ calculations. Filtration method was not employed to separate the soil to prevent its loss during filtration and transfer.

Table 3.1 lists the results from 5 day soaking. Here, the initial pH refers to the aqueous layer pH measured immediately after adding HCl or water or NaOH to the soil and final pH refers to the aqueous layer pH after it was in contact with the soil for 5 days. It is worth to point out that the final soil pH is assumed to be the same as the final pH of the solution in which it was soaked. Considering that the soil was in contact with the pH altering reagent in a sealed vial for five days and the typical recommendation is to make a soil-water slurry in ~1:2 ratio and measuring its pH^[17], the assumption is reasonably valid. It is quite interesting to note that after 5 days, the final pH values seem to “converge” towards $\sim 7 \pm 3$ pH units, with the middle three values being nearly identical. While an exact reason for this behavior is not clearly understood at this time, it may be attributed to the nature of soil used in this project which has various amendments and fertilizers (see Fig. 3.4).

It is well established that soil pH can have a significant effect on its ability to adsorb ionic pollutants.^[14,16] Specifically, an ‘S-curve’ type behavior is observed for various soils and cations^[14,16] and as an example effect of pH on Cu^{2+} adsorption in two types of clays is shown in

Table 3.1. Absorbance, initial and final pH values of soil collected at room temperature and atmospheric pressure. Initial pH refers to the pH of source at time 0 and final pH refers to the pH of the aqueous layer measured after 5 days of equilibration. See Table 2.2 for further details.

Source to alter pH	Initial pH	Final pH	A _{max} of [Cu(BCA) ₂] ⁺ at 562 nm
0.1 M HCl _(aq)	1	4.47	1.536
0.001 M HCl _(aq)	3	7.11	1.198
DI water	~7	7.17	1.326
0.1 M NaOH _(aq)	11	7.22	1.426
0.001 M NaOH _(aq)	13	9.18	0.788



Figure 3.4. Fertilizers and ingredients present in Vigoro all-purpose garden soil. These contents are attributed to soil’s “buffering” nature observed in the experiments measuring pH. See text for further details.

Fig. 3.5a.^[16] This behavior suggests that the copper adsorption rate is expected to steadily increase until a certain pH, rise sharply in a narrow pH range and finally level off steadily at high pH values. Fig. 3.5b depicts the equilibrium constant, K , as a function of final soil pH from 5 day soaking experiment. K values are evaluated using Eq. 3.2. K vs. pH trend observed for Vigoro garden soil is consistent with Cu²⁺ adsorption in clays.^[16]

$$K = \frac{[\text{Cu}^{2+}]_{\text{soil,equb}}}{[\text{Cu}^{2+}]_{\text{aq,equb}}} \quad (3.2)$$

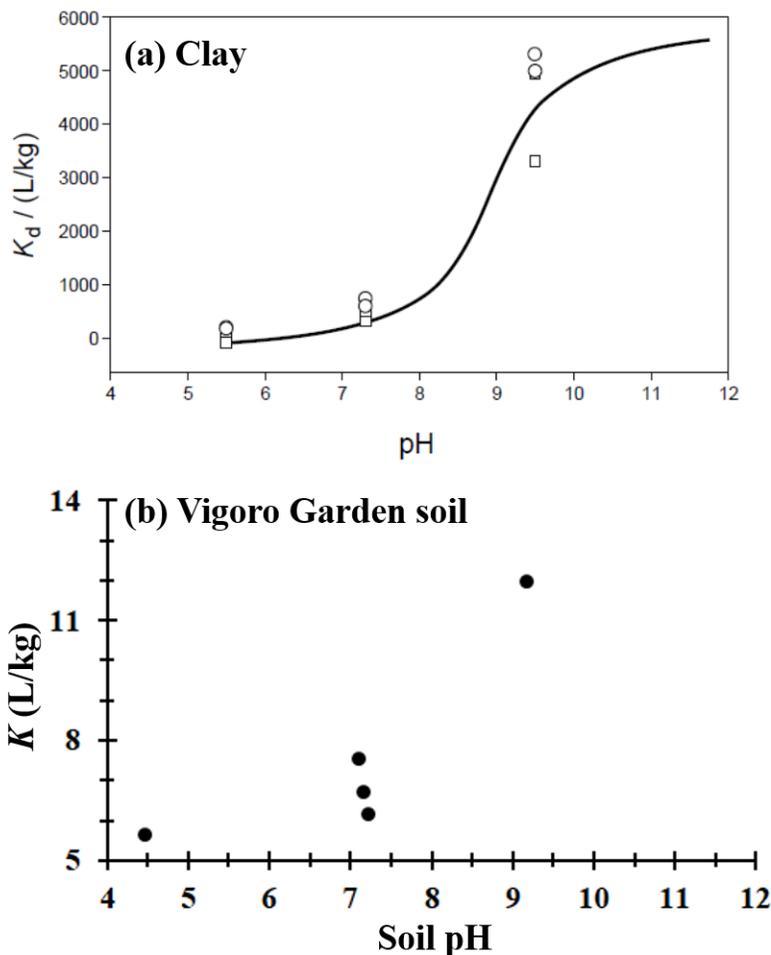


Figure 3.5. K vs. pH graph for: (a) Cu^{2+} ions adsorption by two types of clay^[16]. Circles represent Kaolin, squares represent Montmorillinite and the solid line depicts the expected ‘S’ type pH dependence behavior^[14,16] and (b) Cu^{2+} adsorption by Vigoro Garden soil used in this project. Data represents the results from 5 days soil soaking in pH altering agent. See text for further details.

In Eq. 3.2, $[\text{Cu}^{2+}]_{\text{aq,equb}}$ and $[\text{Cu}^{2+}]_{\text{soil,equb}}$, respectively, represent the equilibrium concentration of Cu^{2+} ions in aqueous phase and in the sorbent soil. Difference in the magnitude of K values is tentatively attributed to the difference in the soil types. At the molecular level, pH dependence can be understood by examining the interactions between soil and ionic contaminants. According to Thompson et. al.^[14], as the solution pH increases, $-\text{OH}$ and $-\text{COOH}$ functional groups present in the soil deprotonate thereby increasing the density of the negative charge in the soil. This enhanced negative charge density facilitates the adsorption of cations such as Cu^{2+} . Leveling of K at high pH values suggest that all the $-\text{O}^-$ and $-\text{COO}^-$ groups being ‘occupied’ by Cu^{2+} ions.

3.5. Temperature effect on copper adsorption by soil

Thermodynamics of the copper adsorption process by soil can be understood by examining the equilibrium $\text{Cu}_{\text{aq}}^{2+} \rightleftharpoons \text{Cu}_{\text{soil}}^{2+}$ and by analyzing the associated partition coefficient, or equilibrium constant shown in Eq. 3.2. Further, since the equilibrium constant is connected to Gibbs free energy and thereby to enthalpy and entropy by the relation^[18] shown in Eqs. 3.3 and 3.4, one can obtain the latter values by monitoring K as a function of temperature, T . Specifically, according to the Van't Hoff equation^[18] shown in Eq. 3.4, slope of $\ln K$ vs. $(1/T)$ gives enthalpy changes associated with the adsorption process, while the y-intercept gives the entropy associated with the adsorption of copper by soil. It should be noted that the Van't Hoff equation assumes that ΔH° and ΔS° do not change significantly as the temperature changes.

$$-RT \ln K = \Delta G^\circ_{\text{sorp}} = \Delta H^\circ_{\text{sorp}} - T \Delta S^\circ_{\text{sorp}} \quad (3.3)$$

$$\ln K = \frac{-\Delta H^\circ_{\text{sorp}}}{RT} - \frac{\Delta S^\circ_{\text{sorp}}}{R} \quad (3.4)$$

Adsorption of copper ions by soil at its natural pH was studied at four different temperatures: 0 °C, 22 °C, 40 °C and 60 °C, with an error of about ± 2 °C. This temperature study was repeated three times to ensure the reproducibility of the expected linear trend. At each temperature, the soil and aqueous copper solutions were allowed to equilibrate for 60 minutes before analyzing the left over $[\text{Cu}^{2+}]$ in the aqueous phase. Further experimentation in which soil is pre-equilibrated at a desired temperature, say for 30 minutes, before the addition of copper solution will ensure that the soil temperature is 'truly' changed without any interference from copper ions. Figure 3.6 shows the $\ln K$ as a function of $(1/T)$ for one of the data sets along with a straight line fit. As can be seen from the figure, data fits very well ($R^2 = 0.9985$) to:

$$\ln K = \frac{-2045.6}{T} + 10.77 \quad (3.5)$$

Comparison of Eq. 3.5 with Eq. 3.4 suggests that $\Delta H^\circ_{\text{sorp}} = +17.0$ kJ/mol, $\Delta S^\circ_{\text{sorp}} = +89.5$ J/mol and at 25°C $\Delta G^\circ_{\text{sorp}} = -9.67$ kJ/mol. These results indicate that the copper adsorption process is entropy driven and as suggested by authors in references [5,19], this may be attributed to the fact that in the aqueous phase Cu^{2+} ions are surrounded by water molecules, represented as

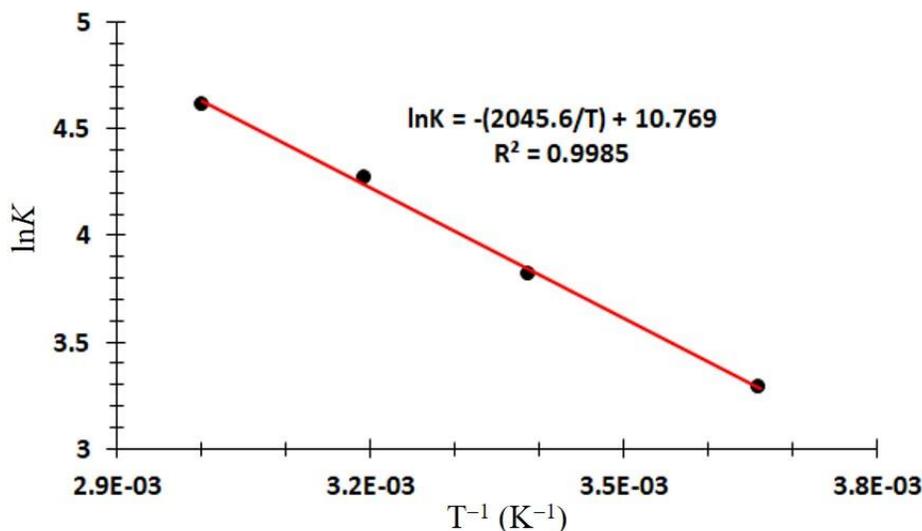


Figure 3.6. $\ln K$ is plotted as a function of inverse temperature. Data were collected soil's natural pH using 1.0 g of soil and monitoring the maximum absorbance of $[Cu(BCA)_2]^+$ complex at $\lambda_{max} = 562$ nm peak. Solid red line represents a linear fit to Van't Hoff equation shown in Eq. 3.4.

Table 3.2. Comparison of thermodynamic data obtained in this work with that of the literature reports.

Parameter	This work	Cessna et.al. [5]	Yavuz et.al [19]
ΔH°_{sorp} (kJ/mol)	+17.0	+10.9	+39.5
ΔS°_{sorp} (kJ/mol·K)	+0.0895	+0.0891	+0.117
$-T\Delta S^\circ_{sorp}$ (kJ/mol) @ 298 K	-26.7	-26.6	-34.9
$(\Delta G^\circ_{sorp})_{25}$ (kJ/mol)	-9.67	-15.7	+4.61

$[Cu(H_2O)_n]^{2+}$, and upon coordination to soil surface, the water molecules are released thereby increasing the entropy of the system.

As can be seen from Table 3.2, while the sign and magnitude of ΔH°_{sorp} , ΔS°_{sorp} and $(\Delta G^\circ_{sorp})_{25}$ match the data reported by Cessna et.al.^[5], they do not match, except for the sign of ΔH°_{sorp} and ΔS°_{sorp} , the ones reported by Yavuz et.al.^[19]. As Cessna et.al.^[5] suggested, the disagreement between the magnitudes and sign of $(\Delta G^\circ_{sorp})_{25}$ could be due to the use of garden soil containing multiple components instead of pure clay used by Yavuz et.al.^[19] Nevertheless, this experiment provides an excellent way to teach equilibrium thermodynamics associated with adsorption processes and allow students an opportunity to learn about data analysis using Van't Hoff relation.

4. Conclusions

In conjunction with the increasing need to better prepare students to the ever-demanding new work environments, skills such as critical and independent thinking, scientific reasoning, and problem-solving are essential to their success in the workplace. Project-based (PBL) or inquiry-based (IBL) laboratory experiences can provide excellent opportunities for students to learn the skills necessary to thrive in any situation. These types of pedagogical methods pose questions, problems, or scenarios with the goal of facilitating learning and understanding of concepts covered in a course.

The results from the work described in this thesis can be summarized as follows:

- (a) Calibration curve for determination of aqueous copper concentration using BCA and glucose was successfully constructed. Results yield the Beer Lamberts equation as $A = 0.0102 [\text{Cu}^{2+}]_{\text{aq}}$ with R^2 of 0.9977 for copper concentrations between 0-100ppm.
- (b) Increasing the mass of soil shows the expected trend of increased amount of copper adsorbed by the soil. Specifically, results suggest an exponential relation between Vigoro garden soil and the adsorption of copper ions. It should be noted that this behavior might be exclusive to the type of soil used in this project. However, in general the adsorption of copper ions is expected to increase proportionally with the increase of mass of the soil.
- (c) Preliminary results reported in this work suggests that adsorption of copper by Vigoro garden soil increases as its pH increases. The graph of copper adsorbed by the soil as a function of final soil pH has a rudimentary S-shaped format which is somewhat consistent with the results found in the literature^[14,16], but further experimentation is needed to determine the right parameters for this study and to confirm that the adsorption of copper by Vigoro soil is truly 'S' shaped.
- (d) As the soil temperature increases, the amount of copper adsorbed by the soil increases. Van't Hoff analysis showed that adsorption of copper by the Vigoro commercial garden soil is endothermic and driven by favorable entropy changes resulting from the release of water molecules surrounding the copper ions when the latter are adsorbed by the soil.^[5,14]

While the adsorption of copper by Kaolinite clay is predicted^[19] to be endergonic at 298 K, our results indicate that the adsorption process in the Vigoro garden soil is exergonic because of the TΔS term. This discrepancy, similar to what is observed by Cessna et.al.^[5], is attributed to the differences between clay and commercial garden soil.

The ideas evaluated in this thesis can be implemented as mini inquiry-driven projects that spread over 3 - 4 weeks, with each week focusing on different concepts covered in CHEM 141 and CHEM 142 courses. Specifically, students would be exploring the effects of mass, pH, and temperature of a commercial garden soil, such as VigoroTM All-purpose Garden Soil, on its adsorption capability for copper ions. These experiments can be implemented in CHEM 142L course at BSU under the theme of 'Environmental Contamination'. We believe that doing so should attract students' attention, encourage their active participation as well as promote a better conceptual understanding of chemical equilibria and related concepts and develop critical thinking and important laboratory skills. A proposed 3-week timeline is as follows.

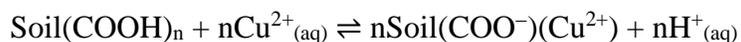
The first week should focus on the development of a calibration curve using UV-Vis spectroscopy because students will need this to assess the amount of copper adsorbed by the soil. Additionally, this gives the students an opportunity to learn and practice with solution preparation, collecting spectra on Perkin Elmer Lambda XLS+ table-top UV-Vis spectrometer, applying Beer Lambert law to analyze the data and using Microsoft Excel for plotting and trend line fit.

The second week should focus on understanding the effect of mass on the adsorption equilibrium $[Cu_{aq}^{2+}] \rightleftharpoons [Cu_{soil}^{2+}]$. A variation that may be useful is to ask one half of the class to change soil mass by keeping initial copper ion concentration at 1000 ppm and ask the other half of the class to change the initial copper ion concentration by keeping soil mass constant. While the former effect is tested in this work, the latter was not tested due to time limitations. These experiments will allow students to learn about the relation between mass and the copper adsorption rate. In addition, they can understand the adsorption process at a molecular level by explaining their results in terms of physical and chemical properties of soil.

The third week can focus on evaluating the temperature effects in terms of thermodynamics of adsorption processes. Specifically, students will experimentally find the value of the equilibrium

constant (K), defined in Eq. 3.2 at various temperatures and analyze the data in terms of Van't Hoff equation given in Eq. 3.4. This will give the students a comprehensive way to understand the relation between K and change in standard Gibbs free energy (ΔG°) as well as the relation between K , change in standard enthalpy (ΔH°), and change in standard entropy (ΔS°) associated with adsorption of copper ions by the soil. Further they will learn about enthalpy driven vs. entropy driven processes and correlate these to the molecular interactions between soil and copper ions.

The effect of pH on the $\text{Cu}_{\text{aq}}^{2+} \rightleftharpoons \text{Cu}_{\text{soil}}^{2+}$ equilibrium was the most difficult to study probably due to complex nature of commercial garden soil's chemical and physical structure, its 'buffering' nature against pH changes, and soil amendments such as fertilizers and organic matter and even traces of copper. Hence, time permitting, this study may be introduced as the 4th week mini-project in CHEM 142L course. Varying the soil pH and monitoring the $[\text{Cu}^{2+}]_{\text{soil}}$ will allow students to see the Le Chatelier's principle in action, according to the qualitative chemical equation shown below.



Instead of a full-scale evaluation of pH (i.e., generating a plot shown in Fig. 3.5b) using aqueous HCl and NaOH solutions, soil could be mixed with agents such as lime to raise soil pH to a desired value and/or use salts such as iron or aluminum sulfate to lower soil pH to a desired value. But it should be noted that these processes require careful planning and extensive experimentation since their use can alter the binding process of copper, thus affecting the results. Another option to illustrate the Le Chatelier's principle is to alter the pH of Cu^{2+} and BCA reaction. Again, further experimentation is needed to ensure that altering the pH doesn't drastically effect the formation of $[\text{Cu}(\text{BCA})_2]^+$ complex or (λ_{max} , A_{max}) doesn't go out of range of the table top UV-Vis spectrometer. Finally, the generation of a curve similar to Fig. 3.5a and evaluation of buffering capacity of soil could be a nice project for sophomore and upper-level chemistry laboratory courses such as Quantitative Chemical Analysis (CHEM 351L) and Physical Chemistry I (CHEM 381L).

Appendix 1 provides step by step instructions for each of the activities described above and can be modified, as needed, on the inquiry-driven spectrum i.e., anywhere between "cookbook-style" to completely "open-ended style".

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Appendix 1

Step by step instructions for three week timeline proposed in Conclusions

Week 1:

Materials:

- a. Balance, pipettes, volumetric flasks, hot water bath @60 °C
- b. 1000ppm Cu²⁺, BCA and 100g/L Glucose stock solutions
- c. Syringes, 0.25 µm syringe filters, syringe needles, and scintillation vials

Standard curve generation

Students will be required to construct a standard curve for copper concentration in aqueous phase, by changing the Cu²⁺ concentration between 0 – 100 ppm.

1. The calibration curve must contain at least 5 points in the 0 – 100 ppm range.
2. After the desired concentrations in this range are determine, students can make the desired volume of these concentrations by diluting an initial 1000ppm Cu²⁺ solution prepared. Alternately, students can be asked to make the stock solution to be used by the class.
3. Then, 2 mL of BCA and 2 mL of the 100g/L Glucose must be added to each of the testing vials.
4. 0.2 mL of each Cu²⁺ concentration should be added to a different testing vial of BCA and Glucose.
5. The solutions testing vials should then be carefully shaken and inserted in the hot bath for approximately 15 minutes.
6. After the 15 minutes the solutions should be allowed 5-10 to cool down to room temperature before being analyzed using the Uv/Vis spectrometer and have the absorbances recorded.

Week 2:

Materials:

- a. Balance, pipettes, volumetric flasks, hot water bath @60 °C
- b. 1000ppm Cu²⁺, BCA and 100g/L Glucose stock solutions
- c. Syringes, 0.25 µm syringe filters, syringe needles, and scintillation vials

Evaluating the relation between soil mass and amount of copper sorbate

1. 4 soil samples (0.100-1.000g) of provided soil samples must be measured and added to glass vials.
2. A 25 mL of 1000ppm Cu²⁺ copper solution must be prepared.

3. 5 mL of the 1000ppm Cu^{2+} must be added to the soil samples and left undisturbed for 60 minutes.
4. In the meantime, a 10 mL of 100g/L Glucose must be prepared.
5. A hot bath @60 °C must be prepared and ready before the 60 minutes are completed.
6. After the 60 minutes are completed the remaining aqueous solution of Cu^{2+} must be removed and filtered using a different syringes and filters and added to a labeled beaker.
7. 2 mL of BCA and 2 mL of the 100g/L Glucose must be added to 4 separated testing vials.
8. Then, 0.2 mL of each filtered remaining Cu^{2+} aqueous solution should be added to a different testing vial of BCA and Glucose.
9. The solutions testing vials should then be shaken and inserted in the hot bath for approximately 15 minutes.
10. After the 15 minutes the solutions should be allowed 5-10 to cool down to room temperature before being analyzed using the Uv/Vis spectrometer.

Week 3:

Materials:

- a. Soil, balances, pipettes
- b. 1000ppm Cu^{2+} , BCA and 100g/L Glucose stock solutions
- c. Filters and syringes
- d. Hot water bath @40 °C and @60 °C; Ice bath (~0 °C)

Evaluating the temperature effect on copper adsorption by soil

1. Students must prepare 2 hot baths, 1 @40 °C and another @60 °C and An Ice bath at approximately 0 °C.
2. 4 soil samples of 1.000g each of the provided soil must be measured and added to a glass vial.
3. A 25 mL of 1000ppm Cu^{2+} copper solution must be prepared.
4. 5 mL of the 1000ppm Cu^{2+} must be added to the soil samples.
5. 1 sample must be left at room temperature and the remaining 3 must be added each to one of the hot/ice baths and left undisturbed for 60 minutes.
6. In the meantime, a 10 mL of 100g/L Glucose must be prepared.
7. After the 60 minutes are completed the remaining aqueous solution of Cu^{2+} must be removed and filtered using different syringes and filters and be added to a labeled beaker.
8. 2 mL of BCA and 2 mL of the 100g/L Glucose must be added to 4 separated testing vials.
9. Then, 0.2 mL of each filtered remaining Cu^{2+} aqueous solution should be added to a different testing vial of BCA and Glucose.
10. The solutions testing vials should then be shaken and inserted in the hot bath @60 °C for approximately 15 minutes.
11. After the 15 minutes the solutions should be allowed 5-10 to cool down to room temperature before being analyzed using the Uv/Vis spectrometer.

Appendix 2

Data tables corresponding to the figures presented in the Results chapter of this work

Table A2.1. Summary of experimental data from the study of the relation between soil mass and amount of copper adsorbed. Standard curve

Mass of soil (g)	Vol. of Cu ²⁺ added (mL)	Conc. Of Cu ²⁺ added (ppm)	Soaking time (min)	A _{max} of [Cu(BCA) ₂] ⁺ complex at 562 nm	Equilibrium [Cu ²⁺] in aqueous layer (ppm)*	Equilibrium [Cu ²⁺] adsorbed by soil (ppm)
0.1015	5	1000	60 min	3.66	359	641
0.3019	5	1000	60 min	1.70	167	833
0.7001	5	1000	60 min	0.461	45.2	955
1.0016	5	1000	60 min	0.145	14.2	986

* Values are obtained from the standard curve $A = 0.0102[\text{Cu}^{2+}]_{\text{aq,equb}}$

Table A2.2. Summary of experimental data from the study of the effect of soil pH on the equilibrium constant *K*. Soil was soaked in 5 mL of the pH altering agent for 5 days. After the aqueous layer was removed, 5 mL of 1000 ppm Cu²⁺_(aq) solution was added to the pH altered soil followed by 60 mins of incubation.

Soil Mass (g)	Initial pH of soil	Final pH of soil	Conc. of aqu. Cu ²⁺ added (ppm)	A _{max} of [Cu(BCA) ₂] ⁺ complex at 562 nm	Equilibrium [Cu ²⁺] in aqueous layer (ppm)*	Equilibrium [Cu ²⁺] adsorbed by soil (ppm)	<i>K</i> [§] (L/kg)
1.000	1	4.47	1000	1.536	151	849	5.64
1.000	3	7.11	1000	1.198	117	883	7.51
1.000	7	7.17	1000	1.326	130	870	6.69
1.000	11	7.22	1000	1.426	140	860	6.15
1.000	13	9.18	1000	0.788	77	923	11.9

* Values are obtained from the standard curve $A = 0.0102[\text{Cu}^{2+}]_{\text{aq,equb}}$

§ *K* is obtained from Eq. 3.2

Table A2.3. Summary of experimental data from the study of the effect of temperature on the equilibrium constant K . 5 mL of 1000 ppm $\text{Cu}^{2+}_{(\text{aq})}$ solution was added 1.00 g soil followed by 60 mins of incubation at the desired temperature.

T^\ddagger (Kelvin)	$1/T$ (Kelvin ⁻¹)	Conc. Of Cu^{2+} added (ppm)	A_{max} of $[\text{Cu}(\text{BCA})_2]^+$ complex at 562 nm	Equilibrium $[\text{Cu}^{2+}]$ in aqueous layer (ppm)*	Equilibrium $[\text{Cu}^{2+}]$ adsorbed by soil (ppm)	K^\S (L/kg)	$\ln K$
273.15	0.00366	1000	0.369	36.2	964	26.6	3.28
295.15	0.00339	1000	0.219	21.5	979	45.6	3.82
313.15	0.00319	1000	0.141	13.8	986	71.3	4.27
333.15	0.00300	1000	0.100	9.80	990	101	4.62

* Values are obtained from the standard curve $A = 0.0102[\text{Cu}^{2+}]_{\text{aq,equb}}$

§ K is obtained from Eq. 3.2

‡ Error is about $\pm 2^\circ\text{C}$