

Incorporation of Phytoremediation Strategies into the Introductory Chemistry Laboratory

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Abstract: An undergraduate laboratory exercise appropriate for introductory chemistry courses at the high school or college level is presented. The objective of the laboratory is to introduce the idea that plants can be used to remove contaminants from the environment—a process called phytoremediation. This laboratory connects the disciplines of chemistry and biology while enabling students to learn the skills of measurement, titration, calculation of molarity of an unknown solution, graphing, and interpretation of data.

Introduction

Contamination of aquatic environments by toxic metals poses multiple severe hazards [1]. Toxic metals, unlike organic pollutants, are not degradable by chemical or biological processes, and thus require remediation. Zinc is one example of these metals. Industries use zinc and zinc compounds in making steel, dry cell batteries, pharmaceutical products, paint, rubber, dyes, wood preservatives, and alloys such as brass and bronze. This has introduced hazardous levels of zinc and zinc compounds throughout our environment. Zinc attaches to soil, sediments, and dust particles in the air, and zinc compounds are known to rapidly move into groundwater, lakes, and waterways where they are absorbed or ingested and retained by fish and other organisms [2, 3].

While zinc is an essential element in the human diet, the lack of which can cause health problems, ingestion or inhalation of amounts in the 100–250 mg/day range are known to be health hazards [3]. Long-term ingestion of these levels of zinc can cause anemia and pancreatic damage, while short-term ingestion of large amounts can induce nausea, vomiting, and intense stomach cramps.

Phytoremediation, a process of using plants to remove contaminants from the environment, is an alternative approach to current remediation strategies [4]. Phytoremediation is an efficient and economical method of contaminant removal without further damaging the environment. Once removed, the metals can be re-extracted for proper disposal or possibly for reuse. This laboratory exercise introduces phytoremediation as a solution to a real-world problem pertinent to the students' lives [5]. Specifically, it demonstrates how an aquatic plant can effectively remove zinc from a solution.

All too often, introductory science courses are perceived as dull, difficult, and useless because students cannot relate the material to their personal experiences [6]. In an attempt to personify such courses, the chemistry and biology faculty at Northern Arizona University are working together to revamp our curriculum. Walls that exist between scientific disciplines present difficulties in accommodating the needs of individual students and in helping them to achieve their professional goals [7]. Examining the issue of remediating metal-contaminated water by using a biological system will provide our students with an understanding of the unity of the

disciplines of chemistry and biology. It will also provide students with a relevant, real-life laboratory experience.

Several unique features exist within this laboratory. Unlike most introductory laboratories, delving into the realms of environmental chemistry that focus on analysis and detection of contaminants [6], this experiment demonstrates a strategy aimed at the *removal* of a contaminant—it offers a solution to a actual situation. Use of the aquatic macrophyte, water hyacinth (*Eichhornia crassipes*) presents a real-time strategy for removal of a representative contaminant metal ion from water. Water hyacinth has been shown to accumulate high concentrations of metals [8–13]. Information on the current use of plants to remediate sites contaminated with metals [14–18] can be provided to students to further stimulate their interest in this issue that is relevant to their lives. Use of water hyacinth also provides many advantages in the laboratory because the plants are easy to grow, they propagate readily, and their large biomass facilitates handling and tissue manipulations.

The goals of this laboratory were to; introduce the concepts of phytoremediation, heighten students' visions of the connections between biology and chemistry, and provide a forum where students can learn the skills of measurement, titration, calculation of the molarity of an unknown solution, graphing, and interpretation of data. This laboratory was successful in meeting these goals.

Description of the Laboratory Exercise

This experiment was conducted over three 2-hour laboratory sessions in a section of 24 students enrolled in the first-semester general chemistry laboratory. All chemicals required for this laboratory were commercially available, and the equipment needed included 50-mL burets, stir plates, stir bars, 10-mL plastic vials with caps, a vessel to hold one water hyacinth plant, and 2 liters of zinc solution. The water hyacinth, *Eichhornia crassipes*, was obtained from a local nursery and cultivated under aquatic greenhouse conditions [19].

The first session of this laboratory involved a 20-minute discussion on the principles of phytoremediation. The students were given two articles [20, 21] and a series of prelaboratory questions designed to assess their understanding of the chemical principles involved in the laboratory (i.e., calculation



Figure 1. Water hyacinth plant in 2 liters of a zinc solution.



a



b



c

Figure 2. Titration using EDTA and Eriochrome Black T.

of molarity of an unknown solution) and their understanding of the process of phytoremediation (see Supporting Material for this handout [530140cks1.pdf](#)). These questions were due at the start of the next laboratory meeting. After this prelaboratory meeting, the students used the remaining 90 minutes to set up their contamination vessels in the laboratory.

The purpose of the second laboratory meeting was for the students to determine the molarity of zinc in each of their 10-mL samples collected the previous week. The instructor asked the students to predict their results, and a brief discussion of what they witnessed last week was conducted. The students analyzed their samples using methods of complexometric titration [22]. The students were able to finish most of their analyses during this laboratory session.

The third laboratory meeting enabled all students to finish their titrations, calculations, and complete graphing of the data. As students finished with their calculations, each gave a copy of the data to the instructor; the instructor was able to collect the class data and present them as an average during the last 30 minutes of this laboratory session. These last 30 minutes of the laboratory session allowed for a meaningful discussion of the experiment, the class data, and a review of

skills and principles learned. In addition, the students were asked to write a one-page (or more), anonymous, critical essay of this laboratory experience.

Experimental

Materials. The water hyacinth plants, *Eichhornia crassipes*, were obtained from a local nursery. Maintenance of the plants was best achieved when the aquatic environment was not cleaned regularly, preserving a more natural habitat. Thus, when the plants were to be used experimentally it was necessary to clean them thoroughly with deionized, distilled water. Caution was necessary when rinsing the plants to prevent damage to the delicate root system.

The following were purchased from Aldrich Chemical and used without further purification: EDTA disodium salt, dihydrate crystal; zinc nitrate, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; sodium hydroxide; buffer solution (pH 10); and Eriochrome Black T indicator. The indicator was prepared using 0.2 g Eriochrome Black T in 15.0 mL deionized, distilled water and stored in a dark bottle to protect it from light.

Procedure. Water hyacinth plants with comparable root mass were chosen for this experiment. Each student placed a water hyacinth plant in 2 liters of a zinc solution with a concentration of approximately 0.05 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (concentration unknown to the student) for one hour (Figure 1). The plants were placed so that the roots were completely under the water—the way the plant is found in its natural environment. The students also set up two control vessels—one with the zinc solution and no plant and one with a plant placed in deionized, distilled water. Aliquots of 10.0 mL were removed at time zero to determine the initial concentration of the zinc solution and after 10 min, 30 min, and 60 min of incubation. After removal of each aliquot, 10.0 mL of deionized, distilled water was added to the solution. These samples were labeled and stored in tightly capped plastic vials at room temperature for analysis during the next laboratory meeting.

Each of these aliquots was then transferred to a small beaker containing a stir bar. In preparation for titration, 1 mL of 1.0 M NaOH was added to the sample to make the solution slightly basic (pH ~8), along with 0.5 mL of buffer (pH 10), and one small drop of Eriochrome Black T indicator solution (Figure 2a). This solution was placed on a magnetic stir plate and stirred throughout the titration. A buret was filled with 50.0 mL of 0.01 M EDTA.

Upon addition of EDTA to the zinc solution, the color changed from a light purple to blue (Figure 2b). Small amounts (1 mL is recommended) of EDTA were added as the solution turned to a pink color (see Figure 2c for the color of this solution). The endpoint was determined to be that point at which the solution remained blue for greater than 5 minutes before returning to pink (blue color persists). The volume of EDTA titrated to reach the endpoint was then used to determine the molarity of the zinc aliquot.

The zinc solutions and plants were treated as zinc waste and labeled properly for appropriate disposal.

Common Student Problems and Sources of Error

This laboratory exercise was an overwhelming success. There were limited student problems and sources of error. Only 5% of the total trials resulted in a “mistrial”—meaning that the solution never turned blue upon addition of the EDTA when titrating. This can occur when a student adds too much Eriochrome solution, NaOH, and/or buffer to the solution before titrating. Another source of error can occur if the students store their samples for analysis at a later time, as ours did. Students need to cap their sample vials tightly, as evaporation of water from the sample vial will cause a change in the molarity of solution over time. In our class, some students noticed an increase in the molarity of zinc

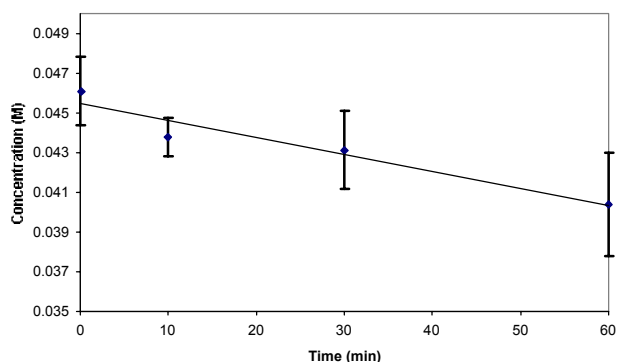


Figure 3. Graph of the average calculated molarity of zinc in a sample at a given interval.

concentration over time. Upon inspection of the students' vials we noticed that they had stored their samples uncapped, allowing evaporation to occur. Finally, we recommend that the students initially titrate their samples at a rate of 1 mL at a time to avoid overshooting the endpoint.

Results and Discussion

Students' Results from This Laboratory Experiment.

The data graphed in Figure 3 were compiled from the average calculated molarity of zinc in a sample at a given interval. For example, after the plant had been immersed in zinc solution for 10 minutes, the average data for the class show a 0.043 M sample solution of zinc. This illustrates removal of zinc from solution by the plant over time.

The students were able to demonstrate that the water hyacinth is capable of removing 0.005 M zinc from solution, or 10% of the zinc from the initial solution, over 60 minutes. These data coincide with those determined by the instructor and research students, before implementing the laboratory, using atomic absorption spectroscopy and complexometric titration to measure the zinc concentrations.

During the final laboratory meeting we discussed the environmental impact of this rate of removal of zinc from solution as well as concepts of saturation. The students came up with solutions to saturation—harvest the plants at a determined interval and replace with new plants to maintain a constant rate of removal of metal ions.

Assessment of the Laboratory. Curriculum-reform efforts clearly require assessment methods that credibly determine improvements in skill development and student learning [23]. The data collected from the 24 students participating in this laboratory exercise provide the basis for an observational study to qualitatively determine whether this laboratory could offer educational advantages over the current laboratory experiments. The following factors were considered as points of observation:

Student Attitudes. According to the constructivist view of knowledge, education occurs only when students are willing to actively engage in their learning experiences. Therefore, student attitudes greatly impact the degree to which learning can occur [24]. In order to collect information about students' attitudes, during their last meeting we collected a one-page (or more), anonymous, critical essay of each student's view of the laboratory experience. We used these essays to generate a list of frequently recurring themes:

- This laboratory exercise was more interesting and enjoyable than the other exercises completed thus far.
- This laboratory exercise was much more relaxing than the other exercises completed thus far.
- This was a simple, yet effective way of learning how to measure, titrate, calculate molarity, and graph results.
- The real-life applications of this laboratory exercise were apparent as well as the connections to biology.
- This laboratory exercise should be incorporated into future sections of general chemistry.

Student Achievement. It can be argued that student achievement is of higher importance in assessment than student attitudes. Clearly this idea has merit, since there are students who are successful in subjects for which, by their own admission, they lack interest and/or enthusiasm. While a positive attitude may lay the foundation for a successful learning experience, one's achievement ultimately determines one's educational and professional fate.

This laboratory exercise replaced for these students an exercise with the same achievement objectives: obtaining measurement and titration skills, and understanding how to calculate the molarity of an unknown solution, how to graph the data calculated, and how to interpret these data. All of the students in this laboratory section completed these achievement objectives successfully.

Conclusion

This laboratory exercise was an overwhelming success. Both the students' positive attitudes and their achievements in attaining the goals that we put forward contributed to the success of the exercise. Each student gained knowledge of the concepts of phytoremediation, had an enhanced vision of the connections between biology and chemistry, and was able to successfully measure solutions, titrate a sample, calculate the molarity of an unknown solution, graph these calculations, and interpret these data. Furthermore, the results from the students' attitudes illustrated some weaknesses in our current program (e.g., laboratory experiments that are stressful and not very interesting). Thus we have a new perspective for evaluation our current introductory chemistry laboratory experiments.

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