ACR (2024-25)
Summer Assignment #1
Basics of Scientific Research
(40 points)

You will need to read two papers (both will be emailed to you).

Due date: By 11:00 pm on August 10, 2024, provide me with an electronic file (via email) with the following file name:

ACR–Research Basics(xxx).doc, **.docx, or .pdf** (where "xxx" are your initials)

Here are the references and the assignments:

1. Alon, Uri (2009). How to choose a good scientific problem. Molecular Cell 36(6), 726-728.

From this paper, quote three (3) passages that you find interesting or that raise questions for you. Write a brief (2-4 complete sentences) response to each of your chosen quotes.

Here is a link to a Ted Talk from Dr. Alon which might help clarify some of the ideas in his paper:

https://www.ted.com/talks/uri_alon_why_science_demands_a_leap_into_the_unknown#t-95423

2. Stewart, Anna (2009). A Research Guide for Students and Teachers. Syracuse: State University of New York College of Environmental Science and Forestry.
(Read Part 1: Beginning a Research Project, from page 1 to page 15.)

Write answers to the following questions (2-4 complete sentences for each question):

1. *What is the difference between primary and secondary research?*
2. *How do the two types of research work together in the overall research process?*
3. *What are three (3) reasons to spend a good deal of time doing quality secondary research?*

After looking over the possible research ideas from the list that begins on page 7 of the Stewart document, choose one possibility that might interest you or come up with another idea based on your interests. Pose two additional related questions that further develop the initial idea presented.

If you find a paper that you would like to review and you can't retrieve a full-length version, please email me – I may be able to get it for you.

Have a great summer, and if you have any questions, don't hesitate to ask.

Mr. Smith
ssmith@rvgs.k12.va.us

ACR (2024-25)
Summer Assignment #2
How To Read A Scientific Research Paper
(50 points)

You will need to read two papers (both will be emailed to you).

Due date: First thing in class on August 27, 2024, submitted to TurnItIn. Also provide me with an electronic file (via email) with the following file name:

ACR–Research Papers – Figure Facts(xxx).doc, **.docx, or .pdf** (where "xxx" are your initials)

Here are the references and the assignment (digital files will be emailed to you):

1. Maureen A. Carey et al (2020). *Ten Simple Rules for Reading a Scientific Paper.* PLOS Computational Biology, 16(7)

*You will need to read this paper in its entirety. Make notes on each of the ten rules, paraphrasing (in your own words) why each of the rules are important when reading a research paper. (This will **not** have to be turned in.)*

2. Amaris, Zoe et al (2017). *Using Mung Beans as a Simple, Informative Means to Evaluate the Phytotoxicity of Engineered Nanomaterials...* Journal of Chemical Education, 1428-1433.

You will need to read this paper, paying close attention to the figures in it (there are six of them).

*You will also need to fill out the **Figure Facts Template** for the paper (.docx). This will be submitted to TurnItIn on the first day we meet as a class (most likely August 27th). If you are absent that day, you will still need to submit it to TurnItIn no later than the beginning of elective class that day).*

If you find a paper that you would like to review and you can't retrieve a full-length version, please email me – I may be able to get it for you.

Have a great summer, and if you have any questions, don't hesitate to ask.

Mr. Smith
ssmith@rvgs.k12.va.us

PLOS COMPUTATIONAL BIOLOGY

EDITORIAL

Ten simple rules for reading a scientific paper

Maureen A. Carey*, Kevin L. Steiner, William A. Petri Jr

Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia, United States of America

* mac9jc@virginia.edu

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Introduction

"There is no problem that a library card can't solve" according to author Eleanor Brown [1]. This advice is sound, probably for both life and science, but even the best tool (like the library) is most effective when accompanied by instructions and a basic understanding of how and when to use it.

For many budding scientists, the first day in a new lab setting often involves a stack of papers, an email full of links to pertinent articles, or some promise of a richer understanding so long as one reads enough of the scientific literature. However, the purpose and approach to reading a scientific article is unlike that of reading a news story, novel, or even a textbook and can initially seem unapproachable. Having good habits for reading scientific literature is key to setting oneself up for success, identifying new research questions, and filling in the gaps in one's current understanding; developing these good habits is the first crucial step.

Advice typically centers around two main tips: read actively and read often. However, active reading, or reading with an intent to understand, is both a learned skill and a level of effort. Although there is no one best way to do this, we present 10 simple rules, relevant to novices and seasoned scientists alike, to teach our strategy for active reading based on our experience as readers and as mentors of undergraduate and graduate researchers, medical students, fellows, and early career faculty. Rules 1–5 are big picture recommendations. Rules 6–8 relate to philosophy of reading. Rules 9–10 guide the "now what?" questions one should ask after reading and how to integrate what was learned into one's own science.

Rule 1: Pick your reading goal

What you want to get out of an article should influence your approach to reading it. **Table 1** includes a handful of example intentions and how you might prioritize different parts of the same article differently based on your goals as a reader.

Rule 2: Understand the author's goal

In written communication, the reader and the writer are equally important. Both influence the final outcome: in this case, your scientific understanding! After identifying your goal, think about the author's goal for sharing this project. This will help you interpret the data and understand the author's interpretation of the data. However, this requires some understanding of who the author(s) are (e.g., what are their scientific interests?), the scientific field in which they work (e.g., what techniques are available in this field?), and how this paper fits into the author's research (e.g., is this work building on an author's longstanding project or controversial idea?). This information may be hard to glean without experience and a history of reading. But don't let this be a discouragement to starting the process; it is by the act of reading that this experience is gained!

Table 1. Reading intentions and how it might influence your approach.

Examples	Intention	Priorities
1	You are new to reading scientific papers.[1]	For each panel of each figure, focus particularly on the questions outlined in Rule 3.
2	You are entering a new field and want to learn what is important in that field.	Focus on the beginning (motivation presented in the introduction) and the end (next steps presented in the conclusion).
3	You receive automated alerts to notify you of the latest publication from a particular author whose work inspires you; you are hoping to work with them for the next phase of your research career and want to know what they are involved in.	Skim the entire work, thinking about how it fits into the author's broader publication history.
4	You receive automated alerts to notify you of the latest publication containing a set of keywords because you want to be aware of new ways a technique is being applied or the new developments in a particular topic or research area.	Focus on what was done in the methods and the motivation for the approach taken; this is often presented in the introduction.
5	You were asked to review an article prior to publication to evaluate the quality of work or to present in a journal club.[2]	Same as example 1. Also, do the data support the interpretations? What alternative explanations exist? Are the data presented in a logical way so that many researchers would be able to understand? If the research is about a controversial topic, do the author(s) appropriately present the conflict and avoid letting their own biases influence the interpretation?

[1] Yay! Welcome!

[2] A journal club is when a group of scientists get together to discuss a paper. Usually one person leads the discussion and presents all of the data. The group discusses their own interpretations and the authors' interpretation.

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A good step toward understanding the goal of the author(s) is to ask yourself: What kind of article is this? Journals publish different types of articles, including methods, review, commentary, resources, and research articles as well as other types that are specific to a particular journal or groups of journals. These article types have different formatting requirements and expectations for content. Knowing the article type will help guide your evaluation of the information presented. Is the article a methods paper, presenting a new technique? Is the article a review article, intended to summarize a field or problem? Is it a commentary, intended to take a stand on a controversy or give a big picture perspective on a problem? Is it a resource article, presenting a new tool or data set for others to use? Is it a research article, written to present new data and the authors' interpretation of those data? The type of paper, and its intended purpose, will get you on your way to understanding the author's goal.

Rule 3: Ask six questions

When reading, ask yourself: (1) What do the author(s) want to know (motivation)? (2) What did they do (approach/methods)? (3) Why was it done that way (context within the field)? (4) What do the results show (figures and data tables)? (5) How did the author(s) interpret the results (interpretation/discussion)? (6) What should be done next? (Regarding this last question, the author(s) may provide some suggestions in the discussion, but the key is to ask yourself what you think should come next.)

Each of these questions can and should be asked about the complete work as well as each table, figure, or experiment within the paper. Early on, it can take a long time to read one

article front to back, and this can be intimidating. Break down your understanding of each section of the work with these questions to make the effort more manageable.

Rule 4: Unpack each figure and table

Scientists write original research papers primarily to present new data that may change or reinforce the collective knowledge of a field. Therefore, the most important parts of this type of scientific paper are the data. Some people like to scrutinize the figures and tables (including legends) before reading any of the "main text": because all of the important information should be obtained through the data. Others prefer to read through the results section while sequentially examining the figures and tables as they are addressed in the text. There is no correct or incorrect approach: Try both to see what works best for you. The key is making sure that one understands the presented data and how it was obtained.

For each figure, work to understand each x- and y-axes, color scheme, statistical approach (if one was used), and why the particular plotting approach was used. For each table, identify what experimental groups and variables are presented. Identify what is shown and how the data were collected. This is typically summarized in the legend or caption but often requires digging deeper into the methods: Do not be afraid to refer back to the methods section frequently to ensure a full understanding of how the presented data were obtained. Again, ask the questions in Rule 3 for each figure or panel and conclude with articulating the "take home" message.

Rule 5: Understand the formatting intentions

Just like the overall intent of the article (discussed in Rule 2), the intent of each section within a research article can guide your interpretation. Some sections are intended to be written as objective descriptions of the data (i.e., the Results section), whereas other sections are intended to present the author's interpretation of the data. Remember though that even "objective" sections are written by and, therefore, influenced by the authors interpretations. Check out **Table 2** to understand the intent of each section of a research article. When reading a specific paper, you can also refer to the journal's website to understand the formatting intentions. The "For Authors" section of a website will have some nitty gritty information that is less relevant for the reader (like word counts) but will also summarize what the journal editors expect in each section. This will help to familiarize you with the goal of each article section.

Rule 6: Be critical

Published papers are not truths etched in stone. Published papers in high impact journals are not truths etched in stone. Published papers by bigwigs in the field are not truths etched in stone. Published papers that seem to agree with your own hypothesis or data are not etched in stone. Published papers that seem to refute your hypothesis or data are not etched in stone.

Science is a never-ending work in progress, and it is essential that the reader pushes back against the author's interpretation to test the strength of their conclusions. Everyone has their own perspective and may interpret the same data in different ways. Mistakes are sometimes published, but more often these apparent errors are due to other factors such as limitations of a methodology and other limits to generalizability (selection bias, unaddressed, or unappreciated confounders). When reading a paper, it is important to consider if these factors are pertinent.

Critical thinking is a tough skill to learn but ultimately boils down to evaluating data while minimizing biases. Ask yourself: Are there other, equally likely, explanations for what is observed? In addition to paying close attention to potential biases of the study or author(s), a

Table 2. The structure of a primary research article.

Section	Content
Title	The "take home" message of the entire project, according to the authors.
Author list	These people made significant scientific contributions to the project. Fields differ in the standard practice for ordering authors. For example, as a general rule for biomedical sciences, the first author led the project's implementation, and the last author was the primary supervisor to the project.
Abstract	A brief overview of the research question, approach, results, and interpretation. This is the road map or elevator pitch for an article.
Introduction	Several paragraphs (or less) to present the research question and why it is important. A newcomer to the field should get a crash course in the field from this section.
Methods	What was done? How was it done? Ideally, one should be able to recreate a project by reading the methods. In reality, the methods are often overly condensed. Sometimes greater detail is provided within a "Supplemental" section available online (see below).
Results	What was found? Paragraphs often begin with a statement like this: "To do X, we used approach Y to measure Z." The results should be objective observations.
Figures, tables, legends, and captions	The data are presented in figures and tables. Legends and captions provide necessary information like abbreviations, summaries of methods, and clarifications.
Discussion	What do the results mean and how do they relate to previous findings in the literature? This is the perspective of the author(s) on the results and their ideas on what might be appropriate next steps. Often it may describe some (often not all!) strengths and limitations of the study: Pay attention to this self-reflection of the author(s) and consider whether you agree or would add to their ideas.
Conclusion	A brief summary of the implications of the results.
References	A list of previously published papers, datasets, or databases that were essential for the implementation of this project or interpretation of data. This section may be a valuable resource listing important papers within the field that are worth reading as well.
Supplemental material	Any additional methods, results, or information necessary to support the results or interpretations presented in the discussion.
Supplemental data	Essential datasets that are too large or cumbersome to include in the paper. Especially for papers that include "big data" (like sequencing or modeling results), this is often where the real, raw data is presented.

Research articles typically contain each of these sections, although sometimes the "results" and "discussion" sections (or "discussion" and "conclusion" sections) are merged into one section. Additional sections may be included, based on request of the journal or the author(s). Keep in mind: If it was included, someone thought it was important for you to read.

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reader should also be alert to one's own preceding perspective (and biases). Take time to ask oneself: Do I find this paper compelling because it affirms something I already think (or wish) is true? Or am I discounting their findings because it differs from what I expect or from my own work?

The phenomenon of a self-fulfilling prophecy, or expectancy, is well studied in the psychology literature [2] and is why many studies are conducted in a "blinded" manner [3]. It refers to the idea that a person may assume something to be true and their resultant behavior aligns to make it true. In other words, as humans and scientists, we often find exactly what we are looking for. A scientist may only test their hypotheses and fail to evaluate alternative hypotheses; perhaps, a scientist may not be aware of alternative, less biased ways to test her or his hypothesis that are typically used in different fields. Individuals with different life, academic, and work experiences may think of several alternative hypotheses, all equally supported by the data.

Rule 7: Be kind

The author(s) are human too. So, whenever possible, give them the benefit of the doubt. An author may write a phrase differently than you would, forcing you to reread the sentence to understand it. Someone in your field may neglect to cite your paper because of a reference count limit. A figure panel may be misreferenced as Supplemental Fig 3E when it is obviously Supplemental Fig 4E. While these things may be frustrating, none are an indication that the quality of work is poor. Try to avoid letting these minor things influence your evaluation and interpretation of the work.

Similarly, if you intend to share your critique with others, be extra kind. An author (especially the lead author) may invest years of their time into a single paper. Hearing a kindly phrased critique can be difficult but constructive. Hearing a rude, brusque, or mean-spirited critique can be heartbreaking, especially for young scientists or those seeking to establish their place within a field and who may worry that they do not belong.

Rule 8: Be ready to go the extra mile

To truly understand a scientific work, you often will need to look up a term, dig into the supplemental materials, or read one or more of the cited references. This process takes time. Some advisors recommend reading an article three times: The first time, simply read without the pressure of understanding or critiquing the work. For the second time, aim to understand the paper. For the third read through, take notes.

Some people engage with a paper by printing it out and writing all over it. The reader might write question marks in the margins to mark parts (s)he wants to return to, circle unfamiliar terms (and then actually look them up!), highlight or underline important statements, and draw arrows linking figures and the corresponding interpretation in the discussion. Not everyone needs a paper copy to engage in the reading process but, whatever your version of "printing it out" is, do it.

Rule 9: Talk about it

Talking about an article in a journal club or more informal environment forces active reading and participation with the material. Studies show that teaching is one of the best ways to learn and that teachers learn the material even better as the teaching task becomes more complex [4–5]; anecdotally, such observations inspired the phrase "to teach is to learn twice."

Beyond formal settings such as journal clubs, lab meetings, and academic classes, discuss papers with your peers, mentors, and colleagues in person or electronically. Twitter and other social media platforms have become excellent resources for discussing papers with other scientists, the public or your nonscientist friends, or even the paper's author(s). Describing a paper can be done at multiple levels and your description can contain all of the scientific details, only the big picture summary, or perhaps the implications for the average person in your community. All of these descriptions will solidify your understanding, while highlighting gaps in your knowledge and informing those around you.

Rule 10: Build on it

One approach we like to use for communicating how we build on the scientific literature is by starting research presentations with an image depicting a wall of Lego bricks. Each brick is labeled with the reference for a paper, and the wall highlights the body of literature on which the work is built. We describe the work and conclusions of each paper represented by a labeled brick and discuss each brick and the wall as a whole. The top brick on the wall is left blank: We

aspire to build on this work and label this brick with our own work. We then delve into our own research, discoveries, and the conclusions it inspires. We finish our presentations with the image of the Legos and summarize our presentation on that empty brick.

Whether you are reading an article to understand a new topic area or to move a research project forward, effective learning requires that you integrate knowledge from multiple sources ("click" those Lego bricks together) and build upwards. Leveraging published work will enable you to build a stronger and taller structure. The first row of bricks is more stable once a second row is assembled on top of it and so on and so forth. Moreover, the Lego construction will become taller and larger if you build upon the work of others, rather than using only your own bricks.

Build on the article you read by thinking about how it connects to ideas described in other papers and within own work, implementing a technique in your own research, or attempting to challenge or support the hypothesis of the author(s) with a more extensive literature review. Integrate the techniques and scientific conclusions learned from an article into your own research or perspective in the classroom or research lab. You may find that this process strengthens your understanding, leads you toward new and unexpected interests or research questions, or returns you back to the original article with new questions and critiques of the work. All of these experiences are part of the "active reading": process and are signs of a successful reading experience.

In summary, practice these rules to learn how to read a scientific article, keeping in mind that this process will get easier (and faster) with experience. We are firm believers that an hour in the library will save a week at the bench; this diligent practice will ultimately make you both a more knowledgeable and productive scientist. As you develop the skills to read an article, try to also foster good reading and learning habits for yourself (recommendations here: [6] and [7], respectively) and in others. Good luck and happy reading!

Acknowledgments

Thank you to the mentors, teachers, and students who have shaped our thoughts on reading, learning, and what science is all about.

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JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

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Using Mung Beans as a Simple, Informative Means To Evaluate the Phytotoxicity of Engineered Nanomaterials and Introduce the Concept of Nanophytotoxicity to Undergraduate Students

Shailise S. Ross,[†] Matthew J. Owen,[‡] Brian P. Pedersen,[†] Gang-yu Liu,*[,†] and William J. W. Miller*[,‡]

[†]Department of Chemistry, University of California, Davis, California 95616, United States

[‡]Department of Chemistry, Sacramento City College, Sacramento, California 95822, United States

Supporting Information

ABSTRACT: This work presents a lecture and lab series that focuses on teaching the concept of nanophytotoxicity to undergraduate students in a relatively simple experiment. In this experiment, students evaluated the phytotoxicity of engineered nanomaterials (ENMs) using mung beans (i.e., *Vigna radiata*) and industrially relevant, commercially available ENMs, silicon dioxide (SiO_2) and zinc oxide (ZnO) nanoparticles (NPs). In comparison to the control system using solutions of Nanopure water, the growth of mung beans in solutions of ZnO NPs with a concentration of 20 mg/L was severely stunted, showing clear evidence of a high level of nanophytotoxicity. The growth of mung beans in solutions of SiO_2 NPs with the same concentration was intermediate to, though statistically separate from, the aforementioned solutions, showing clear evidence of a lower level of nanophytotoxicity than for the ZnO NPs. The simplicity of the experiment and the clear phytotoxic results should make this experiment of interest to many types of students including science majors, nonmajors, and high school students.

KEYWORDS: *First-Year Undergraduate/General, High School/Introductory Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Collaborative/Cooperative Learning, Nanotechnology, Plant Chemistry, Toxicology*

INTRODUCTION

Nanomaterials are generally classified as those materials having at least one dimension less than 100 nm, which leads to the possibility that they can exhibit different properties than their respective bulk materials.[1] Engineered nanomaterials (ENMs) are materials created with nanoscale dimensions. There are over 1800 consumer products reported to include ENMs. Two of the top 10 ENMs in terms of both the number of commercial products and estimated worldwide production are ENMs made from silicon dioxide (SiO_2) and zinc oxide (ZnO).[2]

Although there have been great advances in the production and uses of ENMs, their impacts on health and the environment are not completely clear and often depend on the transport and exposure pathways as well as the subjects into which ENMs enter.[3] This complexity regarding nanotoxicity often leads to seemingly conflicting conclusions, causing confusion among scientists and especially among nonscientists.[4] In one example, recent reports in the media have focused on the possible nanotoxicity of titanium dioxide (TiO_2) nanoparticles (NPs), a component in sunscreens and the confections of Dunkin Donuts. Dunkin Donuts has since announced that it is removing all TiO_2 from its products.[5]

Given the massive industrial quantities of SiO_2 and ZnO ENMs being produced,[6] it is important to determine the impacts of these materials on the environment. One important aspect of their interactions with plants, via accumulation in soils and subsequent entry into plants, is through the application of biosolids, sewage sludge that has been treated, to agricultural fields. In the U.S., approximately 3.36 million tons of biosolids (representing 60% of the sewage sludge in the U.S.) are applied to over 70 million agricultural acres annually as fertilizer.[7] The concentration of zinc in a sample of biosolids was measured to be 620 mg Zn/kg dry mass of biosolid, still 10 times less than the limit set by the Environmental Protection Agency but a factor of 30 times more enriched than typical soil[8] (and a factor of more than 30 times more concentrated than some of the solutions used in this experiment).

As researchers and educators in the area of nanotechnology and nanoscience, we believe one of the best means to deal with the complexity regarding the topic of nanotoxicity is to introduce the concept of nanophytotoxicity, as well as the objective means to investigate this concept, to students. This vision is shared by the U.S. National Nanotechnology Initiative (NNI), as indicated in one of the NNI's primary goals: to develop and sustain educational resources, a skilled workforce, and a dynamic infrastructure and toolset to advance nanotechnology.[1]

Complementary to other work that has created courses that focus on nanotechnology for undergraduate students,[9] this work introduces nanophytotoxicity as part of a one-unit seminar-style course that includes eight lecture/laboratory series on topics in several areas of nanotechnology[10] without the typical demands of a majors science course. This course has a prerequisite of first semester general chemistry, but is not a required course. Anecdotally, the majority of students who take the course are interested in advanced laboratory and research experiences before transferring from community college.

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While many educators have developed experiments that focus on the synthesis and characterization of ENMs,[11] past reports have also detailed lecture/laboratory experiments that focused on the toxicity of coinage metal NPs to Wisconsin Fast Plants (*Brassica rapa*)[12] and brine shrimp,[13] respectively. While these reports provided educational protocols to expose students to the preparation of ENMs and the procedures to study their toxicity, a significant toxic effect seemed difficult to visualize and extract due, perhaps, to the systems and exposure conditions selected. To enable the students to clearly observe a toxic effect, we carefully selected ENM systems and exposure conditions that enabled students to visualize and measure the effects of phytotoxicity.

This work introduces the concept of nanophytotoxicity during a 1-h lecture and enhances students' understanding of nanophytotoxicity via a laboratory sequence. For the laboratory portion, students set up the experiment during the first lab period, monitor and record data over 2 weeks while the plants grow, and then analyze and draw conclusions about their results during a second lab period. In comparison to prior approaches, our method uses commercially available NPs and is, therefore, relatively simple to implement in a variety of courses at the undergraduate level. With data taken by students, the toxicity results are definitive qualitatively (visually) and quantitatively.

EXPERIMENTAL OVERVIEW

This experiment was completed by two classes of students during the Spring 2014 semester (18 students; within 10 days) and the Spring 2015 semester (12 students; within 14 days). Students attended a 50 min lecture introducing them to the topic of nanophytotoxicity. The lecture covered the basic types of ENMs, the ways in which ENMs can affect plants (both positively and negatively), and the importance of knowing how ENMs affect the environment. In addition, basic statistics (i.e., mean and standard error) were covered along with an overview of what would be introduced in the lab.

The initial laboratory portion of the experiment involved completion of the setup of the experiment, instruction in the protocols for acquiring and analyzing the images of the mung beans, and practice analyzing the digital images of the mung beans and the NPs. The complete experimental protocol is available in the Supporting Information.

Mung beans were obtained from the bulk section of a local grocery store. Five mung beans were placed in each Petri dish. In general, this number of beans allowed for relatively easy visual observation and analysis of the growth of each bean. Each experiment included four Petri dishes using the same conditions. Hence, for four experimental conditions, using Nanopure water and solutions of 20 mg/L SiO_2 NPs, 20 mg/L ZnO NPs, and 2000 mg/L ZnO NPs, the experiment included 16 Petri dishes, and each data point, generally, represented the length of mung bean growth averaged over 20 mung beans.

Exposure to the NPs was attained by soaking the mung beans in aqueous solutions containing a designated concentration and type of NP. The students worked in groups to prepare the solutions by diluting premade stock solutions. Premade stock solutions were used instead of powdered NPs to minimize students' exposure to the NPs. The solutions were then sonicated for 15 min immediately before being used to submerge the mung beans in solution. The covered dishes were placed on a counter, away from windows, and under 24-h overhead room lighting.

The students, with each student signing up to monitor the mung beans for at least 1 day, monitored growth of the mung beans for 10−14 days. Monitoring the mung beans included sonicating each solution, refilling the Petri dishes as necessary to maintain submersion of the mung beans and their growth, and recording a digital image of each Petri dish (approximately 2 h total). Pictures were taken at the same time, daily, within a ±2 h window. A cell phone, mounted on a home-built apparatus, was used to capture the images.

During the second lab period, each student then analyzed his/her images of the mung beans using ImageJ, a free image analysis program. A ruler positioned in the background of each image allowed for straightforward calibration of distance. The "segmented line" tool was then used to trace the length of growth of each mung bean. Figure 1 shows what a typical image of the mung beans looks like before and after analysis with ImageJ, with a yellow line approximating the segmented line used to determine the length of mung bean growth for a mung bean. The length of mung bean growth was defined as the length of the hypocotyl and the root together, the sum of the length of what has been called the "shoot + root".[14]

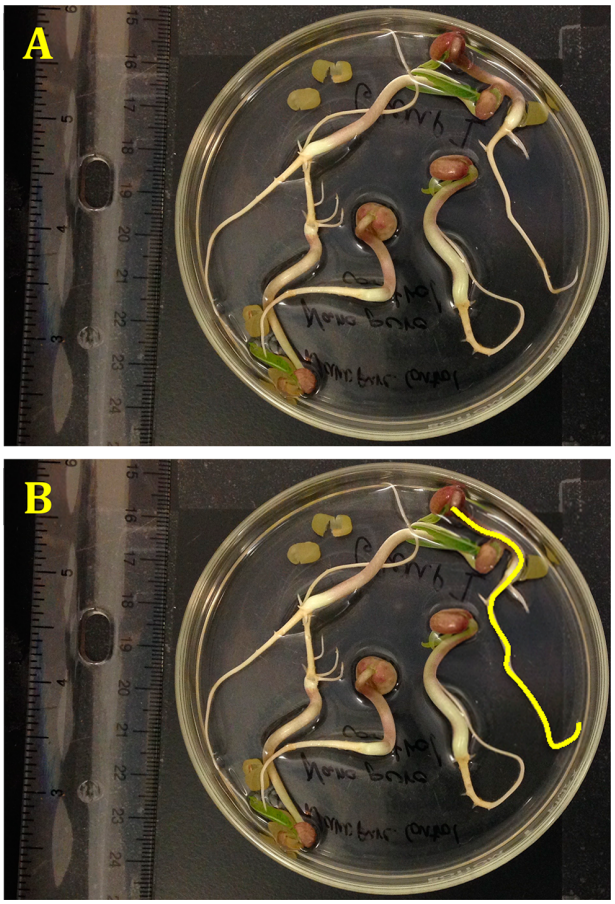

Figure 1. Digital image of five mung beans grown in Nanopure water for 7 days during Spring 2015. (A) Original photo. (B) Same photo with a yellow line approximating the segmented line drawn in ImageJ.

HAZARDS

Dry silicon oxide and zinc oxide NPs become airborne easily and care should be taken to prevent inhalation. Dry NPs should

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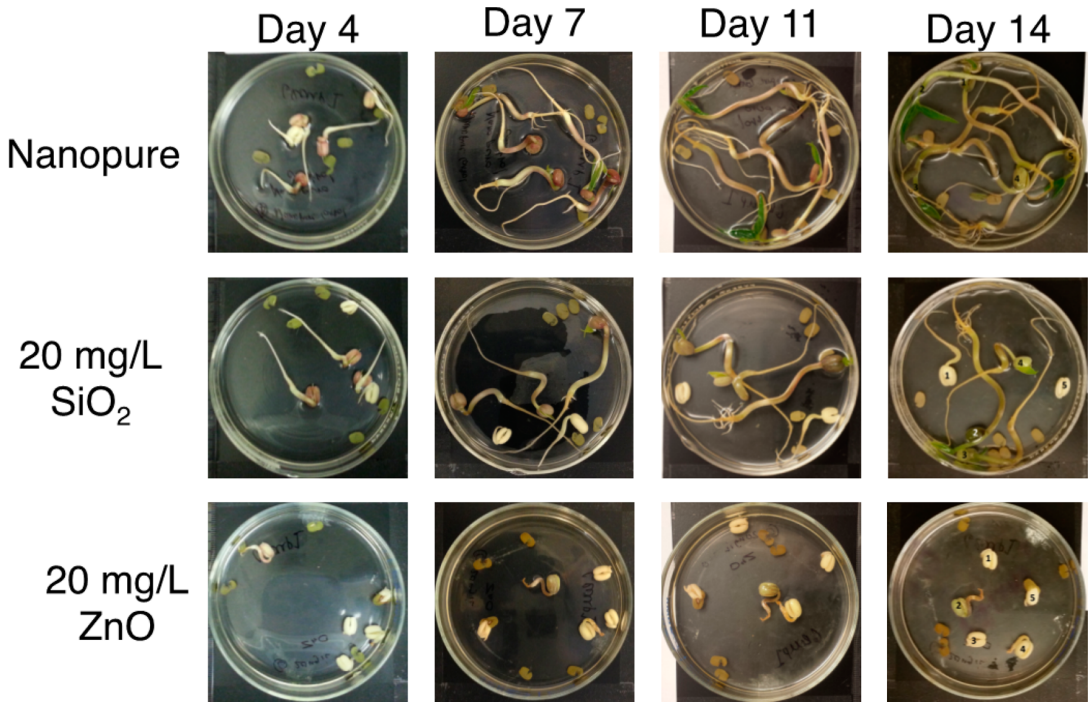

Figure 2. Digital images of typical mung bean growth over a 14-day period during Spring 2015 while submerged in Nanopure water and in 20 mg/L solutions of SiO_2 NPs and ZnO NPs. Images were taken by students using their cell phone cameras resulting in different colors and resolutions.

be handled only in a fume hood. Zinc oxide NPs are very toxic to aquatic life. Waste containers must be available for the collection of all discarded solutions containing NPs. Students should wear goggles and gloves and should follow all general chemistry laboratory safety precautions while performing this laboratory. To ensure safety, solutions containing high concentrations of NPs were prepared by the laboratory instructor prior to the student sessions. In class, students took a designated amount of concentrated solution and diluted it to the required concentration for their experiments.

■ QUALITATIVE RESULTS

Figure 2 illustrates three series of experiments that represent important scenarios in the study of nanophytotoxicity: a control experiment, evidence of a lower level of toxicity, and evidence of a higher level of toxicity, respectively. Out of the 14-day experiment during Spring 2015, images from 4 days are shown in Figure 2. One representative Petri dish from each system, of the four replicates, is shown as an example.

The control, mung beans grown in Nanopure water, was used as a reference to assess the phytotoxicity of the solutions of nanoparticles. By visual inspection, as shown in Figure 2, the students concluded directly that solutions of SiO_2 NPs gave rise to only mild toxicity because the length of mung bean growth appeared shorter than the control. In contrast, the solutions of ZnO NPs gave rise to a much higher level of toxicity as demonstrated by the severe stunting of the growth of the mung beans. On the basis of these highly visual results, it was straightforward for students to comprehend the concept of nanophytotoxicity.

■ QUANTITATIVE RESULTS

Figure 3 contains two plots of the length of mung bean growth versus time as collected and measured by students. Each of the data points represents the mean and standard error values of 20 mung beans except for the Figure 3B-Nanopure data starting on day 3, which represents the results of 15 mung beans due to contamination of one Petri dish on that day. Decreases in the mean length of mung bean growth in one particular type of solution from day to day are due to small differences in determining the starting and ending points of each growth (from student to student), to the application of the segmented line tool, and to movement of the mung beans in the Petri dish that change, in small ways, the appearance of the three-dimensional nature of the mung bean growth. Prior to measuring their data, students practiced measuring mung bean growth using a sample image of a Petri dish with five mung beans. Their measurements had a standard deviation of ±1.4% for all but the shortest lengths for which small differences led to larger relative percentages.

The data in Figure 3 clearly show that the lower phytotoxic effect of SiO_2 NPs and the higher phytotoxic effect of ZnO NPs are statistically significant from each other and from the control experiment in Nanopure water. Mung beans grown in solutions of SiO_2 are significantly shorter than those grown in Nanopure water. The lengths of mung bean growth in ZnO NPs are significantly shorter than mung beans grown in solutions of SiO_2 NPs. In addition, increasing the concentration of ZnO NPs from 20 to 2000 mg/L further inhibits the growth of the mung beans, as shown in Figure 3A.

Between panels A and B of Figures 3, the trends in the lengths of mung beans are consistent, but the absolute lengths are different. As discussed below, several experiments were conducted with the same group of mung beans used during Spring 2014. Each of the data sets using mung beans obtained at the same time had similar lengths of mung bean growth. For Spring 2015, a new batch of mung beans was obtained, which likely accounted for the difference in absolute lengths.

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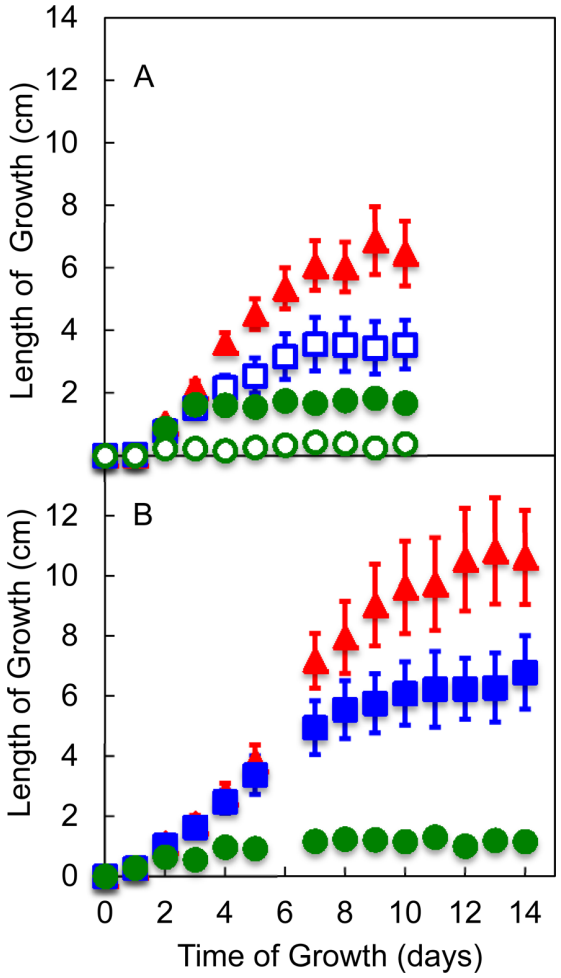

Figure 3. Mean and standard error values for the length of mung bean growth versus time grown in Nanopure water (filled red triangles), solutions of SiO_2 NPs (filled blue squares are 20 mg/L and open blue squares are 2000 mg/L), and solutions of ZnO NPs (filled green circles are 20 mg/L and open green circles are 2000 mg/L). (A) Data collected by students during Spring 2014. (B) Data collected by students during Spring 2015. Error bars not visible fall within the size of each marker.

Previous studies[14] of the effects of ZnO NPs on the growth of mung beans using different conditions (seeds that were first sterilized, germinated in wet cotton, and then placed in agar solutions for 60 h) have found, similarly, that growth is inhibited when increasing the concentration of ZnO NPs from 20 to 2000 mg/L. Contrary to our findings, these authors found that the length of mung bean growth was 67% higher for mung beans grown in solutions of 20 mg/L ZnO NPs than in deionized water. The ZnO NPs were also shown to be incorporated into the plants. Possible reasons for the differences in results include the sizes of the NPs (mean size of 20 nm versus mean size of 102 nm in this study, see below), growth conditions, and the sources of the NPs (commercially available versus synthesized).

Previous studies on other plant systems have found that ZnO NPs can either promote or inhibit growth, oftentimes dependent upon the concentration of the ZnO NPs.[15] As mentioned above, the results reported here do find concentration dependence for the length of mung bean growth.

Both within and outside of the class, two other sets of experimental conditions were studied. Mung beans were grown in solutions of 20 mg/L single-walled carbon nanotubes and 20 mg/L multiwalled carbon nanotubes. Results of two experiments using these nanotubes did not yield consistent results from experiment to experiment with regards to the growth of the mung beans in comparison to the control (data included in the Supporting Information).

As a result of their data analyses, statistical analyses, and conclusions formed about the data shown in Figure 3, students deepened their understanding of nanophytotoxicity.

CHARACTERIZION OF ENMs

During the first lab period, each student was given one scanning electron microscopy (SEM) image of each type of NP to determine the sizes of the NPs using a protocol similar to that used to determine the growth of the mung beans. The results presented are the averages of all of the student data.

Figure 4 shows representative SEM images of SiO_2 and ZnO NPs used in the experiments. Students observed that SiO_2 NPs were agglomerated, as shown in Figure 4A. The size of the SiO_2 NPs was measured to be 21 ± 4 nm, within reasonable agreement with the manufacturer's specifications of a particle size of 10−20 nm (Sigma-Aldrich, cat. no. 637238). The size of the ZnO NPs was measured to be 102 ± 58 nm, significantly different than the manufacturer's specifications of an average particle size of ≤35 nm (Sigma-Aldrich, cat. no. 721077), as shown in Figure 4B. This exercise helped students to understand the concepts of the heterogeneity and evolution of NPs in their life cycle before reaching plants, including the conclusion that the nanoparticles' status could be different from the manufacture's specifications.

STUDENT LEARNING

With the use of a simple survey (see Supporting Information for details), student learning was assessed before the lecture, after the lecture but before the experiment, and after the experiment. Among other topics, students were asked to self-evaluate and rank their understanding of nanophytotoxicity and characterizing nanomaterials. Before the lecture, the vast majority of students reported that they knew nothing about either nanophytotoxicity or characterizing nanomaterials. After the experiment, the vast majority of students reported at least a "basic understanding" of these topics, and a simple majority of students reported feeling "familiar" with them.

The terminal survey also contained open-ended questions. Several students were surprised at the differences in growth using the solutions of SiO_2 and ZnO NPs. Many also felt that being an experienced user of ImageJ was a useful skill to have. Finally, students said this project gave them first-hand experience at how challenging and interesting scientific research can be.

SUMMARY

A lecture and laboratory series was developed to introduce undergraduate students to the concept of nanophytotoxicity. This lecture and laboratory series taught students basic methods used to investigate and assess the effects of ENMs on the environment and allowed students to clearly measure nanophytotoxic effects. Due to the focus of this series being on the methods used to identify nanophytotoxicity, this experimental setup can be easily adapted to assess the effects of

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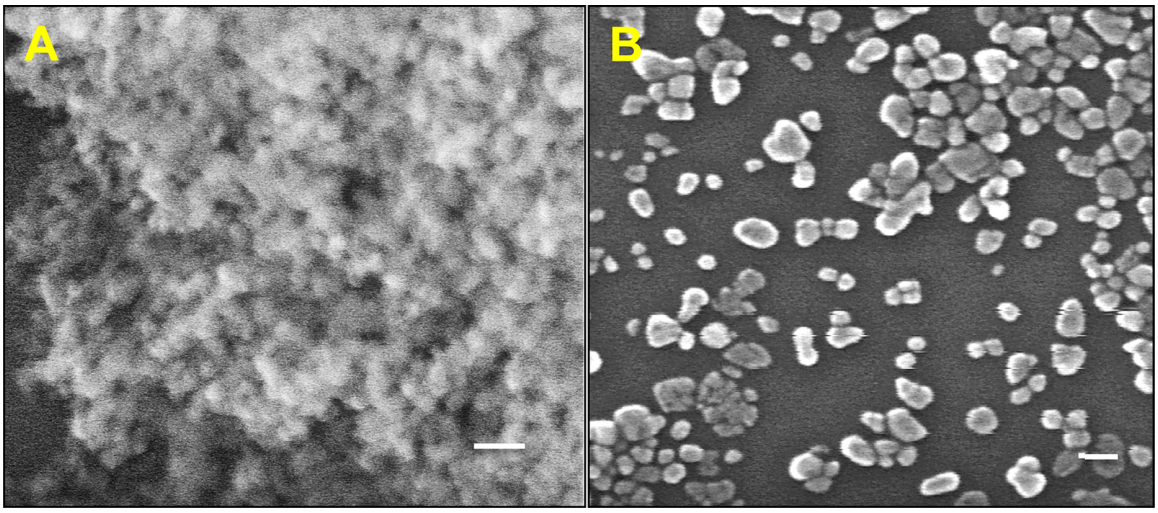

Figure 4. Example SEM images of the ENMs used for students to practice measuring the sizes of the nanoparticles: (A) SiO_2 NPs and (B) ZnO NPs. Scale bar, 50 nm.

alternative ENMs on a variety of plant species. Moreover, this lecture and laboratory series will help cultivate students' interest in nanophytotoxicity early in their academic careers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b01038.

Complete experimental protocol, data from experiments involving carbon nanotubes, and complete results from the student surveys (PDF, DOCX)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: gyliu@ucdavis.edu.
*E-mail: millerw@scc.losrios.edu.

Notes

The authors declare no competing financial interest.

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