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Laboratory Experiment

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Detecting Microplastics in Soil and Sediment in an Undergraduate Environmental Chemistry Laboratory Experiment That Promotes Skill Building and Encourages Environmental Awareness

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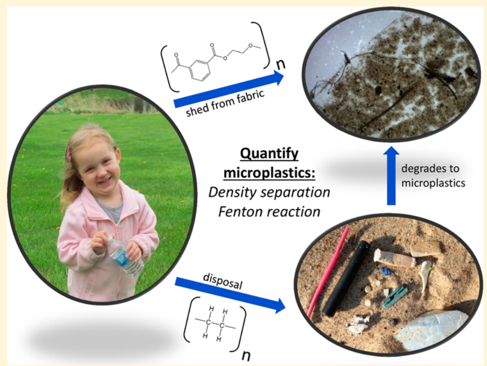
Supporting Information

ABSTRACT: Environmental pollution is both a worldwide and a local issue, and microplastic pollution in particular is receiving increased attention due to its prevalence and bioaccumulation potential affecting the food chain. This laboratory experiment uses current, research-based methods such that the students can determine the extent of microplastic pollution in local soil samples. This laboratory experiment can be used as either a 2 or 3 week mini-research-project for first-year undergraduate students in either an introductory chemistry course for nonmajors or a general chemistry course for majors. The laboratory experiment gives students exposure to sieving, density gradients, and exposure to the Fenton reagent to isolate microplastics from soil samples, which are then analyzed and quantified under stereomicroscope magnification. Several general chemistry topics common to most first-year chemistry courses (density and solution concentration calculations, etc.) are emphasized during the laboratory experiment. From postexperiment assessments, students showed a marked improvement in select skill sets and knowledge of the microplastic pollution problem, and some students recognized their misconceptions concerning research following the completion of this laboratory experiment.



KEYWORDS: *First-Year Undergraduate/General, Environmental Chemistry, Interdisciplinary/Multidisciplinary, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Separation Science, Nonmajor Courses, Applications of Chemistry*

INTRODUCTION

Human-made pollution comes in many forms, and one of the more prevalent forms in modern society is plastics. Plastic materials are remarkably resistant to biodegradation upon exposure to a wide variety of conditions.[1] Plastic materials tend to persist, in one form or another, in the environment for a very long time. Although pure plastics are usually biologically inert and are considered nontoxic to living organisms, many compounds that leach from plastics during their breakdown are carcinogenic or endocrine disruptors, and other toxic environmental pollutants will tend to "stick" to plastics in the environment.[2−5] Plastic pollution, especially in waterways and oceans, has been studied for years and is well-known to many (but not all) people, but a less well-known problem is that of microplastics pollution. Microplastics are plastics less than 5 mm in diameter and are a class of pollutants of emerging concern due to their immense prevalence in water and soil, and possible effects on ecosystems and food chains due to their bioaccumulation.[6−11]

This laboratory experiment focuses on isolating and quantifying microplastics and microfibers found in local creek soil.[12,13] During this particular laboratory experiment students study soil samples that are collected from areas downstream from a local wastewater treatment plant, and from a different control location, in order to assess the degree to which the wastewater treatment plant efflux may contribute to local microplastics contamination.[14] However, this laboratory experiment can be used to study soil or sediment samples from any local site of interest, in order to make the analysis and results more tangible and meaningful to the students. The primary aim of this laboratory experiment is to engage first-year undergraduate students as active researchers exploring a current question relevant to the world around them, giving them experience in a research-based laboratory experiment.[15]

Many of the laboratory experiments undergraduate students are exposed to can be categorized as "cookbook" experiments that attempt to reinforce and demonstrate concepts learned in lecture through the repetition of experiments that have been previously completed numerous times by other students, although there is little evidence that these laboratory types succeed in reinforcing lecture-based concepts.[16] Both the American Association for the Advancement of Science (AAAS)

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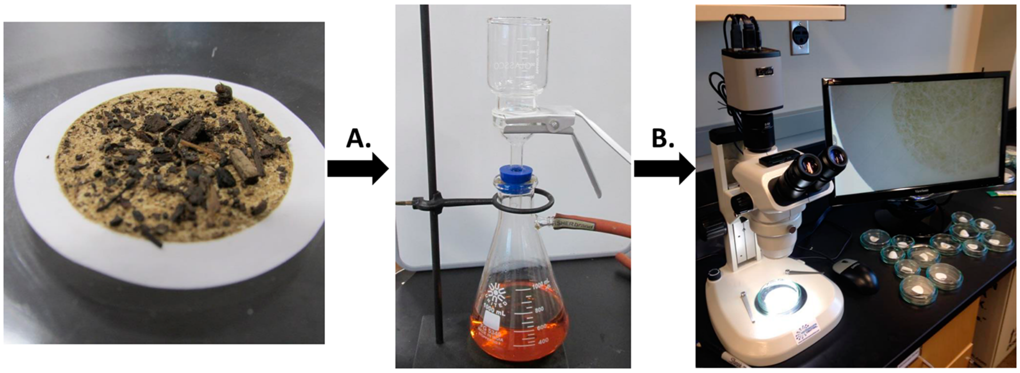

Figure 1. Work flow of the experiment: Soil is sieved and plastics are separated from the majority of organic matter using density separation and vacuum filtration. The 47 mm nylon filter on the far left is an example of the extent of organic matter that may remain on the nylon filter after density separation. This matter remaining on the nylon filter is then subjected to the Fenton reagent, an oxidation reaction that degrades organic matter, and refiltered (A). The remaining nylon filter is then analyzed with a microscope (B) in order to visualize and quantify microplastics in the form of microplastic particles and microfibers (Figure 2).

and the National Research Council (NRC) state that "science as inquiry" is a fundamental aspect of scientific literacy that should be a content standard for undergraduate students, as well as K−12 students.[17,18] Moreover, in many cases it has been found that switching from traditional laboratories to more inquiry-based or research-based laboratories increases student interest in science and increases their understanding of the connection between science and everyday life.[16]

Therefore, the aim of this laboratory experiment was to introduce (mainly) first-year undergraduate students in an introductory chemistry course to a short course-based research experience module that was relevant to their everyday life by analyzing microplastics in local soil during either a 2 or 3 week mini-research-project. The student perceptions of pollution, science, and research were assessed before and after the experiment, with significant perceptual changes occurring following the completion of the project. Additionally, several specific pedagogical goals related to solution concentration and density calculations were assessed pre- and postlaboratory experiment completion, with a 16−53% increase in the number of students correctly answering select questions following completion of the laboratory experiment.

The novelty of this laboratory experiment is 3-fold. First and foremost, to our knowledge, it is the first laboratory experiment for undergraduate students to quantify microplastic (with an emphasis on microfibers) pollution in soil samples. Environmental concerns are of major importance to many students, and although there are many published environmental chemistry laboratory experiments, some of which deal with plastics, there are none that allow students to quantify microplastics pollution in their environment.[19−25] Second, this laboratory experiment incorporates several key topics (pH, solution concentration calculations, etc.) learned in virtually any nonmajors' or majors' chemistry course within a single laboratory experiment. Finally, the methods employed in this laboratory experiment are very similar to current research methods in the field, such that students are participating in scientific research during this laboratory experiment.[13,26,27]

■ EXPERIMENT

Equipment and Material

Spades and buckets (a variety of brands) were used to collect soil samples. Plankton sieves with 3.36 mm pore size were purchased from Carolina Biological. Nylon filters with a 47 mm diameter and 0.45−5.0 μm pore size were purchased from GVS Life Sciences. Zinc chloride, hydrogen peroxide, hydrochloric acid, and iron(II) chloride tetrahydrate were purchased from Sigma-Aldrich and were prepared with filtered deionized water from a Milli-Q Advantage A-10 Q-Pod water filtration system using a 0.22 μm filter. A variety of different brands of pH paper and vacuum filtration apparatus were employed. A Central Accuscope (0.5× stereolens) microscope from Microscope Central with an Excelis HD camera software adaptor was employed to produce microplastic and microfiber images found in this laboratory experiment, whereas students used a Leica DM500 with a 4×/0.10 lens to visualize their microplastics and microfibers on the nylon filter.

Experimental Overview

This experiment was broken into three different sections that were undertaken over three consecutive weeks in a nonmajors' chemistry course in which the laboratory period was 1 h and 50 min long. Additionally, adoption into a 3 h per week laboratory course was piloted for a first-year chemistry course for majors in which the laboratory experiment was completed in 2 weeks. Prior to students beginning the experiment, the instructor must first dig up an appropriate amount of soil from the selected environment(s), considering the number of students that will undertake the experiment (assume 100 g of soil per student group). The soil should be dried in an oven at 100 °C for several hours prior to students handling it during the first week for accurate mass measurements and for the sieving process. Note that the term "soil" is used in this laboratory experiment, although the "soil" from the local creek bank was likely a soil/sediment mixture. Any soil or sediment could potentially be analyzed for microplastic pollution using this method.

The soil is pulverized with a mortar and pestle and passed through a sieve as the first step in the isolation of microplastics (under 5 mm) during the first week of the experiment (task time = 10−15 min).[10,12] Students then prepare a high density solution of zinc chloride (density ∼ 1.4 g/mL) in order to separate the plastics (density < 1.4 g/mL) in the sample from the rest of the soil sample using a density separation step (task time = 30 min to 1 h).[26,28−30] This is allowed to settle overnight, or for the week. During the second week of the experiment, the liquid portion of the sample containing

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suspended plastics is vacuum filtered and the collected solid is exposed to the Fenton reagent to digest the organic soil matrix and natural fibers (task time = 1−2 h) (Figure 1).[31−33]

For the Fenton's reagent reaction, ferrous iron reacts with hydrogen peroxide and the liquid sediment sample that has been pH adjusted to 2−3; the reaction mixture is subjected to mild heat with stirring until froth formation ceases. The froth formation is a result of carbon dioxide gas escaping following secondary oxidation reactions, and it indicates the end of the reactions that digest the organic soil matrix.[34] During the final week of the experiment (3rd week for the 3 week lab, second week for the 2 week lab), the solution is vacuum filtered again in order to isolate the remaining debris on the nylon filter (task time = 10−15 min). This nylon filter is then visualized under the stereomicroscope, and microplastics and microfibers are identified and quantified by the students via visual inspection (task time = 30 min to 1 h) (Figures 1 and 2).[12] Students record the number of microplastics and microfibers on the board so that the class can get an overall number of microplastics and fibers found by the class. Students then added the total number of microplastics and microfibers found in all Soil A samples and divided that number by the total grams of Soil A analyzed by the class. This calculation was repeated for Soil B samples so that an average number of microplastics + microfibers per gram of soil was determined for both Soil A and Soil B using data from the entire class. The students also set up a laboratory control sample since microfiber contamination is common. The laboratory control sample will be a beaker containing no soil at all which is then subjected to all the experimental steps as the actual soil sample (density separation, filtration, Fenton reagent, filtration, and microscopic analysis), except for the mortar and pestle and sieving initial steps. Logically, the soil containing more microplastics and fibers per gram would be the downstream sediment, since laundry produces a great deal of microfibers; this gray water is processed in a wastewater treatment plant, and wastewater treatment plants do not completely screen out microplastics.[14] Exceptions to this result introduce an opportunity to discuss the importance of multiple sampling sites, multiple replicates, potential contamination of samples, and the limitations and difficulties encountered in research.

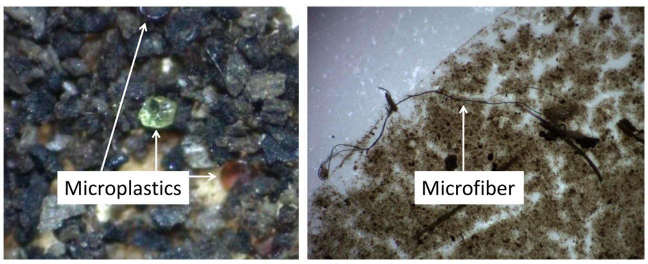

Figure 2. Microscopic images of a plastic microfiber (right) and several microplastics (left) from soil downstream of a wastewater treatment plant on a 47 mm nylon filter. Image was taken after Fenton reagent and final filtration step using a Central Accuscope with a 0.5× lens.

This laboratory experiment was performed by students during four consecutive semesters in an introductory chemistry course for nonmajors, with over 200 students participating. After each semester the procedural steps were altered slightly to increase the simplicity and relevance of the experiment, with the final iteration included in the Supporting Information. This procedure was completed by 66 students in a nonmajor course in the spring of 2018. The laboratory was completed over a 3 week period, in which students had a 1 h and 50 min laboratory period each week. During spring semester 2018, this laboratory was piloted in a general chemistry course for majors. There were 18 students who completed the experiment using a 2 week schedule and a lab period spanning 2 h 50 min per week.

The student perceptions and pedagogical and learning goal assessments discussed in the Learning Experience section were collected during the semester just prior to the submission of this paper (Spring 2018).

■ HAZARDS AND DISPOSAL

Students should use appropriate personal protective equipment at all times. Special care should be taken with the handling of the hydrochloric acid and hydrogen peroxide. Acids and oxidants are corrosive, toxic, and irritating, and students should add these substances to their samples under a fume hood. It is recommended that gloves, a lab coat, and face protection (i.e., goggles) be used for the hydrogen peroxide addition. For disposal, soil and nylon filters can be disposed of in regular garbage; liquid waste following the zinc chloride density separation step should be disposed of in the hazardous waste (or zinc heavy metal should be precipitated out of solution), and Fenton reagent solution can be disposed of down the drain. Prior to drain disposal, check the Fenton reagent solution pH to ensure the pH is between 5 and 9. If the pH is outside this range, adjust it accordingly with HCl or NaOH prior to disposal. When disposing of solid soil, students should be reminded to scoop out the solids from the containers and deposit them in the regular garbage can, and not down the sink, as large quantities of soil down drains can cause clogged pipes in the laboratory.

■ RESULTS AND DISCUSSION

Students completed all steps of the experiment within their 1 h and 50 min time frame each week during the 3 week experiment, including the completion of the student handout (Supporting Information) for that week. Most of the instrumentation and chemical reactions used for this experiment were fairly straightforward for the students, with a few exceptions. First, almost all students needed initial help using the microscope to identify microplastics and microfibers from the organic soil matrix in their samples (Figure 2 and Supporting Information). Additionally, several students needed to be reminded how to properly prepare a solution and test its density. The correct preparation of the initial zinc chloride solution is essential for the entire laboratory experiment as the solution must be dense enough (1.3−1.4 g/mL) in the first week so that microplastics will float in the upper layer of the solution that is decanted onto the nylon filter.

Students analyzed either a soil sample that was collected downstream from a wastewater treatment plant (Soil A), or from a location that was not downstream of a wastewater treatment plant effluent (Soil B). However, students were not told which soil was which until the completion of the laboratory experiment. Student groups wrote the total number of microplastics and microfibers found in their sample and the mass of soil they analyzed on the board during the final week of the laboratory experiment. This class data was required to answer several questions on the final lab sheet. Two of the

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three laboratory sections determined that Soil A had more microplastics per gram then Soil B using a total of 12 Soil B samples and 8 Soil A samples. One section, however, found that Soil A and Soil B both had approximately the same concentration of microplastics/fibers per gram of soil from 6 samples of Soil A and 3 samples of Soil B. However, using the average of all three sections data (14 Soil A samples and 15 Soil B samples), it was found that Soil A, which was downstream from the wastewater treatment plant, did have more microplastics/fibers per gram than Soil B (0.34 microplastics/fibers per gram of Soil A versus 0.28 microplastics/fibers per gram of Soil B). Although the results confirmed what was expected, that sediment downstream from a wastewater treatment plant had more microplastic contamination than a "cleaner" sediment sample, the difference was not significant. Student analysis of statistical significance was beyond the scope of a first-year chemistry course, although more advanced students could potentially apply the *t* test to the data in order to assess significance. However, the lack of dramatic difference between sampling sites presented a perfect opportunity to discuss the importance of uncertainty, multiple sampling sites, multiple replicates, potential contamination of samples, and the limitations and difficulties encountered in research with the students.

■ LEARNING EXPERIENCE

The three primary learning goals of this laboratory experiment were to

1. Increase student awareness of how scientific research is performed.
2. Increase student awareness of microplastics pollution and the potential effect it has on the environment.
3. Demonstrate to students how knowledge learned in the chemistry classroom (solution concentration calculations, pH determination, etc.) is directly used in environmental research.

During the final semester of this laboratory experiment, an anonymous questionnaire covering the students' perception related to the three learning goals was distributed and completed by 60 students (91% return rate, 60/66 students), in three different laboratory sections (Supporting Information). Of the students, 95% agreed that the laboratory increased their awareness of how scientific research is used to solve real world problems (Learning Goal 1), and 93% of students agreed that the laboratory increased their awareness of challenges that are sometimes encountered in scientific research (Learning Goal 1) (Figure 3). In terms of Learning Goal 2, 98% of students agreed that the laboratory increased their knowledge of plastic pollution and its effect on the environment, while 93% went on to state that completing the laboratory experiment made them consider reducing the amount of plastic pollution they personally produce (Figure 3). Of the students polled, 85% agreed that this laboratory experiment was a worthwhile and/or meaningful activity for them to complete (Figure 3). Overall, the majority of students gave very positive comments with regards to the completion of this laboratory experiment, with one of the most common themes being that they had no idea how prevalent microplastic pollution was, and now understood how important it is to control this pollution.

Learning Goal 3 was also concomitant with the pedagogical goals of this laboratory experiment, which were to increase

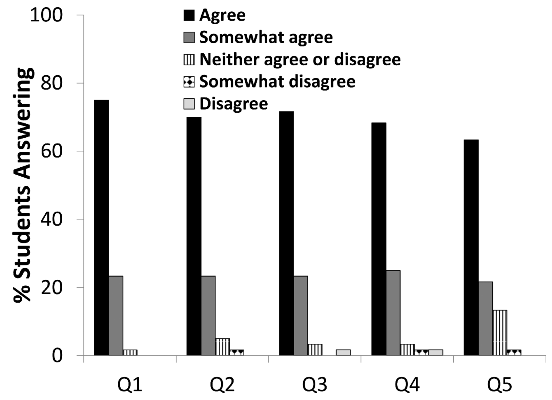

Figure 3. Student perceptions of laboratory experiment. Q1 = The microplastics project we finished increased my knowledge of plastic pollution and its effect on the environment. Q2 = The microplastics project we finished made me consider reducing the plastic waste/pollution I create. Q3 = The microplastics project we finished increased my awarenesss of how science in used to solve real world problems. Q4 = The microplastics project we finished made me realize the difficulties/challenges that are sometimes encountered in scientific research. Q5 = I feel it was worthwhile and/or meaningful to complete the microplastics lab/research project.

student mastery of solution concentration calculations, solution preparation, and density concepts. Specifically, a % (m/m), a % (v/v), a molarity question concerning solution preparation, and two density problems were given to students before they completed the laboratory experiment and after they had completed the experiment. The exact questions are given in the Supporting Information, and the same questions were given both before and after the laboratory experiment was completed. It is important to note that although these questions were given to the students just prior to the laboratory experiment, the topics and problem types had already been discussed in lecture and in assigned homework, in an attempt to isolate the laboratory experiment effect on the comprehension and application of these key concepts. Compared to results just prior to the experiment, more students answered the % (m/m) and % (v/v) questions correctly, with a 19.6% and 16.3% improvement, respectively. Prior to the experiment only 8.8% of students answered the molarity and solution preparation question correctly, versus 61.7% of students answering this question correctly after the laboratory experiment (a 52.9% increase). Student improvements in accuracy for the last two questions, concerning density calculations and concepts, were 21.6% and 18.4% more students answering density related questions correctly after completing the laboratory experiment.

Finally, in reference to Learning Goal 1, student misconceptions about research were assessed before and after completion of the laboratory experiment, in order to determine if participating in a guided mini-research-project altered misconceptions they may have had about research. The student conception of research inventory (SCoRI) developed by Meyers was used in the questionnaire both before and after the laboratory experiment.[35]

The questions in this inventory address whether or not students agree or disagree with statements such as "If followed correctly research procedures will always yield positive results" (Q3) and "It is quite acceptable to modify research data if it does not look exactly right" (Q6). Question 7 in the inventory, "If research is properly conducted then contradictory research

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Table 1. Comparative Pretest and Post-Test Results of the Student Misconceptions of Research Inventory Administered before and after Completing the Laboratory Experiment

Statements for Student Response	Item	Test	Percentage of Students (N = 66) Indicating They: Agree or Somewhat Agree	Neither Agree or Disagree	Disagree or Somewhat Disagree
Good research specifically gathers data that will support the researchers preconceived ideas.	Q1	Pre	70.1	7.0	22.7
		Post	55.0	26.7	28.3
Research becomes true after it is published.	Q2	Pre	19.3	33.3	47.3
		Post	14.9	13.3	63.3
If followed correctly, research procedures always yield positive results.	Q3	Pre	14.0	19.3	66.7
		Post	13.3	11.7	68.4
When qualified people do research, the results are always unbiased.	Q4	Pre	8.7	19.3	71.9
		Post	10.0	8.3	65.0
Research is about collecting data that back your argument.	Q5	Pre	61.4	10.8	28.1
		Post	48.4	21.7	36.6
It is quite acceptable to modify research data if it does not look exactly right.	Q6	Pre	19.3	7.0	73.6
		Post	23.3	16.7	68.3
If research is properly conducted, then contradictory research findings will never occur.	Q7	Pre	NA[a]	NA[a]	NA[a]
		Post	10.0	8.3	78.4

[a]Item not administered in the pretest.

findings will never occur", was only assessed after the laboratory experiment as it was accidentally omitted in the prelaboratory questionnaire. As can be seen in Table 1, completion of the laboratory experiment corrected some misconceptions about research, but not to a great extent.

For example, after completing the laboratory experiment, the following changes were observed: 15% fewer students agreed with the statement "Good research specifically gathers data that will support the researchers preconceived ideas" (Q1), 16% more students disagreed with the statement "Research becomes true after it is published" (Q2), and 13% fewer students agreed with the statement "Research is about collecting data which back your argument" (Q5), as compared to results from the questionnaire given just prior to starting the laboratory experiment. Promisingly, a full 73% of students disagreed with the statement "If research is properly conducted then contradictory research findings will never occur" (Q7). However, for Q3, Q4, and Q6, there was less than a 10% change in student misconceptions of research following completion of the laboratory experiment. These findings suggest that completion of a mini-research-project in a classroom setting begins to change student misconceptions about research but may not be enough to significantly change the majority of student misconceptions about research, and that a direct discussion of these misconceptions would likely be useful.

■ CONCLUSION

This laboratory experiment utilizes current research methodologies to isolate and quantify microplastics in local sediment/soil samples. These environmental chemistry methods not only were suitable for first-year undergraduate students to complete, but also corresponded with many general chemistry topics typically covered in a first-semester chemistry course. The completion of this laboratory experiment not only made students more aware of current plastic pollution issues and how scientific research is completed to solve relevant problems outside the laboratory, but also allowed students to analyze their local pollution issues in a hands-on service learning way that simultaneously reinforced key chemistry concepts encountered in the classroom. Finally, this kind of experiment may enable students to participate in fruitful citizen science work in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.8b00392.

Student handout for the 3 week (1 h 50 min sections) iteration of the laboratory experiment for an introductory nonmajors' chemistry course, including extensive introductory material, step-by-step instructions, and laboratory questions (PDF, DOCX)
Prelab questionnaire (PDF, DOCX)
Postlab questionnaire (PDF, DOCX)
Additional instructor information with additional notes on laboratory experiment and photos of experimental setup and sample microscopic images, and suggestions for truncating the experiment to a 2 week laboratory experiment for a 2 h 50 min laboratory session (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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