

RUNNING HEAD: Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

**Differentiation and Quantification of Microplastic Polymers in Water
through the Use of Spectrophotometry and Fluorometry**

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Abstract

As a relatively new pollutant, microplastics are an emerging threat. This small plastic detritus is a widespread ubiquitous contaminant which pollutes ecosystems around the globe. Microplastics are defined as plastics being smaller than 5 mm in diameter. The purpose of this experiment was to determine whether or not it is possible to differentiate and quantify microplastics within a water sample. This project proposes a method for the identification and quantification of microplastic polymers. The hypotheses suggest it is possible to differentiate and quantify microplastics in water samples according to absorbance of light and fluorescence. If successful, this technique will most certainly be useful in further investigations and experimentation dealing with identification and removal of microplastics from our environment. This experiment encompassed testing of eight distinct polymers across four concentrations. The experiment took place over two rounds of testing. Experimental procedures began with microplastic production through a sanding technique. After sanding, sixty mesh micron sieves were used to ensure microplastics were smaller than 120 nm in diameter. Suspension samples of the eight polymers were created at four concentrations. A Vernier SpectroVis Plus was used to collect absorbance and fluorescence data. Data was organized into spreadsheets. T-tests and analysis of variance were used to analyze data and test the null hypothesis. Results from t-test and analysis of variance tests provided significant statistical support in declining the null. Therefore based on statistical evidence the conclusion was made to reject the null hypothesis and support the validity of the hypothesis.

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Introduction

The goal of this research is to determine whether or not the physical properties, absorbance and fluorescence, may be useful in differentiating polymers, and determining the concentration of microplastics within a water sample. Plastics are a well known pollutant and are of rising concern in our society. In fact 60-90% of marine litter is plastic waste (Global Citizen). However, microplastics (MPs) are not as widely



recognized. The common majority of people have never heard of such a term. Much less understand where they originate from, or be aware of the possible negative ramifications microplastics may have on the

well-being of the environment, the human body, and its microbiome.

As a relatively new pollutant, microplastics are an emerging threat, and are likely to pose many challenges. When plastic weathers down to 5 millimeters (0.2 in) or less in diameter, the debris is called microplastics (National Geographic Society, 2019). This small, sometimes even microscopic, plastic detritus is a widespread ubiquitous contaminant which currently pollutes our planet. Microplastics come from a variety of sources such as: cosmetics, clothing, washing machines, physical erosion, and industrial processes. Two classifications of MPs currently exist, primary and secondary. Primary MPs are manufactured and are a direct result of human material and product

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use. An example of such being microbeads added to cosmetics and toothpastes. Secondary MPs on the other hand are plastic fragments derived from the degradation of larger plastic debris over time, such as the bottles and plastic bags commonly found on beaches all over the world. Regardless both types exist in the environment at high levels and have been reported nearly everywhere. From the depths of the Mariana Trench to the heights of the tallest peak on Earth, Mount Everest, MPs are surprisingly prevalent (Microplastic pollution... The Guardian). Some of the most remote and once pristine locations on Earth have now been infiltrated by this pollutant .

There are many unknowns about the possible detrimental effects MPs have on living organisms and their environments, but in recent years global attention has been focused on the increasing presence of MPs in marine ecosystems. It is estimated 51 trillion MPs litter our oceans. With an estimated weight between 93,000 and 236,000 metric tons (LawSea Education Association) there is an unavoidable amount of MPs in our oceans. While many are shocked to learn there are currently more MP particles in the ocean than stars in the Milky Way galaxy (Smith, Kat, 2019), they fail to realize MPs are more than just a marine issue. There exists a MP problem within our own homes.

It may come as a surprise but we in fact drink these particles. A 2017 study found 94% of tap water in the U.S. is contaminated with MPs (Tyree, Chris, 2017). Turning to the seemingly “easy” solution, bottled water, may not be much help either as a 2018 study found bottled water also contains MPs (Plus Plastic). On average bottled water contains 325 bits per liter. Not to mention use of plastic water bottles will only create

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more pollution. Microplastics are so widespread that evidence of MP presence in the human placenta has been found. For the first time MPs have been reported in all placental portions.

An extensive review of relevant literature did not result in finding research on what the long-term negative effects of plastics on humans or other biota may be. This is a major concern as MPs are foreign bodies in host organisms and may be carriers of various chemicals and substances which may trigger local immunoreactions as well as possibly even act as endocrine disruptors. These may become the cause of negative long-term effects on the human body. The war against MP pollution is far from over. It has but only began. Currently no filter for completely removing MPs exists. It is my belief MP pollution is one the biggest predicaments of our time, and is an impending disaster. This is why finding a solution soon is crucial.

Overcoming this challenge will require an advanced and innovative solution. Moreover, an understanding of how to identify polymers and determine concentrations of MPs, efficiently, is a necessity in order to help address the MP crisis and attempt to mitigate the repercussions our world now faces as a consequence of our impetuous usage of single use plastic. Currently existing technology is time consuming, expensive, as well as inaccessible to the public for citizen science.

In an attempt to create a process or technology capable of accurately differentiating the plastic polymer and determining the concentration of a MP sample, my research seeks to find whether or not the physical properties of absorbance and

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fluorescence are useful in both differentiating polymers, and determining the concentration of MPs within a water sample. My research focuses on MPs 250 microns ($\frac{1}{4}$ mm) or smaller. More specifically, MPs of 8 distinct polymers across 4 concentrations. Various plastics were sanded, then sieved in order to produce MPs. Microplastic suspensions were then created for all 8 polymers at 4 different concentrations, then tested using a portable SpectroVis Plus SpectroPhotometer with fluorescence capabilities. Absorbance and fluorescence data was collected for Polyethylene Terephthalate 1, High-Density Polyethylene 2, Polyvinyl Chloride 3, Low-Density Polyethylene 4, Polypropylene 6, Nylon 7, and Polyester 7. Data was transferred into spreadsheets, from 11 previously determined wavelengths, across the 4 concentrations (2.5, 5.0, 7.5, and 10.0 g/L) of all 8 polymers. Later on data was interpreted and the null hypothesis tested with the use of various statistical analyses.

Being able to identify the polymer and its concentration within a water sample is a step in the correct direction, and something which must be done in order to address the bigger challenge at hand. We must stop avoiding the MP situation. Taking action to engineer technologies and methods which accurately and rapidly identify/quantify specific MP polymers and concentrations in water samples is a priority. To address worldwide pollution levels we must inform the public and get them involved in citizen science. This will help the public understand the course of action necessary in order to minimize humankind's impact on our planet.

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Engineering Goal
Develop a process to identify and quantify microplastics within a water sample.

Research Questions	
Can microplastics in water samples be differentiated and quantified according to their absorbance of light?	
Spectrophotometry Test (Absorbance)	Does the absorbance spectra graph produced by a suspension of a set plastic polymer differ from that of other polymers?
	Does the absorbance spectra graph produced by a suspension of a set plastic polymer differ from that of itself at other concentrations?
Can microplastics in water samples be differentiated and quantified according to their fluorescence?	
Fluorometry Test (Fluorescence)	Do fluorescence levels produced by the excitation of a plastic polymer suspension sample at 500 nm, differ from that of other polymers excited at the same wavelength?
	Do fluorescence levels produced by the excitation of a plastic polymer suspension sample at 500 nm, differ from that of itself at other concentrations?

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Hypotheses: Spectrophotometry Test (Absorbance)	
Microplastics in water samples can be differentiated and quantified according to absorbance of light.	
Experimental	If the absorbance spectra graph produced by a suspension of a set plastic polymer differs from the spectra produced by other polymers at the same concentration, then it is possible to differentiate MP polymer samples according to absorbance.
	If the absorbance spectra graph produced by a plastic polymer suspension at a set concentration differs from the spectra produced by the same polymer at other concentrations, then it is possible to determine the concentration of a MP sample by its absorbance.
Microplastics in water samples cannot be differentiated or quantified according to absorbance of light.	
Null	If the absorbance spectra graph produced by a suspension of a set plastic polymer does not differ from the spectra produced by other polymers at the same concentration, then it is not possible to differentiate MP polymer samples according to absorbance.
	If the absorbance spectra graph produced by a plastic polymer suspension at a set concentration does not differ from the spectra produced by the same polymer at other concentrations, then it is not possible to determine the concentration of a MP sample by its absorbance.

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Hypotheses: Fluorometry Test (Fluorescence)	
Microplastics in water samples can be differentiated and quantified according to fluorescence.	
Experimental	If the emission spectra produced by a suspension of a set plastic polymer differs from the emission spectra produced by other polymers at the same concentration, then it is possible to differentiate MP polymer samples according to fluorescence.
	If the emission spectra produced by a plastic polymer suspension at a set concentration differs from the emission spectra produced by the same polymer at other concentrations, then it is possible to determine the concentration of a MP sample by its fluorescence.
Microplastics in water samples cannot be differentiated or quantified according to fluorescence.	
Null	If the emission spectra produced by a suspension of a set plastic polymer does not differ from the emission spectra produced by other polymers at the same concentration, then it is not possible to differentiate MP polymer samples according to fluorescence.
	If the emission spectra produced by a plastic polymer suspension at a set concentration does not differ from the emission spectra produced by the same polymer at other concentrations, then it is not possible to determine the concentration of a MP sample by its fluorescence.

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Experimental Design

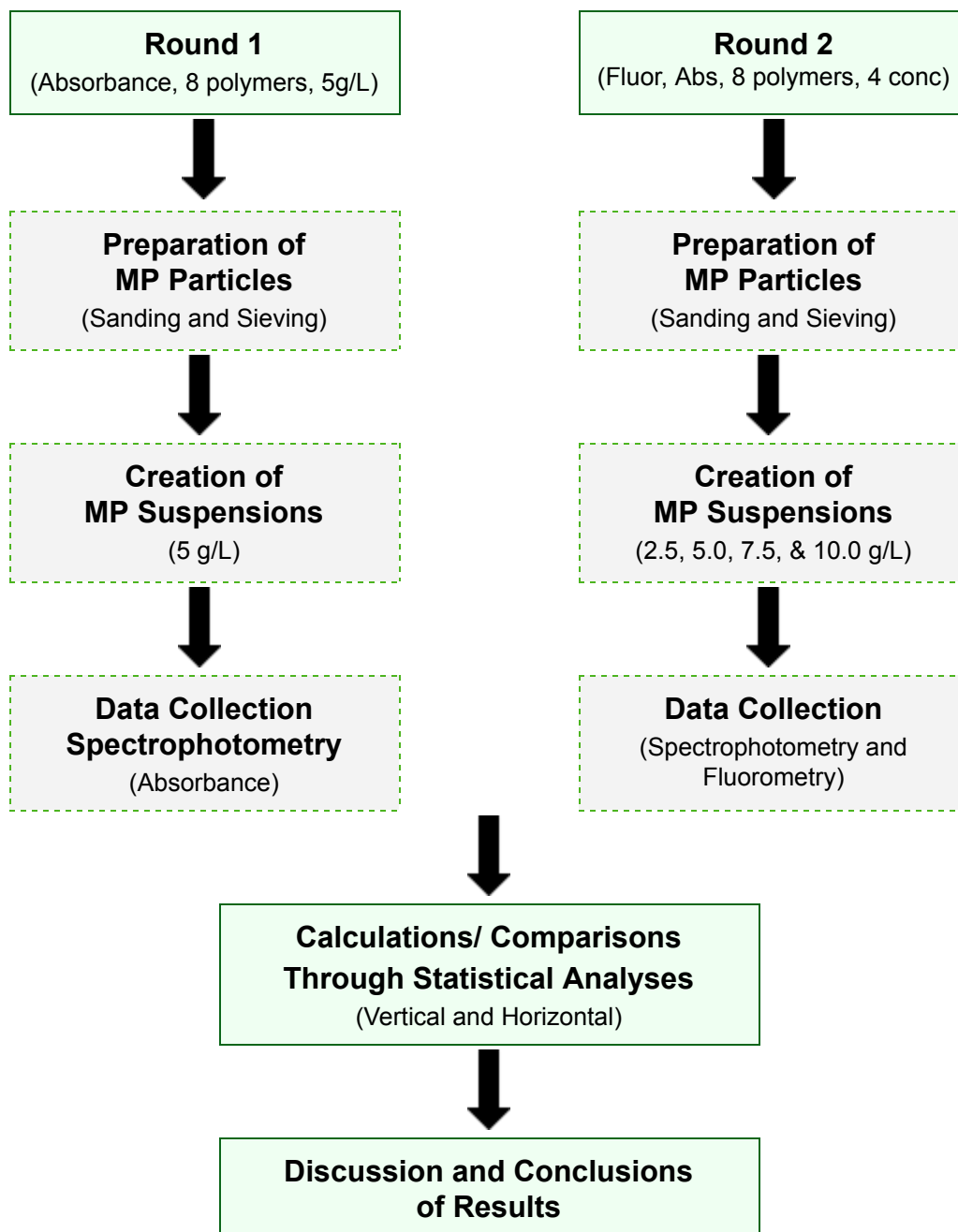
Research was conducted over two testing rounds. The first round consisted of testing the absorbance levels of 8 polymers at only one concentration. The second round encompassed a wider range of sampling. Again the same 8 polymers were tested, but across 4 different concentrations. In addition Fluorescence data was also collected alongside absorbance data. The following page contains a flow chart of the experimental design.

It illustrates experimentation commencing with the requisite preparation of MP suspensions. These suspensions were then transferred into cuvettes and data collection went underway. Data gathered was subject to various statistical analyses and superimposed in scatter graphs for visual aid. Conclusions were drawn and discussed from these analyses.

Please note this is a continuation project. Previous and recent data, results, and conclusions may be compared. Experimental procedures have followed the same general format, thus making prior data comparable to current data.

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Flow Chart of Experimental Design



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Review of Literature

Ever since plastic particles in the sea were first reported in 1972, plastic pollution in aquatic environments has drawn increasing worldwide concern. In particular, plastic particles, designated as microplastics, have been the focus of recent research interests. Although there is no agreeable standard, microplastics (MPs) are commonly defined as plastic particles with sizes less than 5 mm in diameter. These plastics pose a potential threat to our environment and possibly even us. The literature referenced is all current and relevant. All research referenced in this review may be found within the bibliography. This review will enlighten you on current methods for quantification and identification of microplastics.

Microplastics are plastic particles with a diameter less than 5 mm. Particles smaller than 100 nanometers (nm) have been dubbed nanoplastics. Microplastics are categorized into two groups, primary and secondary. Primary MPs are created as particulates in commercial products, facial cleansers for example. Other manufactured microscopic plastic particles such as those in drilling fluids and industrial abrasives are also considered primary MPs. Secondary MPs are defined as fragmentation of large plastic debris through physical, chemical, or biological processes which create smaller plastic particles. As an emerging environmental contaminant, MPs pose possible risks to the aquatic ecosystem due to their widespread occurrence and potential biological effects.

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To better understand the distribution and effects of MPs, identification and quantification are the main concerns of most studies to date. Yet there is a lack of standard protocols for MP sampling and analysis. Therefore, an up-to-date and comprehensive evaluation for analytical methods of MPs in aquatic environments is necessary. Small-sized MPs are the most important for future investigations; hence, MPs larger than 5 mm are not discussed further in this review or experimented on in my research.

Some common methods of identification are visual sorting, Pyrolysis-GC/MS, TDS-GC/MS, and Raman spectroscopy. In visual sorting, large plastics can be sorted out directly, while smaller-sized ones need further observation under a dissection microscope. While this method is suitable for the sorting of large MPs, it is not always applicable and it is necessary to use other instrumental techniques such as Raman spectroscopy. Visual identification may not be accurate and may under- or overestimate the abundance of MPs. Although visual sorting is a time-saving method for enumeration of MPs, more reliable technologies are urgently desirable for evaluating the abundance and chemical composition of MPs.

Next we will review pyrolysis gas chromatography with mass spectrometric detection (Pyr-GC/MS) and thermal desorption gas chromatography with mass spectrometric detection (TDS-GC/MS). A key benefit of Pyr-GC/MS is it can simultaneously analyze both the polymer type and organic additives of MPs. However, this technique allows only one particle at a time to pass through the pyrolysis tube,

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which in turn is both time-consuming and limited by the aperture size of the tube. Moving on the TDS-GC/MS method has been used in analysis of MP composition in environmental samples. TDS-GC/MS can process larger sample masses and measure more complex matrices. Pyr-GC/MS and TDS-GC/MS may be complementary with FT-IR or Raman spectroscopy for comprehensive analysis of MPs.

Finally the last identification procedure this review will discuss is Raman spectroscopy. It is another promising analytical technique frequently used for MP detection. Raman spectroscopy relies upon inelastic scattering of photons, known as Raman scattering. Typically, a sample is illuminated with a laser beam. Electromagnetic radiation from the illuminated spot is collected with a lens and sent through a monochromator. Elastic scattered radiation at the wavelength corresponding to the laser line (Rayleigh scattering) is filtered out by either a notch filter, edge pass filter, or a band pass filter, while the rest of the collected light is dispersed onto a detector. The name "Raman spectroscopy" typically refers to vibrational Raman using laser wavelengths which are not absorbed by the sample. There are multiple variations of Raman spectroscopy including but not limited to: surface-enhanced, tip-enhanced, resonance, polarized, stimulated, transmission, spatially-offset, and hyper.

In conclusion many possible ways to sample and analyze MPs exist but standard protocols for MP sampling and analysis do not exist within the scientific community. This is a major concern. Steps need to be made, further investigations need to be held, and more experimentation is necessary.

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Experimental Materials

Personal Protective Equipment

- Safety goggles
- Laboratory coat
- Dust mask
- Hair ties

Microplastic Preparation

- PET 1 - Plastic water bottles
- HDPE 2 - Plastic milk jugs
- PVC 3 - Plumbing pipes
- LDPE 4 - Squeeze bottle
- PP 5 - Twine rope
- PS 6 - Styrofoam cups
- Nylon 7 - Zip ties
- Polyester 7 - Rope
- 220 Grit sandpaper
- Acetone
- Large beaker
- Stirring rod
- Tin foil
- Wood clamps
- Sanding block
- Microplastic storage containers
- Ample work area
- Safe storage location

Sieving

- 250 micron 60 mesh sieve
- 8 microplastic polymer MPs
- Weighing trays
- Dishwashing soap and Water
- Cleaning brush (soft bristled)
- Permanent marker (labeling)

MP Suspensions Preparation

- Various 80 ml flasks (or larger)
- Sieved MPs (8 polymers)
- Pipettes
- Cuvettes and lids
- Distilled water
- Sieved microplastics
- Electronic balance
- Electricity source
- Weighing trays

Spectrophotometry Test

- Vernier SpectroVis Plus
- Computer with Vernier Spectral Analysis Software (must be previously downloaded)
- Microplastic suspensions (cuvettes)
- Miscellaneous laboratory tools

Calculations

- Software with spreadsheet capabilities
- Analysis software is necessary (must be capable of conducting t-tests and two way ANOVAs)
- Graph Pad Prism recommended

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Plastics Listed in Materials

Before perusing through the rest of this report, taking a look at this table of plastic polymers is recommended. This table gives the resins and specifications of the plastics involved in this experiment. Plastics were chosen due to prevalence in the environment.

SPI Code:	Resin:	Qualities:	Source Used to Test
	Polyethylene Terephthalate (PET)	Strong and clear Heat resistant	Plastic water bottles
	High-Density Polyethylene (HDPE)	Stiff and strong	Plastic milk jugs
	Polyvinyl Chloride (PVC)	Rigid and flexible Chemical resistant	Plumbing pipes
	Low-Density Polyethylene (LDPE)	Flexible and soft	Squeeze bottle
	Polypropylene (PP)	Strong and flexible Oil and heat resistant Low chemical release	Twine rope
	Polystyrene (PS)	Can be inflated Packing material	Styrofoam cups
	Nylon	Common in rope and clothing	Zip ties
	Polyester	Common use in rope and clothing	Rope

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Experimental Procedures

(Please note since experimentation was divided into two rounds these procedures will be split into two parts, each for its respective round of experimentation.)

Round 1

(Absorbance data of 8 polymers at the concentration of 5g/L)

Wear laboratory coat, gloves, and hair ties for all aspects of experimentation to prevent contamination. Wear a dust mask and safety goggles when necessary. Make sure materials listed previously are available and a clean, ample working space in a well ventilated area is accessible.

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Microplastic Preparation

Sanding Plastics

Wear a protective mask during this portion of testing to prevent inhalation of plastic particles. Manually sanding plastics will be necessary. This is because the use of any mechanical techniques will blow away product and make it difficult to gather. Prepare the workspace and ensure the area is clean.

Special Instructions (Dissolving Polystyrene)

Styrofoam is the name of expanded polystyrene (EPS). Styrofoam is more than 90% air. PS is a polymer manufactured from the polymerization of styrene monomers. There is no chemical reaction between EPS and acetone when combined in ambient conditions. EPS easily dissolves in the acetone solvent. Dissolved PS has a goop-like consistency after the physical change. Since styrofoam is mainly made of air, the air escapes when dissolved. Styrofoam must be dissolved before sanding commences.

1. Rip 4 styrofoam cups into small pieces and place in a large beaker.
2. Slowly pour acetone until it covers the bottom of the beaker
3. Use a glass stirring rod to facilitate the process if needed.
4. The foam will then be in an aqueous state.
5. Let it harden for about 2 days in a well ventilated area.
6. Remove from the flask once all acetone has evaporated.

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Making MP Particles

1. Prepare the workspace and ensure the area is clean.
2. Lay tin foil on the work area.
3. Flatten plastic if needed (if in bottle form).
4. Securely clamp the plastic to the table top (where foil has been laid down).
5. Grab 220 grit sandpaper (cut to size) and sanding block.
6. Proceed to manually sand all eight of the plastics.
7. Sand one plastic at a time and replace tinfoil and sandpaper for each resin.
8. Wash hands with soap and water between working with different resins.
9. Sand until 2 grams of MP product has accumulated on tin foil.
10. Unclamp plastic, fold tin foil in half, and empty MPs into storage clean container
11. Label all containers correctly using a permanent marker.
12. Repeat steps until all plastic resins have been sanded and correctly stored.

Next samples must be sieved using stackable micron sieves. The use of 60 mesh sieves shall ensure MP particle diameter remains less than 250 microns. Ensure the work area, hands, and sieves are clean.

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Sieving

1. Sieve **one** resin at a time.
2. Stack the 60 mesh sieve on top of a sieve collection pan (see photo below).
3. Place a weighing tray in the collection pan to collect MP particles sieved.
4. Select a microplastic resin and pour it into the sieve stack.
5. Sieve MP particles until any able to pass through have done so.
6. Place the weighing tray (contains MPs) in a secure container for later use.
7. Label the container correctly with the polymer (resin) it contains.
8. Wash the sieve and hands every time before sieving new plastic polymers.
9. Wash the sieve with a warm soap and water solution to remove near-size particles possibly lodged in the mesh.
10. Brush the underside of the sieve carefully in the water.
11. Dry completely before next use.
12. Repeat steps 2-11 until all 8 MP polymers have been processed.



Next sieved MPs will be used to create suspensions. These suspensions are used to fill cuvettes which will later undergo testing. Again ensure the work area, hands, and any other materials are clean.

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Spectrophotometry

Suspension of MPs

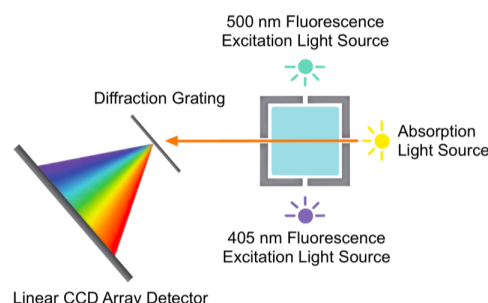
1. Gather sieved microplastics (8 polymers within different containers).
2. Follow these steps to create eight suspensions, one for each polymer.
3. Suspensions will have a concentration of 5.0 g/L.
4. Select a MP polymer.
5. Measure 50 mL of distilled water.
6. Empty into an appropriate sized flask.
7. Precisely weigh $\frac{1}{4}$ g. of microplastics with an electronic balance.
8. Store excess microplastics.
9. Pour microplastics into the water-filled flask.
10. Rotate the flask to ensure the microplastics are properly suspended.
11. Set up 10 cuvettes with lids.
12. Fill all 10 cuvettes $\frac{3}{4}$ full using a pipette.
13. Cover each cuvette with a lid (label if deemed necessary).
14. Rotate flask in between filling each cuvette to ensure MPs are evenly distributed.
15. Repeat steps 4-14 for the other 7 microplastic polymers.

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Intro to Absorbance with SpectroVis

SpectroVis Plus is a portable and affordable visible light spectrophotometer which measures wavelengths from 380 nm to 900 nm and analyzes results in full color. It consists of a spectrometer which produces and measures light, and a photometer which indicates the photoelectric detector that measures intensity of light.

The SpectroVis determines absorbance through the use of a linear CCD array detector. A CCD (Charge Coupled Device) is a highly sensitive photon detector divided into a large number of light-sensitive areas, or pixels. Pixels are used to produce the graph images. The CCD detects visible and near-infrared light.



Absorbance levels are calculated by measuring the intensity of light that passes through a sample. The mathematical definition of Absorbance, denoted A , is as follows.

$$A = \log_{10} \frac{\Phi_e^i}{\Phi_e^t}$$

In this equation the numerator is the radiant energy transmitted by the sample, and the denominator is the radiant energy originally received by the material.

Absorbance is simply a ratio. It is important to discuss transmittance's relationship to absorbance. This is the equation to calculate transmittance. $T = \Phi_e^t / \Phi_e^i$ Transmittance and absorbance are inversely related. The more a wavelength of light is absorbed the less it is transmitted. Moreover, this inverse relationship is not linear, but logarithmic.

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Spectrophotometry Test (Absorbance)

1. Open the Vernier Spectral Analysis software
2. Connect the spectrometer to a computer using the standard USB cable.
3. Open a new experiment under “*Absorbance vs. Wavelength (full spectrum)*.”
4. Proceed to calibrate the spectrometer, select “*calibrate spectrometer*” in menu.
5. Fill a cuvette $\frac{3}{4}$ full with distilled water and place it in the cuvette holder.
6. Follow the instructions in the dialog box to complete calibration, then click “*ok*.”
7. A warm up countdown will appear before testing is available.
8. After the lamp warms up, select the first 10 cuvettes to test (all same polymer).
9. Invert chosen cuvette 5 times since microplastics have likely settled within it.
10. Quickly and efficiently place the cuvette into the spectrometer.
11. Once the cuvette has been securely placed in the spectrometer, collect data.
12. Click the “*Collect*” button at the top of the computer screen.
13. A full absorbance spectrum alongside a value table will appear on screen.
14. Proceed to save data.
15. Title collections by polymer type and cuvette number.
16. Repeat steps 3-15 for remaining 9 cuvettes of chosen polymer.
17. Once the 10 cuvettes of a certain polymer have been processed, select another polymer and test its 10 cuvettes.
18. Do this until all 8 polymers (40 cuvettes) have been tested
19. Clean all flasks and cuvettes and properly dispose of suspensions.

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Calculations

Preparation

T-tests were chosen as an analysis method for this round of testing. Before t-tests can be performed, a spreadsheet (table of values) for each polymer must be created. These sheets contain data for polymer absorbance at wavelengths ranging from 400-900 nm. Data must be entered from all saved cuvette tests in increments of 50. For example, wavelengths 400 nm, 450 nm, 500 nm, etc. Ensure there are 8 sheets (all under one primary sheet), one for each polymer. This is important as cell ranges will later be referenced. Below is an example spreadsheet. In this spreadsheet PS data has been color coded according to its wavelengths.

PS 6	400	450	500	550	600	650	700	750 (Infrared)	800 (Infrared)	850 (Infrared)	900
1	0.6104	0.5356	0.4337	0.4178	0.4012	0.3912	0.3831	0.3906	0.3911	0.4157	0.4337
2	0.5452	0.4650	0.4731	0.4797	0.4652	0.4554	0.4481	0.4574	0.4634	0.4873	0.4993
3	0.4827	0.4922	0.4557	0.4672	0.4564	0.4473	0.4418	0.4474	0.4480	0.4686	0.4829
4	0.4175	0.5062	0.4257	0.4160	0.4011	0.3945	0.3882	0.3900	0.3920	0.4045	0.4086
5	0.4988	0.4854	0.4029	0.3964	0.3843	0.3748	0.3663	0.3706	0.3685	0.3895	0.4039
6	0.5629	0.5197	0.4371	0.4549	0.4480	0.4375	0.4323	0.4393	0.4363	0.4522	0.4521
7	0.5345	0.4796	0.4381	0.4234	0.4138	0.4050	0.3968	0.4042	0.4020	0.4267	0.4467
8	0.5422	0.5263	0.4537	0.4699	0.4601	0.4521	0.4443	0.4509	0.4483	0.4626	0.4688
9	0.5314	0.4805	0.4296	0.4664	0.4596	0.4453	0.4365	0.4499	0.4526	0.4869	0.5179
10	0.6301	0.5178	0.4566	0.4281	0.4162	0.4081	0.4001	0.4041	0.3972	0.4163	0.4294

What follows is an introduction to the t-test, what type was chosen, and an explanation of how t-test results are calculated.

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Introduction to the T-Test

The t-test is a type of inferential statistic used to determine if a significant difference in group means exists. T-tests are commonly used in statistics for the purpose of hypothesis testing. T-tests require three key data values. These are the mean difference, standard deviation, and the number of data values of each group. Many types of t-tests exist. The t-test applicable depends on the data and analysis type required.

T-Test Type Chosen

In this case a two-tailed, independent (unpaired) T-Test is necessary. This type of test was chosen because of the nature of measurement sets being compared. This test compares two plastic data sets by looking at both ends of the standard deviation curve. In other words, this is not just a directional study that only looks at only one end. This test will be used to compare wavelength data sets of one polymer to the corresponding wavelength data sets of the other seven polymers. This type of t-test was conducted for all 11 wavelength increments of all 8 plastic polymers. There are two main steps in performing a T-Test, they are explained in the following pages.

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Conducting a T-Test

The first step is finding the t-value. Start off by calculating the mean for each of the two data sets being tested. Proceed to find the standard deviation and variance of the two data sets. The variance is simply the standard deviation squared. Finally data

being collected must be counted. In this case there are 10 data points in each of the two sets. Now the equation at the right will be

$$\text{t-value} = \frac{|\bar{X}_1 - \bar{X}_2|}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

used to find the t-value. This equation finds the t-value by dividing the signal by the noise. The signal is the difference between group means. If we calculate the mean in both samples and call them X bar one and X bar two, then the absolute value of their difference will equal the signal. In other words it shows how much difference there is between the two sets.

Next, calculate the noise. The noise is represented by the bottom portion of this equation. First find S_1^2 and S_2^2 . These are the variance, or standard deviation squared. Simply square the standard deviation (S). An increase in variance will lower the t-value because it will increase the noise. The second factor in this equation is n. This is the number, or count, of samples taken. As the number of samples goes up, the signal will also increase, up to a point. Finally calculate the noise by imputing the previously calculated values into the denominator of the formula.

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Now that the calculations for signal and noise have been done, simply divide the signal by the noise. This will give a t-value. If the t-value is higher than one, there is more signal than noise.

Next comes the second main step, the actual T-Test. This tests the null hypothesis. In this experiment the null hypothesis states there is no significant statistical difference between plastic sample groups. Start off by identifying a critical value. If the t-value is lower than this, we do not reject the null hypothesis. If we get a t-value which is higher than the critical value, the null hypothesis is rejected; therefore, giving the hypothesis statistical support. Use a t-table to find this critical value.

H ₀			
Degrees of Freedom	p=0.05	p=0.025	p=0.01
1	12.71	25.45	63.66
2	4.30	6.20	9.92
3	3.18	4.17	5.84
4	2.78	3.50	4.60
5	2.57	3.16	4.03
6	2.45	2.97	3.71
7	2.36	2.84	3.50
8	2.31	2.75	3.36
9	2.26	2.68	3.25
10	2.23	2.63	3.17
11	2.20	2.59	3.11
12	2.18	2.56	3.05
13	2.16	2.53	3.01
14	2.14	2.51	2.98
15	2.13	2.49	2.95

T-tables usually look similar to the one at the left. This particular table is for a two-tailed test. First a probability must be chosen. Generally in science $p=0.05$ is used. This is the second column in the table. This is an inferential statistic. It signifies that if this sample was taken a hundred times, then 95 of those times would reject the null hypothesis, and 5 would accept it.

Next, the row used must be decided. To do this knowing the number of samples collected and calculating the degrees of freedom is necessary. Below is the equation used to calculate the degrees of freedom. The degrees of freedom are going to be the samples of n_1 and n_2 minus 2. Since 10 cuvette samples were taken from

$$df = n_1 + n_2 - 2$$

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each sample group, then degrees of freedom are $20 - 2$, or 18 degrees. This will decide the critical value. Though not illustrated by the table, the critical value for this experiment is 2.10.

If the previously determined t-value is higher than the critical value, 2.10, then the null hypothesis is rejected. This means there is something statistically significant between two sample sets. On the other hand, if the t-value is lower than the critical value then the null hypothesis will be accepted. In other words, any difference found between two data sets is simply due to chance.

During the first round of testing a total of 616 T-tests were conducted. Data tables and t-test results are shown in the results section of this report and discussed in the discussion and conclusions section. Also note all raw data spectra graphs from spectrophotometry can be found within the appendices of this report.

The next section of this report discusses the experimental procedures for the second round of testing. This includes a step by step account of procedures. Also included is background information on fluorescence and analysis of variance (ANOVA).

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Experimental Procedures

(Please note experimentation was divided into two rounds, these are procedures for the second round. First round procedures can be found prior to this page.)

Round 2

(Absorbance and fluorescence of 8 resins at 4 concentrations: 2.5, 5, 7.5, and 10g/L)

Wear laboratory coat, gloves, and hair ties for all aspects of experimentation to prevent contamination. Wear a dust mask and safety goggles when necessary. Make sure all necessary materials are available as well as clean, ample working space in a well ventilated area is accessible.

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Microplastic Preparation

Sanding Plastics

Wear a protective mask during this portion of testing to prevent inhalation of plastic particles. Manually sanding all plastics will be necessary. This is because the use of any mechanical techniques will blow away product and make it difficult to gather. Prepare the workspace and ensure the area is clean.

Dissolving Polystyrene

Styrofoam is the name of expanded polystyrene (EPS). Styrofoam is more than 90% air. PS is a polymer manufactured from the polymerization of styrene monomers. There is no chemical reaction between EPS and acetone when combined in ambient conditions. EPS easily dissolves in the acetone solvent. Dissolved PS has a goop-like consistency after the physical change. Since styrofoam is mainly made of air, the air escapes when dissolved.

1. Rip up 4 styrofoam cups into small pieces and place pieces in a large beaker.
2. Slowly pour acetone into the beaker.
3. Use a glass stirring rod to facilitate the process if needed.
4. The foam will then be in an aqueous state.
5. Let it harden for about 2 days in a well ventilated area.
6. Remove it from the flask once all of the acetone has evaporated and the polystyrene is fully hardened.

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Making Microplastic Particles

1. Wear a protective mask during this portion of testing to prevent inhalation of plastic particles.
2. Manually sanding all plastics is necessary. This is because the use of any mechanical sanders will blow away products and make them difficult to gather. This is concluded based on previous failed attempts at sanding by using a mechanical technique.
3. Prepare the workspace and ensure the area is clean.
4. Lay down tin foil on the sanding area.
5. Flatten plastic if deemed needed (bottle form).
6. Clamp plastic to the tabletop with foil previously laid down.
7. Grab sandpaper and sanding block, then manually sand all 8 polymers.
8. Sand one polymer at a time and replace tinfoil and sandpaper for each polymer.
9. Wash hands with soap and water between working with different plastic types.
10. Sand until 3 grams of product has accumulated on the tinfoil.
11. Unclamp plastic, fold tinfoil in half, and empty MPs into storage containers.
12. Label MP containers correctly using a permanent marker.
13. Repeat steps until all 8 plastic polymers have been sanded and correctly stored.

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Sieving

Sanded MPs must be sieved down to less than 250 microns in diameter. This is done using stackable micron sieves. Ensure the bottom sieve is 60 mesh, and that the work area, hands, and sieves are clean.

1. Sieve **one** resin at a time.
2. Stack the 60 mesh sieve on top of a sieve collection pan.
3. Place a weighing tray in the collection pan to collect MP particles sieved.
4. Select a microplastic resin and pour it into the sieve stack.
5. Sieve MP particles until any able to pass through have done so.
6. Place the weighing tray (contains MPs) in a secure container for later use.
7. Label the container correctly with the polymer (resin) it contains.
8. Wash the sieve, hands, and work area before sieving a new plastic polymer.
9. Wash the sieve with a warm soap and water solution to remove near-size particles possibly lodged in the mesh.
10. Brush the underside of the sieve carefully in the water.
11. Dry completely before next use.
12. Repeat steps 2-11 until all 8 MP polymers have been processed.

Next sieved MPs will be used to create suspensions. Suspensions are used to fill cuvettes which later undergo testing. Again ensure the work area, hands, and any other materials are clean.

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Spectrophotometry

Making Microplastic Suspensions

Gather sieved microplastics and create 32 total suspensions. Select only one polymer first and work through the other 7 when finished. Four suspensions will be created for each microplastic polymer. These suspensions will be used to fill 10 cuvettes each. This means 320 total cuvettes will be created. Forty cuvettes for each polymer (10 cuvettes for each concentration).

1. Prepare 4 four flasks
2. Fill each with 50 mL of distilled water.
3. Select a polymer and separately weigh out $\frac{1}{8}$, $\frac{1}{4}$, $\frac{3}{8}$, and $\frac{1}{2}$ grams on trays.
4. Store excess MPs in a safe location.
5. Empty each MP filled weighing tray into its own water-filled flask.
6. Label each flask according to its concentration and polymer content.
7. Rotate each flask 20 times to ensure the microplastics are properly suspended.
8. Set up 10 clean cuvettes and lids for each flask (40 total).
9. Fill cuvettes $\frac{3}{4}$ full using a pipette then cover with a lid.
10. Rotate flasks between each cuvette to ensure microplastic is evenly distributed.
11. Keep cuvettes organized by their respectful polymer and concentration.
12. Repeat steps 1-11 for the other 7 microplastic polymers.



(Do not to misplace or incorrectly label suspensions or cuvettes)

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Intro to Fluorescence with SpectroVis

The SpectroVis Plus spectrophotometer combines fluorescence capabilities with a linear CCD array detector (refer to pg 23). It offers two excitation wavelengths. For this experiment the 500 nm excitation wavelength was selected.

Fluorescence is defined as the emission of light by a substance that has absorbed light or electromagnetic radiation. Fluorescence is a form of luminescence.



Fluorescent materials cease to glow nearly immediately when the radiation source stops. Fluorescence has many applications, like fluorometry. Fluorescence happens naturally in nature, but it also occurs in

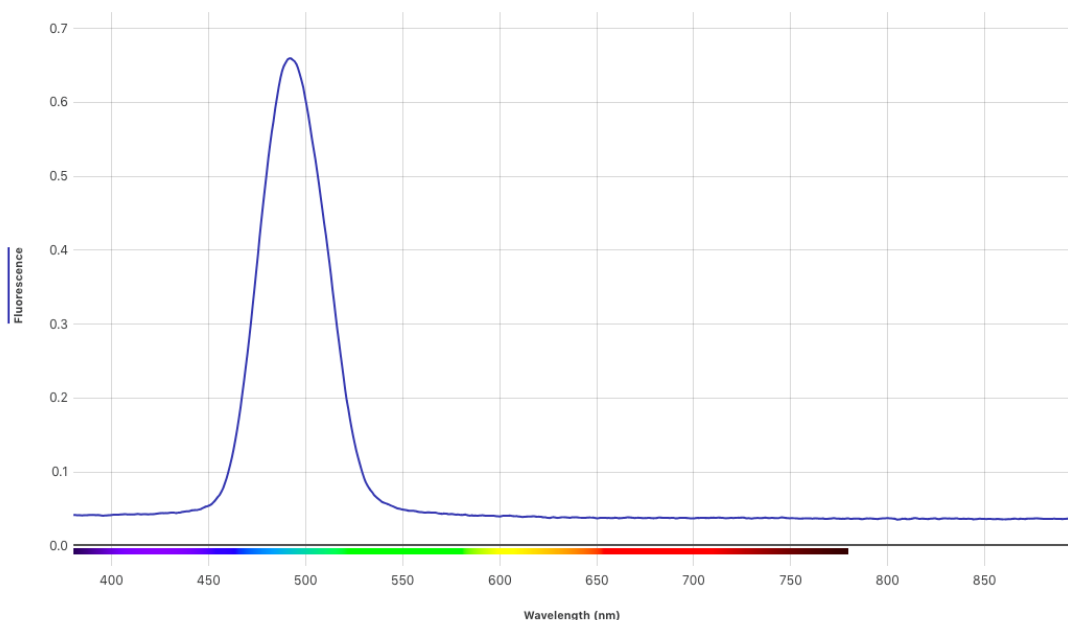
man-made products such as plastics. Many plastics show the characteristic fluorescence when illuminated with a radiation source.

There are two types of fluorometers, filter fluorometers and spectrofluorometers. They are similar but the main difference is filter fluorometers use filters to select wavelengths of incident light, and spectrofluorometers use grating monochromators. The SpectroVis utilized during testing contains a monochromator and therefore is a spectrofluorometer. Spectrofluorometers are more sensitive and have better resolution than filter fluorometers. Another advantage of monochromators is their capability to

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operate over a wide range of wavelengths, as opposed to filter fluorometers which can only operate at the wavelengths of their filters. The SpectroVis fluorometer measures the parameters of visible spectrum fluorescence: or the intensity and wavelength distribution of emission spectrum after excitation at a chosen wavelength, in this case 500nm. A fluorescence emission spectrum is produced when the excitation wavelength is fixed and then scanned to get a plot or graph of intensity vs. wavelength.

In the case of plastics incoming light raises the energy of the electrons in the molecule to an excited state, or higher orbital. The electrons then lose some energy due to the vibrations of the molecules. Finally electrons return to their ground state by releasing light. We perceive this release of energy in the form of visible light. Here is an example fluorescence emissions spectra. This is the emission spectra of a 2.5 g/L polystyrene sample. The rest of these graphs can be found in the report's appendices.



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Spectrophotometry Test (Absorbance and Fluorescence)

1. Open the Vernier Spectral Analysis software and connect the spectrometer.
2. Open a new experiment under the *“Advanced Full Spectrum”* option.
3. Calibrate the spectrometer by choosing *“calibrate spectrometer”* from the menu.
4. Fill a cuvette $\frac{3}{4}$ full with distilled water and place it in the cuvette holder.
5. Follow the instructions in the dialog box to complete the calibration, click *“ok.”*
6. Select a polymer and start testing the lowest concentration of that polymer.
7. First record absorbance data, refer back to page 24 of this report
8. Do not save data yet, now collect fluorescence data.
9. Select the *“Fluorescence vs. Wavelength”* option at the right side of the screen
7. Remove the cuvette currently in the spectrometer and invert it 5 times.
8. Quickly and efficiently place it back into the spectrometer.
9. Click the *“Collect”* button on the top of the screen.
10. Fluorescence spectra should appear on screen.
11. Now save data, title by polymer content, concentration, and cuvette number.
13. Repeat these steps for the rest of the cuvettes for that polymer (40 total).
14. Then do the same for the other polymers.

(Clean all flasks, cuvettes, and properly dispose of all suspensions)

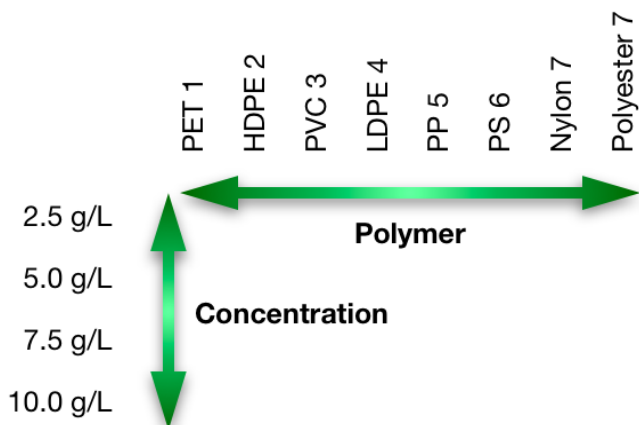
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Calculations

Preparation

T-tests and analysis of variance (ANOVA) were chosen as analysis methods for this round of testing. Before these can be performed, a spreadsheet (table of values) for each polymer and its 4 concentrations must be created. Five primary sheets need to be created for absorbance data and another 5 for fluorescence data. These sheets will contain data for polymer absorbance at wavelengths ranging from 400-900 nm. Data must be entered from all saved cuvette tests in increments of 50. For example, wavelengths 400 nm, 450 nm, 500 nm, etc.

Ensure there are 8 sheets under each primary sheet, one for each polymer. This is important as cell ranges will later be referenced. In this section horizontal and vertical comparisons of data through t-tests will take place. Horizontal comparisons will compare wavelength data of one polymer at a specific concentration to data of the other 7 polymers at that same concentration. Four of the 5 primary sheets will be dedicated to each of the 4 concentrations. The 5th primary sheet will be dedicated to Vertical comparisons. These compare the wavelength data of a polymer at a specific concentration with data of the same polymer but at different concentrations.



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Below is an example spreadsheet. In this spreadsheet PS absorbance data has been color coded according to its wavelengths and ordered by concentration.

The following page will introduce analysis of variance, explain why it was chosen and how ANOVA results are calculated. T-tests were also used to analyze round 2 data.

During the second round of testing a total of 4,928 horizontal comparison T-tests were conducted as well as a total of 1,056 vertical comparison t-tests . Data tables and t-test results are shown with better resolution in the results section of this report and discussed in the discussion and conclusions section.

Also note all raw data spectra graphs from spectrophotometry can be found within the appendices

of this report. As t-tests have already been introduced they will not be further discussed in this section, refer to pages 26-29 of this report for more information on t-tests.

What follows is an introduction to the analysis of variance, how results are calculated, the program used, and other specifications.

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Introduction to Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) is a collection of statistical models and estimation procedures used to analyze the differences among means. ANOVA is a form of statistical hypothesis testing often used in the analysis of experimental data. Tests results are calculated from the null hypothesis and the samples in question. ANOVA calls results statistically significant if it is deemed unlikely to have occurred by chance. If the ANOVA returns a result of statistically significant this means the null hypothesis is able to be rejected and the experimental hypothesis accepted. When the probability, p-value, is lower than the significance level then the rejection of the null hypothesis is justified. There are two types of ANOVA, one-way, and two-way.

ANOVA Type Chosen

In this case a two-way ANOVA was necessary. This type of test was chosen because of the nature of data sets being compared. The two-way ANOVA, or factorial analysis, examines the effect of two factors (polymer type and concentration) on the dependent variable (absorbance). Because the two-way ANOVA considered the effect of two categorical factors, and the effect of these factors on each other, there are three pairs of null hypotheses.

- Polymer type will have no significant effect on absorbance
- Concentration will have no significant effect on absorbance
- Polymer type and concentration interaction will have no effect on absorbance

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Conducting a Two-Way ANOVA

Starting off, means are calculated separately for each factor as well as marginal means for total averages, or grand means. Next the sum of squares of the 1st factor (polymer type), sum of squares of the 2nd factor (concentration), total sum of squares of both factors, and sum of squares within (Error) are calculated. When these squares are added we are given the sum of squares total.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	11.353	21	0.54063	F (21, 288) = 107.53	P<0.0001
Concentration	48.466	3	16.155	F (3, 288) = 3213.3	P<0.0001
Polymer	90.895	7	12.985	F (7, 288) = 2582.7	P<0.0001
Residual	1.4480	288	0.0050277		

Next comes calculating degrees of freedom for each sum of squares. Degrees of freedom is simply the number of samples, n, minus 1. The mean square is calculated by dividing a sum of squares by its degrees of freedom. Lastly the f-score is calculated by dividing the sum of squares by the sum of squares within. Above is a ANOVA table with all of these values calculated.

Next is determining a critical value though the use of a F-distribution table such

		degrees of freedom numerator														
degrees of freedom denominator		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	1	161.5	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	246.0	248.0	249.1	250.1
	2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45	19.45	19.46
	3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.70	8.66	8.64	8.62
	4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.77	5.75
	5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.53	4.50
	6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.84	3.81
	7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51	3.44	3.41	3.38
	8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.12	3.08
	9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.90	2.86
	10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85	2.77	2.74	2.70
	11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.61	2.57
	12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.51	2.47
	13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.42	2.38
	14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.35	2.31
	15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.29	2.25

as this one. The point of intersection between the DF of the numerator and DF of the denominator gives you the critical value.

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Any value greater than the critical value rejects the null hypothesis. Also note there is a 6 th column in the table. The p-value, this is given by ANOVA calculation software. In the case of the table above the p-value is $P < 0.0001$. This also rejects the null, in fact it signifies that there is only a 0.01% chance of getting these results by chance. This is equal to a 1 in 10,000 chance.

Software Recommendations

Taking into account how large the amount of data collection was it is highly recommended and almost imperative that an analysis software is used. Not using one would be tedious and extremely time consuming. GraphPad Prism and Sheets software was used for purposes of ANOVA analysis and creation of visual data representations.

Specifications

A two-way ANOVA was used but more specifically a Tukey comparison test at the confidence interval of 0.05.

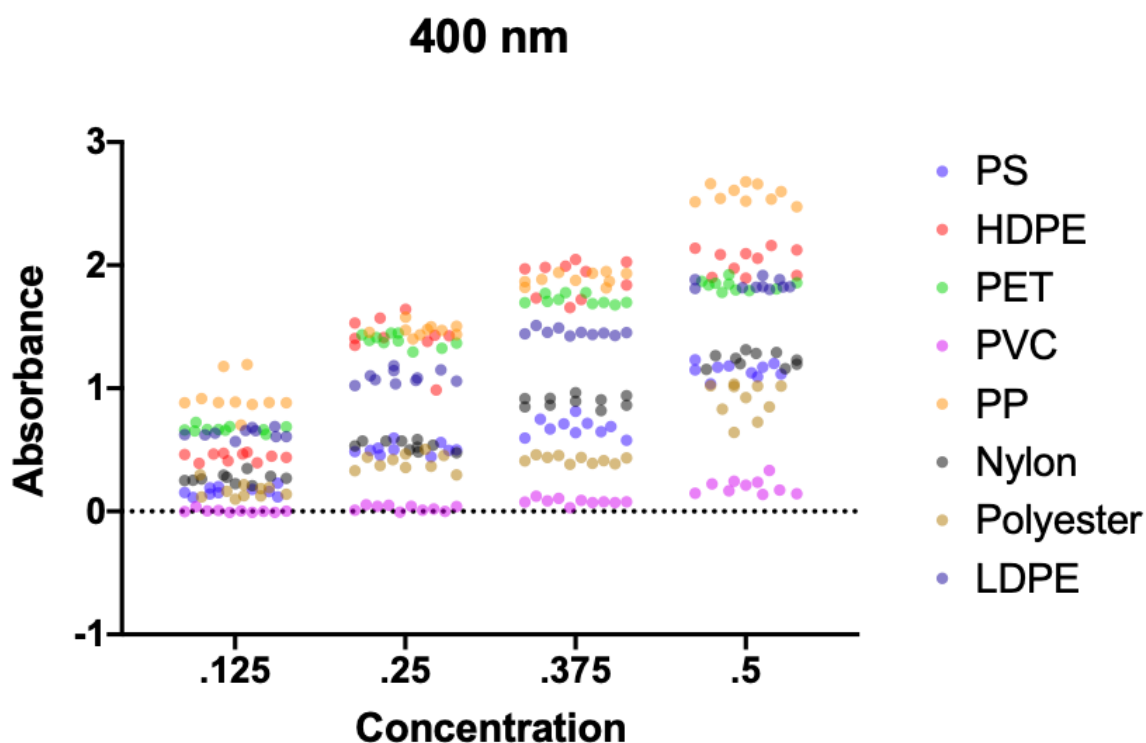
Visual Aids

Graphic representations of data values were also created for each wavelength. Eleven grouped superimposed scatters were rendered to help provide further depiction.

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Results

Please note all of the tables and data graphs used to further analyze the results discussed in this section are included in the appendices. The original spectra captured from the spectrometer are also included.



Also note, in the T-Test results, the value within the red and green colored cells is not the t-value of the T-Test but rather the p-value. The p-value is different because it is a critical value or confidence interval.

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Round 1

(Absorbance and fluorescence of 8 resins at 4 concentrations: 2.5, 5, 7.5, and 10g/L)

Polyethylene Terephthalate (PET 1)

Critical Value 0.05												
p-value PS	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value HDPE	0.2756251	0.0953920	0.0448663	0.0248236	0.1430777	0.1733915	0.1310927	0.2633049	0.2208822	0.4202623	0.7442196	
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PP	0.0000002	0.0000000	0.0000443	0.0000248	0.0000003	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Nylon	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Polyester	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000

Above are the t-tests conducted for PET. The t-tests compared the mean of each PET wavelength increment to that of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis was rejected and red signifies the null hypothesis was accepted. The only instances where the null hypothesis was accepted was when PET was compared to HDPE. Nine out of 11 wavelengths accepted the null in this case. But two rejected it. Moving on to PET compared to the other 6 polymers, the null hypothesis was rejected in all instances.

The t-tests were performed at the 0.05 confidence interval. This gives the hypothesis substantial statistical support when comparing PET to 6 of the 7 polymers. Not only did the t-tests for PET reject the null hypothesis to the critical value of 0.05, but also up to the 0.0004 critical value in all but two cases where the null was rejected. This tells us differences found were not simply due to chance.

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High-Density Polyethylene (HDPE 2)

p-value PS	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PET	0.2756251	0.0953920	0.0448663	0.0248236	0.1430777	0.1733915	0.1310927	0.2633049	0.2208822	0.4202623	0.7442196
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0001748	0.0001055	0.0000029	0.0000007	0.0000006	0.0000018	0.0000020	0.0000014	0.0000010	0.0000002	0.0000002
p-value PP	0.0035612	0.0000491	0.0000661	0.0154594	0.0000302	0.0000010	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Nylon	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Polyester	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000

Above are the t-tests conducted for HDPE. The t-tests compared the mean of each HDPE wavelength increment to that of the other 7 plastics. The t-test results are shown above. Green signifies rejection of the null hypothesis and red signifies acceptance of the null hypothesis. The only instances where the null was accepted was when HDPE was compared to PET. In this case 9 of the 11 t-tests accepted the null hypothesis. On the contrary, the remaining 6 polymers showed a significant inclination towards rejecting the null hypothesis. The t-tests were performed at the 0.05 confidence interval. This gives the hypothesis substantial statistical support when comparing HDPE to six of 8 polymers. Not only did the t-tests for HDPE reject the null hypothesis to the critical value of 0.05, but also up to the 0.01 critical value for all of the other 6 polymers.

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Polyvinyl Chloride (PVC 3)

p-value PS	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value HDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PET	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PP	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Nylon	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Polyester	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000

Above are the t-tests conducted for PVC. The t-tests compared the mean of each PVC wavelength increment to that of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis was rejected and red signifies the null hypothesis was accepted. We can clearly see the null hypothesis was rejected in all instances, by the absence of any red cells.

The t-tests were performed at the 0.05 confidence interval. This gives the hypothesis strong statistical support when comparing PVC to all 7 plastics. Not only did the t-tests for PVC reject the null hypothesis to the critical value of 0.05, but also even higher than the 0.00000001 critical value for all tests. We can see that no non-zero digits even show up until after 7 decimal places or more. This tells us there is a strong statistical difference between the PVC and the other 7 plastics.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Low-Density Polyethylene (LDPE 4)

p-value PS	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value HDPE	0.0001748	0.0001055	0.0000029	0.0000007	0.0000006	0.0000018	0.0000020	0.0000014	0.0000010	0.0000002	0.0000002
p-value PET	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PP	0.0000000	0.0000000	0.0000000	0.0000093	0.0079838	0.7891217	0.0061978	0.0000113	0.0000001	0.0000000	0.0000000
p-value Nylon	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Polyester	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000

Above are the t-tests conducted to compare the mean of each LDPE wavelength increment to that of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis being rejected and red signifies the null hypothesis was accepted. As you can see all but 1 t-test rejecting the null hypothesis. Out of 77 instances only 1 accepted the null. This occurred when comparing LDPE to Polypropylene. The t-tests were performed at the 0.05 confidence interval.

Not only did the t-tests for LDPE reject the null hypothesis to the critical value of 0.05, but in many cases extended well past that. This tells us there is a strong statistical difference between the absorbance levels of LDPE and PET, HDPE, PVC, PP, PS, Nylon, and Polyester.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Polypropylene (PP 5)

p-value PS	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value HDPE	0.0035612	0.0000491	0.0000661	0.0154594	0.0000302	0.0000010	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PET	0.0000002	0.0000000	0.0000443	0.0000248	0.0000003	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0000000	0.0000000	0.0000000	0.0000093	0.0079838	0.7891217	0.0061978	0.0000113	0.0000001	0.0000000	0.0000000
p-value Nylon	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Polyester	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000

Above are the t-tests conducted to compare the mean of each PP wavelength increment to the corresponding increment of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis being rejected and red signifies the null hypothesis was accepted. As you can see all but 1 t-test rejecting the null hypothesis. Out of 77 instances only 1 accepted the null. This occurred when comparing PP to LDPE. The t-tests were performed at the 0.05 confidence interval.

Not only did the t-tests for PP reject the null hypothesis to the critical value of 0.05, but in many cases extended well past that. This tells us there is a strong statistical difference between the absorbance levels of PP and PET, HDPE, PVC, PS, LDPE Nylon, and Polyester.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Polystyrene (PS 6)

p-value HDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PET	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PP	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000001
p-value Nylon	0.4858360	0.4565465	0.6745056	0.8522068	0.7448706	0.9207453	0.9852548	0.9303347	0.9298603	0.9328837	0.8051596
p-value Polyester	0.0021217	0.0012632	0.0000018	0.0000261	0.0000610	0.0000969	0.0001345	0.0001623	0.0002817	0.0002867	0.0003489

Above are the t-tests conducted to compare the mean of each PS wavelength increment to that of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis being rejected and red signifies the null hypothesis was accepted. As you can see the majority of the t-tests showed a significant inclination towards rejecting the null hypothesis. There were only 11 instances where the null hypothesis was accepted out of the 77 t-tests performed for polystyrene. The t-tests were performed at the 0.05 confidence interval.

Not only did the t-tests for polystyrene reject the null hypothesis to the critical value of 0.05, but also up to the 0.02 critical value for all but 11 of the tests. This tells us there is a strong statistical difference between absorbance levels PS and PET, HDPE, PVC, LDPE, PP, and Polyester.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Nylon (7)

p-value PS	0.7077058	0.4630501	0.5493695	0.7860894	0.7221989	0.8995678	0.9667236	0.8956176	0.8587995	0.8303594	0.6875599
p-value HDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PET	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PP	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Polyester	0.0012948	0.0060347	0.0014915	0.0010426	0.0019651	0.0018804	0.0018640	0.0018997	0.0022220	0.0013404	0.0010229

Above are the t-tests conducted to compare the mean of each Nylon wavelength increment to that of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis being rejected and red signifies the null hypothesis was accepted. As you can see the majority of the t-tests showed a significant inclination towards rejecting the null hypothesis. There were only 11 instances where the null hypothesis was accepted out of the 77 t-tests performed for Nylon. These instances have already been discussed. The t-tests were performed at the 0.05 confidence interval.

Not only did the t-tests for Nylon reject the null hypothesis to the critical value of 0.05, but also extended well past this. This tells us there is a strong statistical difference between absorbance levels Nylon and PET, HDPE, PVC, LDPE, PS, PP, and Polyester.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Polyester (7)

p-value PS	0.0021217	0.0012632	0.0000018	0.0000261	0.0000610	0.0000969	0.0001345	0.0001623	0.0002817	0.0002867	0.0003489
p-value HDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PET	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PVC	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value LDPE	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value PP	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
p-value Nylon	0.0012948	0.0060347	0.0014915	0.0010426	0.0019651	0.0018804	0.0018640	0.0018997	0.0022220	0.0013404	0.0010229

Above are the t-tests conducted to compare the mean of each Polyester wavelength increment to that of the other 7 plastics. What is shown in the table above is the t-test results. Green signifies the null hypothesis being rejected and red signifies the null hypothesis was accepted. We can clearly see the null hypothesis was rejected in all instances, by the absence of any red cells.

The t-tests were performed at the 0.05 confidence interval. This gives the hypothesis strong statistical support when comparing Polyester to all 7 plastics. Not only did the t-tests for Polyester reject the null hypothesis to the critical value of 0.05, but extended far past this. This tells us there is a strong statistical difference between the Polyester and the other 7 plastics.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Overall Results Round 1

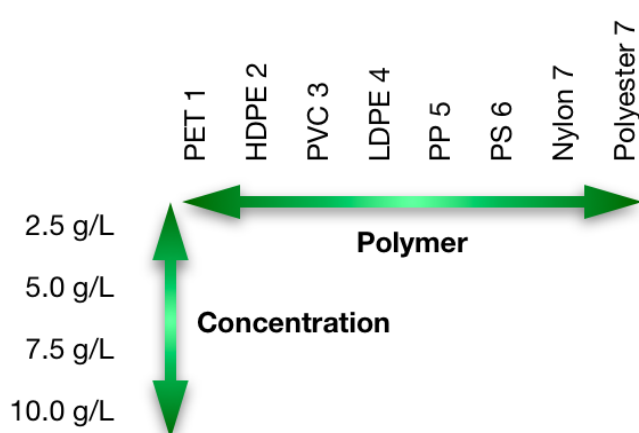
Overall, the t-tests strongly rejected the null hypothesis. Only 42 of the total 616 accepted the null hypothesis. In other words approximately 7% accepted the null. The other 93% rejected it. The t-tests compared the mean of each wavelength increment of a certain plastic to that of the other 7 plastics. In the results above, the tables showed the majority of the t-tests rejected the null hypothesis. Green signified rejection of the null hypothesis and red signified acceptance of the null hypothesis. The t-tests were all performed at the 0.05 confidence interval. In many cases the t-tests rejected the null far beyond the 0.05 confidence interval. This gives the hypothesis substantial statistical support.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Round 2

(Absorbance and fluorescence of 8 resins at 4 concentrations: 2.5, 5, 7.5, and 10g/L)

Results for round two will only include overall results and not detailed results by plastic. This is because the sample size was octupled. Discussing results by plastic would be extremely lengthy and unnecessary. If the reader so chooses they may look at



the appendices for data tables. The appendices include all data tables and t-test results.

Round two results will be split into two categories. These will be absorbance and fluorescence.

Absorbance will contain three sub-sections. These are horizontal comparisons, vertical comparisons, and ANOVA results. Fluorescence will contain two sub-sections, horizontal and vertical. Horizontal refers to the comparison of a constant concentration across different polymers. Vertical comparisons do the opposite. They compare a constant polymer across different concentrations. This is illustrated above. Specifically note the ANOVA absorbance section will go into further detail than the other sections.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Absorbance Horizontal Comparisons

2.5 g/L

When horizontally comparing polymers at the concentration of 2.5 g/L only 18 of the total 616 t-tests accepted the null hypothesis. In other words less than 3% accepted the null, the other 97% rejected it. The only uncertainty came when comparing polypropylene to PET, here 10 of 22 t-tests accepted the null. Nonetheless, the rest of the t-tests still give the hypothesis substantial statistical support.

5.0 g/L

When horizontally comparing polymers at the concentration of 5.0 g/L 28 of the total 616 t-tests accepted the null hypothesis. In other words less than 5% accepted the null, the other 95% rejected it. The only instance when the majority of t-tests did not reject the null occurred when comparing Nylon to Polystyrene, there 16 of 22 t-tests accepted the null. Nonetheless, the rest of the t-tests still give the hypothesis substantial statistical support.

7.5 g/L

When horizontally comparing polymers at the concentration of 7.5 g/L only 8 of the total 616 t-tests accepted the null hypothesis. In other words less than 2% accepted the null, the other 98% rejected it. In this case the hypothesis was supported substantially.

10.0 g/L

When horizontally comparing polymers at the concentration of 7.5 g/L 72 of the total 616 t-tests accepted the null hypothesis. In other words less than 12% accepted the null, the other 88% rejected it. In this case the hypothesis was not supported to the extent of the other concentrations but was still significant.

Overall

126 of 2,464 total t-tests accepted the null. This means a little over 5% accepted the null but approximately 95% rejected it. This is significant in testing the hypothesis.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Absorbance Vertical Comparisons

PET 1

- 0/66 t-tests accepted null. 100% rejected the null hypothesis.

HDPE 2

- 10/66 accepted null. Approximately 85% rejected the null hypothesis.

PVC 3

- 9/66 t-tests accepted the null. Approximately 86% rejected the null hypothesis.

LDPE 4

- 0/66 t-tests accepted the null. 100% rejected the null.

PP 5

- 0/66 t-tests accepted the null. 100% rejected the null.

PS 6

- 0/66 t-tests accepted the null. 100% rejected the null.

Nylon 7

- 0/66 t-tests accepted the null. 100% rejected the null.

Polyester 8

- 10/66 accepted null. Approximately 85% rejected the null hypothesis.

Overall

29 of 528 total t-tests accepted the null. This means a little over 5% accepted the null but approximately 95% rejected it. This is significant in testing the hypothesis.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

Absorbance ANOVA Results

Eleven ANOVA tests were conducted, one for each wavelength increment. The ANOVA tested three null hypotheses (pg. 40). Superimposed scatter graphs of data were also created and can be seen in appendices.

400 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	90.895	12.985
Concentration	3	48.466	16.155
Interaction	21	11.353	0.54063
Residual (error)	288	1.4480	0.0050277
Total	319	152.16	

Does polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 7.4613% of the total variance.

F = 107.53. DFn = 21, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there was no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 59.735% of the total variance.

F = 2582.69. DFn = 7, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 31.852% of the total variance.

F = 3213.28. DFn = 3, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

450 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	79.257	11.322
Concentration	3	41.078	13.693
Interaction	21	9.7722	0.46534
Residual (error)	288	1.3273	0.0046086
Total	319	131.44	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 7.435% of the total variance.

F = 100.97. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 60.302% of the total variance.

F = 2456.82. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 31.253% of the total variance.

F = 2971.11. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

500 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	57.612	8.2303
Concentration	3	31.544	10.515
Interaction	21	7.8568	0.37413
Residual (error)	288	0.68762	0.0023876
Total	319	97.701	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 8.0417% of the total variance.

F = 156.70. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 58.968% of the total variance.

F = 3447.14. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 32.287% of the total variance.

F = 4403.94. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

550 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	46.152	6.5931
Concentration	3	29.996	9.9988
Interaction	21	7.3765	0.35126
Residual (error)	288	0.68160	0.0023667
Total	319	84.206	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 8.7601% of the total variance.

F = 148.42. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 54.808% of the total variance.

F = 2785.80. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 35.623% of the total variance.

F = 4224.84. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

600 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	40.830	5.8329
Concentration	3	28.043	9.3478
Interaction	21	7.0464	0.33554
Residual (error)	288	0.70249	0.0024392
Total	319	76.622	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.1963% of the total variance.

F = 137.56. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 53.287% of the total variance.

F = 2391.31. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 36.6% of the total variance.

F = 3832.35. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

650 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	37.916	5.4166
Concentration	3	27.013	9.0044
Interaction	21	6.7765	0.32269
Residual (error)	288	0.69731	0.0024212
Total	319	72.403	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.3594% of the total variance.

F = 133.28. DFn = 21, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 52.368% of the total variance.

F = 2237.11. DFn = 7, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 37.31% of the total variance.

F = 3718.95. DFn = 3, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

700 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	35.411	5.0587
Concentration	3	26.177	8.7255
Interaction	21	6.5628	0.31252
Residual (error)	288	0.67577	0.0023464
Total	319	68.826	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.5354% of the total variance.

F = 133.19. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 51.45% of the total variance.

F = 2155.91. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 38.033% of the total variance.

F = 3718.62. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

750 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	35.315	5.0450
Concentration	3	26.781	8.9269
Interaction	21	6.6487	0.31661
Residual (error)	288	0.68550	0.0023802
Total	319	69.430	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.5762% of the total variance.

F = 133.02. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 50.864% of the total variance.

F = 2119.54. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 38.572% of the total variance.

F = 3750.46. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Differentiation and Quantification of Microplastic Polymers in Water through the Use of Spectrophotometry and Fluorometry.

800 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	34.062	4.8660
Concentration	3	26.785	8.9284
Interaction	21	6.5682	0.31277
Residual (error)	288	0.67493	0.0023435
Total	319	68.090	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.6464% of the total variance.

F = 133.46. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 50.025% of the total variance.

F = 2076.37. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 39.338% of the total variance.

F = 3809.84. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

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850 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	35.668	5.0955
Concentration	3	27.721	9.2405
Interaction	21	6.7193	0.31997
Residual (error)	288	0.64419	0.0022368
Total	319	70.753	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.4968% of the total variance.

F = 143.05. DFn = 21, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 50.412% of the total variance.

F = 2278.06. DFn = 7, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 39.18% of the total variance.

F = 4131.19. DFn = 3, DFd = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

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900 nm

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean square</u>
Polymer	7	36.188	5.1698
Concentration	3	28.077	9.3590
Interaction	21	6.6703	0.31763
Residual (error)	288	0.63107	0.0021912
Total	319	71.567	

Does Polymer type and concentration interaction have an effect on absorbance?

Interaction accounts for 9.3204% of the total variance.

F = 144.96. DF_n = 21, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant.

Does Polymer affect the result?

Polymer accounts for 50.566% of the total variance.

F = 2359.33. DF_n = 7, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Polymer has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Concentration affect the result?

Concentration accounts for 39.232% of the total variance.

F = 4271.17. DF_n = 3, DF_d = 288

The P value is < 0.0001 meaning the null was rejected and the hypothesis accepted

If Concentration has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant

Overall

- On average interaction accounts for 9% of the total variance.
- On average polymer accounts for 54% of the total variance.
- On average concentration accounts for 37% of the total variance.

Absorbance ANOVA results rejected all three null hypotheses thus accepting,

- Polymer type does have a significant effect on absorbance.
- Concentration does have a significant effect on absorbance.
- Polymer type and concentration interaction has a significant effect on absorbance.

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Fluorescence Horizontal Comparisons

2.5 g/L

When horizontally comparing polymers at the concentration of 2.5 g/L 36 of the total 616 t-tests accepted the null hypothesis. In other words less than 6% accepted the null, the other 94% rejected it. These t-tests provide substantial statistical support.

5.0 g/L

When horizontally comparing polymers at the concentration of 5.0 g/L 270 of the total 616 t-tests accepted the null hypothesis. In other words less than 44% accepted the null, the other 56% rejected it. Although this is close to 50% acceptance of the null please read the discussion section as the significance of these values will be discussed.

7.5 g/L

When horizontally comparing polymers at the concentration of 7.5 g/L 153 of the total 616 t-tests accepted the null hypothesis. In other words less than 25% accepted the null, the other 75% rejected it. In this case the hypothesis had some support. Again read the discussion and conclusions section for further analysis.

10.0 g/L

When horizontally comparing polymers at the concentration of 7.5 g/L 294 of the total 616 t-tests accepted the null hypothesis. In other words less than 48% accepted the null, the other 52% rejected it. In this case the hypothesis was not supported to the extent of the other concentrations but the data is still important to discuss.

Overall

753 of 2,464 total t-tests accepted the null. This means a little over 30% accepted the null but approximately 70% rejected it. It is important to read the discussion section as this will explain the possible reasoning for higher acceptance of the null, as well as which wavelengths are more reliable/should be more relevant when analyzing data.

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Fluorescence Vertical Comparisons

PET 1

- 17/66 t-tests accepted null. Approximately 74% rejected the null hypothesis.

HDPE 2

- 15/66 accepted null. Approximately 77% rejected the null hypothesis.

PVC 3

- 12/66 t-tests accepted the null. Approximately 82% rejected the null hypothesis.

LDPE 4

- 25/66 t-tests accepted the null. Approximately 62% rejected the null.

PP 5

- 16/66 t-tests accepted the null. Approximately 76% rejected the null.

PS 6

- 13/66 t-tests accepted the null. Approximately 80% rejected the null.

Nylon 7

- 11/66 t-tests accepted the null. Approximately 83% rejected the null.

Polyester 8

- 7/66 accepted null. Approximately 89% rejected the null hypothesis.

Overall

116 of 528 total t-tests accepted the null. This means approximately 23% accepted the null but approximately 77% rejected it. See discussion for conclusions

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Discussion and Conclusions

The results displayed in the graphs and tables, both from raw and through statistical analysis, have proven to be very promising. The main goal of this research is to determine whether or not the physical properties, absorbance and fluorescence, may be useful in differentiating polymers, and determining the concentration of microplastics within a water sample. But the purpose of this project extends further than answering this inquiry. This project seeks to help the microplastic crisis not only through facilitating MP differentiation and quantification but by bringing awareness to the MP crisis currently happening under our noses. It is through readers such as yourself that awareness and knowledge can be shared.

Reading countless literature on how MPs are currently analyzed only led to processes which are time consuming, ineffective, expensive, and inaccessible to the public for citizen science. Not to mention there currently are no standard protocols for MP identification and quantification.

This research attempts to find a solution to this problem. It was hypothesized that MPs in water can be differentiated according to absorbance and fluorescence, of course with the use of a SpectroVis Plus. If so this could lead to a process which can differentiate and quantify MPs within water samples. This process could be quick, effective, cost efficient, and available to anyone interested in the MP field. After much planning and research data was collected and analyzed. Through statistical analysis the results provided considerable insight.

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First the round one results will be discussed. Overall, the t-tests during this round strongly rejected the null hypothesis. Only 42 of the total 616 t-tests accepted the null hypothesis. In other words approximately 7% accepted the null. The other 93% rejected it. These results convey the strong difference between the absorbance levels of all 8 polymers. For this round the validity of the hypothesis was supported.

Moving on to round two absorbance testing, 126 of 2,464 total horizontal t-tests accepted the null, and 29 of 528 total vertical t-tests accepted the null. This means in total a little over 5% accepted the null but approximately 95% rejected it. These results convey the strong difference between the absorbance levels of all 8 polymers across 4 concentrations. In addition 11 ANOVA tests also supported rejection of the null hypothesis. Based on these test results the validity of the hypothesis is supported.

Next are round 2 fluorescence tests. Here 753/2,464 total horizontal t-tests accepted the null and 116/528 total t-tests accepted the null. This means approximately 71% rejected it. Now, 71 percent rejection may not provide enough support to reject the null, but if we only look at 400-650 nm t-tests, there are more promising results. At these wavelengths only 257 of 1632 t-tests accepted the null. This is a little over 84% rejection. Disregarding the longer wavelengths is acceptable since the excitation wavelength was 500 nm, meaning that fluorescence levels observed at more distant wavelengths are not as relevant. Because of this the null hypothesis was rejected, but only for wavelengths 400 nm to 650 nm. This means that when analyzing data in future research, wavelengths larger than 650 nm are not as important.

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Based upon the data from the tables, T-Tests, ANOVA tests, and further illustrated by the graphs, there was sufficient statistical evidence to reject the null hypothesis and support the validity of the overall hypotheses.

This is a small step in solving the microplastic problem at hand, but a giant leap in reaching my engineering goal. There are many encounterable challenges when proceeding in completing the engineering goal. This involves the effect of using other colored plastics, and polymer mixtures. This research revolved around only 8 of the numerable plastic polymers, as well as only white or clear plastics. Though there are challenges they can be overcome through time and experimentation.

If this project were to be recreated there are some aspects I would change. Such as testing more cuvettes as this would increase the signal in the T-Tests. Collecting data from more than just 11 wavelengths might also prove useful. Other alterations include the inclusion of more plastic types and varieties as well as maybe even mixtures of more than one polymer. I plan to continue my research on the differentiation and quantification of microplastic polymers in water through the use of spectrophotometry and fluorometry.

Future research may include the use of Beer Lambert's Law, identifying multiple plastics, plastic removal/filtration, and possibly even investigating the negative effects of MPs on the human body and microbiome. Though the possibilities for further experimentation are vast, the ultimate goal of my research is to engineer a process for identifying and quantifying plastic polymers within a water sample.

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