Measuring the Adherence of *Escherichia coli* to Microplastic Surfaces

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ABSTRACT

The existence and increasing concentration of microplastics in the marine environment has become a source of concern over the past few decades; one such concern is the ability of microplastics to act as vectors for pathogens causing gastrointestinal diseases, such as pathogenic *E. coli*. This experiment aims to determine whether *E. coli* adhesion is differentially influenced by microplastic surfaces, with plastics of interest being polycarbonate, polypropylene, and polyvinyl chloride, in order to risk stratify the amount of bacterial adhesion to these common, commercially used plastics, thus informing further decisions on the use of these plastics. A K-12 *E. coli* strain was exposed to predetermined concentrations (0.0001 g/L, 0.001 g/L, 0.01 g/L, 0.1 g/L, 1.0 g/L) of the three microplastics and a spectrophotometer was used to determine approximate adhesion using optical density. The change in bacterial adhesion to each microplastic surface was proven to be insignificant (p-value $\geq$ 0.05) when analyzed using a two-way ANOVA test. When optical density values were analyzed across concentrations for each of the three plastics using a one-way ANOVA test, results were significant at 0.01 g/L and 0.1 g/L, respectively, indicating a dose-response relationship. Experiment results further proved that microplastics can act as adequate vectors for *E. coli*.

Keywords: E. coli, Microplastic, Polycarbonate, Polypropylene, Polyvinyl chloride, Optical Density

1. INTRODUCTION

Synthetic polymers, commonly known as plastics, are frequently used throughout the United States due to their versatility, low cost of production, and resistance to wear over time. Plastic production has steadily increased throughout the last century, jumping from 1.5 million tons of produced plastic in the 1950s to an estimated 299 million tons in 2013 (Li et al., 2016). The increased plastic waste amalgamates in marine environments, creating a persistent pollutant in the world's oceans. The extensive lifespan of plastic is a direct result of its resilience against the degradation process. Plastic that undergoes

photodegradation, in which the plastic absorbs light photons thereby breaking bonds between molecules and separating the plastic into fragments, and thermo-oxidative degradation, where chemical changes occur as the plastic incorporates oxygen atoms into its molecular structure, but remains too large to be metabolized by or consists of polymer chains resistant to microorganisms during biodegradation remains a microplastic (MP), a plastic particle less than 5 mm in diameter.

Microplastics can have numerous ecologically-damaging effects, both directly harming local marine life when ingested, creating gastrointestinal blockage and reproduction problems resulting from decreased production of steroid hormones, and damaging marine ecosystems, leaching out bioactive monomer additives like colorant variants and UV stabilizers (e.g. benzophenones (to protect the plastic from UV light) and benzotriazoles (used as a corrosion inhibitor), among other harmful chemicals) (Webb et al., 2012; Pironti et al., 2021). MPs have further been identified as a possible food contaminant for humans, entering the human diet predominantly through the consumption of seafood, notably crustaceans and commercially significant species of fish. Many chemicals, including phthalates like diethylhexyl phthalate (DEHP), and bisphenol A (BPA), used in the plastic manufacturing process are identified as carcinogenic, neurotoxic, and endocrine disruptors, and can cause substantial harm once ingested by humans (Barboza et al., 2018). MPs can further act as vectors for potential human pathogens, like *Escherichia coli* (*E. coli*)., An extended-spectrum beta-lactamase (ESBL) *E. coli* can cause a variety of diseases resulting from the different pathotypes contained within the organism structure, including intestinal-tract infections and cholecystitis (gallbladder infection). The slow degradation of plastic, its environmental proliferation, and its potential incorporation into the food chain make research on microplastics imperative; that these plastics can also act as a vector for pathogenic bacteria makes the research urgent and vital to human health.

2. LITERATURE REVIEW

2.1 PLASTIC DEGRADATION

Due to their physical characteristics, stemming from molecular shape and structure, synthetic polymers are generally highly resistant to aging and degradation. Estimates on the degradation time for many commonly used plastics (10-20 years for polycarbonate (PC), 20-30 years for polypropylene (PP), and ~450 years for polyvinyl chloride (PVC)) suggest that plastics do not often highly degrade when released into the environment, but the resistance to degradation specifically depends on the chemical composition of the plastic (Moore, 2008). The degradation process can occur through four overlapping processes: photodegradation, thermo-oxidative degradation, hydrolytic degradation, and biodegradation. Generally, the process begins with photodegradation, which leads to thermo-oxidative degradation. Ultraviolet (UV) light accumulated during the initial photodegradation provides the activation energy required by the system to gradually incorporate oxygen atoms into the polymer, causing the plastic to become brittle (Webb et al., 2012). Plastic particles with a sufficiently low molecular weight (hydrolysis increases as the weight decreases due to surface area) are biodegradable and can be depolymerized by microorganisms. However, plastic fragments of insufficiently low molecular weight are unable to go through the biodegradation process and therefore remain microplastics. The existence and uncontrollable accumulation of microplastics in the marine environment has become a source of concern over the past few decades. As of 2015, the current estimate for the number of microplastics floating on the ocean's surface ranged from 15-51 trillion, with over 490,000 tons of microplastics entering the ocean in 2010 alone (Van Sebille et al., 2015; Burns and Boxall, 2018). The increasing amount of plastic in global marine environments, combined with the extensive degradation process by which plastic breaks down, creates a critical need for further research into the risks associated with microplastics.

2.2 POLYCARBONATE

Polycarbonate, a transparent and high-impact resistant plastic, is often used in eyewear and medical supply components like filter cartridges for hemodialysis and blood oxygenators during cardiac surgery. PC is widely used specifically because it can be sterilized in a multitude of ways, including steam

autoclaving and by use of ethylene oxide (Powell, 1998). The various applications of PC ensure that it is commonly used within multiple industries. The amorphous molecular structure of PC creates a flexible, transparent polymer, while the polarity of the molecule, resulting from uneven electron density due to the properties of the structural atoms, creates a more operable surface for molecules to adhere to (Prasanna and Doerksen, 2009).

The most common form of PC is bisphenol A polycarbonate, which uses bisphenol A (BPA) as a hardening agent. Because the carbon rings in a BPA molecule are rigid, when reacted with a carbonyl source in a condensation reaction, they form rigid plastic. When BPA PC undergoes photodegradation or hydrolytic degradation, where bonds within polymer chains react with water molecules, resulting in the fragmentation of chains, the bond formed with BPA molecules breaks, resulting in the chemical leaching of BPA into marine environments. BPA can affect marine life, impacting both overall growth and reproductive viability (Flint et al., 2012). BPA has further been classified as an endocrine disruptor when ingested in humans and can cause the pathogenesis of hormone-dependent tumors, like breast and prostate cancer, as well as polycystic ovary syndrome (PCOS) (Fenichel et al., 2013). Thus, polycarbonate was chosen as a plastic of interest for its molecular structure and properties resulting therefrom, as well as global applications and potentially harmful effects on marine and human life.

2.3 POLYPROPYLENE

Polypropylene, the most widely used form of thermoplastic, a plastic that can retain its molecular structure after being heated and cooled, is a semi-crystalline, flexible polymer. Compared to other commercial thermoplastics, like polyamide and polystyrene, polypropylene has a high melting point and rigidity, but remains highly susceptible to degradation, taking only 20-30 years to completely break down (Maddah, 2016). Additionally, PP is shown to be one of the most common plastics in marine environments, due to its extensive production as a result of its numerous applications in different industries (Gewert et al., 2017). The prevalence of PP exposes marine wildlife to potential risks of

ingestion and entanglement with plastic. Further, because plastic can absorb pollutants from the water, marine fauna are susceptible to ingesting heavy metals they would otherwise remain unexposed to (Andrades et al., 2018).

The properties of polypropylene can vary depending on molecular chain regularity, length distribution, thermal processing conditions, and mixture composition with other polymers. The semi-crystalline structure of common forms of PP implies a high strength and low susceptibility to wear over time (Glüge et al., 2019). The applications of polypropylene range from food packaging to disposable syringes in medical fields. Polypropylene was therefore chosen as a plastic of interest due to its semi-crystalline structure and resulting properties, as well as various applications across industries in the United States.

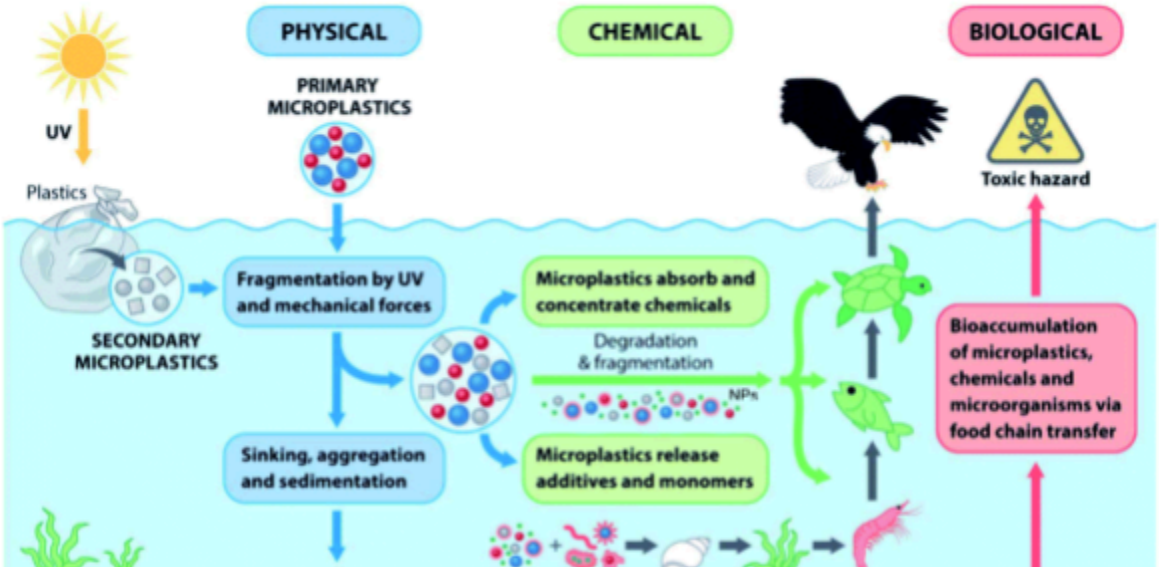
2.4 POLYVINYL CHLORIDE

Polyvinyl chloride is a versatile plastic used for drainage, medical devices, blood storage bags, and cable installation. The hydrophobic nature of the plastic surface, combined with the low surface free energy, poor biocompatibility, and unwanted protein absorption, presents a risk to marine life when exposed to their biological systems. The phthalates used to soften PVC, including diisononyl phthalate (DINP) and DEHP, are considered potential carcinogens and can be exposed to marine environments through chemical leaching during the degradation process. Though recent modifications done to the surface of polyvinyl chloride have resulted in the suppression of bacterial adhesion through plasma treatment and acrylic acid grafting, the majority of PVC used is unmodified, and therefore likely to show greater adhesion of bacteria (Asadinezhad et al., 2012). Polyvinyl chloride was chosen as a plastic of interest for its hydrophobic surface which may affect bacterial adhesion, but specifically for its proven capacity to carry bacteria and its prevalence in marine environments.

2.5 MICROPLASTIC SOURCES

As of 2015, global plastic production has exceeded 300 million tons, with the United Nations Environmental Program considering microplastic contamination as a main global environmental issue (Eerkes-Medrano et al., 2015). Microplastics stem from two sources: primary and secondary. Primary microplastics, MPs manufactured to be less than 5 mm in diameter, include microbeads used in cleansers and face wash products and plastic pellets intended to be used as raw materials for the fabrication of other products. Primary microplastics are often more regulated and traceable. Secondary microplastics, degraded plastic particles from larger plastics, pose a much larger threat and include textile fiber fragments and plastic litter. Secondary microplastics are far more abundant in the ocean, comprising 69%-81% of all plastic in the ocean (Mason et al., 2016).

When non-biodegradable plastics are ingested by marine organisms like zooplankton, lobsters, sea urchins, fish, etc., they are bioaccumulated, where chemical concentration increases gradually within each organism up the food chain. In marine life, microplastics can cause clogging of intestinal tracts, delayed ovulation and fertility, and inhibition of gastric enzyme secretion (Sharma and Chatterjee, 2017). North America, chiefly the United States, is responsible for 19% of global plastic production and 21% of global consumption. Further, North America has the highest plastic consumption per capita in the world at 139 kg per capita^{-1} year^{-1}, as of 2020 (Heller et al., 2020). The high plastic consumption manifests as high plastic waste, contributing to the large amounts of plastic in marine environments. The extensive plastic consumption in North America can result in the ingestion of microplastics into wildlife and humans after the majority of the degradation process is complete, making research into possible microplastic contaminants crucial (Figure 2.1).



2.6 *E. coli*

The growth transition of *E. coli* cultures, from lag-phase to exponential log-phase growth to stationary phase, involves several physiological changes, including cell volume and shape change. Upon the first inoculation into growth media, *E. coli* enters the lag phase, wherein no growth occurs. During the log phase, developed about 6 hours after initial inoculation, cells rapidly divide by binary fission, creating exponential growth and making the log phase the ideal phase in which to conduct experimental research. The bacteria enter the non-growth stationary phase soon after, then the death phase where ~99% of cells die and release their contents into the growth medium (Makinoshima et al., 2003).

The association of genes that encode virulence factors within mobile genetic elements (MGEs) of pathogenic *E. coli* can lead to a multitude of symptoms in humans, including meningitis, UTIs, and dysentery (Figure 2.2). Pathogenic *E. coli* strains, including enteropathic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC), have specific adherence factors that allow them to bind to areas of the body that *E. coli* does not usually inhabit (Kaper et al., 2004). Because of this, pathogenic *E. coli* is likely to bond to other substance surfaces as well. The adhesion of bacteria adhesion to biomedical surfaces depends on a variety of factors. Physicochemical factors, including chemical composition, surface charge, hydrophobicity, and texture of surfaces can affect the adhesion of bacteria. Further, nonspecific interactions between bacteria and substance surface, like Lifshitz-van der Waal forces (intermolecular forces) and

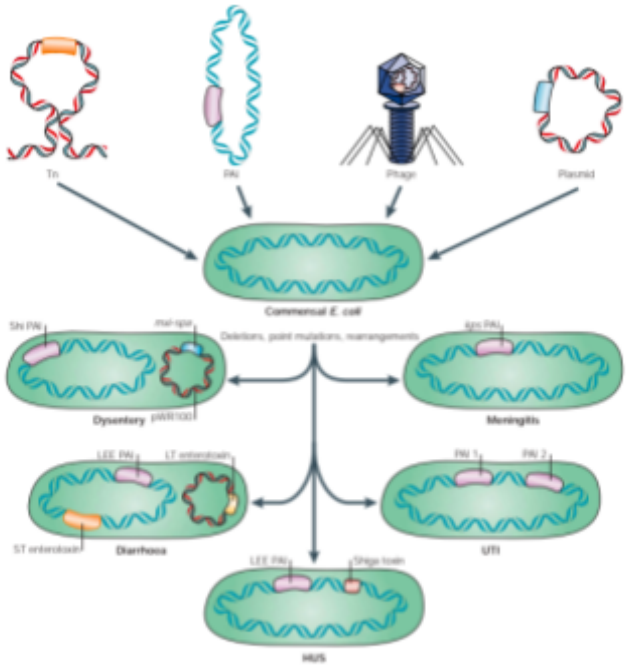


Figure 2.2. This figure shows the effect of virulence factors encoded in mobile genetic elements (shown at the top) on the formation of different symptoms of *E. coli* (Kaper et al., 2004).

acid-base reactions can affect adhesion. Depending on the overall effect of nonspecific interactions, bacteria can either be repelled or attracted (Gomes et al., 2014; Rubio-Armendáriz et al., 2022).

2.7 GAP IN RESEARCH

The considerable lack of knowledge on additives in microplastics and their possible harmful effect on human health continues to make research into microplastics critical. Further, the ability of microplastics to harbor and act as vectors of dissemination for hazardous bacteria remains an under-researched subtopic. Many publications investigating the adherence of bacteria to microplastics are field experiments conducted outside a standard laboratory. Field research studying bacterial adhesion is, therefore, unable to isolate and examine only a particular strain of or type of bacteria, and instead researches a multitude of different organisms found in oceans and freshwater bodies. Additionally, while some previous publications have examined the adherence of different strains of *E. coli* to microplastic surfaces, little comparative research between different types of microplastics with varying molecular structures has been completed. While *E. coli* and its impacts on internal human functions have been researched in depth, comparative research between non-pathogenic *E. coli* strains, and their relative adherence to different microplastic surfaces has not been tested in a standard laboratory.

Data collected from this experiment will fill noticeable gaps in microplastic research, contributing greatly to the scientific understanding of the potential health hazards of MPs in humans and the potential danger of increased global plastic manufacturing and consumption. Similarly, data collection will provide more research into the ability of microplastic to act as a vector for pathogenic bacteria, allowing for more concrete conclusions to be made during MP risk assessment.

2.9 PURPOSE

The purpose of this experiment is to analyze the adhesion of *E. coli* to three commonly used microplastics—polypropylene, polycarbonate, and polyvinyl chloride—after being inoculated in

Luria-Bertani (LB) Broth, used as growth media. The optical density (O.D.) of each microplastic and *E. coli* solution will be used to determine the amount of *E. coli* adhered to the microplastic surfaces. By determining whether or not the surface properties of microplastics affect the adherence of *E. coli*, industries employing plastics in their operations, both corporate and non-commercial, will have available information on the risks associated with each plastic of interest. This research will further help characterize the impact of microplastics on human and marine health.

2.10 RESEARCH QUESTION

To what extent will different microplastics serve as infectious vectors for non-pathogenic *E. coli*?

2.11 HYPOTHESES

Alternative: The adherence of *E. coli* is not the same between polycarbonate, polypropylene, and polyvinyl chloride.

Null: There is no difference in the adherence of *E. coli* between polycarbonate, polypropylene, and polyvinyl chloride.

3. MATERIALS AND METHODS

3.1 MATERIALS

Table 3.1. This table shows the general materials and their quantities used during experimentation.

Materials	
Item	**Quantity**
LB Broth	125 mL
1 cm Light Path Round Glass Cuvettes	20
Spectrophotometer	1
Hemocytometer	1

Table 3.2. This table details the specific materials bought to be used during experimentation, including their quantity, source, and brand.

Materials		
Item	**Quantity**	**Source**
K-12 *E. coli* Strain	1	Flinn Scientific™
Digital-Display Micrometer	1	Beslands
Polycarbonate	3 g	SIBE-R-PLASTIC SUPPLY LLC
Polypropylene	3 g	SIBE-R-PLASTIC SUPPLY LLC
Polyvinyl chloride	3 g	SIBE-R-PLASTIC SUPPLY LLC

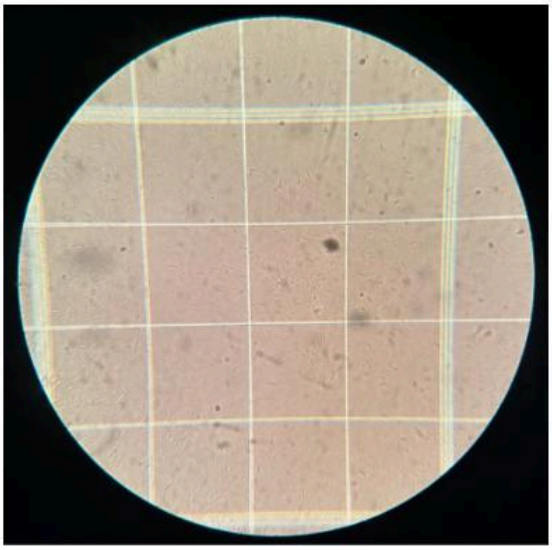
3.2 METHODS

As pre-existing comparative research on the adherence of *E. coli* to different microplastic surfaces does not exist, an experimental research design was the most feasible and accurate method to obtain accurate and valid data on this topic. Though preliminary research supplied a framework for experimental data collection, previous methods of experimentation were expanded upon to account for the inclusion of two independent variables and three overall microplastics of interest.

A lab-safe K-12 *E. coli* strain was used per the working laboratory's safety level. A preparatory experiment to reduce unsupported assumptions on the growth of the *E. coli* strain was performed to find the ideal timeframe by which to add growth media to the bacteria culture to create an exponential fed-batch cell culture before the principal experiment. Within a laminar airflow cabinet to maintain sterility, 50 mL of LB broth was added to a sterile conical flask as growth media. One inoculating loop of stock *E. coli* solution was added to the flask. Immediately, 1 mL of the new stock cell culture was added to a round glass cuvette with a 1 cm light path containing 2 mL of excess LB broth. A second glass

cuvette containing 3 mL of excess LB broth was inserted into a spectrophotometer set to a wavelength of 600 nm to measure the absorbance of the liquid (McBirney et al., 2016). The spectrophotometer was blanked using the LB broth to ensure that the absorbance value of the *E. coli* solution was only attributing the absorbance to the *E. coli*, not the broth. The initial glass cuvette containing *E. coli* was placed in the blank spectrophotometer and the absorbance reading was recorded. The new stock *E. coli* culture was placed in an incubator at 37°C. 20 µg/L of the *E. coli* solution taken from the initial cuvette was placed onto each side of a hemocytometer under a microscope to manually count *E. coli* bacterium. The final cell count was calculated using:

$$\text{Total Cells mL}^{-1} = (\text{Total Cells Counted} \times \text{Dilution Factor} \times 10{,}000 \text{ cells mL}^{-1}) / \text{Number of Squares Counted}$$



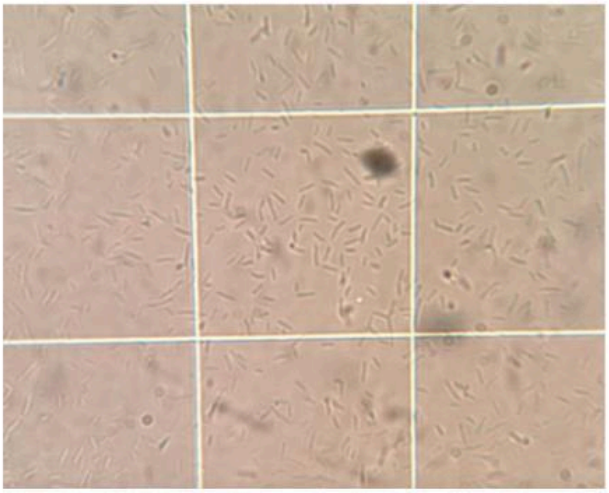


Figure 3.1 (Left). This image shows log-phase *E. coli* on 1/9 of the full grid on the hemocytometer 24 hours after *E. coli* was initially inoculated in LB broth. The exponential increase in rod-shaped bacteria present indicates the phase change from lag-phase to log-phase.
Figure 3.2 (Right). This image displays an enhanced subsquare of the hemocytometer pictured in Figure 1. Dark, rod-shaped bacterium was totaled after the complete calculation had been concluded. Other present bacteria were not included.

The number of counted bacteria seen on each side of the hemocytometer was recorded and an average was taken and officially recorded (Figure 3.1; Figure 3.2). Both cuvettes were then properly

bleached and disposed of. This process was repeated in two-hour intervals across two days, at two, four, six, twenty-four, twenty-six, and twenty-eight hours after initial inoculation.

Once the stock *E. coli* culture became an exponential fed-batch cell culture (created by adding 50 mL of LB broth to the stock culture every 1-2 days), the principal experiment was run. To start, plastic samples from SIBE-R-PLASTIC were ground by two means, depending on the initial size and solidity of the sample. Polycarbonate, due to the flexibility of the acquired sample, was cut into initial sizes and measured with a micrometer to be between 7 mm and 10 mm, before being blended in a grinder to final sizes between 0.20 mm and 0.34 mm. Polypropylene and polyvinyl chloride, due to the solidity and thickness of the samples, were cut using a circular saw to initial sizes between 6 mm and 8 mm, before being blended in a grinder to final sizes between 0.20 mm and 0.44 mm, and 0.19 mm and 0.33 mm, respectively. Two samples of five concentrations (0.0001 g/L, 0.001 g/L, 0.01 g/L, 0.1 g/L, 1.0 g/L) of each of the three plastic types of interest (PP, PC, PVC) were measured on an analytical balance and stored in 20 mL Falcon tubes at room temperature. *E. coli* culture was added to both samples and samples were incubated at 37°C for 24 hours.

Samples were transferred to round glass cuvettes with a 1 cm light path and all microplastic particles were allowed to sink to the bottom of the tube. The spectrophotometer was blanked using LB broth. A singular cuvette containing only *E. coli* culture was inserted as a control and the absorbance value was recorded. All samples were inserted into the spectrophotometer and absorbance values were taken over a period of 20 minutes to account for *E. coli* cell division in log-phase. Absorbance values were converted to Optical Density (O.D.) values by first converting absorbance (A) to percent transmission (%T), then %T to O.D. Absorbance values to percent transmission values were calculated using the:

$$\%T = (1 / 10^{a}) \times 100$$

%T to O.D. was calculated using the:

$$O.D. = \log_{10}(100 / \%T)$$

Two-way Analysis of Variance (ANOVA) and one-way ANOVA tests were performed to determine the statistical significance of plastic-type and plastic concentration differences regarding adherence of *E. coli*, as well as the significance of different plastic types and the significance of different concentrations.

4. RESULTS

4.1 PREPARATORY EXPERIMENT RESULTS

Consistent with previous research, the results of the preparatory experiment showed that *E. coli* enters the log phase of growth about 6 hours after initial inoculation into LB broth as growth media (Figure 4.1). Colony count was consistent with optical density values, with both showing an exponential increase in bacteria roughly 6 hours after initial inoculation. The graph was calculated using data from Table A.2 and Table A.7.

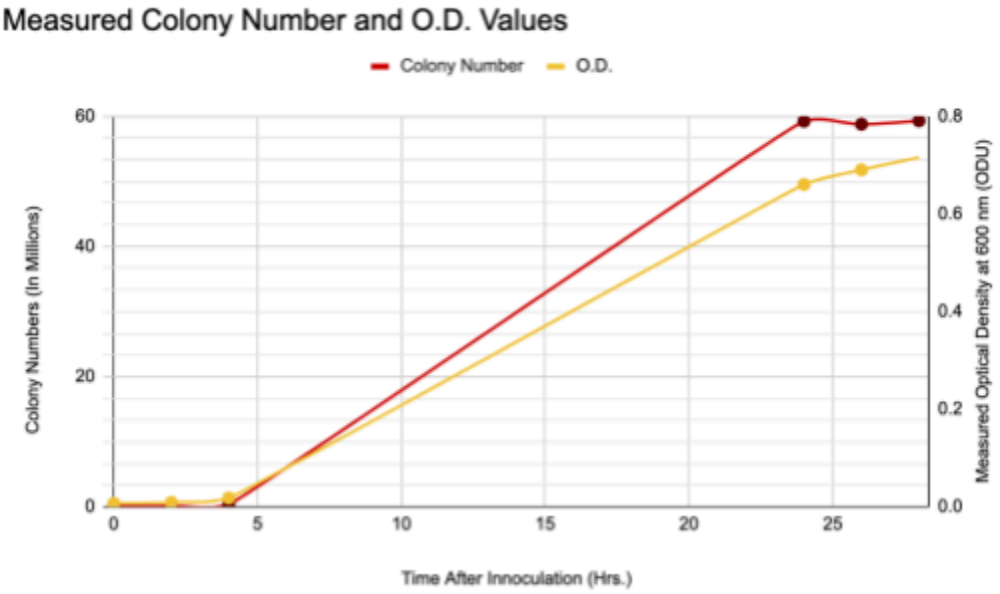


Figure 4.1. This graph depicts the growth stages of E. coli after initial inoculation into LB broth. The E. coli culture entered the log phase of growth around 6 hours after inoculation and remained in exponential growth until about 24 hours after initial inoculation. The death phase was not measured because E. coli would stay in exponential log phase during the duration of the experiment.

4.2

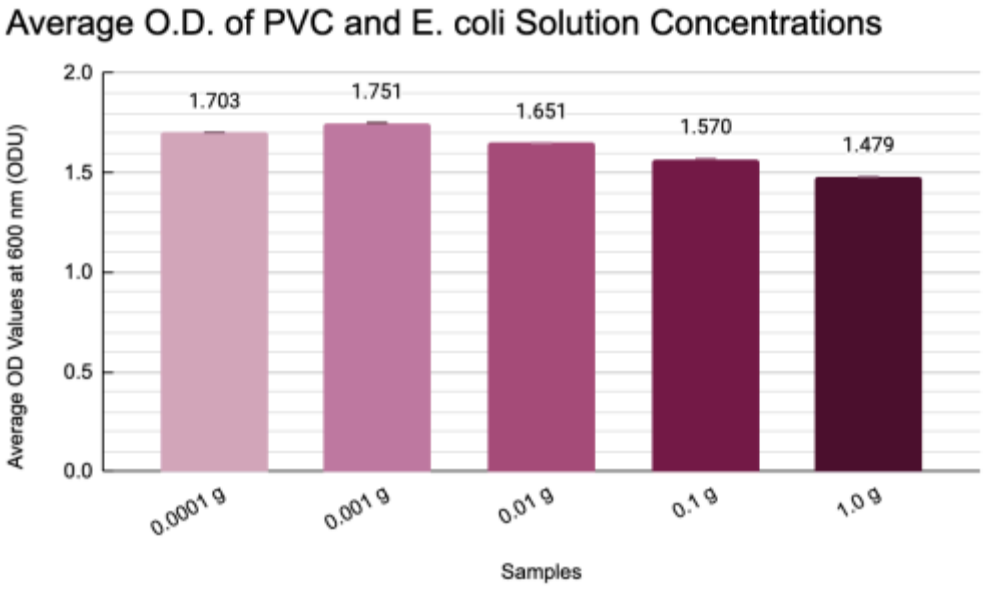


Figure 4.2. This figure shows the O.D. values for polyvinyl chloride, taken from the average of both replicates shown in Table A.5. As plastic concentration increased, O.D. values decreased, indicating greater bacterial adhesion in larger concentrations. PVC showed the greatest average O.D. values across concentrations of all plastics of interest, except at a plastic concentration of 0.0001 g/L.

PRINCIPAL EXPERIMENT

Agreeing with preliminary research, O.D. values decreased as concentration increased, due to a greater amount of bacterial adhesion to microplastics. All three microplastics experienced approximately the same amount of bacterial adhesion, with polypropylene consistently showing the lowest absorbance values across all samples, except 0.0001 g/L and 0.1 g/L replicates. Polycarbonate showed the second-lowest absorbance values, followed by polyvinyl chloride.

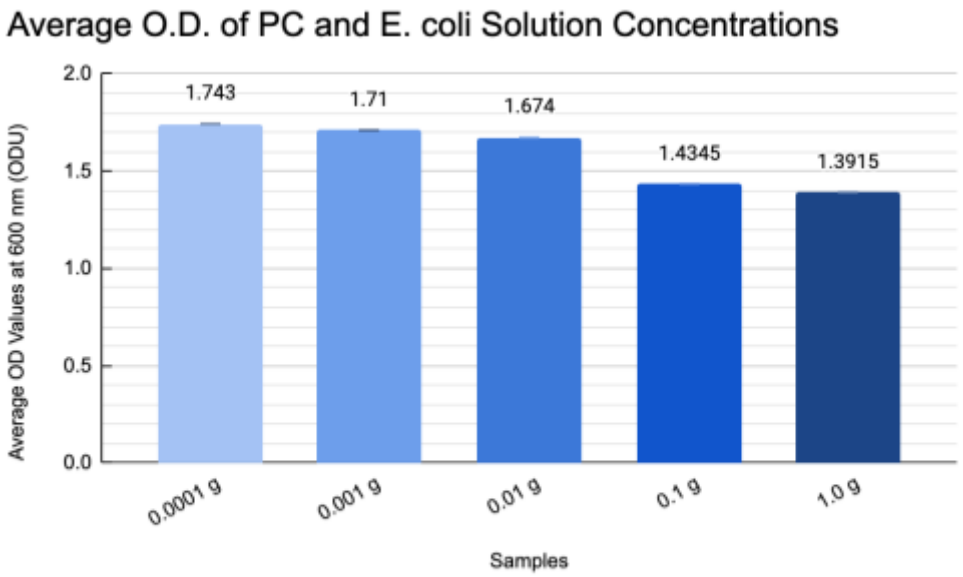
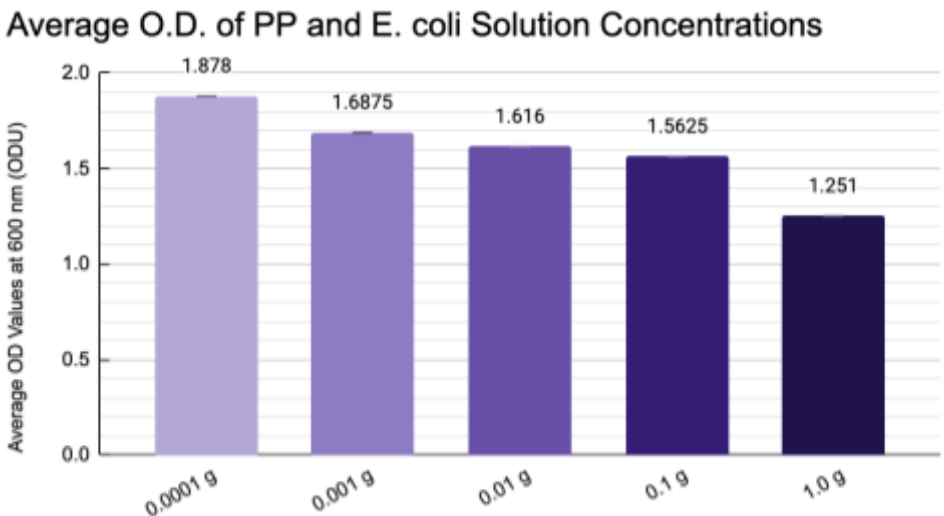


Figure 4.3. This figure shows the graphed calculate average polycarbonate O.D. values, taken from data in Table A.5. As plastic concentration increases, average O.D. decreases, insinuating greater bacterial adhesion in greater concentrations.



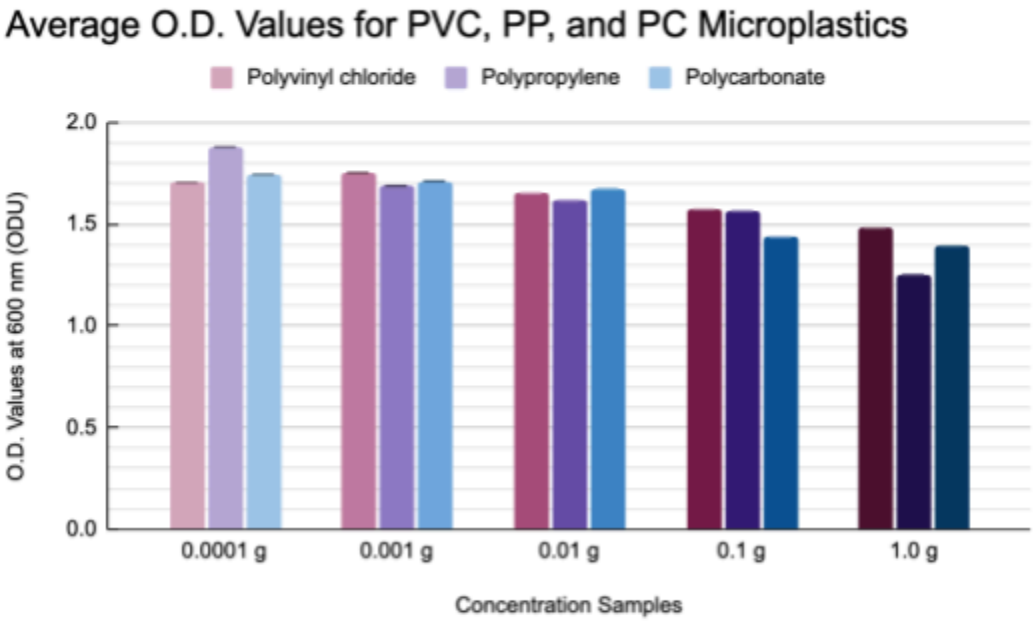


Figure 4.5. This figure shows the average O.D. values for all plastics for all concentrations. Graphed data was taken from Table A.5. Polypropylene is shown to consistently have the lowest absorbance values, except for plastic concentrations at 0.0001 g/L and 0.1 g/L.

The O.D. values of experimental samples are all lower than the control O.D. value of only the *E. coli* sample. This is likely due to the density of the plastics, which were all heavier than the LB broth and

therefore sank to the bottom of each cuvette. *E. coli* bonded to the plastic also likely sank, lowering the overall O.D. value.

4.3 STATISTICAL ANALYSIS

A statistical analysis of optical density results was performed using two-way and one-way ANOVA tests to assess the relationship between microplastic concentration and type of microplastic on *E. coli* adhesion. A two-way ANOVA test was run to account for the two independent variables manipulated during experimentation: type of plastic and plastic concentration. The two-way ANOVA test produced a p-value of 0.32 when using the average O.D. values from all samples across all concentrations (Table A.8). A p-value ≤ 0.05 is determined to be statistically significant, so the differences in O.D. between both independent variables are statistically insignificant. However, one-way ANOVA tests were further run between each plastic type across all five concentrations. Significant p-values, shown in Table A.9, at concentrations of 0.01 g and 0.1 g were 0.003 and 0.04, respectively. This indicates a possible dose-response relationship between microplastic concentration and microplastic type regarding bacterial adhesion of *E. coli*.

5. DISCUSSION

5.1 DISCUSSION

The overall results of this experiment indicate that microplastics do serve as an adequate vector for *E. coli*. Polypropylene experienced the greatest overall adhesion, shown by the consistently low O.D. values, resulting from bonded *E. coli* sinking with microplastics, instead of being suspended in solution. While it is plausible that the lowered O.D. values resulted from the mass death of *E. coli* bacteria, as decayed *E. coli* sink in solution, because the absorbance values for all samples were measured within a 20-30 minute timeframe, it's unlikely that a significant amount of *E. coli* entered the death phase during

data collection. Additionally, all samples analyzed through the spectrophotometer had a lower O.D. value than the control sample of pure *E. coli* culture. This furthers the conclusion that *E. coli* did bind to the microplastic surfaces, demonstrating that MPs are a viable pathogenic vector.

Even though polypropylene experienced the greatest adhesion, because the p-value of the two-way ANOVA test was greater than 0.05, the differential adhesion between plastic types shown in the results fell short of statistical significance. Therefore, the experiment failed to reject the null hypothesis. Future testing may find statistical significance by increasing the amount of each of the respective MP sample sizes or measuring different concentrations within the previously tested range.

It is critical to acknowledge the inconsistent results of the 0.0001 g/L concentration in polypropylene. Because the O.D. calculation is uncharacteristically high, it's likely that all plastic debris wasn't allowed enough time to fully sink to the bottom of the cuvette tube or the absorbance reading was taken too early, before the spectrophotometer had shown a constant value. However, due to the low standard deviation value between both replicates of the 0.0001 g/L concentration, the results may be accurate, but further replicates should be completed to confirm.

5.2 LIMITATIONS

The use of a non-pathogenic strain of *E. coli*, due to the safety level of the working laboratory, may differ from pathogenic bacteria. While working with pathogenic *E. coli* was unfeasible, results reported here can only accurately be applied to non-pathogenic bacteria, with extrapolation to pathogenic *E. coli* affecting human or marine health.

Time constraints further limited the amount of data collected. Wherein the experiment was originally planned to include three replicates, due to a limited amount of lab time and materials, only two replicates were completed, leading to a smaller sample size than originally intended. A larger sample size may have shown statistical significance. Additionally, because *E. coli* divides about every 30 minutes

when in the log phase, it is possible that spectrophotometer data gathered towards the end of the collection window was skewed due to a greater number of *E. coli* bacteria in the solution.

A lack of complete sterilization in the BSL-1 laboratory may have possibly altered results. Lab equipment was frequently shared between those working in the laboratory; therefore, cross-contamination between research projects is possible despite methodical cleaning. As this experiment aims to measure the adherence of bacteria to different surfaces, due to possible contamination it is plausible that some adherence values recorded were partly the result of foreign bacteria in solution.

One of the major possible limitations was the reuse of glass cuvettes during data collection. Due to a lack of resources and an adequate amount of cuvettes for each replicate, one glass cuvette was used for two replicates of each concentration per type of plastic. Cuvettes were rinsed with water and then rinsed with LB broth to avoid dilution of samples, which would skew absorbance values, but cross-contamination between *E. coli* and microplastic solutions across replicates is possible. However, due to the low standard deviation value of replicates per concentration of plastic, it's unlikely that the data was skewed significantly.

6. CONCLUSION

6.1 CONCLUSION

Ultimately, this study did not identify a statistically significant difference between the adherence of *E. coli* to polypropylene, polycarbonate, and polyvinyl chloride surfaces, and therefore the experiment failed to reject the null hypothesis. The possible existence of a dose-response relationship, however, wherein the results between the differences for all three plastics by concentration were significant, is vital to furthering the understanding of microplastics as a potential vector for bacteria. This experiment did unintentionally demonstrate that microplastics can act as an efficient vector for *E. coli*. This is shown by both the trend of decreasing optical density values seen as plastic concentrations increase (Table A.5) and

the lower optical density values of experimental samples when compared to the *E. coli* culture control (Table A.5; Table A.6). Given these conclusions, it's imperative that industries using plastic in their operations fully understand the implications plastic waste and degradation can have on human and marine health.

6.2 FURTHER WORK

To consider data collected from this experiment valid and reproducible, at least two additional replicates should be completed to ensure the reliability of results and accurately determine the influence microplastic surface has on *E. coli* adhesion. Additional concentrations within the dose-response relationship window should be tested to affirm the authenticity of the relationship. Further, other forms of microplastic with varying molecular structures within different polymers could be tested, including polyethylene and polystyrene, to determine differences in adhesion between plastics of varying tensile strength and composition.

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APPENDIX

Table A.1. This table shows the raw absorbance data, in AU, taken from the spectrophotometer during the preparatory experiment. Absorbance values increased exponentially from 2:00 PM Day 1 to 10:00 AM Day 2, indicating that *E. coli* cells entered the log phase around 6-24 hours after initial inoculation into LB broth.

Absorbance (AU)					
	10:00 AM	12:00 PM	2:00 PM	Average	SD
Day 1	0.007	0.009	0.018	0.011	0.0059
Day 2	0.660	0.690	0.715	0.688	0.0275

Table A.2. This table shows the raw colony numbers taken from the hemocytometer during the preparatory experiment. The average number of colonies increased exponentially from 2:00 PM Day 1 to 10:00 AM Day 2, indicating that E. coli cells entered the log phase around 6-24 hours after initial inoculation into LB broth.

Colony Count			
	10:00 AM	12:00 PM	2:00 PM
	4.00E+04	1.50E+05	3.20E+05
Day 1	5.00E+04	4.00E+04	4.50E+05
Average	4.50E+04	9.50E+04	3.85E+05
	5.87E+07	5.85E+07	5.76E+07
Day 2	5.98E+07	5.90E+07	6.10E+07
Average	5.92E+07	5.88E+07	5.93E+07

Table A.3. This table shows the raw absorbance data, in AU, taken from the spectrophotometer during the principal experiment for all replicates of all plastics of interest. Absorbance values decreased as the concentration of plastic increased, indicating that a higher number of bacteria had adhered to plastic at higher concentrations.

Absorbance (AU)						
	Replicate Number	**0.0001 g**	**0.001 g**	**0.01 g**	**0.1 g**	**1.0 g**
	1	1.704	1.717	1.652	1.572	1.480
PVC	2	1.702	1.785	1.650	1.568	1.478
Average		1.703	1.751	1.651	1.57	1.479
SD		0.00141	0.0481	0.00141	0.00283	0.00141
	1	1.896	1.683	1.620	1.576	1.373
PP	2	1.860	1.692	1.612	1.549	1.129
Average		1.878	1.6875	1.616	1.5625	1.251
SD		0.0255	0.0064	0.0057	0.0191	0.1725
	1	1.808	1.640	1.678	1.471	1.375
PC	2	1.678	1.780	1.670	1.398	1.408
Average		1.743	1.710	1.674	1.4345	1.3915
SD		0.0919	0.0990	0.0057	0.0516	0.0233

Table A. 4. This table shows the raw absorbance data, in AU, for the LB broth used to blank the spectrophotometer and the initial control *E. coli* sample.

Absorbance (AU)	
LB Broth:	0.550
E. coli	1.952

Table A.5. This table shows the calculated optical density, in ODU, of the absorbance taken from the spectrophotometer during the principal experiment for all replicates of all plastics of interest. Absorbance values and optical density values are identical due to the use of a 1 cm light path circular cuvette.

Optical Density (ODU)						
	Replicate Number	**0.0001 g**	**0.001 g**	**0.01 g**	**0.1 g**	**1.0 g**
PVC	1	1.704	1.717	1.652	1.572	1.480
	2	1.702	1.785	1.650	1.568	1.478
Average		1.703	1.751	1.651	1.57	1.479
SD		0.00141	0.0481	0.00141	0.00283	0.00141
PP	1	1.896	1.683	1.620	1.576	1.373
	2	1.86	1.692	1.612	1.549	1.129
Average		1.878	1.6875	1.616	1.5625	1.251
SD		0.0255	0.0064	0.0057	0.0191	0.1725
PC	1	1.808	1.640	1.678	1.471	1.375
	2	1.678	1.780	1.670	1.398	1.408
Average		1.743	1.710	1.674	1.4345	1.3915
SD		0.0919	0.0990	0.0057	0.0516	0.0233

Table A.6. This table shows the calculated optical density, in ODU, of the absorbance for the LB broth used to blank the spectrophotometer and the initial control *E. coli* sample.

Optical Density (ODU)	
LB Broth:	0.550
E. coli	1.952

Table A.7. This table shows the calculated optical density, in ODU, of the spectrophotometer absorbance data taken during the preparatory experiment. O.D. values increased exponentially from 2:00 PM Day 1 to 10:00 AM Day 2, indicating that *E. coli* cells entered the log phase around 6-24 hours after initial inoculation into LB broth.

Optical Density (ODU)					
	10:00 AM	12:00 PM	2:00 PM	Average	SD
Day 1	0.007	0.009	0.018	0.011	0.0059
Day 2	0.660	0.690	0.715	0.688	0.0275

Table A.8. This table shows the calculated average O.D. values, in ODU, for all three plastics of interest across all five concentrations. Data was used during the two-way ANOVA test to determine the statistical significance of the results.

Average O.D. Values (ODU)					
	0.0001 g	**0.001 g**	**0.01 g**	**0.1 g**	**1.0 g**
PVC	1.704	1.717	1.652	1.572	1.480
	1.702	1.785	1.650	1.568	1.478
PP	1.896	1.683	1.620	1.576	1.373
	1.860	1.692	1.612	1.549	1.129
PC	1.808	1.640	1.678	1.471	1.375
	1.678	1.780	1.670	1.398	1.408

Table 4.2. This table shows the p-values determined for each concentration of plastic between the O.D. values for the three plastic types of interest. Statistical significance was seen in concentrations of 0.01 g/L and 0.1 g/L, respectively.

	0.0001 g	**0.001 g**	**0.01 g**	**0.1 g**	**1.0 g**
p-values	0.09827	0.64382	0.00261	0.03938	0.21980
Significance	NO	NO	YES	YES	NO