Microplastics in Lettuce

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#### Abstract

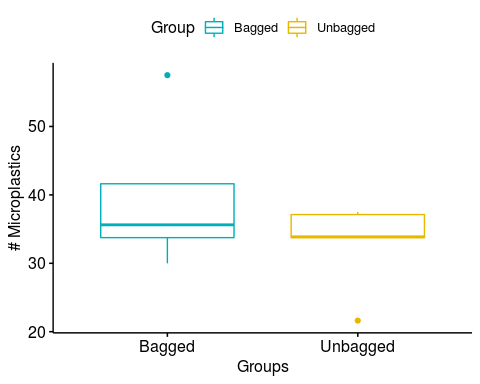
#### Introduction

#### Methods

1. Obtain Romaine lettuce. Purchase *two unpackaged heads of romaine lettuce* and two heads of romaine lettuce in plastic packaging (pre-chopped for salad is acceptable) from 5 nearby stores - Sprouts on Foothill Boulevard, Stater Bros. on N. Garey Avenue, Cardenas on E. Holt Avenue, El Super on E. Holt Avenue, and Super King on Auto Center Drive. Upon purchasing the lettuce, ensure that it is carried out in individual paper bags to avoid contamination. Seal the bags to avoid contamination with airborne particulates, however, some contamination will likely be unavoidable.
2. In the lab, obtain a glass blender. If unbagged, thoroughly wash one head of lettuce with deionized water, and then fill the blender with leaves until they reach the top. If bagged insert leaves into blender until they reach the top without washing, as the lettuce is labeled pre-washed.
3. Add 200 mL of Milli-Q deionized water to the blender. Blend for 30 seconds on the slower “grind” setting, then for 60 seconds on the faster “cream” setting.
4. Strain the contents of each beaker through a stainless steel filter with a mesh size of 5 mm to capture larger organic fibers while allowing all particles meeting the qualification of “microplastic” (>5mm) to pass through until 100 mL of solution is obtained. Place this solution in a glass beaker covered with aluminum foil to avoid airborne contamination.
5. Extract 5 mL of the blended lettuce solution using a glass pipette and place in a 100 mL glass beaker. Due to the large quantities of cellulose in lettuce, use the enzyme cellulase from Niger Aspergillus niger to digest the remaining organic material while leaving any microplastic particles intact. Add 5 mL of cellulase and 10 mL of a phosphate-buffered saline (PBS) solution (1 L prepared with 8 g sodium chloride, 200 mg potassium chloride, 1.44 g disodium phosphate and 240 mg monopotassium phosphate in deionized water, set to pH 5.0 using hydrochloric acid). Cover beaker with aluminum foil.
6. Incubate the solution at 50° C for 4 days.
7. Repeat steps 2-6 for every purchased head of lettuce.
8. Add NaCl in solution with deionized water (density=1.2g/mL, stirred for 10 minutes) to the incubated solution until the beaker has 100 mL of solution in it. Allow solution to settle for 30 minutes, then use a vacuum system to collect the top 40 mL of each sample and any floating microplastics within it. 9. Add 5 mL of 0.08 g/mL Nile red dye solution to this extracted solution and wait 30 minutes to allow staining to occur.
9. Run this extracted and stained solution through vacuum filtration with an entirely glass apparatus to separate stained fibers from their liquid matrix. Place the resulting filter paper in a glass beaker, cover with aluminium foil, and allow it to dry overnight.
10. Use RStudio to generate random coordinate points. Create a grid on the dried filter paper, and plot four random points onto this grid on each paper.
11. Use the digital Revolve microscope with fluorescent light (4x zoom lens, TXRED fluorescent/FL overlay at 100% brightness, 110 ms capture) and use ocular techniques to count the number of fluorescent particles present at each marked location on the paper.
12. Repeat steps 8-12 with each incubated solution.
13. In order to mitigate the impacts of contamination or procedural error, corroborated the results through the use of blanks. 3 water “samples” should go through steps 2-11 alongside the experimental samples, but with 100 mL of deionized water rather than solution material. All equipment used should be washed thoroughly before and after touching any sample, and all water used to wash should be deionized so as to avoid contamination from the water.
14. The methods of Löder et al. 2017, Maes et al. 2017, Wang and Wang 2018, Karlsson et al. 2017, and Quinn et al. 2018 were consulted in developing these proposed procedures.

#### Results

The data collection yielded five replicates and five pseudo-replicates for both bagged and unbagged lettuce, as two bagged and unbagged heads were purchased at five different stores. In order to more accurately reflect these replicates, the number of microplastics found on each head of lettuce was averaged with its pair. For example, the number of microplastics found on each bagged lettuce head purchased at Cardenas were averaged with one another. The graph below shows the box plots of these averages for bagged and unbagged lettuce.

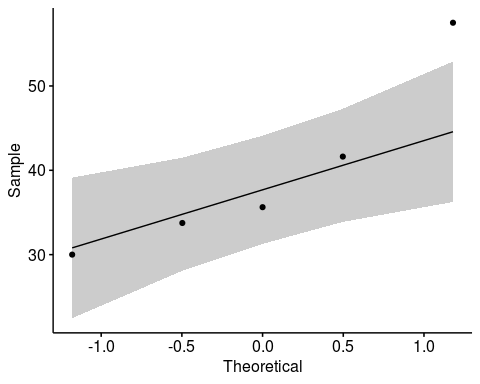


#### Normality Tests

This experiment was designed to test the null hypothesis of “there is no difference in microplastic quantities in romaine lettuce with or without plastic packaging.” In order to determine whether or not to reject the null hypothesis, whether or not the data fell within normal distribution was first assessed, using Q-Q Plots and the Shapiro-Wilk normality test.

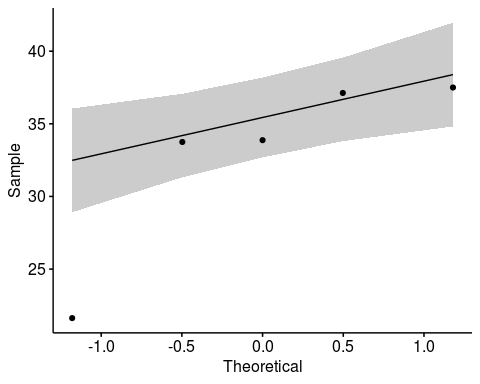
##### Bagged

For bagged lettuce data, the Shapiro-Wilk normality test yielded a p-value of 0.2768, indicating that it is within the bounds of normal distribution. The Q-Q plot, shown below, also demonstrates that most points fall within the expected bounds.



##### Unbagged

For unbagged lettuce data, the Shapiro-Wilk normality test yielded a p-value of 0.04526, indicating that it is not within the bounds of normal distribution. The Q-Q plot, shown below, also demonstrates that, while most points fall within the expected bounds, some are much further out of normal distribution than they were for the bagged lettuce data.



#### Statistical Analysis

As the unbagged data fell outside of normal distribution, a Paired Samples Wilcoxon Test was used to evaluate the data. Using this test, a p-value of 0.5839 was yielded. This p-value does not demonstrate statistical significance, meaning that the null hypothesis may not be rejected. A Two-Sample T-Test was also conducted, yielding a p-value of 0.2538. This also does not demonstrate statistical significance, indicating that statistical significance likely would not be demonstrated even if the data fell within normal distribution.

#### Discussion

#### Conclusion