JOURNAL OF
EMERGING INVESTIGATORS

Article

Lettuce seed germination in the presence of microplastic contamination

Pravit Kochar[1], Giselle Gomes[2]
[1]Bergen County Academies, Hackensack, New Jersey
[2]Biology, Bergen County Academies, Hackensack, New Jersey

SUMMARY

Microplastic pollution is a pressing environmental issue, particularly in the context of its potential impacts on ecosystems and human health. Given the prevalence of microplastics in the environment and their known effects on altering plant physiological processes, such as water retention and chlorophyll balance, understanding their interaction with edible plants is crucial. In this study, we explored the ability of plants, specifically those cultivated for human consumption, to absorb microplastics from their growing medium. We hypothesized that microplastics would be detectable within the plant roots, particularly under stress conditions induced by root damage. However, we found no evidence of microplastic absorption in both intact and mechanically damaged roots. This outcome suggests that microplastics larger than 10 µm may not be readily absorbed by the root systems of leafy crops such as lettuce (*L. sativa*). The study acknowledges limitations in the particle size and growth period examined, indicating that future research with a broader range of microplastic sizes and extended growth periods could provide further insights. The findings underscore the importance of continued investigation into the interactions between microplastics and plant systems, offering a reassuring perspective on the safety of consuming leafy greens in the context of microplastic pollution. This study contributes to the growing body of knowledge on the environmental dynamics of microplastics, emphasizing the need for comprehensive strategies to address their widespread presence.

INTRODUCTION

Microplastics, plastic particles less than 5mm in size, are pollutants that pose significant environmental and health risks.These particles originate from various sources, including plastic waste, synthetic clothing fibers, and industrial processes, and are now pervasive in the environment, contaminating water, air, and soil. The pressing matter of microplastic pollution lies within the issues it has already caused. The amount of microplastics found in the ocean accounts for 11% of the total plastic pollution in the ocean (1). The growing amount of microplastics results in microplastics being found all over the globe and even within humans (1). Out of the 10,000 chemical additives found in microplastics, 2,400 are estimated to be potentially harmful (2). Some of these chemicals can lead to issues regarding neurodevelopment, bone density, obesity, and potentially multiple types of cancer (2).

Microplastics have been found in soils all across the globe. These microplastics usually fall within the range of 100-300 µm (3). Since the decomposition rate for microplastics is unknown, it is assumed that microplastics will continue to accumulate. This results in microplastics interfering with soil nutrient cycling and soil aggregates (3). These microplastics also alter the nutrients present and the bioavailability of the soil by increasing pH and reducing soil bulk density (4). This continued accumulation of microplastics decreases soil quality, which could result in negative effects on plants growth and qualities including reduced root development, impaired nutrient uptake, and stunted overall plant health.

There are many types of microplastics, each with varying effects on the soil, and potentially the plant. Microplastics are often produced with different polymers depending on the desired flexibility, resistance, roughness, and durability of the plastic (5). When these polymers break down into monomers, they can potentially be damaging to the environment as well as to humans. For example, polyurethane, commonly found in foam-based products, can break down into toxic monomers, posing health risks (3). The extent of polyurethane degradation into microplastics and subsequent absorption by plants, potentially impacting human health, warrants further research to elucidate these pathways and effects (3).

One of the most prominent ways to detect microplastics in plants is the use of fluorescence. In nature, microplastics are not inherently fluorescent; therefore, in experiments, microplastics are often fluorescently labeled to make them easier to identify. Since fluorescence can produce bright and stable emission signals that are easily distinguishable, it is not only simple, yet efficient (6). Another less common way to detect microplastics is by Scanning Electron Microscroscopy (SEM), a technique that scans the surface with the use of a beam of electrons, but microplastics in plant tissue tend to be harder to identify with SEM (7), due to the difficulty in distinguishing from other.

Several studies have been conducted to determine the effects of microplastics on plant growth. One experiment aimed to determine the effect of microplastics on tomato plants using sewage sludge containing microplastics (10). The results indicated that sewage sludge with microplastics have a negative impact on the growth of the plant and delay or diminish fruit production (10). However, the use of sewage sludge leaves a variety of possible factors that could influence plant growth, aside from microplastics. A similar study done specifically observing the effect of microbeads in leaf veins of lettuce (*L. sativa*) found no uptake of microplastics by SEM (7). SEM is limited in differentiating microplastics from other small particles based solely on morphology (7). This study investigated whether rice seedlings can absorb and transport

https://doi.org/10.59720/24-005

nano-sized (about 80 nanometers) and micro-sized (about 1 micron) fluorescently labeled polystyrene (PS) microspheres, which aimed to establish if these plastic particles could move from the roots to the aboveground parts of the plants, including stems and leaves (7). The focus was on understanding the pathway and extent of microplastic uptake and translocation within these food crops, but they were unable to come to a conclusion because they could not determine if the plastic particles were within or on the surface of the plant (7). Furthermore, it is thought that microplastics could lead to limited root permeability causing limited water and poor nutrient uptake (9). Building upon previous research has been crucial to this study. By ensuring that the environment is controlled and the ideal microscope type is used, the data represents an accurate measure of the hypothesis, that lettuce will uptake microplastics.

In previous studies, there have been contradictory answers as to the effect of microplastics in soil due to their shape, structure, additives, and concentration (5). In this study, we aimed to determine the presence of microplastic uptake in commonly consumed crops, using *L. sativa* lettuce as a model. The microplastics used in this experiment were designed for regional blood flow studies in tissues and organs (8). These microplastics are designed to fluoresce more than those found in nature(8). We chose to use polystyrene (PS) microplastics, one of the three most common microplastics found in pollutants(9).We initially hypothesized that microplastics would be visible in *L. sativa* roots in an agar media under a fluorescent microscope. However our results indicated that the lettuce do not uptake the microplastics.

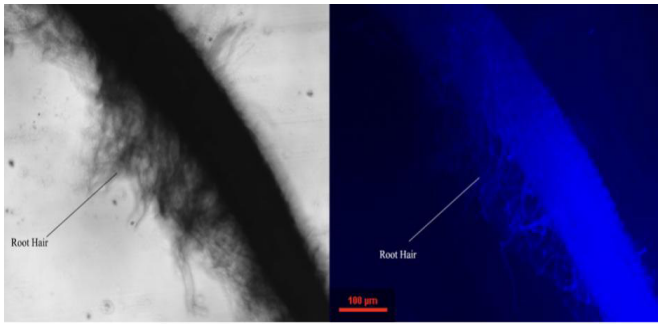

Figure 1. Microplastics in Nondamaged *L. sativa* With Microplastics. Root image of nondamaged *L. sativa* grown with microplastics in the agar-agar solution. Photo taken under brightfield and DAPI blue fluorescence. Root hairs are indicated.

Lettuce (*L. sativa*) Seeds in Agar-Agar with Microplastics			
Sample Number	Microplastics in Experimental Agar-Agar	Microplastics in Experimental L. sativaRoot	Microplastics in Control L. sativa Root
1	41	0	0
2	44	0	0
3	33	0	0

Table 1: Microplstics in Lettuce (*L. sativa*) Seeds in Agar-Agar without Microplastics. Comparison of the microplastics found in the non-cut experimental agar-agar, the microplastics found in the non-cut experimental *L. sativa* roots, and the microplastics found in the non-cut control *L. sativa*.

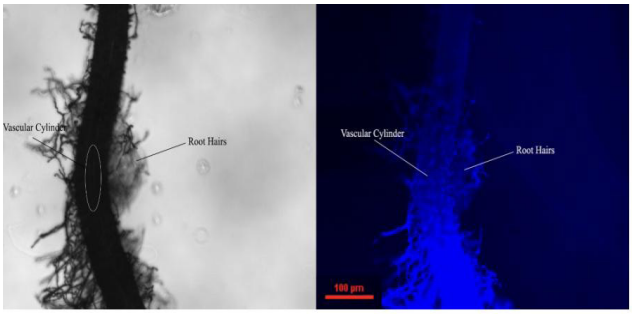

Figure 2: Microplastics in Nondamaged *L. sativa* Control. Root image of nondamaged *L. sativa* control group. Photo taken under brightfield and DAPI blue fluorescence. Vascular cylinder and Root hairs are indicated.

Thus the hypothesis was altered later to be if the roots were damaged the microplastic uptake would increase.

RESULTS

The experiment aimed to explore the uptake of microplastics by lettuce (*L. sativa*) plants grown in agar media. We hypothesized that the roots of the *L. sativa* plants grown on PS-containing agar media would take up the microplastics through the xylem. In the experiment, the control groups were solutions without the presence of microplastics, and the experimental group had microplastics, of size 10 micrometers in the agar solution. After a growth period, we examined samples of both the agar media and plant roots by fluorescence microscopy. Microplastics were counted across 10x magnitude, and then the microplastis in one frame of refrence was used as the average for the agar solution. Fluorescent imaging of the plant roots demonstrated that the lettuce (*L. sativa*) roots did not uptake these microplastics (**Figure 1, Table 1**). This lack of microplastic uptake was identical to that of plant roots grown in control media (**Figure 2, Table 1**). Images of the media with microplastics confirm that the microplastics were still intact and in the agar media (**Figure 3**).

Then we hypothesized that the microplastics were too large for the xylem to uptake them directly. In an effort to potentially increase xylem size to thereby increase microplastic presence, we hypothesized that damaged roots would lead to an increase in microplastic uptake, explaining the varying conclusions from studies that have been

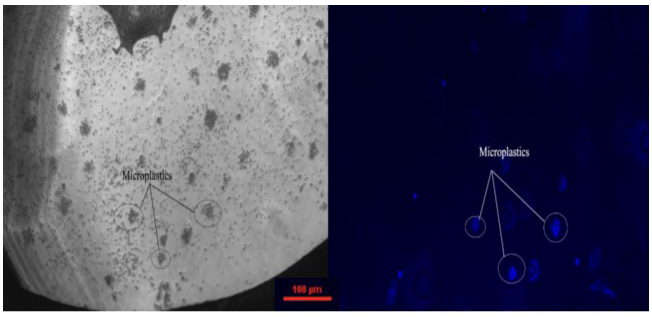

Figure 3: Image of agar with microplastics. Images of agar with microplastics in the solution for uncut *L. sativa*. Photo taken under brightfield (left) and DAPI blue fluorescence (right). Examples of microplastics have been circled.

https://doi.org/10.59720/24-005

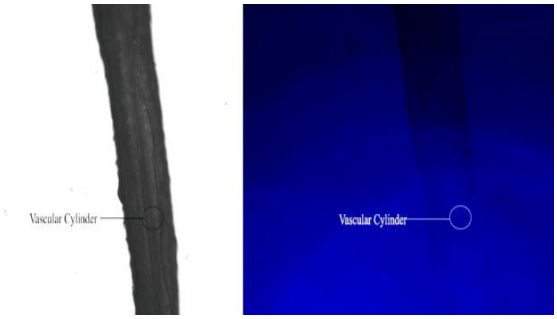

Figure 4. Damaged *L. sativa* With Microplastics. Root image of cut *L. sativa* with microplastics in the agar-agar solution. Photo taken under brightfield and DAPI blue fluorescence. Vascular cylinder and cut line are indicated.

Lettuce (*L. sativa*) Seeds in Agar-Agar with Microplastics and Cut Root			
Sample Number	Microplastic in Experimental Agar-Agar	Microplastics in Experimental Cut Root	Microplastics in Control Cut *L. sativa* Root
1	23	0	0
2	38	0	0
3	34	0	0

Table 2: Microplastics in *L. sativa* Seeds in Agar-Agar with Microplastics and Cut Root. Comparison of the microplastics found in the cut experimental agar-agar, the microplastics found in the cut experimental *L. sativa* roots. No microplastics found in the *L. sativa* root.

conducted on the subject. In the second set of experiments, we attempted to increase the entry portal for microplastic uptake by damaging the roots through scalpel incisions in both the experimental and control groups. Yet once again, we did not detect microplastics in the *L. sativa* roots (**Figure 3 and 4, Table 2**). No microplastics were found in the *L. sativa* roots, indicating that under these conditions, the plant did not absorb the 10-micrometer microplastics, and the growth of the lettuce with and without microplastic were consistent with one another (**Figure 5**). Had there been microplastics in the plant, due to the high fluorescence of the microplastics when compared with the lettuce, there would have been points that shown throw the slight natural fluorescence of the lettuce, similar to how it did with the agar. Overall, we conclude that it is unlikely for lettuce, and with further research, crops as a whole to absorb microplastics of size 10 micrometers.

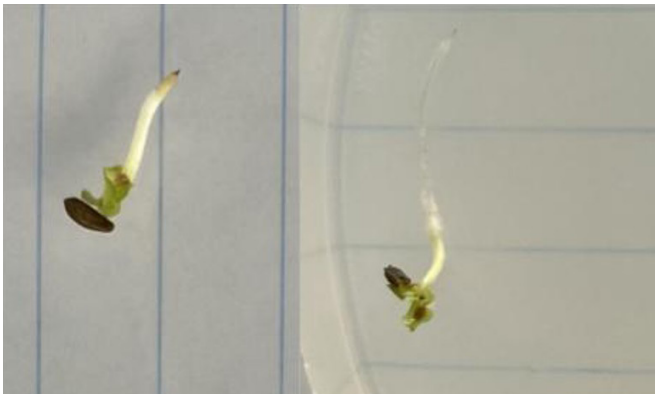

Figure 5: *L. sativa* With and Without Microplastics Side by Side. *L. sativa* root image in agar. *L. sativa* root image without microplastics on the left. *L. sativa* root with microplastics on the right.

DISCUSSION

The presence and potential impacts of microplastics in agricultural systems have become a critical environmental concern, particularly due to the increasing quantities of discarded plastics, their degradation into microscopic particles, and the subsequent implications for human health as these particles make their way into the food chain. Our investigation sought to explore whether microplastics, specifically those of 10 µm diameter- as this was slightly smaller than the size that plastics in nature commonly break down to- could be absorbed by the roots of *L. sativa*, echoing concerns raised in current literature about the impact of microplastics on plant health and agricultural productivity.

Contrary to our initial hypothesis that microplastics would be taken up by the lettuce roots, specifically through the xylem, our findings revealed no absorption of microplastics, even when the roots were artificially damaged to potentially facilitate entry. We hypothesize that the size of the microplastic particles at 10 µm diameter might be too large for absorption by the root hairs into the xylem tissue. To further investigate this, the roots were "damaged" by cutting them with a sterile scalpel blade in the zone of differentiation. Thus, if the *L. sativa* roots were damaged in some way and the xylem vessels or sieve tubes were open to media, the microplastics would have a larger conduit in which to enter. However, the xylem still did not uptake the microplastics.

It is important to note when assessing this data that there were some limitations to the experiment. The microplastics are 10 µm in diameter and had smaller microplastics been used the xylem may absorb some microplastics due to their smaller size. However, the type of plastics discarded that degrade into microplastics do not usually degrade to particles smaller than 10 µm (3).

Reflecting on the broader scientific discourse, it appears our findings diverge from the anticipated impacts of microplastics on plant systems. While the literature suggests potential negative effects on plant growth and soil health due to microplastic pollution, our experiment, focused solely on the uptake of microplastics by plant roots, does not directly address these growth aspectsThis conclusion might overextend the findings, as the assessment of plant growth was not directly measured or compared to controls beyond root development.

Given these considerations, future research should aim to address these gaps, potentially by utilizing microplastics of varying sizes, shapes, and chemical compositions to more closely mimic environmental conditions. Additionally, extending the duration of plant growth before analysis could provide insights into the long-term effects of microplastics on plant health and yield. Investigating the potential for microplastic accumulation and translocation in plants over a complete growth cycle and testing different imagin techniques, including segmenting the roots and taking cross sections, could further elucidate the risks posed to food security and human health.

In conclusion, while our experiment contributes to the growing body of research on microplastics and their

https://doi.org/10.59720/24-005

interaction with plant systems, it underscores the complexity of these interactions and the need for further studies. The results suggest that lettuce plants are capable of germinating and developing root systems in the presence of 10 µm microplastics, without immediate evidence of harm. However, the broader implications of microplastics on plant health and productivity, as well as their potential entry into the food chain, remain areas for further exploration.

MATERIALS AND METHODS

Agar plates were prepared by mixing 15 grams of nutrient agar per liter. The agar was autoclaved at 15psi, 121°C, for 15 minutes, then plating in sterile petri dishes. 1mL of microplastics was added to experimental plates. The final concentration of microplastics per petri dish was 0.167 mL which is equal to 601,200 beads. 3-4 seeds of lettuce (*L. sativa*), which were from Non-GMO, open-pollinated, USA were added into each petri dish, both experimental and control plates, and then they were left to grow for 2 weeks. The experimental plates were the plates with microplastics in the agar and the control plates did not have microplastics in the agar. An aqueous NPK fertilizer solution was added to the Petri dishes to allow for growth. A separate series of trials were done for the cut lettuce seeds. For the cut lettuce (*L. sativa*), shortly after germination, both control and experimental lettuce (*L. sativa*) were cut using a scalpel at the apical meristem to maximize microplastic uptake.

After the full growing period was complete, fluorescent microscopy was completed on samples of both agar media and plant roots. Forceps were used to obtain the sample of lettuce, and a 5mL pipette was cut along the base in order to extract an equal cylidracil sample of agar- After a brief rinsing period to remove any microplastics on the surface, these would then be observed under the fluorescent microscope. After looking at the sample under the brightfield mode (black and white) and taking an image, these would then be observed under fluorescence to observe the microplastics more clearly. The fluorescent microscope used has a Nikon Eclipse TS100, with a DS-U3 camera with a Lumen 200 mercury lamp, and a DAPI filter cube (Excitation/Emission wavelengths are 375/435 nm).

Received: December 21, 2023
Accepted: April 8, 2024
Published: December 9, 2024

REFERENCES

1. Katanich, Doloresz. “You Eat a Credit Card’s Worth of Plastic Every Week, Says a New Study.” *Euronews*, 11 Apr. 2022, www.euronews.com/green/2022/04/11/how-much-plastic-do-you-eat-it-could-be-as-much-as-a-credit-card-a-week#:~:text=It%20could%20be%20as%20much%20as%20a%20credit%20card%20a%20week.
2. Ordoñez, Maya. “Microplastics and Health Risks: What Do We Really Know?” *WebMD*, 28 Oct. 2022, www.webmd.com/a-to-z-guides/news/20221028/microplastics-health-risks-what-do-we-really-know.
3. Uurasjärvi, Emilia, et al. “Microplastic Concentrations, Size Distribution, and Polymer Types in the Surface Waters of a Northern European Lake.” *Water Environment Research*, vol. 92, no. 1, 12 Sept. 2019, pp. 149–156, https://doi.org/10.1002/wer.1229.
4. Huang, Danlian, et al. “Research Progress of Microplastics in Soil-Plant System: Ecological Effects and Potential Risks.” *Science of the Total Environment*, vol. 812, 15 Mar. 2022, p. 151487, https://doi.org/10.1016/j.scitotenv.2021.151487.
5. Lozano, Yudi M., et al. “Microplastic Shape, Polymer Type, and Concentration Affect Soil Properties and Plant Biomass.” *Frontiers in Plant Science*, vol. 12, 16 Feb. 2021, https://doi.org/10.3389/fpls.2021.616645.
6. Li, Lianzhen, et al. “Confocal Measurement of Microplastics Uptake by Plants.” *MethodsX*, vol. 7, 1 Jan. 2020, p. 100750, https://doi.org/10.1016/j.mex.2019.11.023.
7. Liu, Yingying, et al. “Uptake and Translocation of Nano/Microplastics by Rice Seedlings: Evidence from a Hydroponic Experiment.” *Journal of Hazardous Materials*, vol. 421, Jan. 2022, p. 126700, https://doi.org/10.1016/j.jhazmat.2021.126700.
8. “FluoSpheresTM Polystyrene Microspheres, 10 Mm , Blue Fluorescent (365/415), for Blood Flow Determination.” *Thermofisher.com*, 2022, www.thermofisher.com/order/catalog/product/F8829.
9. Ullah, Raza, et al. “Microplastics Interaction with Terrestrial Plants and Their Impacts on Agriculture.” *Journal of Environmental Quality*, vol. 50, no. 5, 25 Aug. 2021, pp. 1024–1041, https://doi.org/10.1002/jeq2.20264.
10. Hernández-Arenas, Ricardo, et al. “The Effect of Sewage Sludge Containing Microplastics on Growth and Fruit Development of Tomato Plants.” *Environmental Pollution*, vol. 268, Jan. 2021, p. 115779, https://doi.org/10.1016/j.envpol.2020.115779.