

A Pilot Study on How Microplastic Polluted Rainfall Infiltrates Commonly Eaten Leafy Vegetables

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Abstract

Microplastics found in the human body have been reported to compromise tissue integrity, damage organs, and contribute to inflammation and carcinogenesis. Recent research indicates that microplastics also reduce photosynthetic efficiency by approximately 10% across terrestrial, aquatic, and freshwater ecosystems. This reduction has significant implications, potentially leading to an annual loss of up to 360 million metric tons of crops and nearly 24 million tons of seafood, which could impact food availability for roughly 123 million adults. In response to these concerns, this pilot study employed a floating disc assay to quantitatively assess the infiltration of rigid micro- and nano thermo-amino particles (MNPPs) into the foliage of four representative vegetable species: *Basella alba* (Malabar Spinach), *Capsicum chinense* (Habanero), *Brassica rapa* (Bok Choy), and *Phaseolus vulgaris* (String Beans). The crops were grown organically in Amityville, New York, an area marked by substantial industrial activity. The primary aim was to determine whether rainfall facilitates the entry of MNPPs, ranging in size from 1000 nanometers to 5 micrometers, into leaf tissue through photosynthesis, respiration, and cuticular absorption. Both adaxial and abaxial surfaces of leaf discs were analyzed before and after exposure to MNPPs using a LABOMED CxL clinical microscope. The findings indicate that MNPPs can penetrate leaf structures during both photoperiod (photosynthesis) and scotoperiod (respiration), irrespective of whether the stomata are closed. These findings reveal a previously unidentified route for plastic pollutants to enter the human food chain.

Keywords

Vegetables, Atmospheric Pollution, Rainfall Pollution, Micro Nano Plastics, Photosynthesis, Cellular Respiration, Foliar Absorption

1. Introduction

Global plastic production is estimated to reach between 902 megatons and 1124 megatons by 2050 [1]. Microplastics derive from larger plastic particles measuring 5 millimeters or smaller, while nanoplastics are even smaller, ranging from 1 to 1000 nanometers in size [2]. These dimensions reveal a remarkable fineness that is nearly impossible to discern with the naked eye, highlighting the complexity of detecting micro and nano particles only on the microscopic level. Collectively, they are referred to as micro- and nano-plastic particles (MNPPs) [3]. Recent studies have demonstrated that global micro- and nanosized plastic particles (MNPPs) have detrimental effects on the human immune system [4], as well as on aquatic [5] [6] and terrestrial ecosystems, including plant species [7]. These impacts result from both ingestion and atmospheric exposure pathways.

The atmospheric characteristics of MNPPs—encompassing their chemical composition, morphology, size distribution, and coloration—are influenced by anthropogenic factors, including geographic location, population density, and local meteorological conditions [8] [9]. Atmospheric MNPPs include thermoplastics and thermoset plastics that stem from a variety of sources. Moreover, they may consist of such items as cigarette filters, textile fibers, cleaning products, personal care items, resin fibers, weathered plastics, and even dust generated by tires [9] [10]. Both thermoplastics and thermoset plastic substances contribute to the formation of persistent pollutants, leading to a significant increase in their presence in land, water, food, and air, posing environmental risks and severe threats to human health [10] [11].

2. Background

2.1. Rainfalls and Leaves

Recent research suggests that as many as 100 trillion microplastics may enter the atmosphere each year through the consequences of rainfall [12] [13]. Due to their velocity and the high density of water, raindrops can exert a significant force upon impact with a surface, despite their relatively small size [12]. Thus, this force can influence the process of foliar absorption. Foliar absorption is crucial for water supply and diffusion in terrestrial plants [14]-[18]. It refers to the uptake of water, nutrients, and substances like pesticides through the leaf lamina, which supports photosynthesis, respiration, and metabolism [19] [20]. The lamina also regulates gas exchange, stomatal conductance, turgor pressure, and transpiration, and its structure enhances sunlight capture and absorption efficiency [21]. MNPPs present challenges to plants through both root and foliar pathways [22]. The roots absorb and accumulate MNPPs from the soil, which are subsequently distributed throughout plant tissues [22] [23]. Foliar uptake of MNPPs can induce adverse effects at morphological, physiological, biochemical, and molecular levels [22]-[24]. Internal exposure to MNPPs also triggers excessive production of reactive oxygen species (ROS), leading to inhibited photosynthesis, lipid membrane oxi-

dation, and disruptions in metabolic processes [7]. Although plants have developed defense mechanisms such as antioxidant enzymes and cell wall barriers, these strategies have demonstrated limited effectiveness against MNPPs exposure [7] [23].

2.2. Pilot Study

This pilot study is founded on the hypothesis that micro-nano plastic particles (MNPPs) are absorbed into edible vegetables through foliar absorption and cuticular penetration during the day and night when photosynthesis and respiration occur [20]. Recent research has indicated the presence of MNPPs in plants [15], rainfall [13], but also in atmospheric air [25] [26], highlighting their pervasive distribution and potential to contaminate plants under atmospheric influence.

3. Material and Methods

Leaf Assay Setup

Sixteen glass jars (200 mL capacity) were used, with two jars per vegetable assigned to daytime and nighttime treatments to ensure replication and separation of conditions. Each assay included 20 leaf discs (6 ± 0.5 mm diameter), prepared using a standard hole punch. Five discs were allocated to each treatment condition.

Solutions and Reagents

Four sodium bicarbonate solutions were prepared per vegetable: two controls and two containing micro- and nanoplastic particles (MNPPs). Each solution consisted of 200 mL distilled water with one teaspoon of sodium bicarbonate. For MNPP treatments, Cospheric Red Fluorescent microspheres (Lot #300-45-5337) were added and stirred until uniformly dispersed.

Equipment and Materials

- Sixteen paintbrushes and mini bottles of clear nail polish for stomatal imprinting, ensuring no cross-contamination.
- Clear adhesive tape for transferring imprints to microscope slides.
- LABOMED CxL clinical microscope for analysis with high magnification.
- Sixteen syringes for vacuum infiltration of solutions into leaf discs.

Floating Disk Assay

Air was removed from leaf discs using a syringe vacuum, allowing infiltration of the test solution and causing discs to sink. Discs were then placed in sunlight for 16 minutes, and the time taken to float was recorded as an indicator of photosynthetic activity. The procedure was repeated under dark conditions to assess respiration.

Stomatal and MNPP Analysis

After treatment, leaf discs were air-dried for 20 minutes. A thin layer of clear nail polish was applied, dried, and lifted using adhesive tape. Imprints were mounted on labeled microscope slides and examined for stomatal structure and MNPP presence under magnification.

4. Results

MNPPs were detected throughout the leaf anatomy—veins, stomata, and xylem—under both diurnal and nocturnal conditions.

Capsicum chinense (*C. chinense*) Habanero,

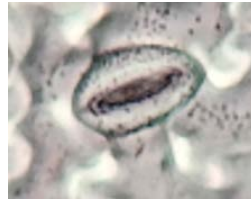


Figure 1. Night 40× MNPPs in stomata No Float.

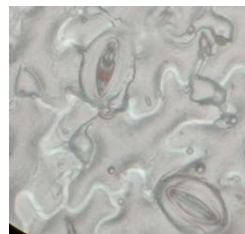


Figure 2. Day No float but MNPPS (bright reddish balls) in stomata and veins 40×.

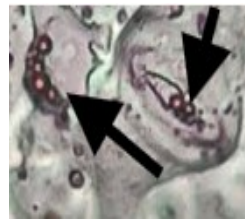


Figure 3. Day MNPPS in stomata and veins 100× float.

Basella alba (*B. alba*) Malabar Spinach, Poi

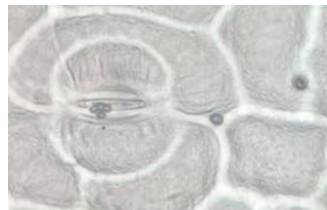


Figure 4. Day MNPPs in mostly n stomata than vein float.



Figure 5. Night MNPPs mostly in veins than stomata no float.

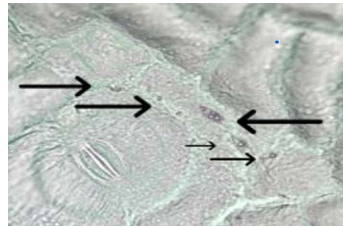


Figure 6. Night MNPPs mostly in veins than in stomata no float.

Phaseolus vulgaris (*P. vulgaris*) (string bean)

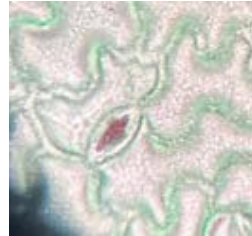


Figure 7. Day MNPPs in stomata no float.



Figure 8. Day no float but MNPPs cluster in xylem.



Figure 9. Night no float MNPPs in xylem near hair follicle.

Brassica rapa (*B. rapa*) (Bok Choy)

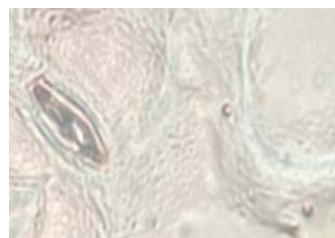


Figure 10. Day MNPPs mostly in veins and caught around and in stomata guard cells.



Figure 11. Night Bok Choi have stomata on both sides of leaves one is closed (abaxial) and the other (adaxial) is open holding MNPP also MNPPs in veins.

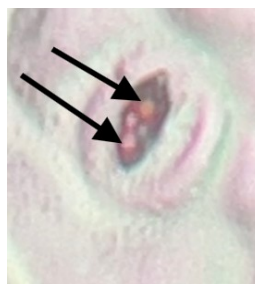


Figure 12. Night Bok Choi MNPPs in stomata.

5. Discussion

Amityville, NY, experiences annual rainfall ranging from 43 to 46 inches. During a typical rainstorm, it is estimated that approximately three million raindrops fall over our garden area of 144 square feet, corresponding to an average precipitation of 0.5 inches. In the case of a heavier storm with one inch of rainfall, nearly seven million raindrops would impact the same area. Leaves exposed to rainfall ranged in size from approximately 2 to 36 square inches (as shown in **Table 1**). The hydraulic pressure exerted on each leaf was calculated to be about 50,000 pascals (Pa) for a 2-square-inch habanero leaf and up to roughly 8,333,333 Pa for poi leaves measuring 36 square inches, based on the formula $Pressure (P) = Force (F)/Area (A)$.

Table 1. Stomata per leaf, vegetable, and MNPPs found during day and night per vegetable leaf discs.

| Vegetable Leaf Discs | Stomata per 2 mm | Σ MNPPs Day | Σ MNPPs Night | Leaf size |
|--|------------------|--------------------|----------------------|--------------------|
| <i>Capsicum chinense</i> (Habanero) | 50 | 40 | 1 | 6in ² |
| <i>Brassica rapa</i> (Bok Choy) | 30 | 6 | 15 | 12 in ² |
| <i>Phaseolus vulgaris</i> (String Beans) | 23 | 12 | 5 | 4 in ² |
| <i>Basella alba</i> (Malabar Spinach) | 15 | 6 | 6 | 36in ² |

In our experimental setup, leaf discs were submerged in 200 milliliters of sodium bicarbonate solution, equating roughly 3084 raindrops (using the conversion factor of 1 milliliter to 15.42 drops). The hydrostatic pressure exerted by this

200 ml volume on the leaf discs was determined to be approximately 39.23 Pa, which is notably less than the pressure applied during a typical rainstorm, where three to seven million raindrops contact the leaves within the garden area. Our experimental design effectively demonstrates how pressure dynamics facilitate the entry of MNPPs into leaves, confirming their ability to infiltrate foliar tissue in the presence of aqueous media.

Cuticular penetration refers to the movement of substances through the cuticle, which serves as the protective outer layer for plants and certain organisms [27]. This process is significant because the cuticle functions as the primary defense against external factors, with its permeability dictating the absorption efficiency of water, nutrients, or chemicals [27]. To determine whether rainwater containing MNPPs could be absorbed via leaf surfaces, we applied a drop of distilled water at room temperature onto the adaxial surface of the various vegetable leaves. Except for poi leaves, the water was absorbed within 20 minutes; poi leaves, characterized by a waxy coating, required approximately 10 additional minutes for complete foliar absorption and cuticular penetration. These findings indicate that microscopic particles present in rainwater can readily penetrate through leaf cells. Each vegetable species was then exposed to natural sunlight to induce photosynthesis, while a separate group was maintained in darkness for comparison.

Under normal conditions, leaf cells regulate water movement between intracellular and intercellular spaces by controlling plasma membrane hydraulic conductivity [21]. This process is influenced by leaf anatomy, size, stomatal aperture, vein arrangement, and transpiration dynamics [21] [28] [29]. After removal from the oxygen-depleted photosynthetic chamber and placement in a high-CO₂ solution, all leaf discs sank regardless of species.

Following infiltration, water traverses' intercellular airspaces to the mesophyll cell wall, providing a conduit for MNPPs to penetrate leaf tissues [19] [20]. Microscopic examination confirmed widespread distribution of MNPPs across multiple anatomical regions, supporting oxygen accumulation within intercellular spaces and sustaining photosynthetic activity.

Heightened photosynthetic activity was observed across all the leaf discs, demonstrating that the intensity of light plays a vital role in driving this essential biological process. As sunlight strength intensifies, plants efficiently convert light energy into chemical energy, causing the leaf discs to float to the surface. The stomata allow carbon dioxide to enter and oxygen to exit. This gas exchange is crucial for photosynthesis and affects the buoyancy of leaves, which tend to float in water due to the gases within the intercellular spaces. The stomatal process causes the entire leaf disc to resurface as oxygen levels were restored. The distribution of MNPPs occurs throughout the leaf discs, regardless of the vegetable type. During this brief gaseous phase, when the MNPPs are effectively incorporated, the leaf discs gradually float to the surface of the distilled solution with the MNPPs encased in their lamina.

The sodium bicarbonate mixture in this experiment, NaHCO₃ + H₂O, yields

NaOH and H₂CO₃. Plants do not directly utilize carbonic acid in the process of photosynthesis [30]. Instead, they rely on carbon dioxide (CO₂) or bicarbonate ions (HCO₃⁻), both of which originate from carbonic acid [30]. The enzyme carbonic anhydrase plays a crucial role [30] in facilitating the conversion between CO₂, carbonic acid, and bicarbonate, thereby making a significant amount of CO₂ accessible for in vivo photosynthesis. While carbonic acid acts as a transient intermediate formed when CO₂ dissolves in water, it is not directly employed by plants in photosynthesis. It then decomposes to produce H₂O and CO₂↑, providing a large supply of CO₂ to simulate molecules used in vivo photosynthesis. Countless bubbles formed within the various vegetable leaf discs examined to illustrate the process of gas exchange occurring in all specimens, thereby demonstrating the efficiency of leaf structure.

As large volumes of water encounter the plant veins, they must navigate through intercellular airspaces before reaching the mesophyll cell walls [19] [20]. The MNPPs effectively navigate this pathway with water, allowing them to infiltrate various regions of the leaf discs. Their presence can be clearly observed in different parts of the leaf anatomy depicted in the figures, highlighting their role in traversing the leaf anatomy. The MNPPs, measuring between 1000 nanometers and 5 micrometers, exploited the hydrodynamics of water movement within leaves (as shown in **Figures 1-10**). They likely utilize common stomatal architecture to facilitate the selective and reversible translocation of water across both the plasmalemma and organelle membranes [31]-[34].

This study revealed variations in nighttime water flow among different plant species, showcasing their unique adaptations for growth and survival [35]. Furthermore, the regulation of stomata differed among species, with some maintaining open stomata during the night. For example, Bok Choy's stomata remained open and were obstructed by MNPPs when subjected to dark conditions (**Figure 11, Figure 12**). During the night, when stomata are generally expected to close, MNPPs blocked their pores, preventing them from doing so.

This stomatal mechanism allows plants to effectively manage abiotic stressors while optimizing nutrient uptake [35]. By enhancing transpiration, the nighttime opening of stomata facilitates the movement of soil water toward the roots, thereby promoting the overall health and vitality of the plant [35]. The transport of micro-nano plastic particles (MNPPs) occurred with less intensity in the evening in some vegetables. During this time, several leaf discs floated to the surface within 16 minutes, while most of the various leaf discs remained submerged in the water.

During both diurnal and nocturnal conditions, MNPPs were caught in the stomata. Since the stomatal pore and its guard cells are larger than the applied MNPPs, which range from 1000 nm to 5 μm compared to the 10 to 80 μm of the pore sizes [36] there was little resistance preventing the MNPPs from distributing through the leaf disc stomata. In this experiment, the majority of MNPPs did not exit the leaf as CO₂ does during photosynthesis but remained within the leaf. The

larger aggregates of MNPPs encountered obstructions in their efforts to proceed in conjunction with the gas molecules and water through the stomata (as shown in [Figure 1](#), [Figure 3](#), [Figure 4](#), [Figure 10](#)). In this study, stomata provided a pathway for the MNPPs to enter the leaf discs and be easily dispersed through gaseous exchange and water distribution. Once inside the leaf, the negatively charged MNPPs also passed through the intercellular airspaces, enabling infiltration of the entire leaf through foliar absorption and diffusion. The findings from our experiment demonstrate that MNPPs, sized between 1000 nanometers and 5 micrometers, can penetrate leaves during both photosynthesis and respiration. Among the leaves examined, the *B. alba* (poi) leaves were the largest and exhibited the highest density of MNPPs per square inch. In contrast, the smaller *P. vulgaris* (string beans) leaves showed the lowest density of MNPPs per square inch, as illustrated in [Table 1](#) and [Figure 13](#).

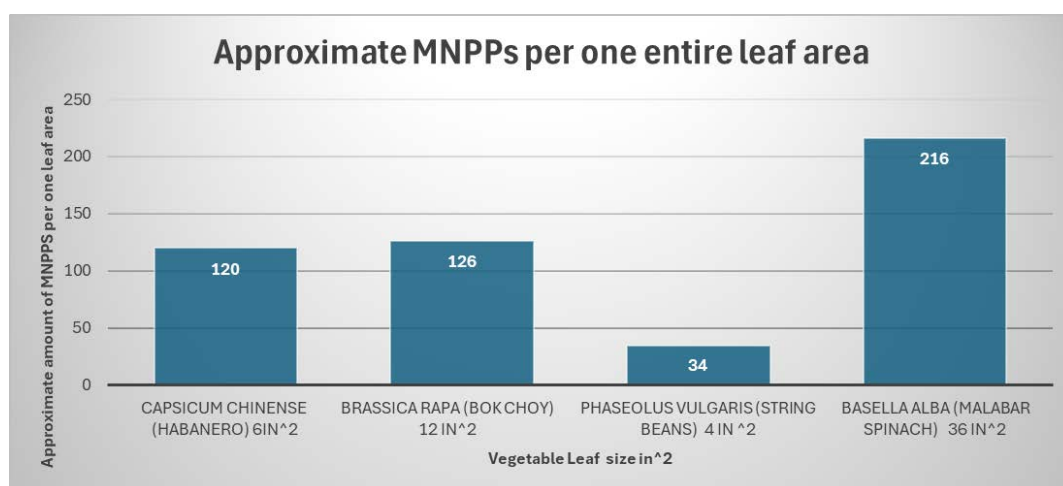


Figure 13. Estimation of MNPPs in each plant leaf per in ².

Aquaporins located in guard cells likely mediate osmotic flux and hydraulic conductivity, possibly further facilitating MNPP translocation throughout the lamina. Aquaporins in guard cells facilitate osmosis and water balance, so they may help with MNPP diffusion throughout leaf discs. These proteins, along with stomata, also contribute to oxygen accumulation in intracellular spaces, supporting photosynthetic processes. Based on previous research for MNPPs to traverse aquaporins effectively [37], they must mix with water pressure imposed on the guard cells [37]. Hence, since aquaporins control water movement through intercellular spaces, it is a concept worthy of further investigation. This phenomenon may be indicated by the slightly reddish and pinkish stains observed in images featuring both MNPPs and water during our experimental procedures. Specifically, when MNPPs are adequately combined with water, they facilitate unobstructed movement through leaf discs under all tested conditions. Analogously, water permeates biological membranes either via simple diffusion or facilitated diffusion mediated by aquaporins, which also play a role in the transport of

MNPPs [26] [38]. When raindrops carry microplastics through the lamina, they can forcefully deposit these particles onto leaves to partake in photosynthesis and cellular respiration—a phenomenon similar to what we demonstrated in our experiment. Rainfall applies pressure on leaves from the atmosphere [13]. In response to rainfall pressure leaves can easily activate a defense mechanism to protect themselves [39].

More comprehensive research into stomatal aquaporins is essential for understanding how plastics move through leaf structures, especially given the likely role of aquaporins in transferring MNPPs as suggested by this experiment. Aquaporins are known to transport water upward from roots to shoots [3], and as bidirectional channels [40], they are likely to facilitate water movement from shoots back to roots as well. These channels can transfer water at rates up to 1×10^9 molecules per second, which allows plants to quickly adjust osmotically to changing environmental conditions. Given that a single water molecule is 0.0275 nanometers long, the length covered by 1×10^9 such molecules would be 2.75×10^9 nanometers, or 2.75×10^9 nm [40]. This suggests that the highly flexible, high-capacity nature of aquaporin pores could enable them to carry plastic particles measuring in nanometers, and possibly even larger particles around micrometers, throughout leaf tissues. Supporting this idea, Li *et al.* in 2020 [41] found that wheat and lettuce can internalize fluorescent polystyrene (PS) beads sized at 0.2 μm and 2 μm . They observed that only the larger, 2 μm (2000 nm) beads were detected in plant roots, whereas the 0.2 μm (200 nm) beads appeared in the roots, stems, and leaves of both species. Further work by Luo *et al.* in 2022 [42] demonstrated that 200 nm polystyrene particles labeled with europium chelate Eu- β -diketonate primarily accumulated in the roots of wheat and lettuce, observed using time-gated luminescence and SEM. Additionally, Giorgetti *et al.*, in 2020 [43] reported finding 50 nm PS beads inside onion root cells after three days of exposure. Recent studies propose several possible pathways for micro- and nano-plastics to enter plants, including stomata, cracks, and aquaporins [16] [41] [44]-[47].

6. Conclusion

The lamina of leaves, which includes veins, intracellular pathways, and stomata, plays a crucial role as a gateway for micro-nanoparticles (MNPPs) to enter leaf discs during foliar absorption. This process is affected by at least several factors, including diffusion, photosynthesis, and cellular respiration. Once MNPPs enter the leaf, they are effectively distributed throughout the leaf tissue by means of hydraulic pressure and osmosis. The MNPPs distribution is not only intertwined with essential gas exchange but also may incorporate the movement of water within the leaf through specialized proteins called aquaporins because all water in leaves is influenced by aquaporins [47]. As MNPPs traverse this pathway and penetrate the leaf tissue, they highlight a potential pathway for plastic particles to enter the human food chain, posing a challenge that needs to be addressed across various types of edible vegetables. Recognizing the potential health risks associ-

ated with MNPPs is an important step in enhancing food safety. By deepening our understanding of their distribution, along with studying the effects of exposure to sunlight, time, and potential contaminants they may carry [5], we can develop effective strategies to mitigate associated risks and ensure safer consumption. This research advances our understanding of the mechanisms by which harmful plastic particles, recognized for their negative impact on the human immune system via dietary intake, can be deposited onto vegetables from atmospheric sources [48] [49].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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