Journal of Undergraduate Chemistry Research, **2025**, 24 (3), 83

VISUALIZATION OF DEGRADED AND NON-DEGRADED MICROPLASTICS OBTAINED FROM CONSUMER PRODUCT WASTE

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Abstract

Microplastics have earned considerable attention due to their high presence in the environment and adverse environmental effects. In this report, microplastics from consumer products were chemically and photochemically degraded prior to exposure to Nile Red and iDye Poly Pink. These dyed microplastics were detected by fluorescence using low-cost equipment, simple methodology, and without optical filters unlike previous reports. Microplastics from polymer classes could be differentiated successfully. Degrading these microplastics prior to staining impacts their fluorescence and provides information on how chemical and photochemical exposure impacts the identification of plastics. Environmental water samples supplemented with microplastics were investigated using iDye Poly Pink coupled with fluorescence detection, demonstrating the possibility to detect microplastic particles from non-specific background fluorescence interference.

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Keywords: Microplastics, Fluorescence, Plastic Degradation, Nile Red, iDye Poly Pink
Received: July 23, 2025 Accepted: July 30. 2025 Revision Received: August 10, 2025 Published: August 22, 2025

Introduction

Microplastics have captured significant scientific investigation due to their negative environmental impact.[1] Through progressive plastic aging, these degraded microplastics[2] are consumed by organisms leading to bioaccumulation[3] or are surfaces for hydrophobic pollutants.[4] Commercially available microplastics typically exist as formulations containing stabilizers, colorants, and other additives. Their impact on the environment also are a concern.[5] Identification and characterization of these microplastics occurs through time-consuming and resource-intensive laboratory equipment such as high-powered scanning microscopes, FT-IR, Raman, size-exclusion chromatography, and thermal analysis.[6] The expense and cumbersomeness of these instruments make them undesirable in environmental field testing.

To identify microplastic pollutants in the field, a simple, yet robust, method for visualizing microplastics should be developed. Stains and dyes provide one method for visualizing microplastics. Nile Red (**1**), a fluorescent solvatochromic dye, is utilized to stain microplastics in bottled drinking water, river water, marine sediment, and seafood.[7,8,9,10] Its spectroscopic properties enable visualization of Nile Red-labeled microplastics with fluorescence microscopy or with a fluorescence imaging camera equipped with wavelength-specific filters. Nile Red facilitates the detection of microplastics derived from various polymer sources. However, false positives are common due to non-specific staining of all organic matter in complex environmental water matrices.[9] Significant effort has focused on optimizing the staining procedure or introducing co-stains to reduce false positives.

As an alternative, Rochman and Sinton disclosed the use of iDye Poly Pink (**2**), a commercially available textile dye, to visualize microplastics.[11] Additional studies by Cizdziel and coworkers confirmed these results and sought to optimize experimental conditions.[12] Although iDye Poly Pink is not well characterized as a visualizing agent, its lower cost relative (Nile Red = $734.00/g, iDye Poly Pink = $0.62/g)[13] and low toxicity make it a practical choice. iDye Poly Pink can stain several types of microplastics and be visualized by a fluorescence imaging camera. Rochman and Sinton studied the stability of iDye Poly Pink-labeled microplastics under environmental stress conditions such as light exposure, KOH digestion, and mineral oil exposure. Results indicated that iDye Poly Pink stained microplastics are more stable than Nile Red-labeled microplastics.

Many lab-based microplastics visualization studies employ microplastics derived from pre-production pellets or beads instead of microplastics derived from consumer products. Although pre-production sources contain additives such as antioxidants, colorants, and stabilizers, these sources are less representative of microplastics from consumer waste. Lab-based visualization efforts also typically neglect the process by which microplastics form in the environment. As stated above, plastic waste, such as packaging waste from consumer products, undergoes environmental stress (UV light, chemical degradation) on its journey to becoming microplastic particles.[2]

Herein we describe the lab-based visualization of microplastic samples derived from consumer product packaging waste using Nile Red (**1**) and iDye Poly Pink (**2**). The microplastics are exposed to UV-light and chemical degradation before treatment with the dyes. The purpose of this study is to understand any increase or decrease in fluorescence due to environmental stress conditions. Rochman and Sinton utilized pre-stained microplastics to establish the fluorescence stability of microplastic particles. In this article, Nile Red and iDye Poly Pink methods were directly compared. Samples from two geographically distinct water sources were tested with Nile Red and iDye Poly Pink to evaluate the method on real-world samples.

These visualization techniques used simple procedures and affordable equipment. The evaluation of Nile Red and iDye Poly Pink on microplastic samples derived from consumer product

packaging will provide information on the performance of these dyes in analyzing microplastics content in environmental water sources such as rivers, ponds, and wetlands.

Experimental Methods

Microplastics Sampling

Microplastics utilized in these experiments were obtained from cutting single-use consumer product containers such as water bottles, soap containers, and packaging. They were categorized based on the recycling code found on the container. These microplastics were rectangular shapes no larger than 5 mm in length, the largest size considered a microplastic.[7] For some plastic types, cutting resulted in amorphous or globular particles shapes. The parent single-use containers were composed of polyethylene terephthalate (**3**, PET), high-density poly(ethylene) (**4**, HDPE), poly(vinylchloride) (**5**, PVC), low-density poly(ethylene) (**6**, LDPE), poly(propylene) (**7**, PP), and poly(styrene) (**8**, PS) (Figure 1). To maintain consistency, microplastics samples had smooth surfaces, although some **3** and **8** microplastics sample surfaces were slightly rough. Table 1 describes the microplastics samples used in this analysis. As noted in Table 1, samples containing different colorants were used to further simulate the analysis of real-world microplastics.

Simulated Photodegradation

Dry microplastics sample **3**-**8** were either not exposed to UV light or exposed to 365 nm light at a 60-min interval, respectively. The microplastic samples then were dyed with Nile Red (**1**) or iDye Poly Pink (**2**) solutions and isolated utilizing procedures reported previously.[7,11] Following filtration with Whatman NC 20 cellulose nitrate filters, washing with filtered de-ionized water, and drying at room temperature for at least 48 h, the microplastics were visualized under an Olympus BH-2 microscope using 254 nm UV light without the assistance of an optical filter. The standard in microplastic visualization studies, Whatman NC 20 cellulose nitrate filters exclude nanoplastics but retains larger sized materials. Each microplastic particle was documented by photography under white light and 254 nm UV light without any optical filters applied.

Chemical Degradation

Microplastics **3**-**8** were exposed to 10 mL of 6 M HCl, 6 M H_2SO_4, and 6 M NaOH, respectively for 2.5 h. Following filtration and washing the microplastics with filtered de-ionized water, the microplastic samples were dyed with Nile Red (**1**) or iDye Poly Pink (**2**) solutions utilizing procedures reported previously.[7,11] Following filtration with Whatman NC 20 cellulose nitrate filters, washing with de-ionized water, and drying at room temperature for at least 48 h, the microplastics were visualized under an Olympus BH-2 microscope using 254 nm and 365 nm UV light without any optical filters.

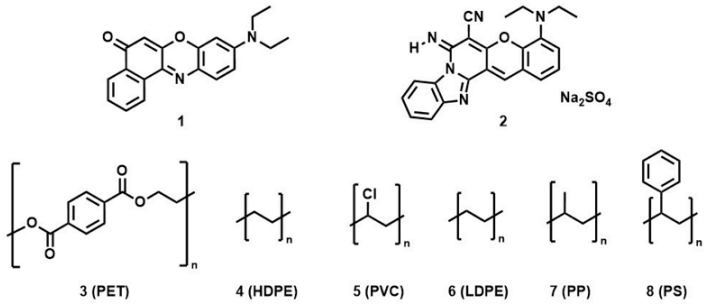

Figure 1: Dyes and polymer types used in microplastic staining experiments

Table 1: Microplastic sample identification table

Sample Identification	Plastic Type	Color	Appearance	Shape
3	PET	blue/green	translucent	rectangle/trapezoid
4	HDPE	yellow	opaque	irregular/globular
5	PVC	colorless	transparent	rectangle/trapezoid
6	LDPE	yellow	opaque	irregular/globular
7	PP	blue	opaque	irregular/globular
8	PS	colorless	transparent	rectangle/trapezoid

Treatment of Environmental Water Samples

Field water samples were collected from local river and stream sources in two locations: Radford, Virginia (New River) and McHenry, Illinois (Fox River Tributary). Field water samples were visualized using iDye Poly Pink by treating 5 mL field water samples in glass vials with 1 µL portions of 100 mg/mL iDye Poly Pink stain prepared in methanol. The glass vials were wrapped in aluminum foil and heated for two hours at 70 °C**.** Following the two-hour incubation period, each water sample was cooled to room temperature then vacuum filtered using Whatman NC 20 cellulose nitrate filters. New River (Virginia) samples were analyzed for microplastics using an Olympus BH-2 simple light microscope. Images were obtained using a Canon DSLR camera affixed to the microscope with a non-telephoto, non-macro lens. Images of Fox River (McHenry, Illinois) samples were obtained using a handheld microscope and portable UV lamp along with a cell-phone camera.

Results and Discussion

UV Irradiation Studies Using Visualization with Nile Red

Microplastics classified in groups **3**-**8** were irradiated with UV light at 365 nm for 60 min to simulate UV light degradation. The

Table 2: Qualitative results for microplastics **3-8** exposed at 365 nm followed by staining with Nile Red

Sample	No UV Exposure (-Nile Red)	No UV Exposure (+Nile Red)	60 Min UV Exposure (-Nile Red)	60 Min UV Exposure (+Nile Red)
3	weak blue fluorescence	strong pink fluorescence	blue fluorescence	red fluorescence
4	no fluorescence	yellow fluorescence	no fluorescence	yellow fluorescence
5	no fluorescence	pink fluorescence	no fluorescence	red fluorescence
6	no fluorescence	strong green fluorescence	no fluorescence	yellow fluorescence
7	no fluorescence	strong green fluorescence	no fluorescence	green fluorescence
8	marginal fluorescence	strong yellow fluorescence	marginal fluorescence	yellow fluorescence

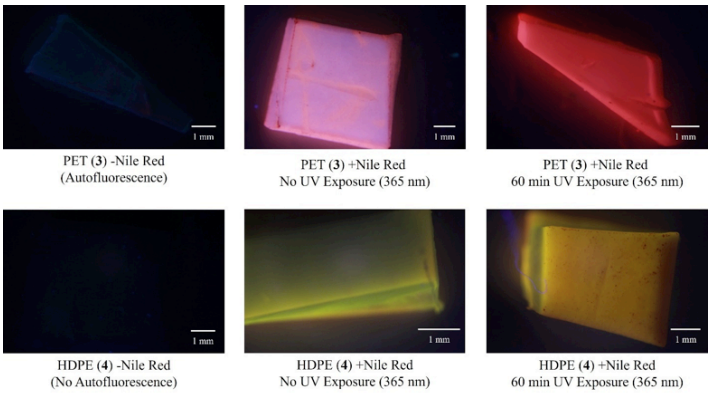

Figure 2: Images of microplastics **3** and **4** exposed at 365 nm followed by staining with Nile Red

Table 3: Qualitative results for microplastics **3-8** exposed at 365 nm followed by staining with iDye Poly Pink

Sample	No UV Exposure (-iDye Poly Pink)	No UV Exposure (+iDye Poly Pink)	60 Min UV Exposure (-iDye Poly Pink)	60 Min UV Exposure (+iDye Poly Pink)
3	marginal fluorescence	red fluorescence	marginal fluorescence	red fluorescence
4	no fluorescence	marginal fluorescence	marginal fluorescence	yellow fluorescence
5	marginal fluorescence	red fluorescence	marginal fluorescence	red fluorescence
6	no fluorescence	marginal fluorescence	no fluorescence	red fluorescence
7	no fluorescence	pink fluorescence	marginal fluorescence	purple fluorescence
8	no fluorescence	pink fluorescence	no fluorescence	purple fluorescence

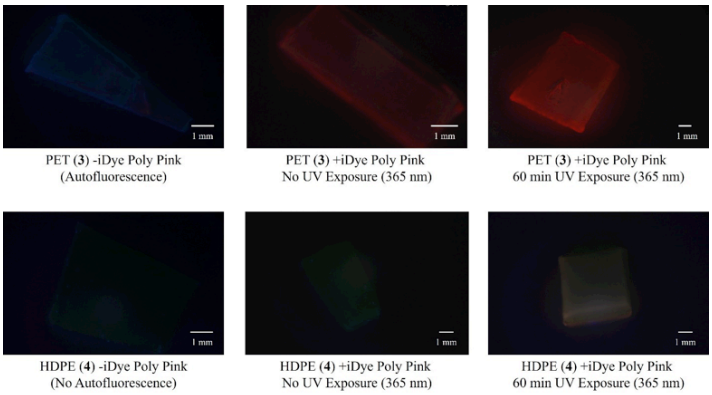

Figure 3: Images of microplastics **3** and **4** exposed at 365 nm followed by staining with iDye Poly Pink

resulting irradiated samples were treated with Nile Red or iDye Poly Pink. Qualitative observations are recorded in Table 2 and Table 3. Representative images of dyed microplastics are shown in Figure 2 and Figure 3. Two control groups were prepared and their fluorescence properties recorded: 1. microplastic particles not dyed but irradiated and 2. microplastics particles not dyed or irradiated.

UV irradiation results showed that PET (**3**) and PVC (**5**) behaved similarly. Both microplastics types are autofluorescent prior to Nile Red dyeing. The autofluorescence may result from the polymer or from antioxidant, colorant, or plasticizer additives included in the plastic formulation. Dyeing **3** and **5** with Nile Red before UV irradiation produced a strong pink/purple fluorescence while dyeing the same microplastics after irradiation at 350 nm for 60 min produced a red fluorescence. Figure 2 displays representative images for a PET microplastics sample. PVC microplastics produced comparable images.

Microplastics composed of HDPE, LDPE, PP and PS (**4** and **6-8**) exhibited different behavior in the presence of Nile Red compared with **3** and **5** (Table 2). Microplastics **4** and **6-7** had little or no observable autofluorescence while **8** had marginal fluorescence. Addition of Nile Red to **4** and **6-8** before irradiation with UV light produced a strong green/yellow fluorescence. Addition of Nile Red to microplastics **4** and **6-8** following irradiation with UV light produced a strong yellow/green fluorescence which qualitatively appeared to be of a different wavelength compared to before UV irradiation. It is noted that **4** and **6-7** are the simplest polyolefins, thus accounting for their similar fluorescence trends when treated with Nile Red. Figure 2 displays images for a HDPE (**4**) microplastics sample, representing results for microplastics samples composed of **4** and **6-7**.

UV Irradiation Studies Using Visualization with iDye Poly Pink

The treatment of microplastics samples with iDye Poly Pink produced fluorescence trends distinct from Nile Red, consistent with previous reports.[11] Prior to UV irradiation, microplastics stained with iDye Poly Pink exhibited weaker intensity fluorescence with pink, red, or purple fluorescence. Nile Red stained samples demonstrated a broader wavelength range of fluorescence emission.

Microplastics **3** and **5** produced moderate red fluorescence with iDye Poly Pink prior to UV irradiation (Figure 3). Following irradiation at 365 nm, **1** and **3** dyed with iDye Poly Pink produced a greater intense red fluorescence. Figure 3 displays images for a PET microplastics sample. Similar results were observed for microplastics composed of PVC.

Microplastics from the groups **4** and **6-8** exhibited weak, but observable fluorescence in the presence of iDye Poly Pink. For example, **4** showed a faint blue-grey color when dyed before UV irradiation (Figure 3). A stronger perceived fluorescence was observed for **4** when the microplastic sample underwent dyeing after UV irradiation at 365 nm for 60 min. Figure 3 displays images of HDPE microplastics samples composed of **4** and **6-8**.

Fluorescence was observed in all stained microplastics samples described above when exposed to a handheld UV lamp without optical filters. Qualitative comparisons of microplastics were achieved by observing fluorescence without optical filters, particularly when distinguishing microplastics **3** and **5** from microplastics **4** and **6-8**. When microplastics samples degraded under a different irradiation wavelength (254 nm), trends comparable to those described above were observed.

Chemical Degradation Studies Using Visualization with Nile Red

Microplastics in groups **3-8** underwent chemical degradation by treatment with 6 M aqueous solutions of sulfuric acid, hydrochloric acid, and sodium hydroxide before staining with either Nile Red or iDye Poly Pink. Control samples consisted of microplastic particles stained with Nile Red or iDye Poly Pink but not subjected to chemical degradation.

Treating degraded microplastics with Nile Red induced fluorescence of red, yellow, and green. Acid-degraded microplastics treated with Nile Red exhibited fluorescence which appeared more intense than respective non-degraded microplastics. The acid type generally did not impact the fluorescence properties (sulfuric acid versus hydrochloric acid). Dyeing base-degraded microplastics with Nile Red led to fluorescence less intense compared to the respective non-degraded microplastics. Different observed fluorescence colors between base-degraded and non-degraded plastics were observed in Table 4. Figure 4 shows representative images for dyed microplastics **5** and **6** without chemical degradation, with acid treatment, and with base treatment. The fluorescence behavior of microplastics following chemical degradation fell into two categories. When dyed with Nile Red, chemically degraded microplastics composed of **3**, **5**, and **8** exhibited red fluorescence while microplastics composed of simple polyolefins (**4**, **6-7**) produced primarily yellow or green fluorescence.

Chemical Degradation Studies Using Visualization with iDye Poly Pink

iDye Poly Pink induced fluorescence in nearly all chemically degraded microplastics, but less intense than Nile Red. Acid-degraded microplastics exhibited greater fluorescence intensity with iDye Poly Pink than with non-degraded microplastics. In contrast, base-degraded microplastics dyed with iDye Poly Pink had weaker fluorescence compared to the untreated microplastics, and produced different fluorescence colors in Table 5. Figure 5 shows representative images for dyed microplastics **5** and **6** without chemical degradation, with acid treatment, and with base treatment. When dyed with iDye Poly Pink, degraded microplastics **3** and **5** produced yellow or pink fluorescence and simple polyolefins (**4** and **6**-**7**) produced red/purple fluorescence (Table 5). A smaller decrease in fluorescence intensity was observed for simple polyolefins after base degradation, and in some examples, acid and base degraded polyolefins showed no qualitative differences.

Table 4: Qualitative results for chemical degradation studies of microplastics **3-8** exposed at 365 nm followed by staining with Nile Red

Sample	Water (+/-Nile Red)	H_2SO_4 (+/-Nile Red)	HCl (+/- Nile Red)	NaOH (+/-Nile Red)
3	**- Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**- Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**- Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**- Nile Red:** blue fluorescence **+Nile Red:** red fluorescence
4	**-Nile Red:** no fluorescence **+Nile Red:** marginal fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** no fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** yellow fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** yellow fluorescence
5	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence
6	**-Nile Red:** no fluorescence **+Nile Red:** yellow fluorescence	**Nile Red:** no fluorescence **+Nile Red:** yellow fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** yellow fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence
7	**-Nile Red:** no fluorescence **+Nile Red:** green fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** green fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** green fluorescence	**-Nile Red:** no fluorescence **+Nile Red:** green fluorescence
8	**-Nile Red:** no fluorescence **+Nile Red:** marginal fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence	**-Nile Red:** blue fluorescence **+Nile Red:** red fluorescence

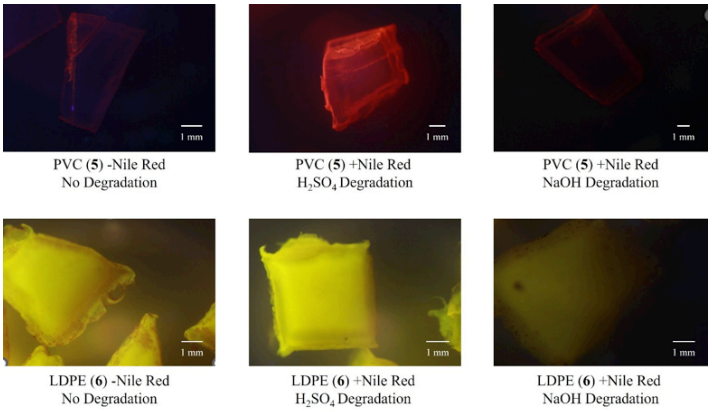

Figure 4: Images of microplastics **5** and **6** exposed to chemical degradation followed by staining with Nile Red

Table 5: Qualitative results for microplastics **3-8** exposed to chemical degradation followed by staining with iDye Poly Pink

Sample	Water (+/- iDye Poly Pink)	H_2SO_4 (+/- iDye Poly Pink)	HCl (+/- iDye Poly Pink)	NaOH (+/- iDye Poly Pink)
3	**-iDye Poly Pink:** green fluorescence **+iDye Poly Pink:** yellow fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** pink fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** yellow fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** yellow fluorescence
4	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** blue fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** pink fluorescence	**-iDye Poly Pink:** red fluorescence **+iDye Poly Pink:** red fluorescence	**-iDye Poly Pink:** red fluorescence **+iDye Poly Pink:** red fluorescence
5	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** yellow fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** yellow fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** yellow fluorescence	**-iDye Poly Pink:** blue fluorescence **+iDye Poly Pink:** red fluorescence
6	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** blue fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** no fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** purple fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** green fluorescence
7	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** blue fluorescence	**-iDye Poly Pink:** blue fluorescence **+iDye Poly Pink:** red fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** purple fluorescence	**-iDye Poly Pink:** blue fluorescence **+iDye Poly Pink:** red fluorescence
8	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** blue fluorescence	**-iDye Poly Pink:** blue fluorescence **+iDye Poly Pink:** red fluorescence	**-iDye Poly Pink:** no fluorescence **+iDye Poly Pink:** purple fluorescence	**-iDye Poly Pink:** blue fluorescence **+iDye Poly Pink:** red fluorescence

Differentiation of Microplastics Classes with Nile Red and iDye Poly Pink

Overall, the results show that Nile Red and iDye Poly Pink can distinguish broad classes of microplastics. Simple polyolefins, such as **4** and **6**-**7**, can be differentiated from other microplastics, including **3** and **5**, by their fluorescence profile, even without optical filters. It is not possible to differentiate microplastics from similar classes such as **4** and **6**.

The results of degradation studies, which examined the dyeing of microplastics pre-treated with UV light, acid, or base, demonstrate circumstances that allow differentiation between degraded and non-degraded microplastics. Greater fluorescence differences were observed when comparing chemically degraded microplastics with nondegraded precursors while more subtle differences existed between UV irradiated samples and non-irradiated microplastics. Being able to identify and distinguish degraded microplastics is critical in anticipating how staining techniques function on real-world microplastics in aqueous environments.

The studies described above employed microplastics derived from formulated plastic consumer goods and packaging. UV fluorescence of microplastics prior to dyeing were different between different samples of the same plastic category (Table 4 and 5, water exposure only). However, this difference in fluorescence was visually indistinguishable after exposure to Nile Red or iDye Poly Pink. How additives such as antioxidants, colorants and plasticizers contribute to the observed changes in fluorescence between sample types is unknown. To a certain extent, the contribution of

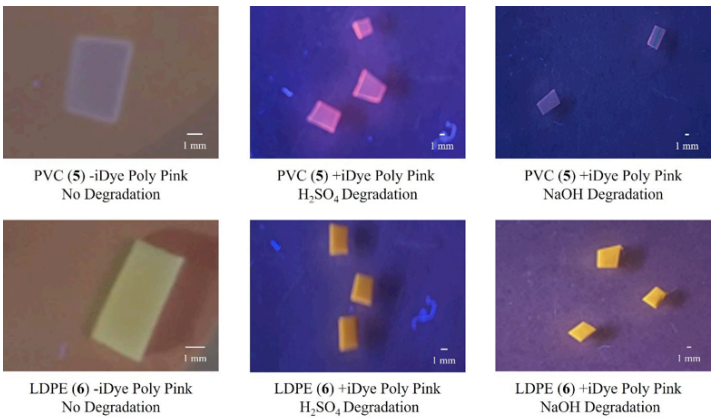

Figure 5: Images of microplastics **5** and **6** exposed to chemical degradation followed by staining with iDye Poly Pink

plastics additives is irrelevant, as all real-world microplastics contain these materials. Nile Red and iDye Poly Pink can classify microplastic particles without fully comprehending the contribution of plastic additives.

These color fluorescent trends likely originate from the solvatochromic properties of Nile Red and similar naphthooxazine dyes, such as iDye Poly Pink. These dyes exhibit a perceived red shift in fluorescence emission in more polar environments compared to non-polar environments.[15] For Nile Red, relatively small changes in polarity induce a significant shift in fluorescence emission. In the presence of nonpolar polymers such as **4**, **6**, and **7**, yellow/green fluorescence occurs. A pink/red fluorescence is observed with the more polar polymer PET and polarizable PVC. These are characteristic of the solvatochromic effects of naphthooxazine dyes. In some cases, degradation of the microplastics qualitatively appeared to produce a red shift, perhaps indicating that degradation leads to increased polarity through oxidation or hydrolysis.

The observed changes in fluorescence result from the interaction between surface binding of the dyes to microplastic particles. Surface interactions produce effects that are more complex than the dissolution of dyes in pure solvents. Fluorescence varies with the strength of hydrophobic interactions between dye molecules and microplastic particles.[16]

Investigation of Microplastics Visualized with iDye Poly Pink in an Environmental Matrix

One challenge to dyeing microplastics in environmental water samples involves the non-specific staining of organic or biological debris that generate interference with the fluorescence related to microplastics.[14] False positives have been reported, especially employing Nile Red.[9] Because our method generated fluorescence without the use of optical filters, it is important to understand what interference could be expected from real-world environmental water matrices. Only iDye Poly Pink was used as a visualization agent because utilization of Nile Red in environmental water samples is well documented.[7]

New River (Radford, Virginia) water samples were split into two groups. One sample set were subjected to the iDye Poly Pink staining procedures. The second group were supplemented with microplastics particles **3** and polyolefins **4** and **6-8** prior to dyeing. After filtering and drying for at least 48 hours, all New River water samples showed a bright red, non-specific background fluorescence due to non-specific dyeing of the Whatman filter. Particles in the shape of strings and amorphous foam fluorescence weakly. In the supplemented New River water samples, PET microplastic particles had strong fluorescence at pink/purple wavelengths and was differentiated from the background (Figure 6). Microplastics derived from other plastic types, especially **4** and **6-8**, were not readily distinguished from the background fluorescence signal. Preliminary results indicate that improved differentiation between microplastics classes when a longer wavelength excitation light source is employed. In general, the New River water experiments indicate that reference samples of environmental water containing added microplastics is advisable to determine whether microplastics exist in non-supplemented water from the same source.

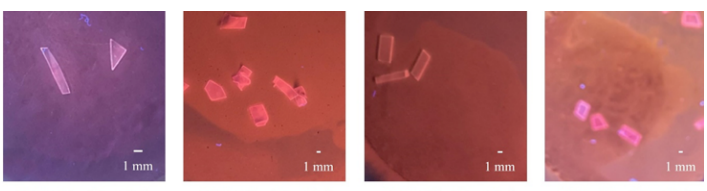

Figure 6: Images of microplastics in environmental water matrix after staining with iDye Poly Pink, filtering, and drying for at least 48 hours.

To assess source-to-source variability, the iDye Poly Pink visualization methodology was extended to non-supplemented environmental water samples from a different source. This experiment challenged the method to detect any microplastics above baseline fluorescence due to non-specific staining. Environmental water samples were collected along a tributary of Fox River in McHenry, Illinois. Following staining with iDye Poly Pink, filtering, and drying for at least 48 hours, the samples were examined for microplastic content. Only bright red background fluorescence due to non-specific staining of the Whatman filter and weekly fluorescent amorphous and string-like particles were observed, similar to the New River water samples. The presence of microplastics in the Fox River water samples could not be confirmed.

Conclusions and Future Work

This article discusses the impact of dyeing and the resulting fluorescence of microplastics obtained from consumer product waste and packaging with Nile Red and iDye Poly Pink. The methodology demonstrates how microplastics from different polymer classes can be differentiated. Both untreated microplastics and chemically/photochemically degraded microplastics can be detected using the dyes. In some examples, degraded microplastics can be distinguished from the non-degraded particles, providing an understanding of how Nile Red and iDye Poly Pink dyeing techniques perform with real-world microplastics.

Low-cost equipment and robust methods were employed in the experiments described above, making the methodology better suited for field samples. Visualization of microplastics without optical filters further advances the simplicity of the Nile Red and iDye Poly Pink staining approaches. By supplementing environmental samples with known microplastics, scientists can distinguish microplastics from non-specific background fluorescence when visualizing samples with iDye Poly Pink.

Future studies will focus on removing background interference by using different fluorescence excitation wavelengths to visualize environmental water samples. Further investigation of degraded microplastics is needed, including samples degraded by environmental stress factors including oxidation, mechanical stress, and temperature extremes. The iDye Poly Pink methodology will be extended to quantitatively analyze microplastics for accuracy, precision, and analytical range of microplastic detection.

Acknowledgements

This study was made possible through support from the Radford University Artis College of Science and Technology, Department of Chemistry.

References

1. Sewwandi, M.; Wijesekara, H.; Rajapaksha, A. U.; Soysa, S.;

Vithanage, M. *Environ. Pollut.,* **2023**, *317*, 120747.
2. Sucharitakul, P. *Nat. Rev. Earth Environ.,* **2024**, *5*, 334.
3. Chormare, R.; Kumar, M. A. *Chemosphere,* **2022**, *302*, 134836.
4. Fu, L.; Li, J.; Wang, G.; Luan, Y.; Dai, W. *Ecotoxicol. Environ. Saf.,* **2021**, *217*, 112207.
5. Hahladakis, J. N.; Velis, C. A.; Weber, R.; Iacovidou, E.; Purnell, P. *J. Hazard. Mater*., **2017**, *344*, 179-199.
6. Prata, J. C.; da Costa, J. P.; Duarte, A. C.; Rocha-Santos, T. *TrAC,* **2019**, 150-159.
7. Maes, T.; Jessop, R.; Wellner, N.; Haupt, K.; Mayes, A. *Sci. Rep.,* **2017**, *7*, 44501.
8. Erni-Cassola, G.; Gibson, M. I.; Thompson, R. C.; Christie-Oleza, J. A. *Environ. Sci. Technol.,* **2017**, *51*, 13641−13648.
9. Stanton, T.; Johnson, M.; Nathanail, P.; Gomes, R. L.; Needham, T.; Burson, A. *Environ. Sci. Technol. Lett.,* **2019**, *6*, 606–611.
10. Gonzalez, M.; Islas, S. M.; Mitton, F. M.; Diaz-Jaramillo, M. In *Chemical Food Contaminants Analysis. Methods and Protocols in Food Science*; Hoff, R.; Molognoni, L., Eds. Springer, Humana, New York, NY **2024** pp. 263-274.
11. Karakolis, E. G.; Nguyen, B.; You, J.B.; Rochman, C.M.; Sinton, D. *Environ. Sci. Technol. Lett.,* **2019**, *6*, 334-340.
12. Gao, Z.; Wontor, K.; Cizdziel, J. V. *Molecules*., **2022**, *27*, 7415.
13. Sigma-Aldrich. https://www.sigmaaldrich.com/US/en (accessed 2025-07-22).
14. Villacorta, A.; Cazorla-Ares, C.; Fuentes-Cebrian, V.; Valido, I. H.; Vela, L.; Carrillo-Navarrete, F.; Morataya-Reyes, M.; Mejia-Carmona, K.; Pastor, S.; Velázquez, A.; Arribas Arranz, J.; Marcos, R.; López-Mesas, M. Hernández, A. *J. Hazard. Mater.,* **2024**, *476,* 135134.
15. Gajo, C.; Shchepanovska, D.; Jones, J. F.; Karras, G.; Malakar, P.; Greetham, G.M.; Hawkins, O. A.; Jordan, C. J. C.; Curchod, B. F. E.; Oliver, T. A. A. *J. Phys Chem B.,* **2024**, *128*, 11768-11775.
16. Meyers, N.; Catarino, A. I.; Declercq, A. M.; Brenan, A.; Devriese, L.; Vandegehuchte, M.; De Witte, B.; Janssen, C.; Everaert, G. *Sci. Total Environ.,* **2022**, *823***,** 153441**.**