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Oil Red O Staining as a Preliminary Visual Screen for Selected Plastic Materials

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ABSTRACT

Microplastic particles less than 5 mm in size are now present in rivers, oceans, soil, air, food and even in the human body. The detection of these particles, however, usually requires special instrumentation such as FTIR and Raman spectroscopy, which may be expensive and not available in many schools and small laboratories. This limitation highlights the need for a rapid screening approach that does not require advanced technology and is cost-effective. We investigated the feasibility of using a dye called "Oil Red O dye", which is generally used to stain fat, to identify different categories of microplastics in our study. Ten plastic and five non-plastic materials were collected from common household products and were chopped into small pieces. Then the samples were treated with isopropanol, stained for 10 minutes with Oil Red O, washed and examined under the visual microscope. Results showed that there were different intensities of staining for various plastics. Strong red staining was observed for polyester, polystyrene, low-density polyethylene (LDPE) and polyvinyl chloride (PVC), and moderate staining was observed for PET film, nylon and HDPE. Polypropylene and oxo-biodegradable polyethylene were stained weakly, while PET fibre was almost not stained. Most non-plastic materials had very little or no staining, except for a moderately staining glossy newspaper, possibly due to its surface coating. The results indicate that Oil Red O may be a quick and cost-effective way to pre-screen samples for microplastics. Some non-plastic materials can still stain, however, and different plastics will stain differently. Therefore, it should only be used as a preliminary screening technique, and positive samples should be confirmed by FTIR or Raman spectroscopy for accuracy. These single-sample visual observations have not been assessed for repeatability or quantified by image analysis; the method requires validation before broader use.

Keywords: *Microplastics, Oil Red O, plastic detection, environmental pollution, screening method, polymers.*

1. INTRODUCTION

The persistence and ubiquity of plastic materials in natural ecosystems have made plastic pollution a significant environmental issue. Microplastics are plastic particles < 5 mm in size, which can be found in freshwater systems, drinking water, sediments, food, and other environmental compartments, and have been reported in a variety of locations, and may have implications for ecosystems and human exposure

[1,2]. Microplastics in drinking water are a major concern, as they are a potential direct pathway to human exposure [1,2].

To understand the environmental occurrence and potential risks of microplastics, accurate identification and quantification are required. Methods like Fourier Transform Infrared (FTIR) spectroscopy and Raman spectroscopy are well known and can be used for the identification of the polymer species and are widely used for the characterisation of microplastics [3]. However, these analytical techniques are generally relatively costly and time consuming, and require special equipment, trained personnel and more complex sample preparation for the routine analysis [3,4]. Thus, the availability of rapid, simple and economical screening methods would allow for initial screening of microplastic contamination, especially in areas where advanced spectroscopic equipment is not readily available.

The use of fluorescent staining with Nile Red has been explored as a fast screening method for microplastics. Nile Red is a hydrophobic dye that can bind to plastic particles and improve their visualization by fluorescence imaging [5–9]. It has been used in a few studies to detect and quantify microplastics, and further studies have investigated modifications of the staining process and fluorescence-based analytical methods [6–9]. While these techniques can offer fast screening, they require fluorescence microscopy or other specialized visualization systems, which may not be suitable for low-resource or field applications.

Oil Red O is a lipophilic dye which is widely used to stain neutral lipids in established histological protocols [10,16]. The interaction of Oil Red O with plastic particles may provide another basis for visual screening, as many of the common plastics have hydrophobic surfaces. The Oil Red O staining method is direct and produces a visible red colour which may be easier to screen for than the fluorescence methods, without the need for specialized equipment. However, its use for different kinds of polymers and its selectivity to non-plastic materials should be tested systematically.

Oil Red O was used in this study as a preliminary screening reagent for the visual detection of microplastics. To compare the staining behaviour of the ten common plastic materials and five non-plastic control materials, the materials were tested. The objective was to determine if Oil Red O could be used as a preliminary screening method followed by advanced methods.

2. LITERATURE REVIEW

The rising occurrence of microplastics in drinking water has raised awareness about the need for reliable and accessible analytical methods. Traditional polymer identification methods like Fourier Transform Infrared (FTIR) and Raman spectroscopy are capable of high-confidence identification but require specific instrumentation and expertise to be used routinely [1–4]. Studies that have investigated bottled mineral water and drinking-water distribution systems have also shown that small plastic particles are present in drinking-water environments, which means that there is a need for practical screening methods that can be used to provide preliminary assessment prior to advanced polymer identification [11,12].

Fluorescence staining has been identified as a potential method for quick microplastic screening. Maes *et. al.* showed that Nile Red has the potential to be used as a fluorescent tagging agent for the rapid detection and quantification of microplastics [5]. Later, Nile Red staining was shown to be a useful tool for the identification and quantification of microplastics according to their fluorescent response by Shim *et. al.* [6]. Other methodological advances have been explored, such as the Nile Red plate method, suggested to make microplastic screening more practical [7]. The use of Nile Red staining in combination with photoluminescence spectroscopy and fluorescence-lifetime measurements has also been performed in other studies, which showed that the fluorescent response can give other information to identify microplastics [8, 9].

In recent years, the mechanisms and limitations of Nile Red staining have also been investigated. Ho *et. al.* conducted a review of the use of Nile Red for microplastic identification and emphasised the role of the fluorescence behaviour, staining conditions and interactions between the dye and the polymer surface in the determination of detection performance [13]. In addition to Nile Red, Lv *et. al.* tested other fluorescent dyes and showed the potential of fluorescent staining to detect and quantify microplastics [14]. Typically, however, fluorescence-based methods require appropriate excitation and visualization apparatus and may not be as readily available for basic laboratories and initial field screening.

The restrictions suggest that simpler colorimetric methods are required that can give a visible response without the need for specialized fluorescence instrumentation. Oil Red O is a lipophilic dye, which is widely used to stain neutral lipids and other hydrophobic substances [10,15]. It has a hydrophobic affinity, which could be used to interact with hydrophobic surfaces, and many plastics have this property. Oil Red O is used mainly for its ability to create a red colouration, which may be visible in normal visible light, as opposed to Nile Red, which is used mainly for its ability to be visualised by its fluorescence. This makes it possible to develop a low-cost preliminary screening procedure.

A peer-reviewed Oil Red O protocol describes imaging and measurement of stained neutral lipids [16], whereas published microplastic staining studies have used image-derived RGB information with Nile Red [17]. These support a future quantitative assessment but do not validate Oil Red O as a polymer-specific stain.

Although there is a lot of literature on Nile Red as a tool for microplastic detection, very little work has been focused on using Oil Red O to screen for various types of polymers. Moreover, there is a possibility of non-plastic hydrophobic materials giving false positive staining, which should be systematically examined.

3. MATERIALS AND METHODS

3.1. Materials

Oil Red O, isopropanol, and distilled water were used in the staining process. The purpose was to observe the effects of the staining on different types of plastic. So, 10 different types of plastic were selected from

the common objects we use daily. These were polyester, polystyrene, low-density polyethylene, polyvinyl chloride, polypropylene and PET fibre. To ensure the test would be fair, 5 non-plastic items were also tested. These were cotton, sewing thread, tissue paper, glossy newspaper and matte newspaper. They were used as controls for comparison with the results.

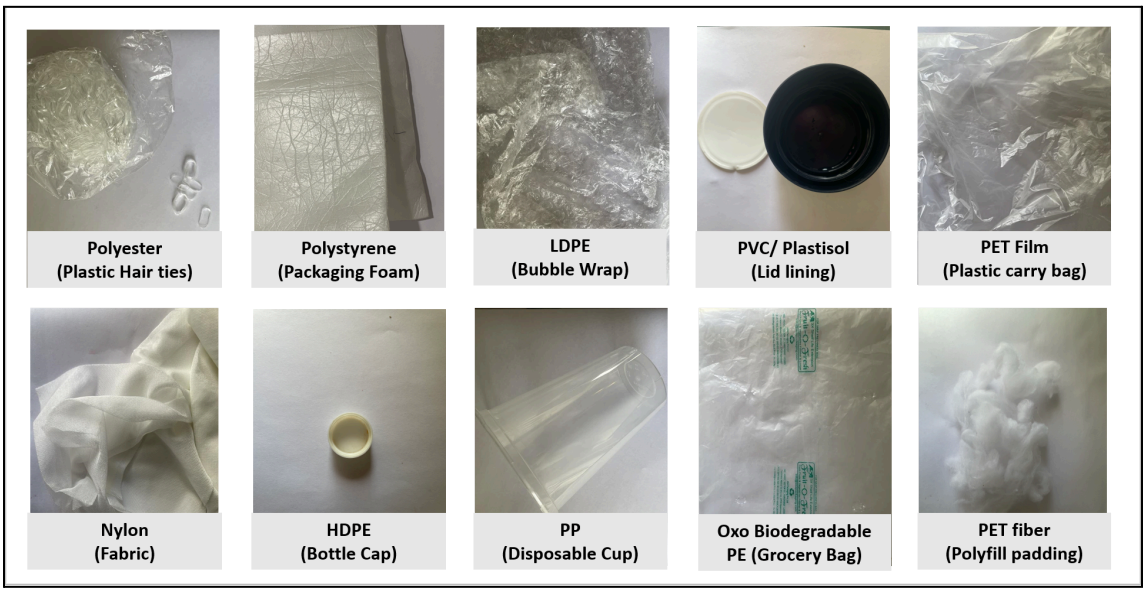


Figure 1: Ten plastic samples selected to represent commonly occurring polymers. The top panel shows polyester, polystyrene, low-density polyethylene, polyvinyl chloride or plastisol and polyethylene film. The bottom panel shows nylon, high-density polyethylene, polypropylene, oxo-biodegradable polyethylene and PET fibre.



Figure 2: Five non-plastic samples selected. The top panel shows cotton fiber, sewing thread and tissue paper. The bottom panel shows matte newspaper and glossy newspaper

3.2 Sample Preparation

The samples were cut into very small pieces (less than 1 mm) to resemble small pieces of plastic that are found in the environment. Approximately 150 mg of each sample was used for staining. All the glass equipment was thoroughly cleaned with pure water prior to use to avoid any possible confusion in the sample.

3.2.1 Preparation of Oil Red O Solution

To prepare the Oil Red O solution, we started by making a stock solution with 0.5% (w/v) Oil Red O in isopropanol. We then made a working solution from this stock solution by diluting with distilled water to 0.3%. The solution was allowed to sit for 10 minutes, then filtered to remove any particles of dye that may have been present. At the same time, we also made a 60% isopropanol solution by mixing isopropanol with distilled water. All this was to make sure we got the correct solutions for our experiment.

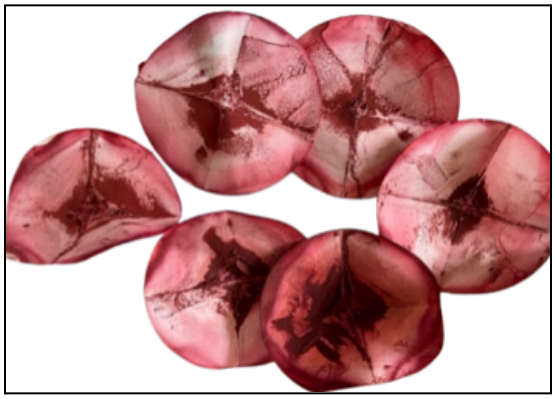


Figure 3: Filter papers with sediment and suspended dye aggregates that were removed from ORO dye solution.

3.3 Staining Procedure

To start with, the samples were cleaned with a mixture of 60% isopropanol—about 3 mL of it was used for each sample, and this process took around 45 seconds. The alcohol was then removed, and 10 mL of the Oil Red O solution was added to each sample. The samples were allowed to remain in this dye for 10 minutes at room temperature. Once the staining time was up, the dye solution was thrown away, and the samples were rinsed with 60% isopropanol to get rid of any extra dye. This was followed by three more rinses with distilled water to make sure the samples were clean. Finally, the samples were placed on clean petri dishes and left to air dry before examination.

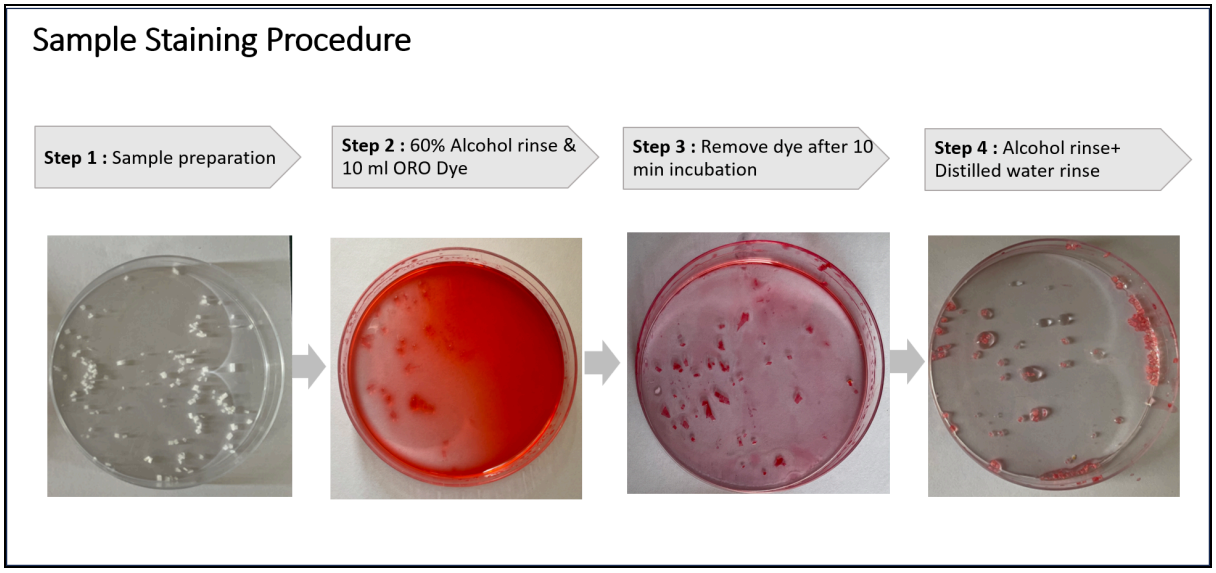


Figure 4: Sample staining procedure followed for ORO staining for every sample. Sample preparation and alcohol rinse were followed by incubation with 10 ml ORO for 10 minutes. The dye was then drained and the sample rinsed with 60% IPA, followed by rinsing with distilled water.

3.4 Staining Assessment

Staining strength was classified into four categories: High (bright red staining over approximately ≥75% of the visible surface), Medium (red staining over approximately 25% to <75%), Low (faint or localized staining over <25%), and Negative (no discernible red staining).
These surface-coverage ranges are a descriptive rubric for the existing visual observations, not measurements obtained by image analysis. Borderline estimates, including the matte newspaper entry in Table 2, should be treated cautiously; the categories have not been validated for inter-observer agreement. Photographs for every sample were taken using the same lighting condition.

Each material was assessed as a single sample in this exploratory comparison (n = 1 per material). Independent replicate staining trials were not performed. Therefore, the observed differences cannot establish reproducibility or account for variation in particle size, surface texture, cutting, illumination, or rinsing. Future tests should prepare at least three independently cut and stained samples per material under matched conditions.

4. RESULTS

Most of the plastic materials used in this study could be stained by Oil Red O, though the staining intensity was different for various polymer types (Table 1; Figures 5-8). The plastics were classified into four groups, based on visual observation, as high stain, medium stain, low stain, and negative stain.

Others, such as polyester, polystyrene, low-density polyethylene and polyvinyl chloride, however, exhibited a very high reaction to staining, taking on a deep red colouration on nearly the whole surface. Polyethylene terephthalate, nylon and high-density polyethylene exhibited some staining, but not as severe as the others, and not as much of the surface was stained.

The staining intensity was low for Polypropylene (PP) and oxo-biodegradable polyethylene, with only small areas of visible red colour. The PET fibre didn't really change or get stained much after we did the staining procedure on it. It was almost unchanged, with only minor variations.

Interestingly, PET behaved differently depending on its form. PET film showed moderate staining, whereas PET fibre showed very little staining under the same experimental conditions.

Table 1. Oil Red O staining results for plastic polymers

Sr. No.	Polymer Type	Sample Source	Staining Intensity	Surface Coverage	Interpretation
1	Polyester	Hair ties	High	≥75% bright red	Hydrophobic, Lipophilic
2	Polystyrene (PS)	Packing foam	High	≥75% bright red	Hydrophobic, Lipophilic
3	Low-density polyethylene (LDPE)	Bubble wrap	High	≥75% bright red	Hydrophobic, Lipophilic
4	Polyvinyl chloride/ Plastisol	Vinyl lining	High	≥75% bright red	Hydrophobic, Lipophilic
5	Polyethylene terephthalate (PET film)	Plastic bag	Medium	25-75% red	Hydrophobic, Lipophilic
6	Nylon (fabric)	Fabric	Medium	25-75% red	Hydrophobic, Lipophilic
7	High-density polyethylene (HDPE)	Bottle cap	Medium	25-75% red	Hydrophobic, Lipophilic

Sr. No.	Polymer Type	Sample Source	Staining Intensity	Surface Coverage	Interpretation
8	Polypropylene (PP)	Disposable cup	Low	<25% red	Hydrophobic, Lipophilic
9	Oxo-biodegradable PE	Grocery bag	Low	<25% red	Hydrophobic, Lipophilic
10	PET (fiber)	Polyfill padding	Negligible	Negligible	No staining, hydrophilic

4.1 Staining of non-plastic materials

Most of the non-plastic materials served as controls and showed little or no staining after treatment with Oil Red O. Cotton, sewing thread, tissue paper, and matte newspaper remained unchanged and showed negligible staining. Glossy newspaper showed exception by moderate red staining, which was stronger than other control materials and some plastic samples. This is a false-positive staining response in a non-plastic control; adsorption to a surface coating is one possible explanation, but the coating composition was not verified.

Table 2. Oil Red O staining results for non-plastic control materials

Sr. No.	Polymer Type	Sample Source	Staining Intensity	Surface Coverage	Interpretation
1	Cotton fiber	Cotton Ball	Negligible	<25% red	No visible staining
2	Sewing thread	Sewing material	Negligible	<25% red	No visible staining
3	Tissue paper	Facial tissue	Negligible	<25% red	No visible staining
4	Matte newspaper	Print media	Low	25% red	Cellulose-based, hydrophilic
5	Glossy newspaper	Print media	Medium	25-75% red	Hydrophobic coating

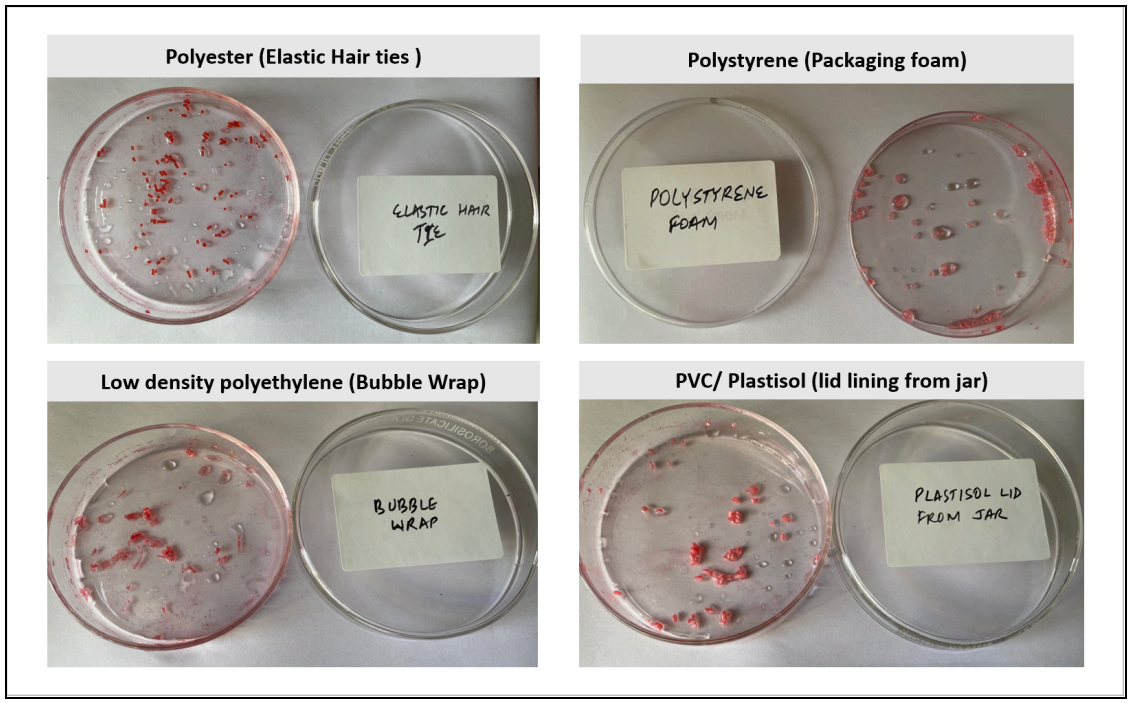


Figure 5: Plastics that show high intensity of staining with ORO. The top panel shows polyester and polystyrene samples. The bottom panel shows low-density polyethylene and PVC/plastisol samples.

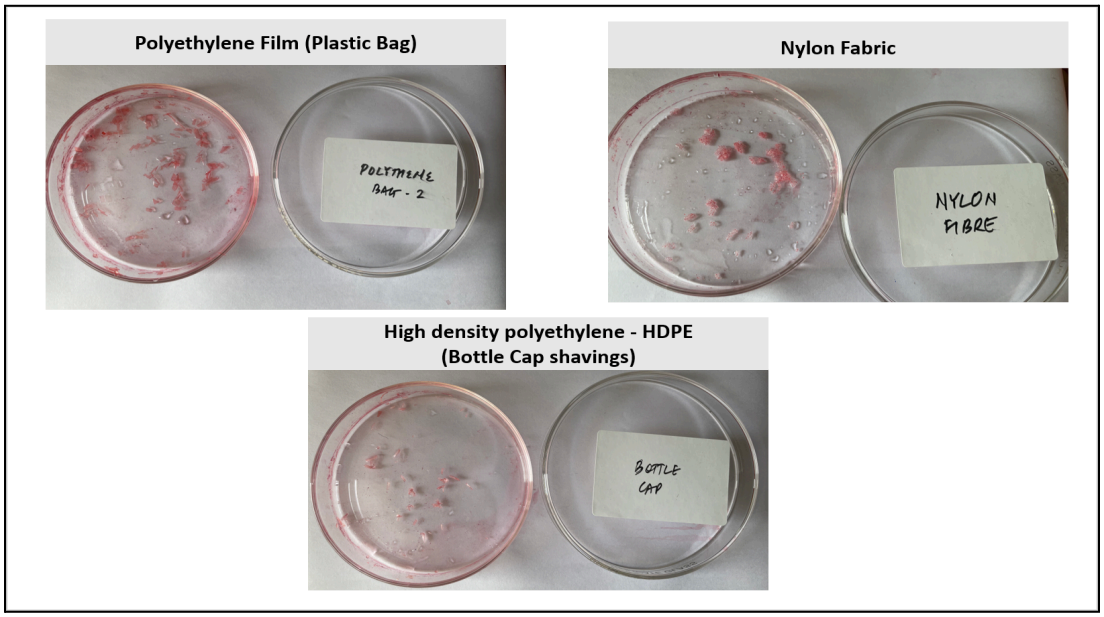


Figure 6: Plastics that show medium intensity of staining with ORO. The top panel shows polyethylene and nylon fabric, while the bottom panel shows high-density polyethylene.

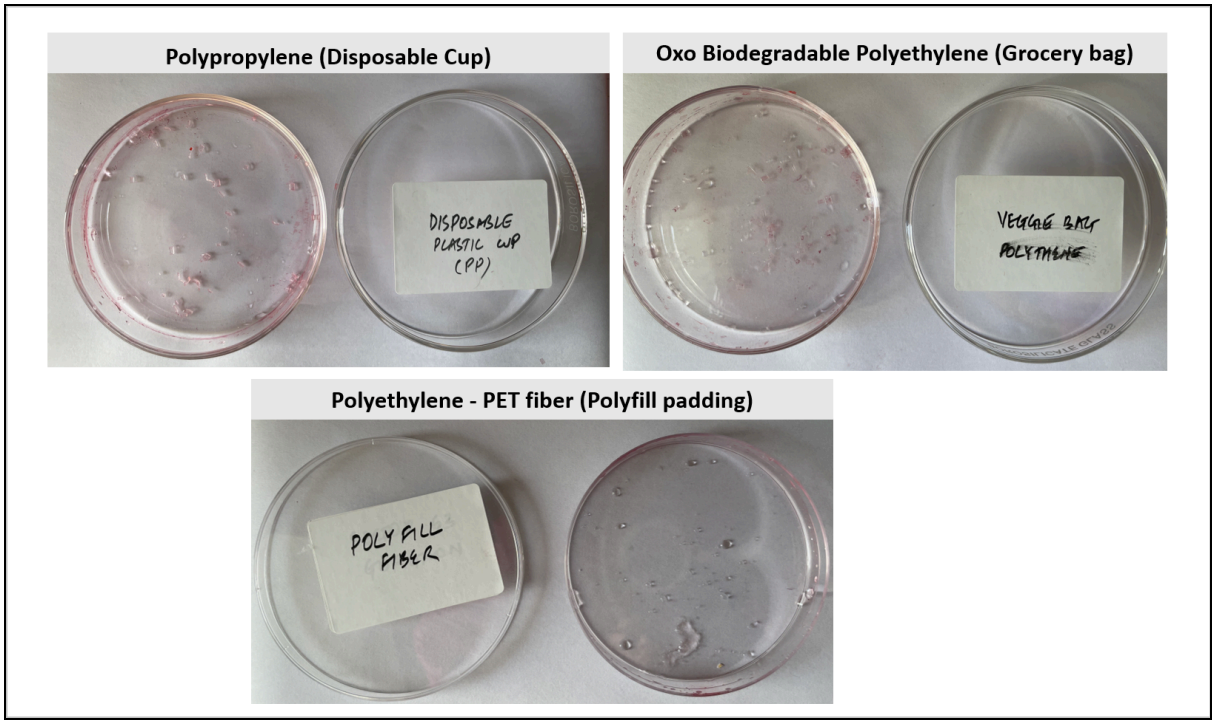


Figure 7: Plastics that show poor staining with Oil Red. The top panel shows polypropylene and oxo-biodegradable polyethylene, and the bottom panel shows polyfill fibers.

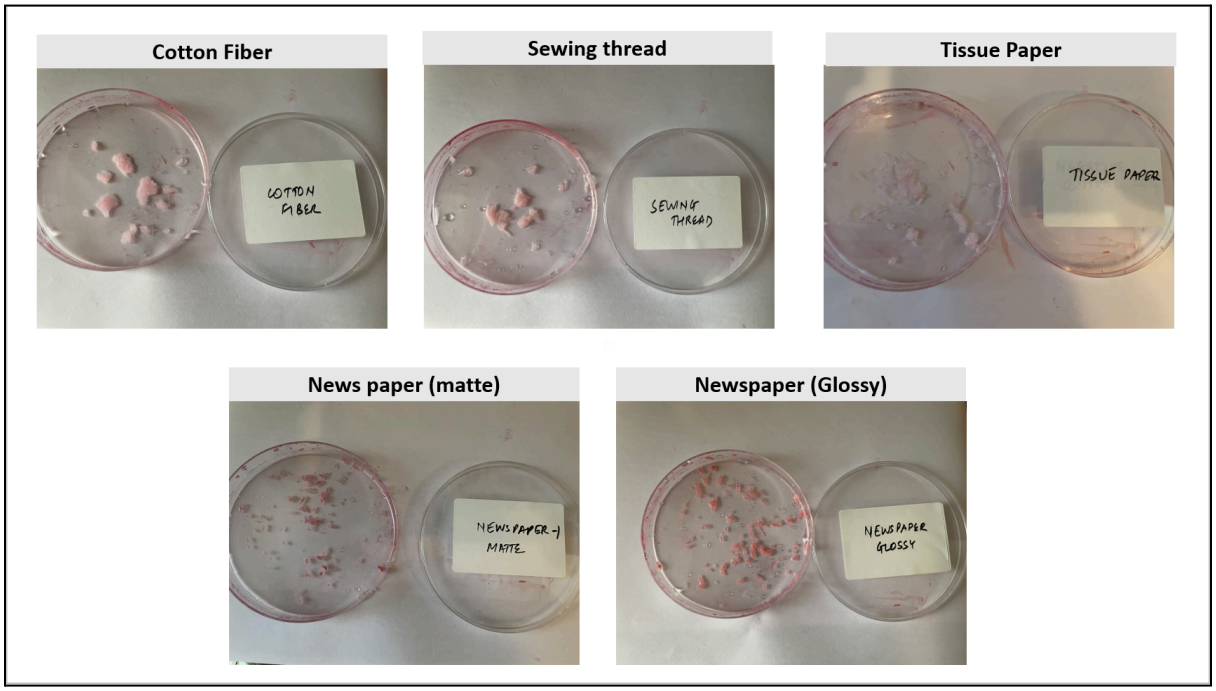


Figure 8: Non-plastic sample staining results. The top row shows cotton fibres, sewing thread and tissue paper with negligible staining observed. The bottom panel shows newspaper samples – both matte and glossy finish. The glossy finish paper showed the highest intensity of staining, comparable to staining in a few plastic samples

The stained samples were again examined after 24 hours of air drying. The high- and medium-staining plastic samples retained their red colour, while the weakly stained samples showed little colour. Samples that were negative remained negative after drying.

DISCUSSION

The results of this study show that Oil Red O can stain many common plastic materials, although the intensity of staining depends on the type of plastic. Some plastics, such as polyester, polystyrene, LDPE and PVC, developed a strong red colour, while others, including polypropylene and PET fibre, showed weak or almost no staining. These differences suggest that Oil Red O does not stain all plastics equally. An interesting observation was the difference between PET film and PET fibre. Although both are made from the same polymer, the film is stained more strongly than the fibre. This could potentially suggest that the physical form of a material may affect how well the dye binds to its surface. A future potential could be to understand how the physical form of the materials affects the dye binding.

Most of the non-plastic materials showed little or no staining, in this limited set of controls. However, glossy newspapers stained more than expected. A surface coating may contribute to this response, although its composition was not established. This moderate false-positive response overlaps with some plastic samples and prevents a reliable plastic-versus-non-plastic distinction based on staining alone. Although Oil Red O cannot replace advanced methods like FTIR or Raman spectroscopy, it does offer some advantages. The staining procedure is simple, cost-effective and takes a short time to complete. Additionally, it does not require specialized instruments and can be used as a preliminary method to screen microplastics.

This study also has several limitations. The first is that only ten plastic materials and non-plastic control samples were tested. This also means that the results are not applicable to all plastic in the environment. Secondly, no FTIR or other analytical tests were performed on materials that tested positive. Third, there were no independent staining replicates, and so there was no assessment of reproducibility. Fourth, the categories of stain and surface coverage were assessed by visual inspection and not from calibrated images. Finally, this study used clean materials rather than weathered environmental microplastics, which could have different properties.

Future studies should include a wide range of plastics made up of different polymers and should also include non-plastic materials to eliminate exceptions. In addition, the materials that tested positive should be analysed and confirmed by FTIR or Raman spectroscopy. To verify this result, a follow-up study should be conducted, taking at least three independent replicates of each material under fixed lighting, camera settings, background, scale, and rinse conditions. A defined sample region of interest could then be segmented against an unstained control to determine the percentage of red-positive pixels and a

background-corrected red-channel intensity, as well as blinded visual scores, replicate values, and dispersion. This is a proposed analysis and was not performed for the present results [17].

Overall, the findings from this work suggest that Oil Red O has the potential to be used as a rapid screening method for microplastics. It is not a method to determine the type of plastic, but it can be used to select samples for further analysis with advanced methods to determine the presence of microplastics.

6. CONCLUSION

This study demonstrates that Oil Red O may be a useful first step in the identification of microplastics. The procedure is low-cost, easy to implement, and suitable for preliminary work in low-resource educational or screening settings. However, as the staining was different for various plastics and non-plastics, Oil Red O cannot be used as the sole test, and further confirmation testing such as FTIR or Raman spectroscopy is still required. Overall, Oil Red O appears to be a useful first step in screening samples for the presence of microplastics, and further detailed analyses could then be performed. The present single-sample, visually scored results require replicate trials and quantitative image analysis before screening performance can be assessed reliably.

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