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Nile Red Fact Sheet

A quick summary of the Nile Red staining technique

Nile Red is a dye that adheres exclusively to hydrophobic substances. Meaning, it stains items that repel water, including fats, lubricants, and plastic. Items stained with Nile Red will fluoresce under a blue-green wavelength light when viewed through an orange filter.

Research documents its application as a stain dating back to 1985. It has been applied to a range of research efforts since that time. Recently, Dr. Andrew Mayes of the University of East Anglia in the United Kingdom is among the a number of researchers who have published peer-reviewed work on the use of Nile Red staining as an accurate method to rapidly identify microplastics.

As published in Nature in March 2017:

> A new approach is presented for analysis of microplastics in environmental samples, based on selective fluorescent staining using Nile Red (NR), followed by density-based extraction and filtration. The dye adsorbs onto plastic surfaces and renders them fluorescent when irradiated with blue light. Fluorescence emission is detected using simple photography through an orange filter. Image-analysis allows fluorescent particles to be identified and counted. Magnified images can be recorded and tiled to cover the whole filter area, allowing particles down to a few micrometres to be detected. The solvatochromic nature of Nile Red also offers the possibility of plastic categorisation based on surface polarity characteristics of identified particles. This article details the development of this staining method and its initial cross-validation by comparison with infrared (IR) microscopy. Microplastics of different sizes could be detected and counted in marine sediment samples. The fluorescence staining identified the same particles as those found by scanning a filter area with IR-microscopy.

Dr. Sherri Mason, Chair of the Department of Geology and Environmental Sciences, the State Univeristy of New York in Fredonia, and a leading microplastics researcher, applied similar methodology to her study, *Synthetic Polymer Contamination in Bottled Water*, which forms the basis for Orb Media's reporting. Dr. Mayes reviewed Dr. Mason's findings prior to publication and said: "This is pretty substantial. I've looked in some detail at the finer points of the way the work was done, and I'm satisfied that it has been applied carefully and appropriately, in a way that I would have done it in my lab."

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The noted difference between testing bottled water for microplastics and testing marine sediment samples is the exclusion of a "digestion step" that is used with debris-filled samples from the ocean or the seashore. This step would remove natural substances such as wood, algae, or chitin [prawn shells] from the sample. Because, unlike in marine samples, the "environmental container" that is packaged drinking water would not contain substances like these, Mason did not use a "digestion step" in her study.

Additional Sources:
https://www.ncbi.nlm.nih.gov/pubmed/3972906; https://www.thermofisher.com/order/catalog/product/N1142; https://www.sciencedaily.com/releases/2017/11/171123095407.htm; https://www.ncbi.nlm.nih.gov/pubmed?cmd=Retrieve&db=PubMed&dopt=AbstractPlus&list_uids=11238460; https://www.ncbi.nlm.nih.gov/pubmed?cmd=Retrieve&db=PubMed&dopt=AbstractPlus&list_uids=23810969; https://www.ncbi.nlm.nih.gov/pubmed?cmd=Retrieve&db=PubMed&dopt=AbstractPlus&list_uids=21767582

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