Marine Microplastics - an educational module for the identification of microplastics in environmental samples with Nile red

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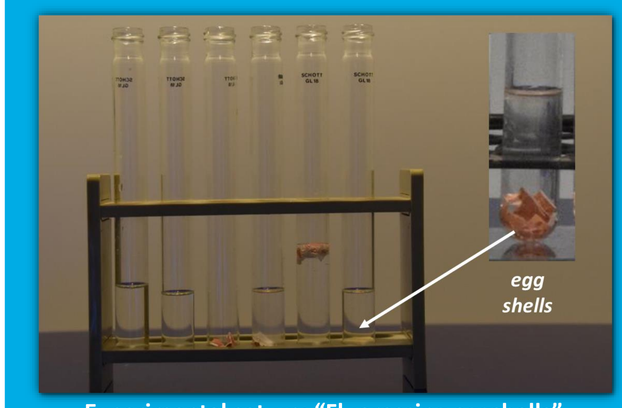
Since the middle of the last century the world production of plastics has increased massively and led to a continuous increase in the amount of plastic waste. The environmental degradation of plastic waste through physical and chemical alterations creates continuous fragmentation of large plastic items into small ones known as microplastics (MP). How the waste gets into the sea, how it is detected and discovered by researchers and what consequences it can have for living organisms are topics of an education program on marine litter implemented in the AWI school lab OPENSEA. We focus on hands-on experiments which are framed by several modules. Our aim is to impart up-to-date scientific knowledge and awareness through scientific experimental work for school classes (age 16-19). We offer experiments to sample plastic waste of different sizes in the environment and characterize the plastic material (microscope, infrared spectrometry). A new module is based on the fluorescent dye Nile red aiming to visualize microplastic particles in environmental samples.

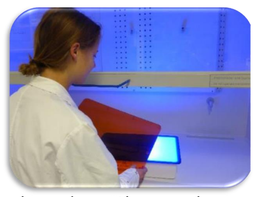


Experimental set-up: “Fluorescing eggshells”

Visualization of fluorescent samples by using a “dark reader”

Environmental samples stained with the fluorescent dye Nile red are examined. When excited with blue light using the “dark reader”, specific fluorescence can be visualized. To introduce the phenomenon and learn about fluorescence in general, we extracted Protoporphyrin IX from brown eggs and visualized auto-fluorescence (emission of red fluorescence) exemplarily. We also can make use of fluorescence for environmental science related questions. Using Nile Red, the dye bound to polymers emits yellow-red fluorescence. The Nile red fluorescence of stained plastic polymers depend on their chemical structure, some of them can be distinguished due to their emission colors. Learning modules and experiments for students were developed in the OPENSEA school lab, to convey how fluorescent staining can be used to analyze environmental samples (see detection of plastic polymers) contaminated with plastics.

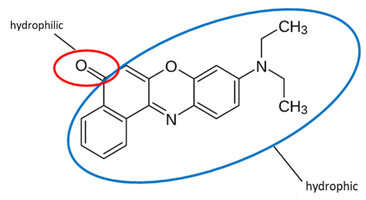
Methods



Dark reader with an amber screen

„Dark reader“

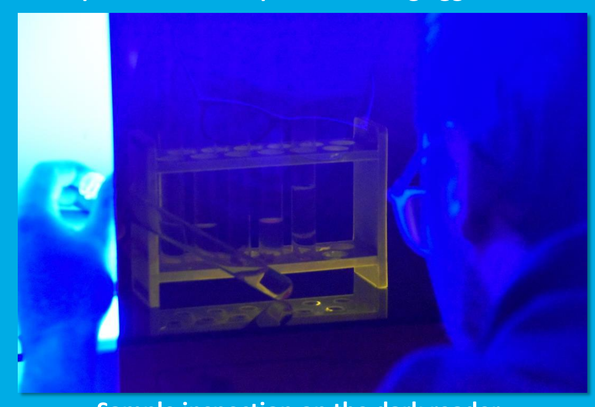
- Blue light transilluminator for fluorescent samples (excitation 440-510 nm)
- Amber screen emission filter (>570 nm)



Structural formula of Nile red

Nile red

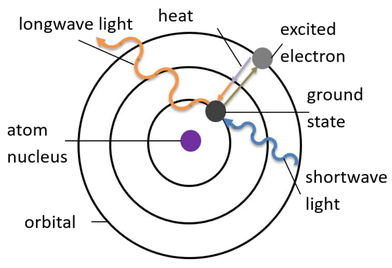
- Fluorescent dye
- Emission ca. 520-600 nm (in EtOH)
- Staining of hydrophobic components



Sample inspection on the dark reader

Students discussing first results

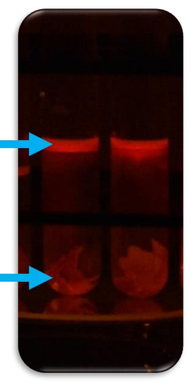
Fluorescing eggshells



The principle of fluorescence

This experiment serves to convey the principle of auto-fluorescence and shows how this phenomenon is made visible. We developed an experiment with auto-fluorescent Protoporphyrin IX from brown egg shells. The students' task is to make this substance visible with the help of the following materials:

- Brown eggshells
- Ethyl acetate (Nail polish remover)
- Acetic acid (Vinegar 5 %)
- Dark reader with an amber screen



„Fluorescing eggshells“

Eggshells contain Protoporphyrin IX, which has fluorescent properties when dissolved in alcohol and excited by a specific wavelength. For an introduction to the topic of fluorescence and to illustrate the phenomenon, the students will receive additional material:

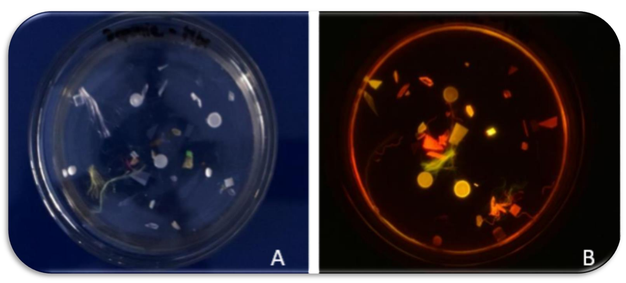
- Video about the principle of fluorescence
- Helpsheets with structural formulas of all important substances used in the experiment
- Atomic model to illustrate the principle of fluorescence

First inspection of plastic particles

Detection of plastic polymers by using fluorescence

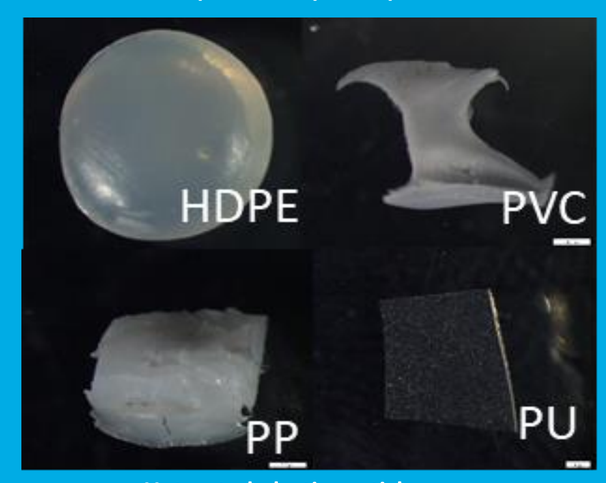
The properties of fluorescence can be used for environmental research. In order to transfer this to laboratory work, the students receive an environmental sample to work with. Various materials are used:

- Nile red on a basis of Ethanol (20 % w/w)
- Different plastic polymers
- Artificial sample (mixed)
- Dark reader with an amber screen
- Compartment dryer (60 °C)

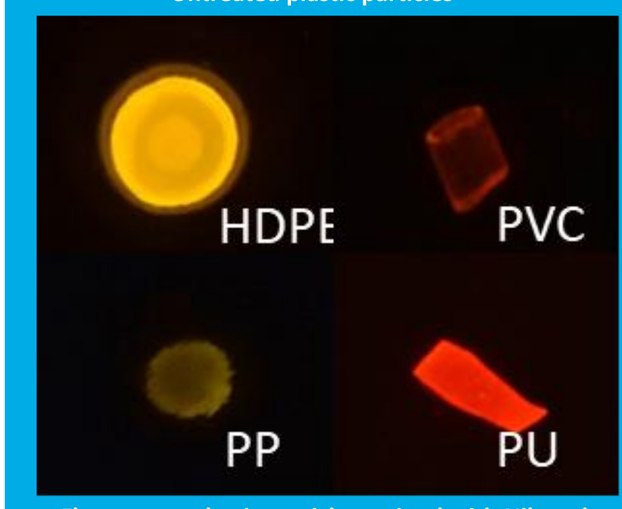


Artificial plastic sample before (A) and after (B) staining with Nile red

By staining with Nile red, plastic polymers can be partially differentiated due to their specific fluorescence. Different plastic polymers in a mixed sample can be distinguished after staining with Nile red, and excited with blue light.



Untreated plastic particles



Fluorescent plastic particles stained with Nile red

Conclusion

This hands- on experimental module is divided into several small experiments aiming to identify plastic particles based on their specific fluorescence. Students work independent, individually or in a team on different scientific questions:
1. How to identify different plastic polymers?
2. Is the fluorescence of stained plastic polymers affected by color or shape of the MP?
3. Can Nile red staining be used to detect and identify different MP’s in an artificial sample?

Finally, students will be able to evaluate the applicability of the method of Nile red staining in comparison to other application methods (microscope, infrared spectrometry).

Acknowledgement
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Photographs: AWI school lab OPENSEA