

Lyceum of Agios Neophytos Paphos, Cyprus



Microplastics detection using fluorescence. Investigation of samples from Marine Organisms in Southwest Cyprus.

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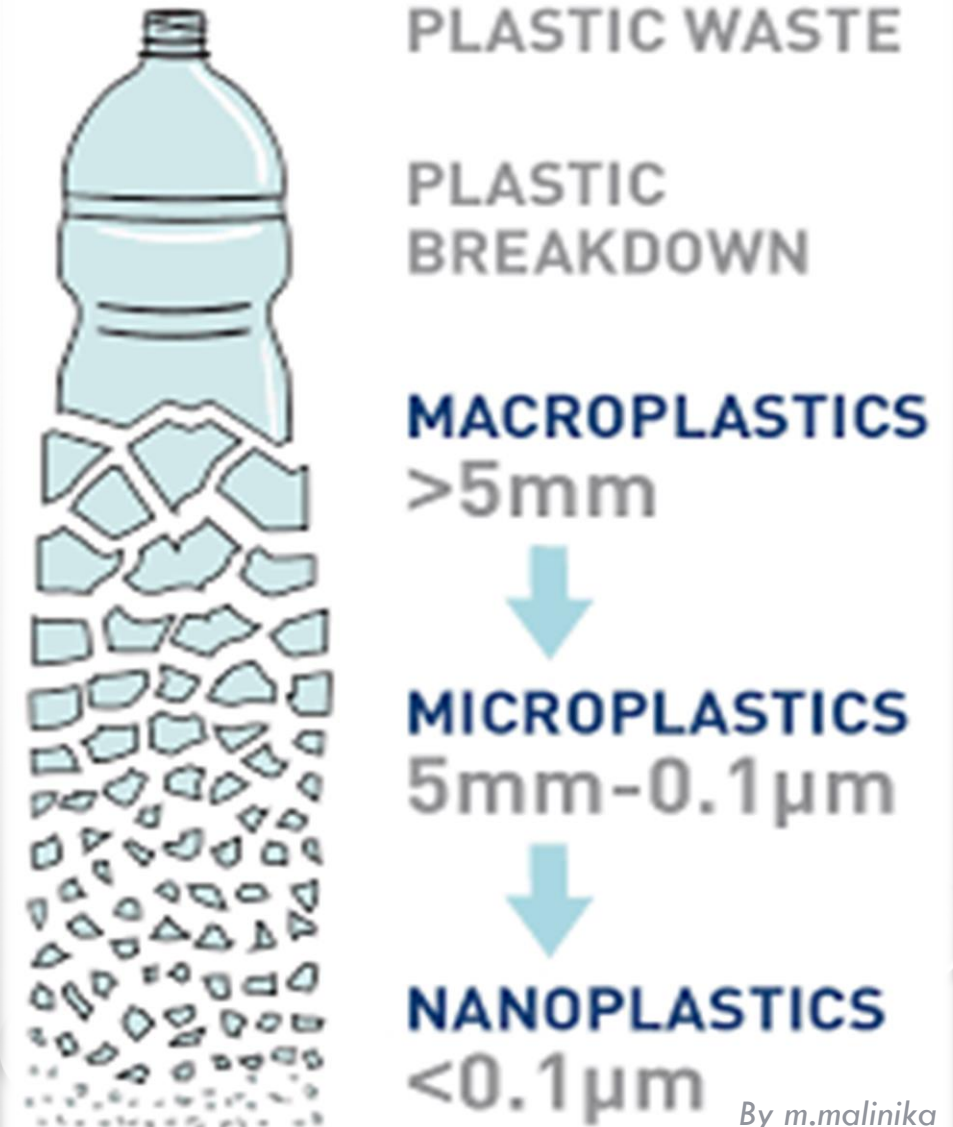
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What are Microplastics?

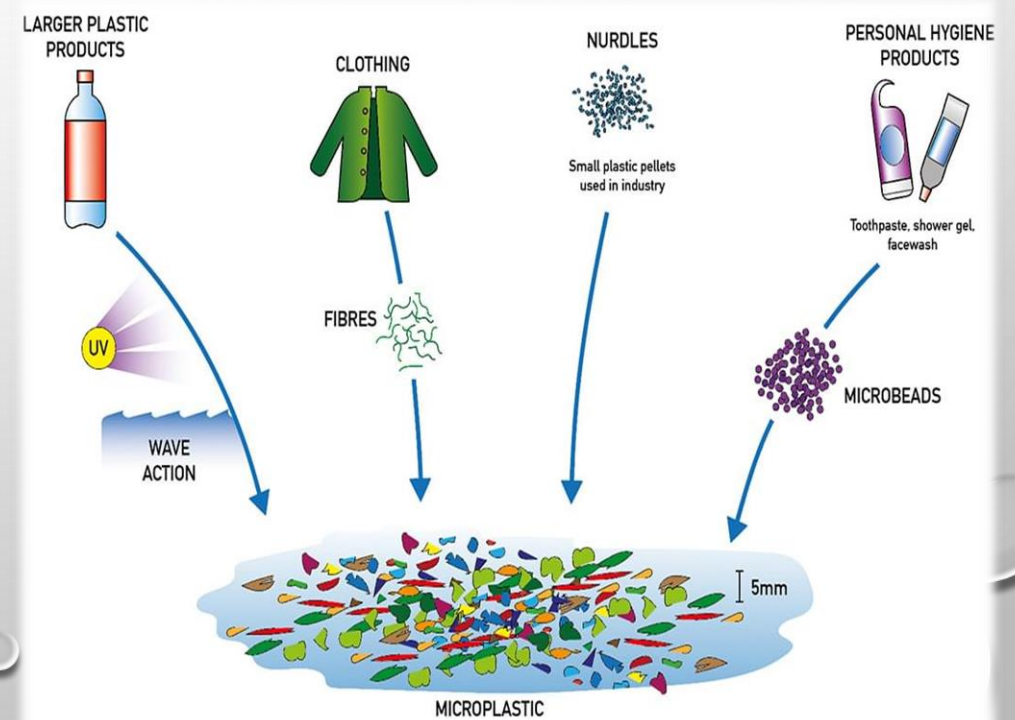
- They are tiny plastic particles usually $0.1\ \mu\text{m}$ - 5mm .
- They result from the break down of larger pieces of plastics (macroplastics $>5\text{mm}$)
- Many microplastics are produced intentionally for industrial and commercial purposes (e.g cosmetics and detergents)

R. Geyer et al. Sci. Adv., 2017
R. Jambeck et al. Science, 2015



Microscopic plastics, huge problem !

- *They pollute terrestrial and aquatic environments*
- *They have already entered our food chain since they are found in human lungs, human blood and human placenta*
- *they are harmful for all living organisms including humans (e.g. hormonal disorders, organ dysfunction, immune system dysfunction)*



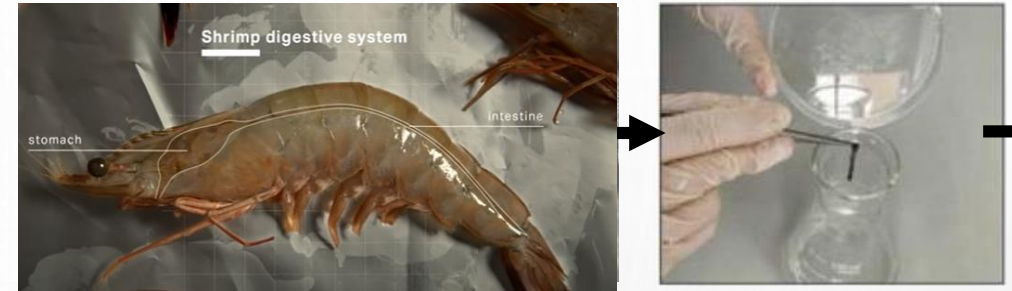
Specific goals of the research project:

- Detection of microplastics in Cypriot Shrimps and sand samples by using fluorescence.
- Building a fluorescent stereoscope «Glowscope» by modifying the basic stereoscope, in order to analyze our samples.



Methodology: Isolation of Microplastics from shrimp samples

Collection of samples (shrimps from local fishermen from the areas: Kato Paphos, Poli Chrysochous, Akamas) and isolation of the digestive system



Isolation of microplastics from shrimp digestion system:

- digestion of guts with H_2O_2 (hydrogen peroxide)
- density separation with $ZnCl_2$ (Zinc Chloride)
- collection of microplastics in a special filter (1.2 μm)

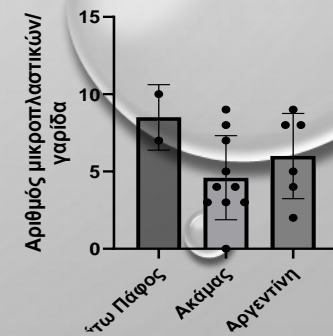
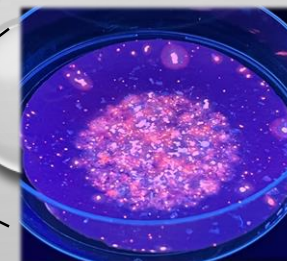


Staining of microplastics with a special fluorescent dye (Nile Red) that binds to microplastics (Thomas et al. Scientific reports, 2017)



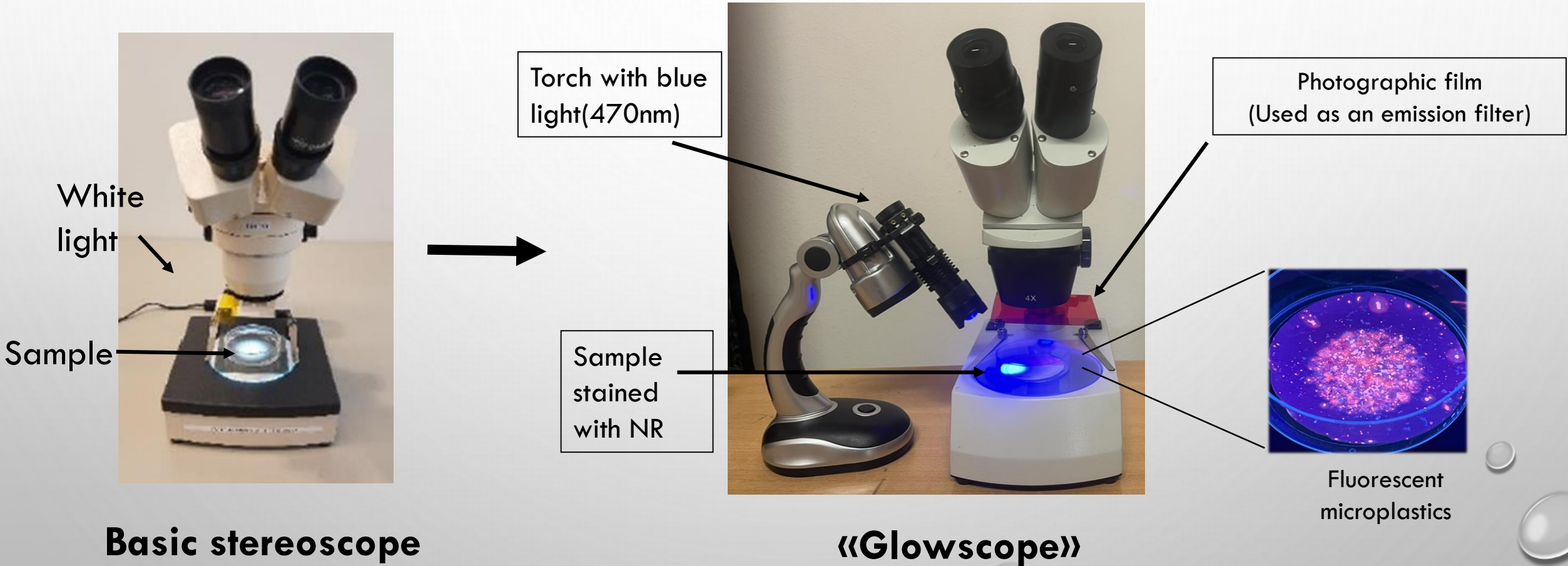
Fluorescence detection with «Glowscope»

Data recording and analysis

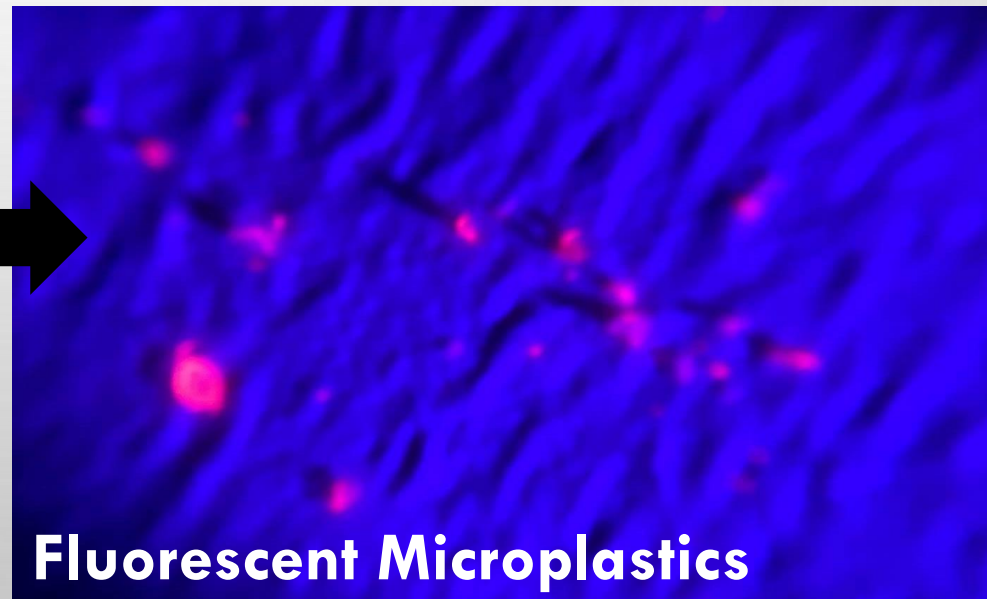


Methodology – Building the «Glowscope»

Simple modifications on the basic stereoscope in order to detect fluorescence



Methodology – Building the «Glowscope»



Fluorescent Microplastics

Data Recording

Number of MPs/sample

Data from shrimp samples

ΔΕΙΓΜΑ	ΕΙΔΟΣ ΔΕΙΓΜΑΤΟΣ	Βάρος Γαρίδας (g)	Βάρος εντέρου (g)	Συνολικός αριθμός ΜΠ	Filament	Fragment	Film	Granule/Bead
1	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	54.91	4.66	3				
2	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	47.99	2.41	8				
3	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	44.18	3.39	2				
4	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	57.99	4.55	5				
5	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	54.60	3.48	4				
6	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	58.28	4.7	8	1	4		3
7	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	47.23	2.74	2				
8	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	48.00	4.55	9		9		
9	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	46.66	4.12	11				
10	ΑΡΓΕΝΤΙΝΗΣ_ΑΤΛΑΝΤΙΚΟΣ-LIDL	45.66	4.7	7				
21	Κυπριακή /ΛΙΜΑΝΙ-ΚΑΣΤΡΟ ΚΠ	62.69	5.86	10		10		
22	Κυπριακή /ΛΙΜΑΝΙ-ΚΑΣΤΡΟ ΚΠ	70.0	1.83	7	1	6		
11	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	25.8	5.00	9		6	1	2
12	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	73.00	5.39					
13	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	36.39	4.00	0				
14	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	41.00	1.00	3		3		
15	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	45.75	2.81	4		3	1	
16	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	120.48	5.10	8		6		2
17	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	62.00	1.67	4		4		
18	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	50.41	2.51	7		6	1	
19	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	28.16	0.26	5		5		
20	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	14.06	0.50	3	1	2		
23	Κυπριακή /ΑΚΑΜΑΣ-ΠΟΛΗ ΧΡ.	22.5	0.27	3		3		

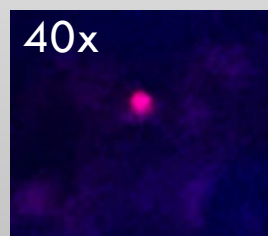
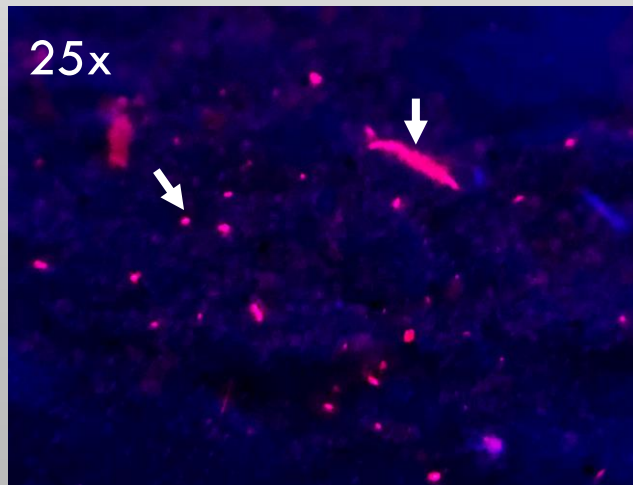
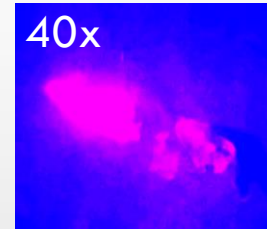
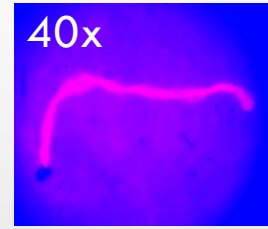
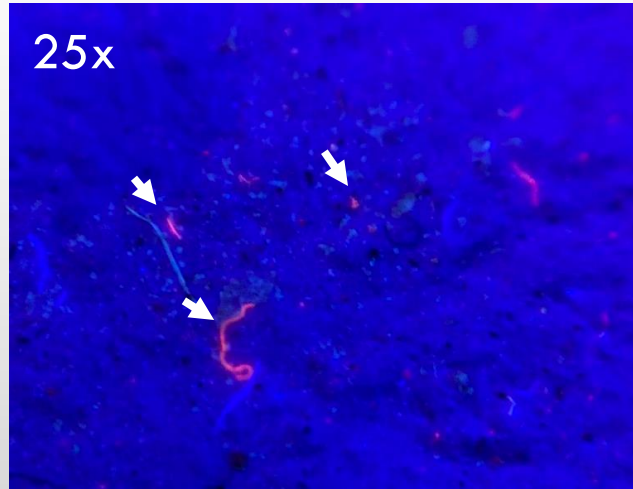
Data from sand samples

Κωδικός Δείγματος	Περιοχή	Γραμμάρια άμμου	ZnCl2	Νερό	Υγρό για φιλτράρισμα	Αριθμος μικροπλαστικών-ΣΥΝΟΛΟ	FILAMENT	FRAGMENT	FILM	BEAD/GRANULE
A1	Φάρος	100g	8g	200ml	100ml	14	4	7		3
A2	Φάρος	100g	8g	200ml	100ml	12	3	7	1	1
A3	Φάρος	100g	8g	200ml	100ml	6				
A4	Σόδαπ	100g	8g	200ml	100ml	58	18	38		2
A5	Σόδαπ	100g	8g	200ml	100ml	27	3	19	2	3
A6	Σόδαπ	100g	8g	200ml	100ml	50				
A7	Riccos	100g	8g	200ml	100ml	11	1	7		3
A8	Riccos	100g	8g	200ml	100ml	25	3	19	2	1
A9	Riccos	100g	8g	200ml	100ml	40	5	25	4	6

Results

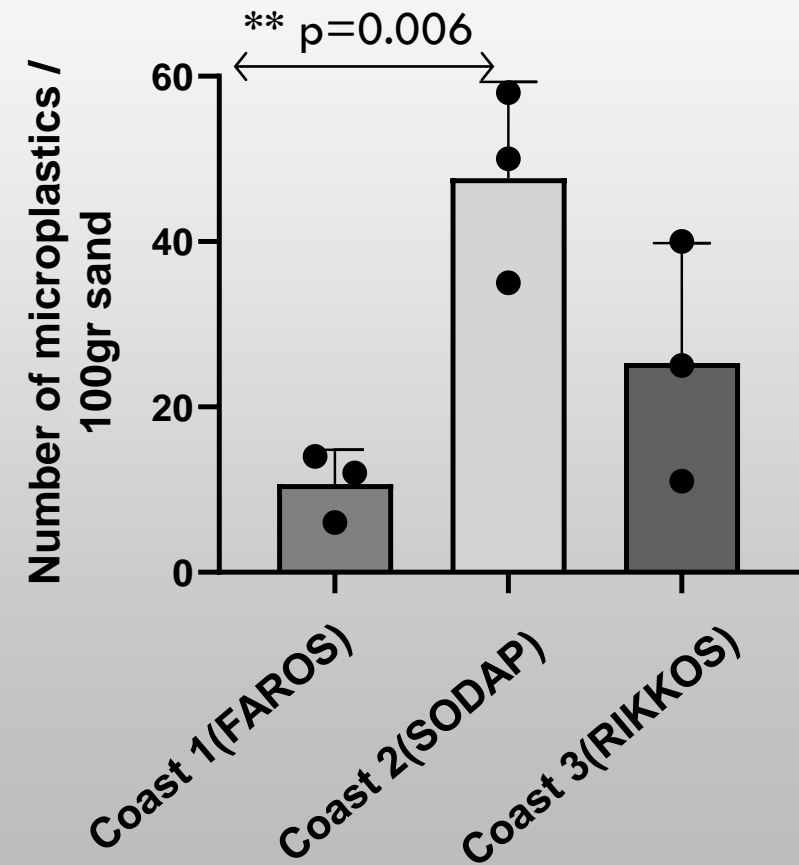
Microplastics are detected in sand samples from different coasts of Paphos

Sand samples under Glowscope:



*25x Macro lense (phone camera)

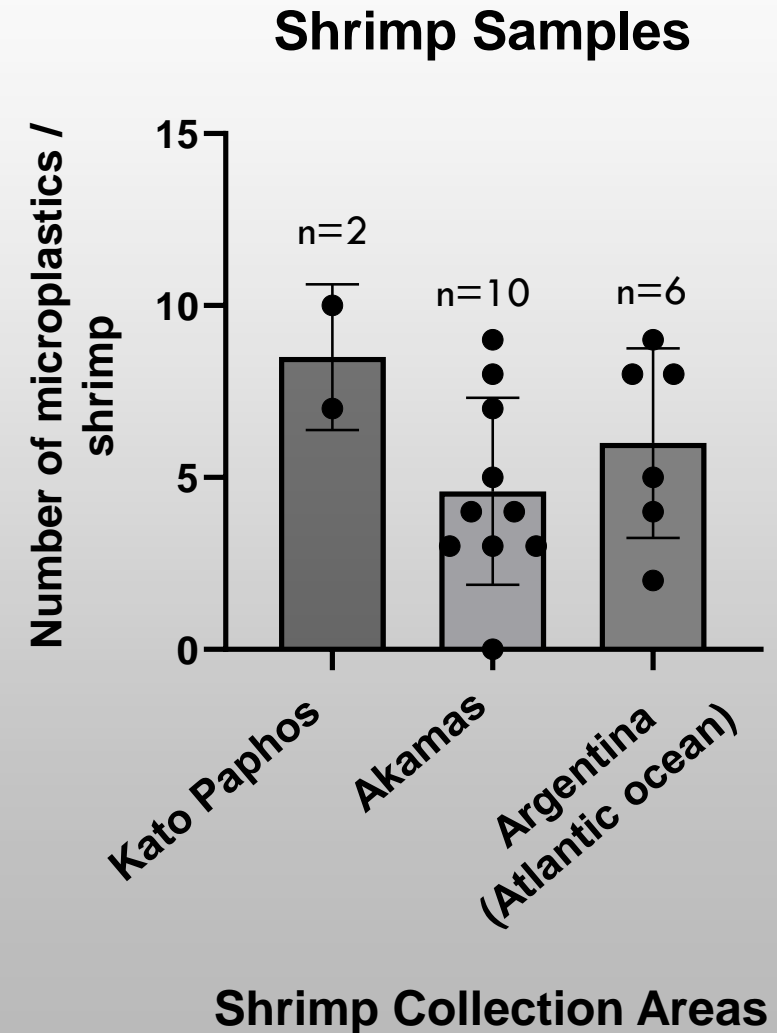
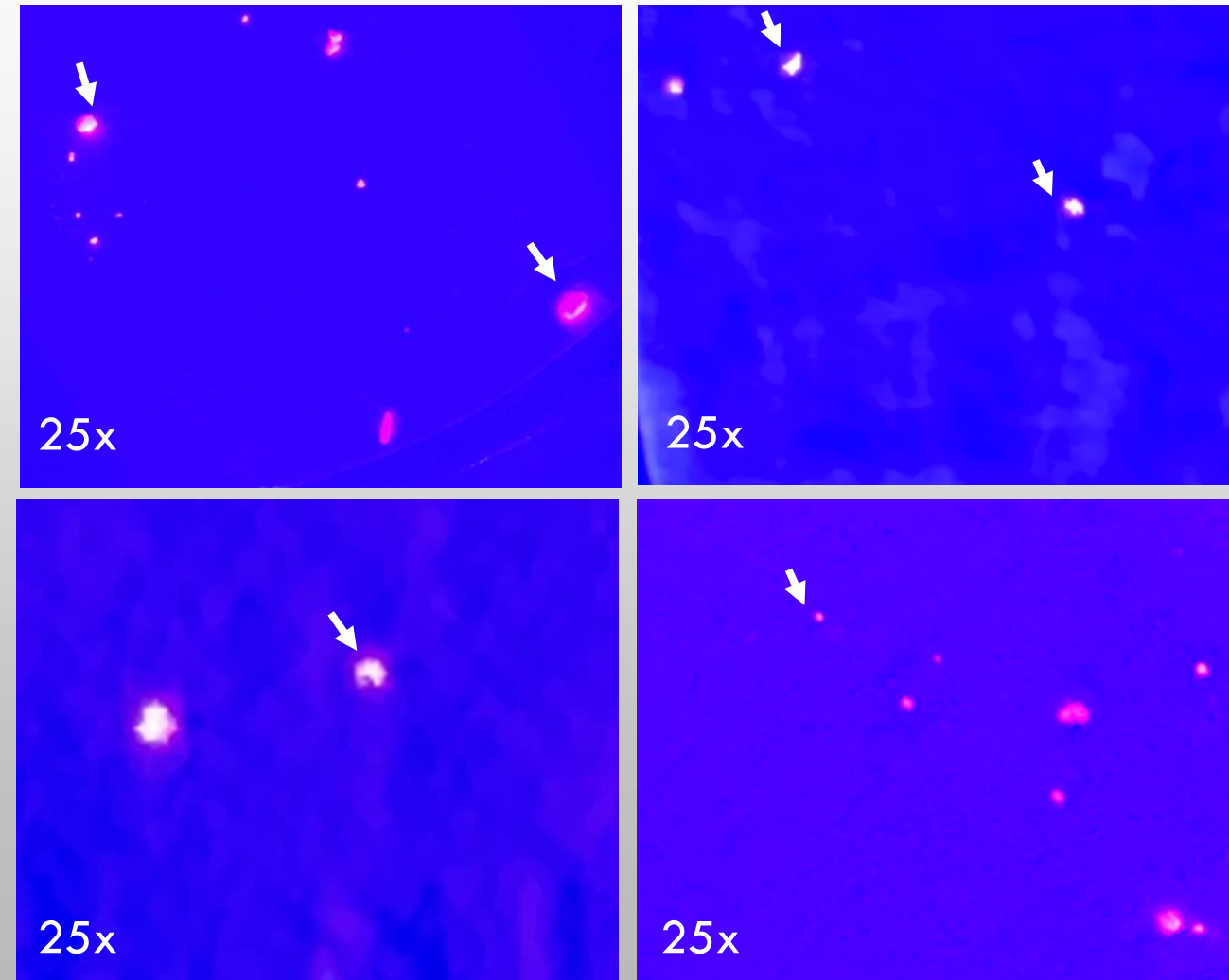
Sand Samples



n= 3, number of samples from each coast

Microplastics are detected in Cypriot shrimps

Shrimp samples under Glowscope:



*n = Number of samples

* Every dot represents a different shrimp

*25x Macro lense (phone camera)

Reliability of the Results

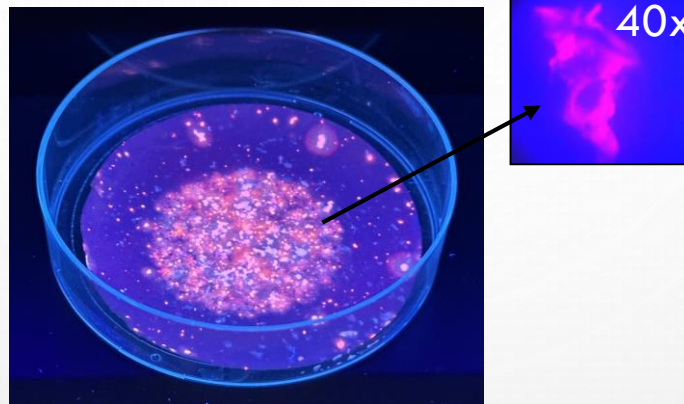
The Importance of using Negative and Positive Control Samples



Negative control

**Sample without plastics
(reagents ONLY)**

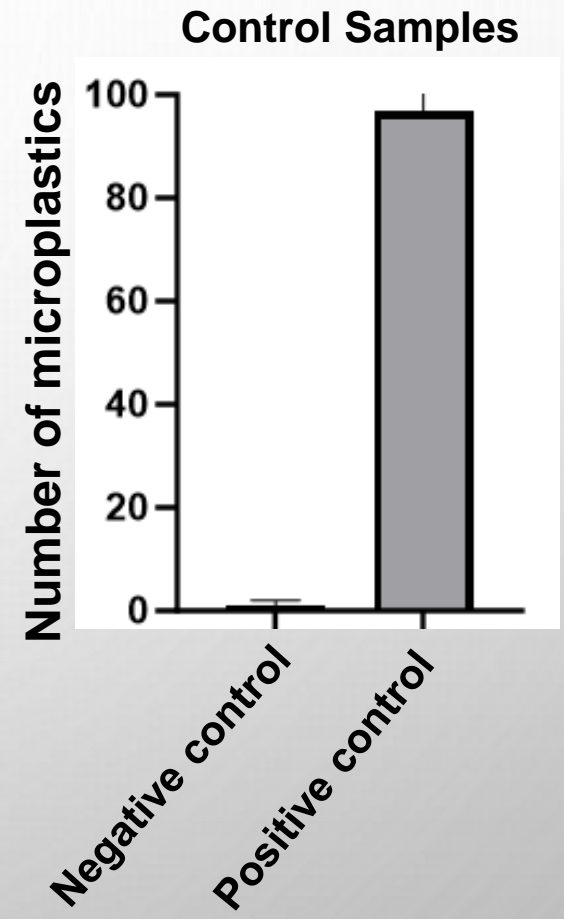
**it shows if there is any
contamination with
plastics from the lab
environment*







Positive control

Sample with various plastics

**confirms that the dye is
working (efficient staining
of plastic particles)*



Summary

-  We detected microplastics in sand samples as well as in the digestive tract of Cypriot shrimps.
-  We builded a fluorescent stereoscope (Glowscope) for the analysis of our samples
-  We confirmed the contamination of marine ecosystems ourselves and how microplastics enter the food chain.
-  Our goal is to disseminate the results to the local community and raise awareness for the environmental pollution in students and citizens of Cyprus.

***The shrimps were not thrown away.
The were reused !***



Thank you!

