A Rapid One-step Field based Method for Nanoplastics in Wastewater Samples

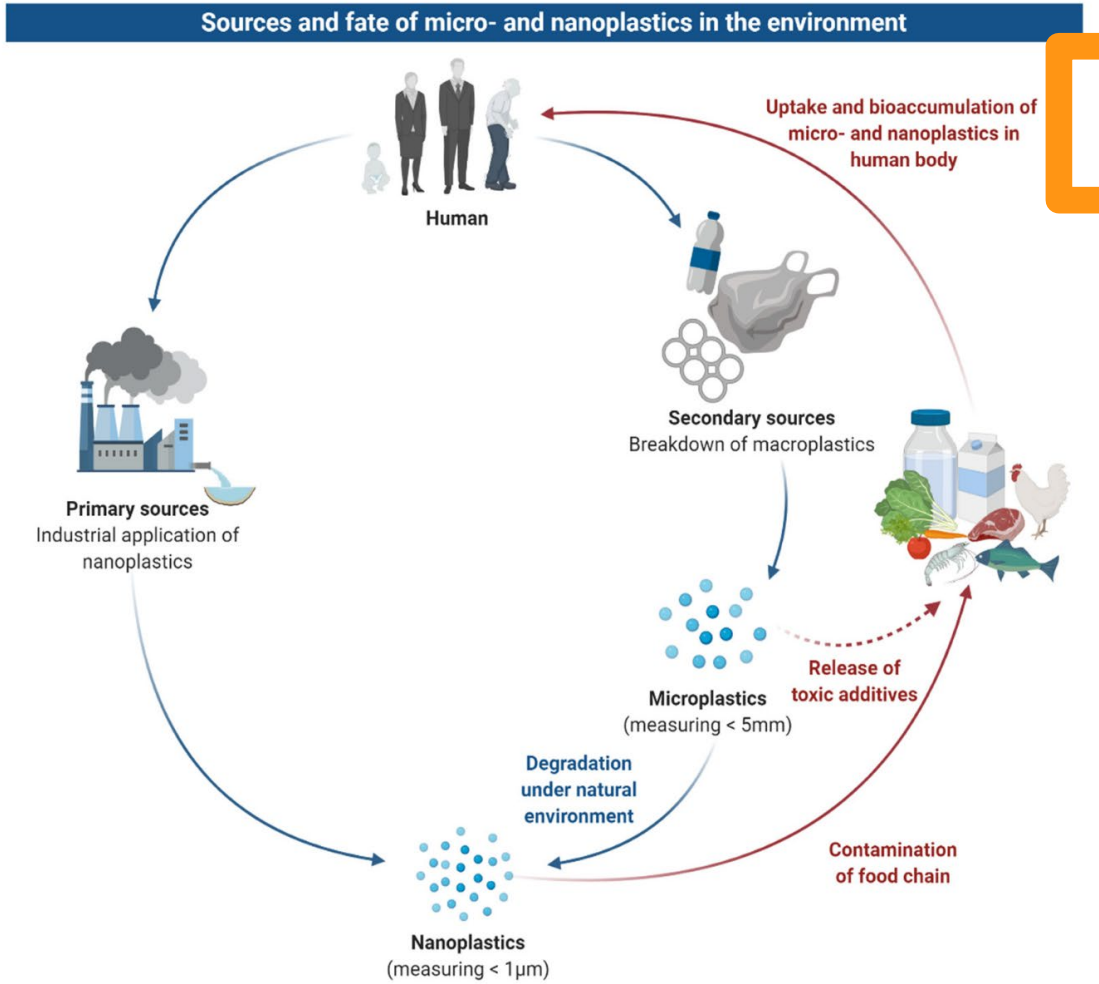
Vidhatri Iyer
University High school of Indiana
Carmel, Indiana

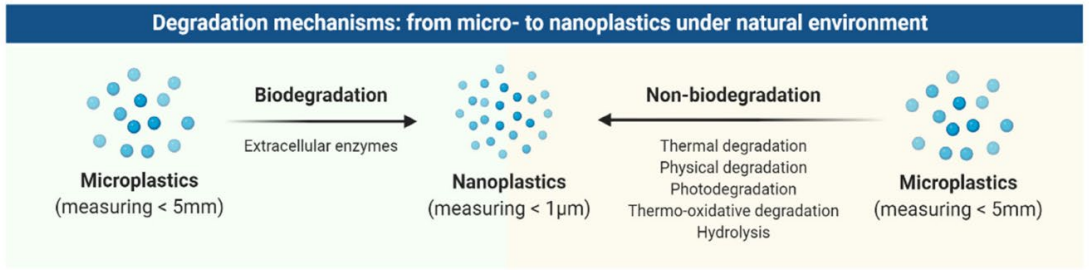
Plastics

- Mismanagement of plastic waste has resulted in 12.5 million tons of toxic plastics entering the ocean.
- The fragmentation of plastics into smaller particles has resulted in microplastics and nanoplastics generation.

https://www.wired.co.uk/article/plastic-waste-packaging

Impact of Microplastics and Nanoplastics on Human Health





https://www.mdpi.com/2079-4991/11/2/496

Classification of Plastics

- **Plastic (>5mm)**

- Visual examination with naked human eye

- **Microplastic (1mm – 5mm)**

- Filtration, staining counting under microscope

- **Nanoplastics (< 1mm)**

- Spectroscopy (infrared, Raman Spectroscopy & Transmission Spectroscopy)

Research Topic

- **Hypothesis:** Nanoplastics can be detected using a plastic binding dye by measuring the emission wavelength with a hand-held fluorometer.

- **Research Question:** Can nanoplastics pollution in wastewater be monitored with Nile Red dye using a single-channel fluorometer?

Methodology

Instrument

- Handheld Fluorometer
- Custom order (AMI Sciences)
- **Absorption** 450nm
- **Emission** 620 nm

Materials

- Milli-Q water
- Lab grade methanol
- 0.3 ml glass mini tubes
- Nile Red dye
- 50nm Polystyrene beads (Polysciences)

https://www.sigmaaldrich.com/US/en/product/sigma/n3013

https://www.polysciences.com/default/polybead-microspheres-005956m

Study Design and Reagents

Nile Red Stock Solution

- Prepare 10 mM Nile Red in Methanol
- Working solution – 40 µM methanol

Bead Prep

- Stock concentration 25 mg/ml
- Serial dilution in water:
- 400 µg/ml, 1:1 dilution

Standard Curve

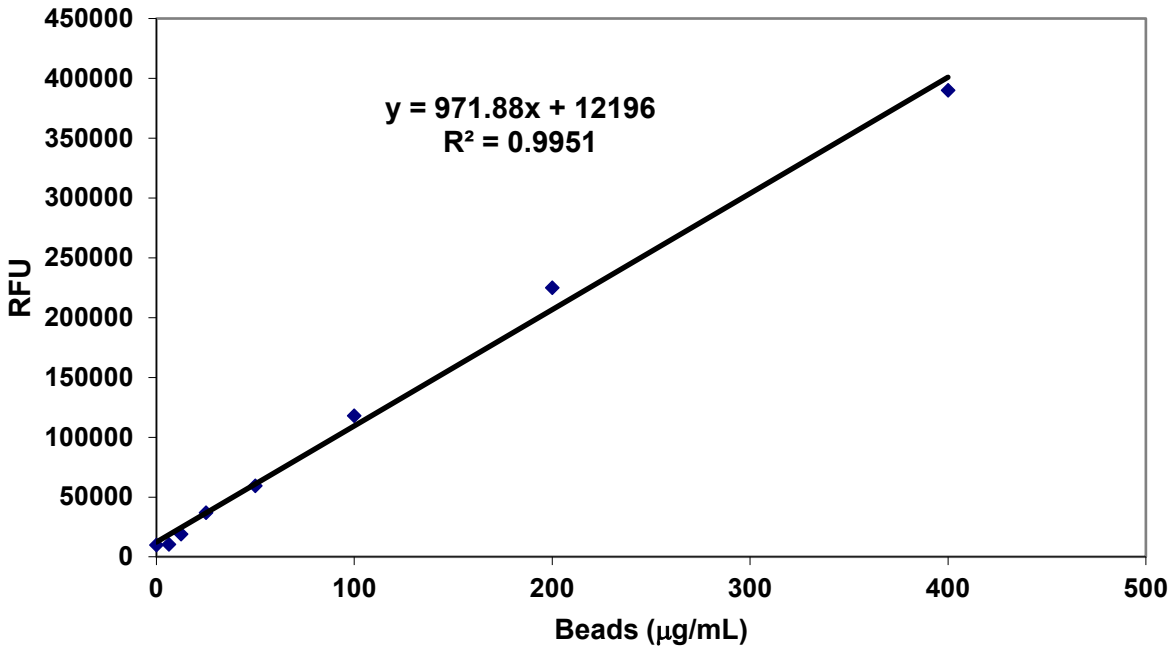
- Mix 160 µL of bead with 40 µL of Dye
- Incubate at room temperature for 10 min
- Read at 450 nm

Blank

- Mix 160 µL of water with 40 µL of Dye
- Incubate at room temperature for 10 min
- Read at 450 nm

Bead Titration Standard Curve Generation

- 160 µl of increasing concentration of beads was mixed with 40 µl of Nile Red Dye.
- Samples were incubated for 10 min and read at 450 nm
- Equation and correlation coefficient associated with linear regression was calculated using excel
- Strong correlation with different bead concentration with limit of detection (LOD) of 35 µg/ml (greater than 95% confidence)



Time-Course Study of Bead Incubation

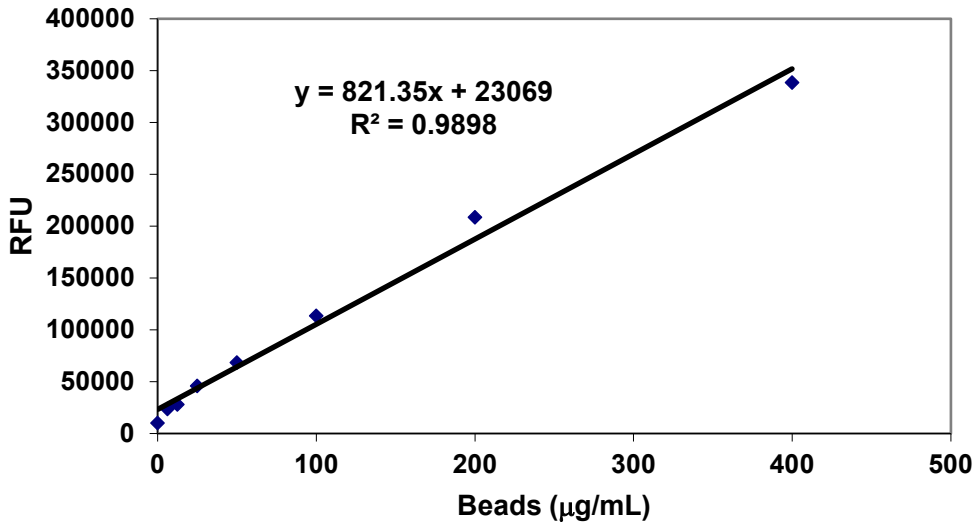
- 160 µl of increasing concentration of beads was mixed with 40 µl of Nile Red Dye.
- Samples were incubated for 10, 20, 30 or 60 min and read at 450 nm
- Similar slopes at all time-points tested

Time (min)	Nile Red	
	Slope	R^2
10	971.88	0.995
20	933.66	0.991
30	964.9	0.990
60	1003.6	0.990

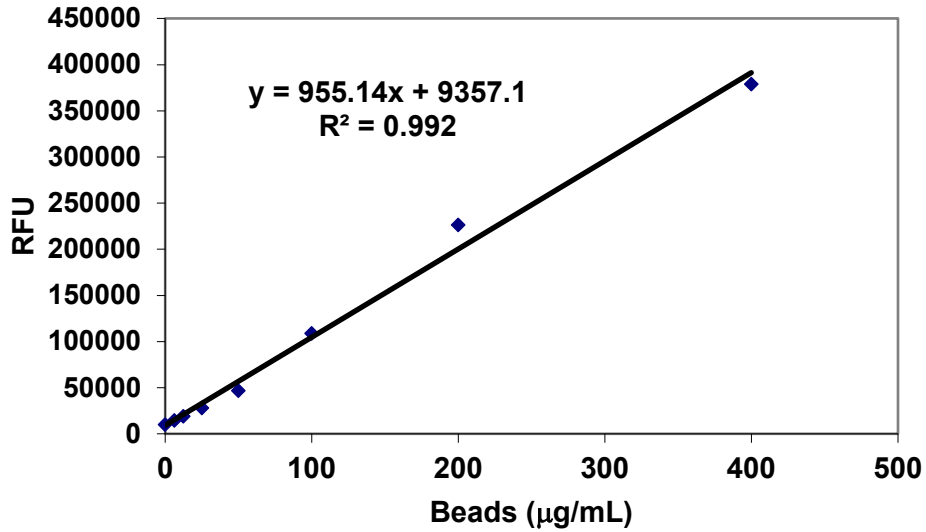
Effect of Shaking on Standard Curve Generation

- 160 µl of increasing concentration of beads was mixed with 40 µl of Nile Red Dye.
- Samples were shaken at 200 rpm for 10 min and read at 450 nm
- Equation and correlation coefficient associated with linear regression was calculated using excel
- Data was comparable in both groups

No Shake



200 rpm Shaking

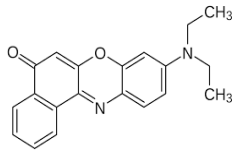


One-Step Nanoplastics Detection Method

Filter water with 0.4 µm syringe

160 µL of water sample to tube

Add 40 µL of Dye (final conc is 8 µM)



10 min incubation

Read at 450 nm

TRICO Sewer Facility Process

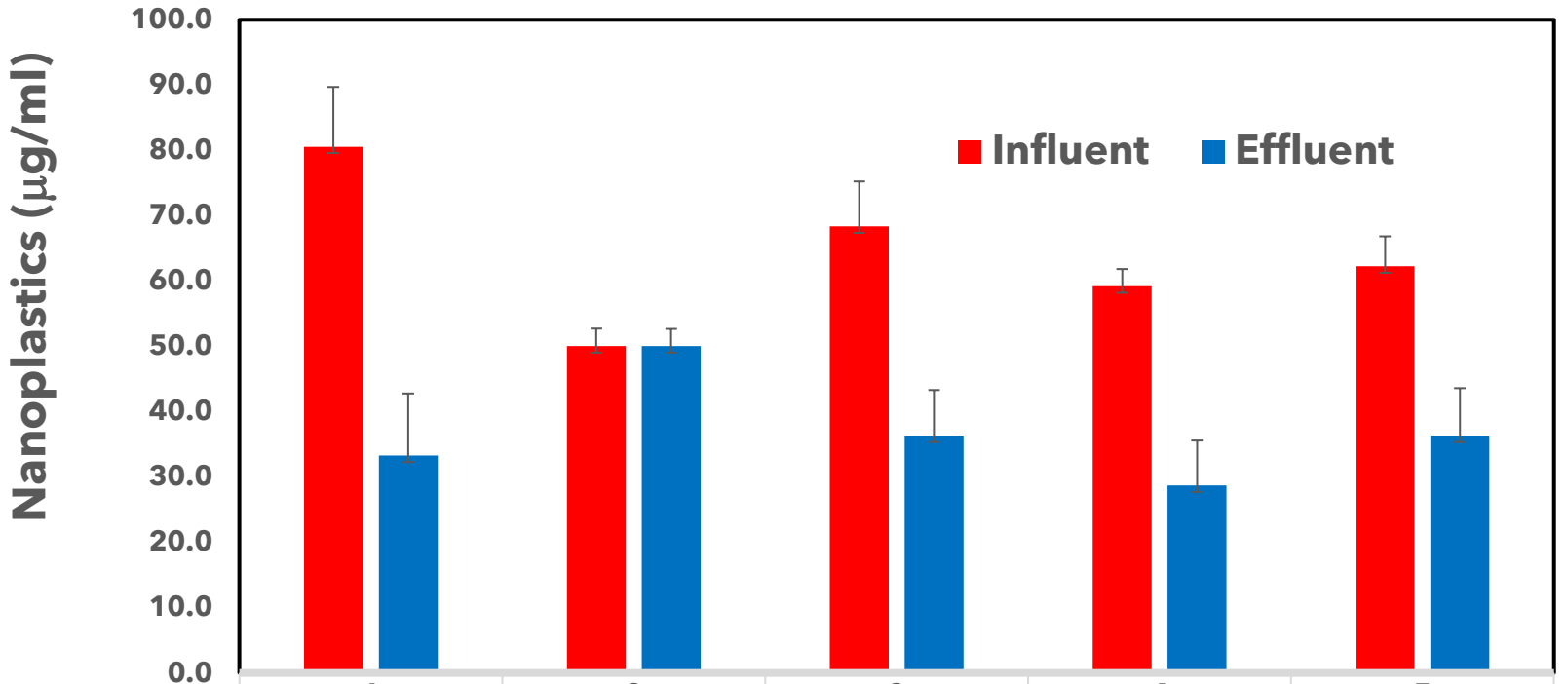
Pretreatment
Facility
(Influent grab)

Primary treatment
(VLR anoxic zone)

Secondary treatment
(Clarifier effluent)

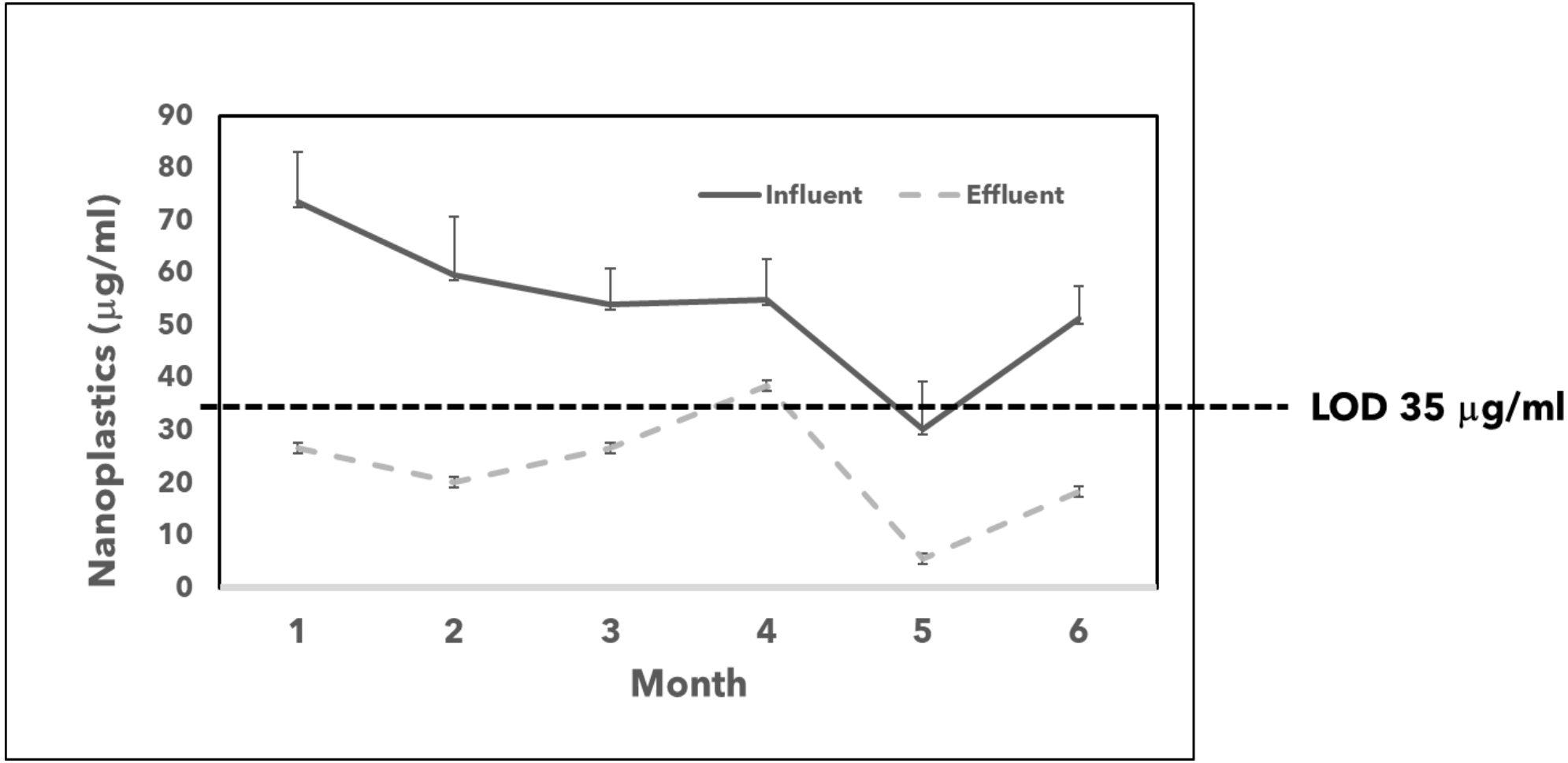
Indiana Water samples
Effluent samples

Reduction in Nanoplastics in Effluent Samples

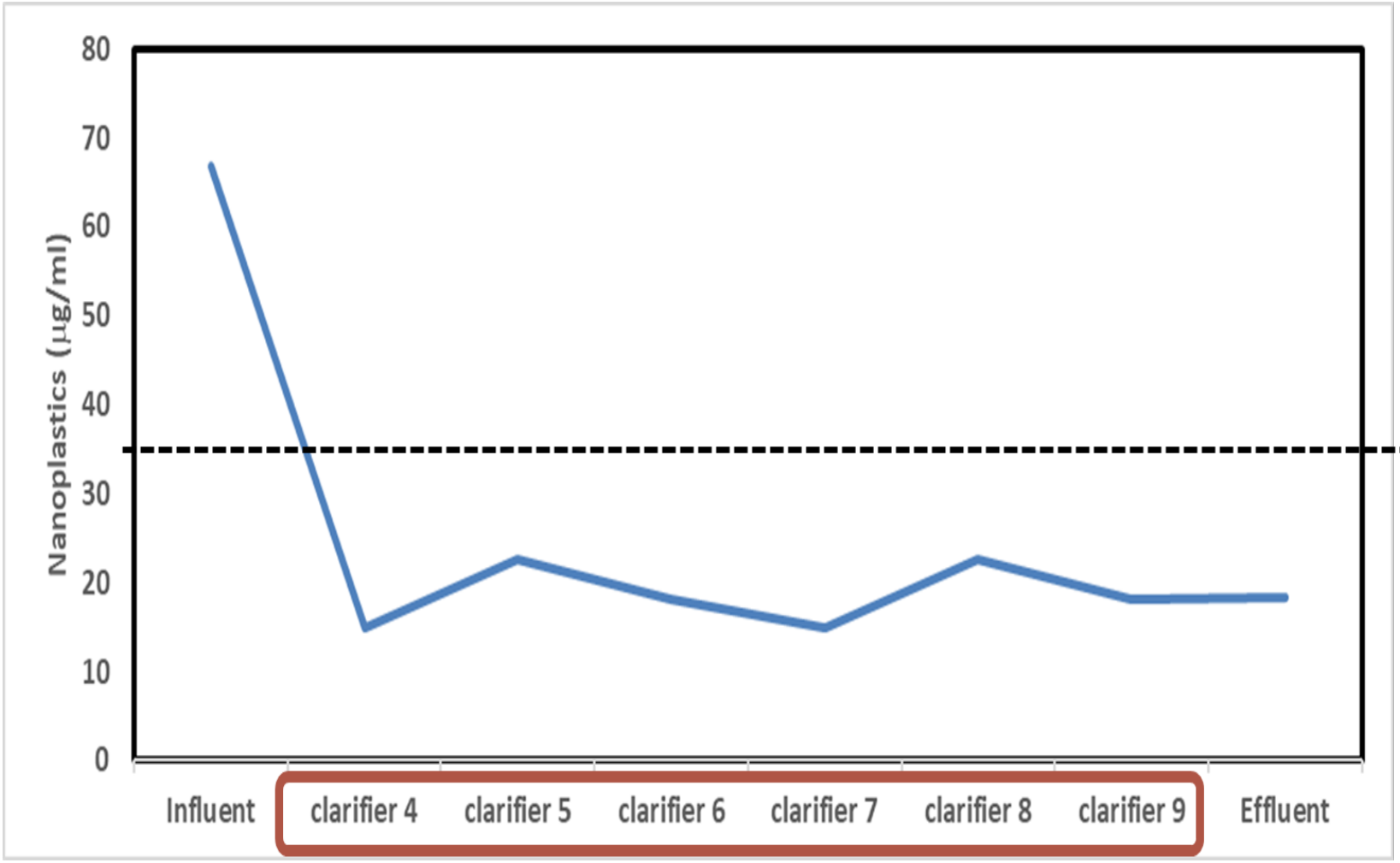


	1	2	3	4	5
Influent	80.6	50.1	68.4	59.2	62.3
Effluent	33.3	50.1	36.3	28.7	36.3

Nanoplastics are reduced in Effluent compared to Influent Wastewater Samples 6-month study



Clarification Reduces Nanoplastics in Effluent Water



Conclusions

- A one-step nanoplastics detection method has been developed using a hand-held fluorometer.
- Results can be obtained within 10 min without additional sample processing.
- Nanoplastics pollution in influent wastewater was monitored over a 6-month period.

Assay Limitations

- Assay does not differentiate different plastic types, size and shape. Need additional confirmation with spectroscopy.
- Only measures plastics in water suspension load not in trapped sludge waste.
- Cannot detect samples below 35 µg/ml.

Ongoing studies and Plans

- River Assessment Field Team Project-Measuring nanoplastic load in Central Indiana water streams. (30+ sites)
- Marion County Public Health Department- Measuring nanoplastic load in Indiana water streams (54 different sites)
- Improve Sensitivity-Test additional dyes such a DCVJ to improve assay sensitivity.