Aquatic microplastics: A unique niche for microbial communities with consequences for human and environmental health

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Abstract

Approximately 12 metric tons of plastic debris enters the ocean per year, all of it teeming with microbes, including potential pathogens and fecal indicator organisms. This study aims to identify if there is a selection driving the colonization of plastics, and due to its unique characteristics, find whether plastics hold a specific microbial composition that differs from that of naturally occurring substrates. We hypothesize that under constant environmental factors, plastics of the same chemical composition with different physical characteristics will select for specific microbial communities due to their shape and roughness. Lastly, we seek to corroborate previous data that anthropogenic input can provide different colonizing microbial communities than naturally occurring microbial colonizers. For our experiments, we constructed two deployments containing the same plastic and naturally-occurring substrates, which were placed in the Huron River by the city of Ann Arbor, Michigan, and in Cordley Lake, a small inland lake located nearby. For controls, existing substrates from Lake Michigan, Huron River, and Cordley Lake were also collected. To further investigate the results of this experiment, DNA extraction for 16S rRNA analysis was performed and samples were prepared for SEM analysis. Resulting SEM images showed a distinct microbial community on the Huron Lake versus Cordley lake, with Cordley Lake exhibiting high microbial diversity. Plastic samples were more densely populated than naturally-occurring substrates. Contrary to what we predicted, rough plastic showed less colonization than smooth plastic with the same chemical composition. 16Ss rDNA sequencing results and nutrient sample data are forthcoming. SEM results indicate that anthropogenic input did impact the MCC that colonized our experimental samples. Based off of visual observations, plastic was colonized more readily than natural occurring substrates.

Introduction

Global production of plastics reached 322 million metric tons in 2015, with a significant amount of plastic entering waterways each year and negatively impacting both aquatic and terrestrial environments[3]. Not only does plastic and its adsorbed toxins threaten public and environmental health, but microorganisms can also be introduced into aquatic environments via land-derived plastic litter[13]. Although extensive scientific work has been done on the "plastic microbiome" in the marine environment[2,3], there is a knowledge gap regarding the potential impact that plastics and their microbial communities have on freshwater systems[7].

Microplastics are ubiquitously found plastics that are less than 5mm either considered primary microplastics—intentionally manufactured in the form of microbeads, small pellets, or fibers—or secondary microplastics, created when larger plastics break down into smaller pieces[3]. Primary microplastics, which are typically washed down drains in residential and commercial settings, eventually enter wastewater treatment plants where they can be exposed to pathogenic microorganisms. Microplastics present a unique niche for microbes, which colonize the surface to form a biofilm. The microbial community composition (MCC) on microplastics has been shown to differ from that of naturally-occurring substrates and the surrounding water column[1,6]. Plastics offer a long-lasting habitat for opportunistic microbial colonizers due to the material's longevity, and its high buoyancy also allows for ease of long-distance transport of both the microplastic and its associated MCC[9].

The MCC on plastics could be a reservoir of pathogenic and fecal microbes that can be transported by using the microplastics as vectors, and because of the diversity of the biofilms, microplastics can introduce non-native species to habitats where they can become invasive. Therefore, plastic debris is a potential vector for the dissemination of microbes, though additional research on microbial transport across marine and freshwater systems is needed considering the potentially harmful effects are not yet known[2,6].

Because of its polymer composition, plastic should select for different microbial communities[7] than natural substrates. If plastics select for different microbial communities, then distinct MCC will show in plastics when compared to natural substrates. Further, we want to corroborate the findings of Keswaniet et al. (2016) and Manca et al. (2017)[2,6] that plastics can act as a vector for pathogenic and fecal microbes by comparing the microbial composition of plastics found and deployed in the Huron River, an area where anthropogenic input prevails, versus the plastics deployed in Cordley Lake, which has low anthropogenic input.

Methods

We compared microbial diversity and composition for biofilms formed on eight various substrates in a river to corroborate findings of plastics acting as vectors for pathogenic and fecal organisms and investigate the effects of anthropogenic input on biofilm composition.

Study sites

To differentiate communities under varying anthropogenic influences, three bodies of water across Michigan were sampled. Cordley Lake in Pinckney, Michigan is a small residential lake that only allows electric motors for watercraft and is considered to be a lake with little anthropogenic input (42.449, -83.869). Suttons Bay was sampled twice during a research cruise in Lake Michigan and has moderate anthropogenic input (44.999, -85.578; 45.123, -85.358). The Huron River flows through six counties within Michigan receiving the anthropogenic input of 650,000 residents[12]. In comparison to our other sampling sites, the Huron River is considered to have high levels of anthropogenic disturbance it starts at Big Lake near Pontiac Michigan, traveling 125 miles before draining into Lake Erie. Our sampling location (42.325, -83.799) was downstream of four of these counties, within Washtenaw county. Levels of anthropogenic input were determined based on the number of inputs to the system and the population size surrounding the body of water, relative to its size.

Collection of environmental conditions

In order to develop a baseline for microbial communities on environmental debris, naturally occurring and anthropogenically added substrates were collected from three bodies of water. In Lake Michigan two manta trawls were conducted, each towing for 30 minutes at an average speed of 3.2 miles per hour. Debris specimens were collected from the cod end of the trawl with sterile tweezers. At the Huron River and Cordley Lake site, detected debris was found and collected by hand, equipped with gloves. All acquired substrates were cut into four separate pieces- three of which were placed into cryovials and covered with 1x Phosphate Buffered Saline (PBS) for DNA extractions, and the fourth piece was put into a cryovial containing 80uL of 25% aqueous glutaraldehyde and 3mL of PBS for scanning electron microscopy (SEM). All samples were flash frozen in liquid nitrogen until returning to the lab for storage at -20°C. Debris was collected based on obtainability, therefore not all substrates were collected at each site.

For analysis of microbial communities living in the water at each of our three sites, 500mL of surface water was collected and filtered using a handheld syringe and 47 mm Swinnex filter

system (SX0001300l MilliporeSigma; MA, USA) outfitted with a 3µm Isopore filter (TSTP04700, Millipore). This filter contained the particle-associated (PA) bacteria. The remaining filtrate from this step was then used to undergo filtration onto a 0.22 µm filter (GSWP04700, Millipore) to capture the free-living (FL) fraction of our sample. Filters were recovered from Suttons Bay, Lake Cordley, and the Huron River.

For basic water chemistry and nutrient analysis of our sampled water, one liter of unfiltered water was collected in acid-washed bottles. These were kept at 4°C until analysis.

Experimental biofilms

The experimental setup consisted of two near identical deployments, one stationed in the Huron River, and the other in Cordley Lake. For each body of water, a wood plate was submerged roughly 4 inches underwater to allow the formation of biofilms on different substrates over a period of 4 weeks. Each plate contained randomly distributed substrates of glass beads, wood dowels, pipette tips (polypropylene), large pore styrofoam (foamed polystyrene), small pore styrofoam (foam polystyrene; Huron River only), twine (polypropylene), hard plastic cups (rigid polystyrene), and biodegradable straws (modified cornstarch). Large pieces were cut to 1cm width. Four strips of each substrate were created so that individually, each strip was nailed down to each edge of the wooden plate. For the Huron River deployment, a rope was threaded through a hole created in the center of the wooden plate. This was fastened to a tree from above and anchored to the river bed for stability against the river current (Figure 1). For the Cordley Lake deployment, an anchor was attached for stability and a buoy was tied to the deployment for identification of positioning.

Figure 1. Deployed experimental setup. (Huron River)

Sequencing and Data Analysis

To prepare for DNA extraction, substrates were cut into smaller pieces using sterile scissors and passed through four successive petri dishes of sterile deionized water and one of DNAse-free water with sterilized tweezers to remove only loosely attached particles, including sediment. Substrates were then placed into a 1.5 ml tube and cells were homogenized using the QIAshredder kit (Qiagen, cat. no. 79656, Germany). DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (cat. no. 69504) and samples were eluted twice in 50 µL Buffer AE and kept at 4C until submitted for sequencing.

DNA sequence data was generated by amplifying the 16S rRNA-encoding gene using barcoded dual-index primers of the V4 region developed by Kozich et al.[4] then sequencing using the Illumina MiSeq system at the University of Michigan Microbial System Molecular Biology Laboratory. Sequencing data was processed using the mothur software package (v. 1.35.1)[5] and analyzed using the phyloseq package in R.

Scanning Electron Microscope (SEM) Imaging

Substrate samples were fixed in 80µL glutaraldehyde with 3 mL PBS and either flash-frozen in liquid nitrogen or stored at -20C. One to three weeks before imaging, samples were rinsed with PBS and serially passed through 9 baths of varying concentrations of ethanol (EtOH) diluted in Milli-Q water and Bis(trimethylsilyl)amine (HMDS): 25% EtOH, 50% EtOH, 70% EtOH, 95% EtOH, 100% EtOH, 100% EtOH, 2:1 EtOH:HMDS, 1:1 EtOH: HMDS, and 100% HMDS. Samples were dessicated under a fume hood for 1 day to 1 week. 1 day to 1 week before imaging, samples were mounted by affixing to double-sided carbon tape and colloidal silver paste conduction was done. Samples were sputter-coated with gold and then imaged using the TESCAN MIRA3 FEG scanning electron microscope at the University of Michigan Microscopy and Image Analysis Laboratory. Fields of view were chosen to optimally view both biofilm and substrate surface structure[4].

Results

To determine whether microbes select for substrates based on chemical or physical properties, the MCC of eight experimental substrates from the Huron River and Cordley Lake were

compared. Based on SEM micrographs, a four-week exposure of experimental deployments to the natural environments revealed heterogeneous biofilms colonized by diatoms.

SEM Images for Experimental Setup Substrates

Plastic substrates: Pipette tip (polypropylene), twine (polypropylene), large pore styrofoam (foamed polystyrene), small pore styrofoam (foamed polystyrene - only on Huron River deployment), and hard plastic cup fragments (rigid polystyrene).

Natural substrates: Biodegradable straws (made from modified cornstarch), glass beads, wooden dowels, and metal.

Pipette Tip (Polypropylene)

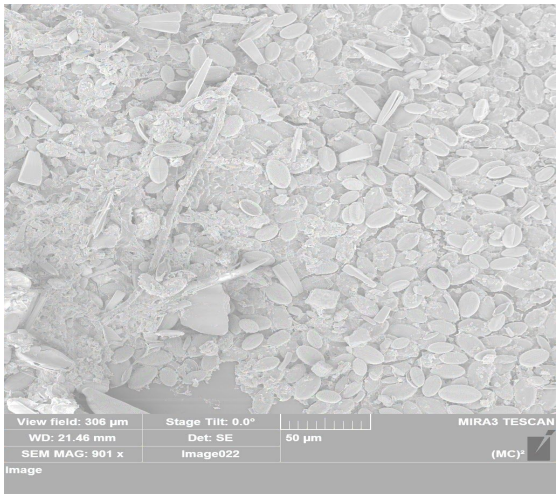

Fig. 2 Pipette tip SEM from Huron River

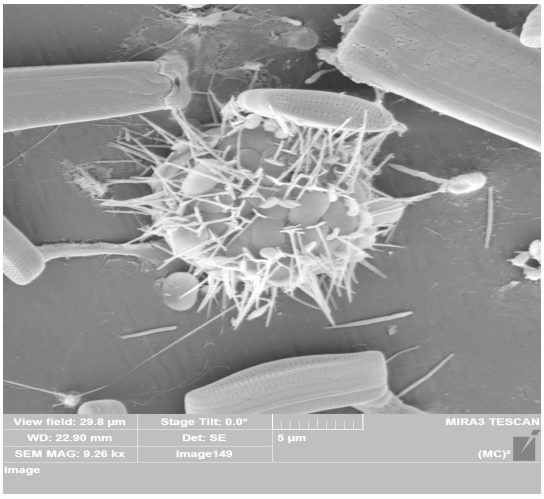

Fig. 3 Pipette tip SEM form Cordley Lake

The SEM results for the pipette tip from the Huron River deployment show the surface being highly populated by the same species of diatoms from the genus *Cocconeis*[15]. The SEM results for Cordley Lake showed more diversity; some diatoms form the genus *Adlafia.* Additionally, the Cordley Lake SEM results shows an agglomeration of cells forming a structure on the biofilm. Some bacterial cocci are found in the biofilm, and the matrix of the biofilm is clearly visible since the microbes are more sparse.

Twine

SEM images from twine samples in the Huron River deployment showed colonization by organisms with a coccobacillus morphology. These may include bacteria based on their observed length and dissimilarity to the diatoms we observed on other substrates. Microbial colonization was seen on the twine fibers alongside extracellular material. Images were not obtained for twine from the Cordley Lake deployment due to samples being lost during SEM preparation.

Fig. 4 Microbial colonization on twine from the Huron River

Large Pore Styrofoam (Foamed Polystyrene)

The SEM images obtained from the large pore styrofoam pieces on the deployments set in both the Huron River and Cordley Lake show a moderate amount of surface colonization by diatoms, although the styrofoam ball from the Huron River shows more microbial growth overall than the ball from Cordley. In both samples, there is more colonization along the curved ridges of the styrofoam surface than the flatter parts of the surfaces. There is a strong distinction in the diatoms' shape and size between the substrates from the two locations.

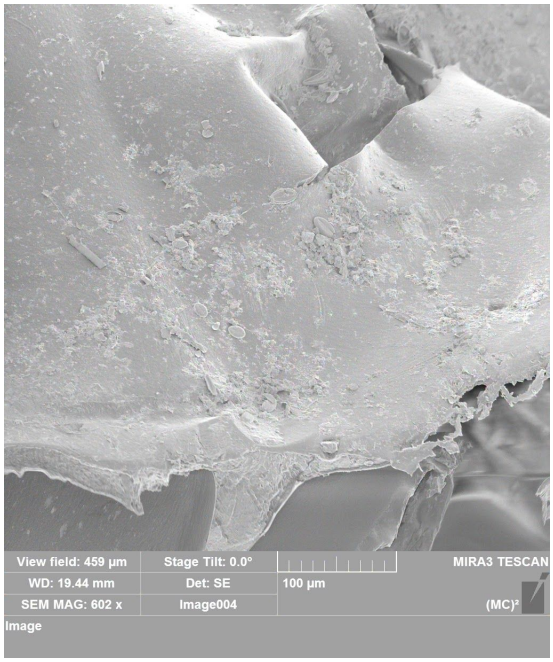

Fig. 5 Large Pore Styrofoam HR - Panoramic

At the Huron River, the biraphid, coffee-bean shaped diatoms found on the surface appear to match those found on other substrates from that location. However, they are much less abundant on the large pore styrofoam sample than other substrates, like the pipette tip (rigid polypropylene) and biodegradable straw (rigid modified cornstarch). Additionally, there is evidence of microbial growth other than diatoms, as observed in Fig. 3.

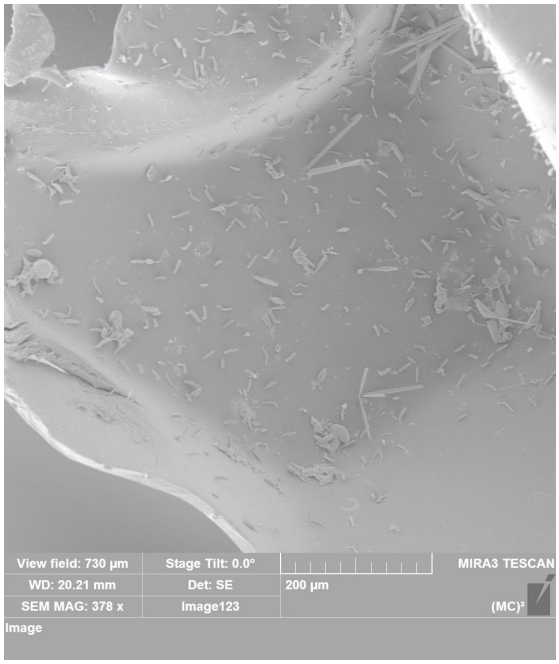

Fig. 6 Large Pore Styrofoam CL - Panoramic

From Cordley Lake, the large pore styrofoam ball used on the deployment showed long rectangular diatoms growing in groups radially in multiple different spots on the surface. We observed the araphid curved rectangular diatoms found on the biostraw in Cordley Lake to also be growing on the surface of the foamed polystyrene sample.

Small Pore Styrofoam (Foamed Polystyrene - only on Huron River deployment)

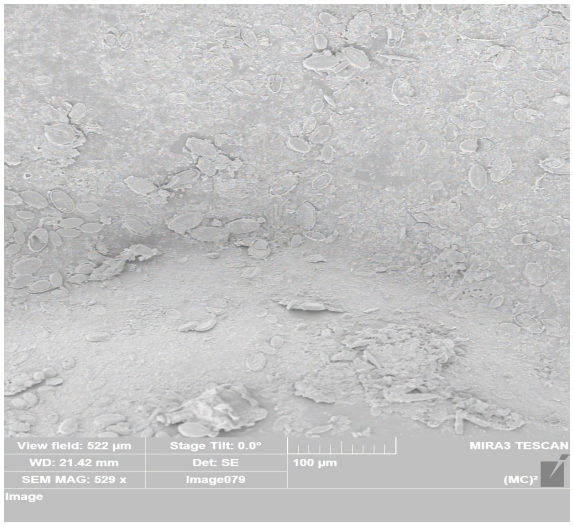

Fig. 7 Small Pore Styrofoam HR - Panoramic

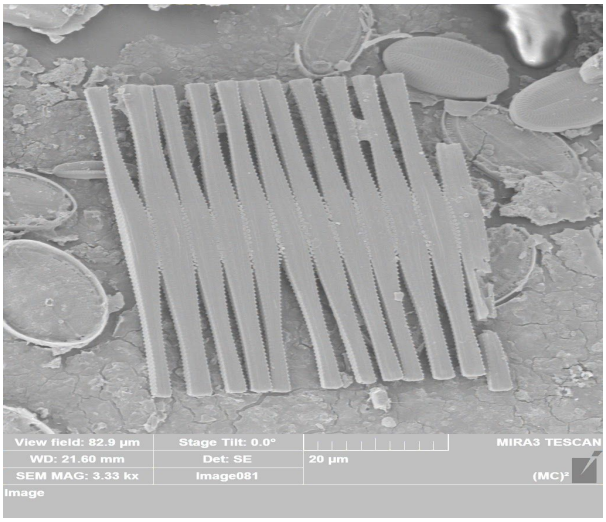

Fig. 8 Small Pore Styrofoam HR - Nanozipper Diatom

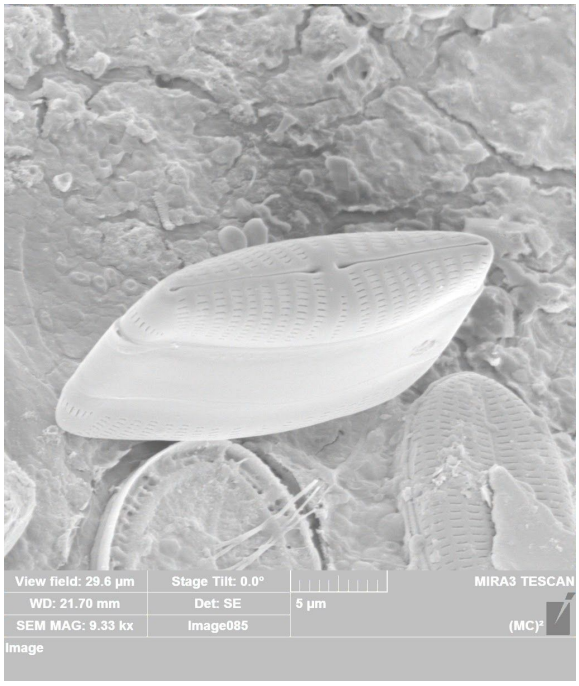

Fig. 9 Small Pore Styrofoam HR - *Navicula*

The SEM images of the small pore styrofoam strip on the deployment in the Huron River shows the surface to be covered in biofilm. There is very little exposed styrofoam surface, and we observed different parts of the surface flaking. The biraphid diatoms commonly found on other substrates in the Huron River were abundant on the surface. However, there were also a variety of other types of diatoms. First, we observed nano-zipper diatoms (Fig. 8), which were not found on other plastic substrates in the Huron River or in Cordley Lake. We also observed symmetric biraphid diatoms we believe to belong to the genus *Navicula*, shown in Fig. 9.

Hard Plastic Cups (Rigid Polystyrene)

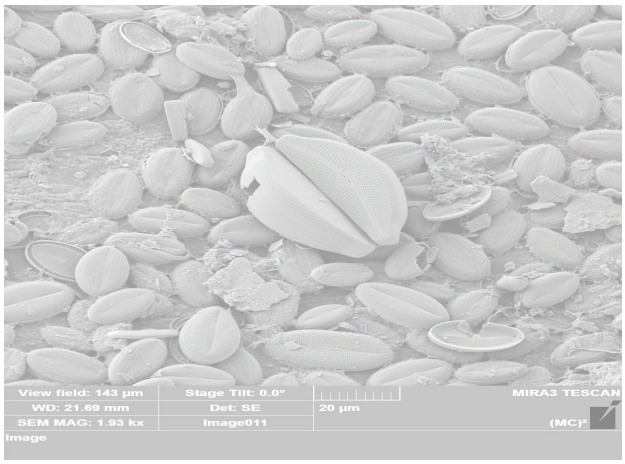

Fig 10. Hard Plastic HR - Panoramic

Fig. 11 Hard Plastic HR - Matrix

From the SEM images of hard plastic cups at the Huron River site, biraphid diatoms with a line down the middle can be observed in high abundance. Beneath them seems to lie a layer of biofilm involving cocci-shaped cells. We also observed a larger diatom with bilateral symmetry from this site. Some diatoms are observed to be linked to each other with what looks to be a stretchy component in between, serving as an anchor.

Fig. 12 Hard Plastic CL - Matrix

From the SEM images of the hard plastic cup obtained from Cordley Lake site, we observed the typical biraphid diatom in our sample, as well as the curved rectangular araphid diatom. Minimal numbers (that can be seen from our images) of coccal cells can be observed in addition to a congregation of rod-shaped microorganisms. One unique observation is the presence of a single long filament embedded into the biofilm matrix on the surface of our hard plastic sample.

Biodegradable Straw (Modified Cornstarch)

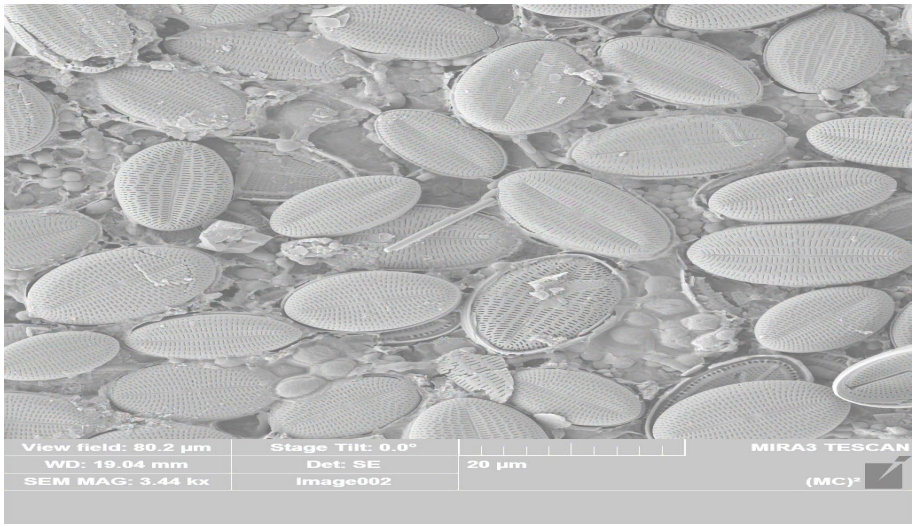

Fig 13. Bio-straw from HR

From the SEM images of the biodegradable straws at the Huron River site, the common biraphid diatoms with a line down the middle can be observed, with biofilm and cocci-shaped cell distributed in some areas under the diatoms. Some diatoms are connected to each other or the biofilm below with filaments. The slits for these diatoms are all throughout its surface.

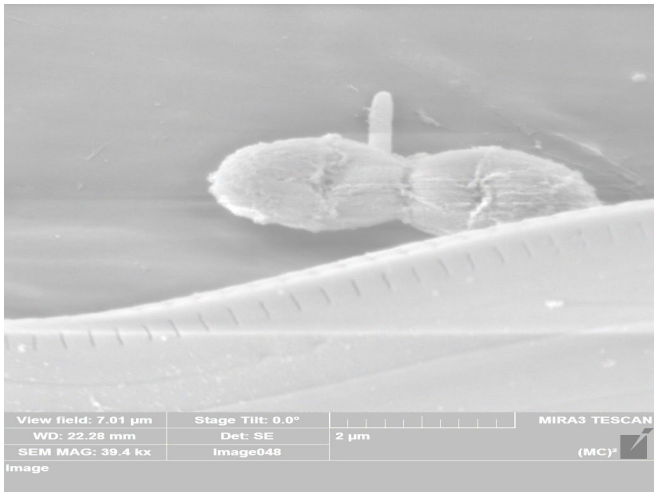

Fig 14. Bio-straw from CL - close up

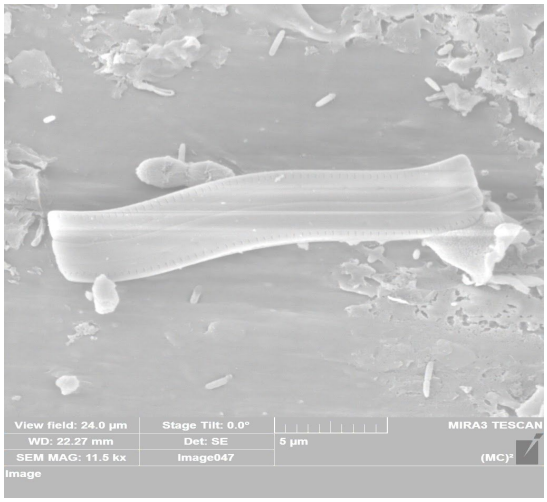

Fig 15. Bio-straw from CL - panoramic

From the SEM images of biodegradable straws that we obtained from the Cordley Lake site, we observed a microbe in which two cells are linked together. In addition to this, we observed an araphid diatom with a curved rectangular shape. The slits for this diatom are on either sides of the length of the diatom. Several rod-shaped microbes can be seen around the single diatom.

Glass

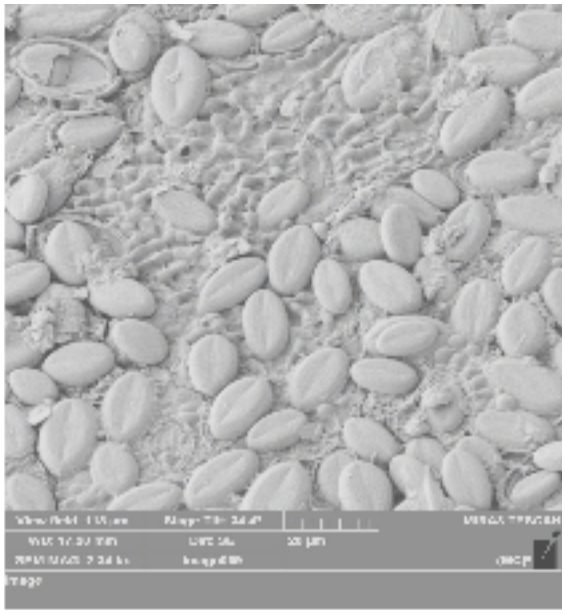

Fig. 16 Diatoms dominate glass bead surface at the Huron River

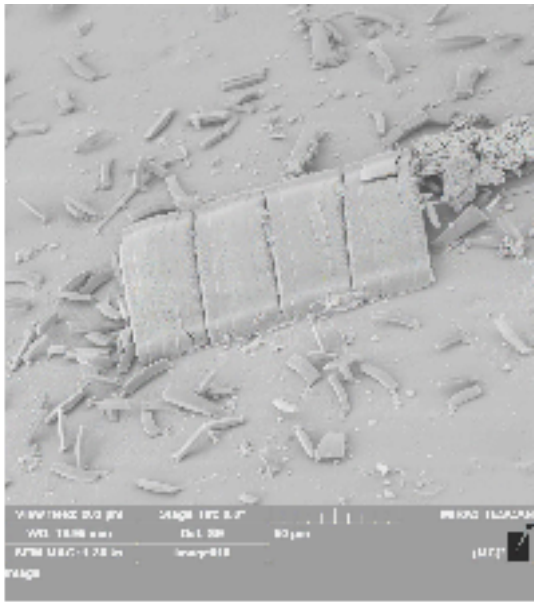

Fig. 17 Diatoms on biofilm on glass bead CL

Diatoms were prevalent on glass surfaces of deployments at both the Huron River and Cordley Lake. Monoraphid diatoms dominated the surface of the glass surface at the Huron River while slightly curved araphid diatoms were present both forming chains and in singular forms. Diatom abundance was relatively higher for the Huron River than Cordley Lake.

Wood

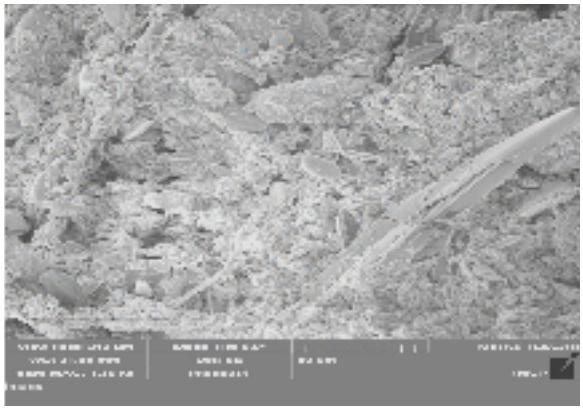

Fig. 18 Monoraphid diatoms found on wood at HR

SEM images of the wooden dowel were only obtained from the Huron River deployment due to samples being lost during preparation. Limited colonization on the surfaces of wood substrates was observed. A mixed matrix of diatoms were seen, within the crevices of the wood surface.

Metal

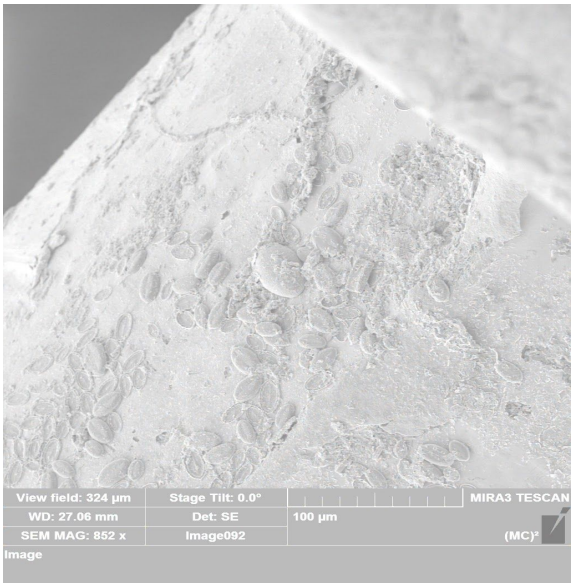

Fig. 19 Image of metal rod from the Huron River.

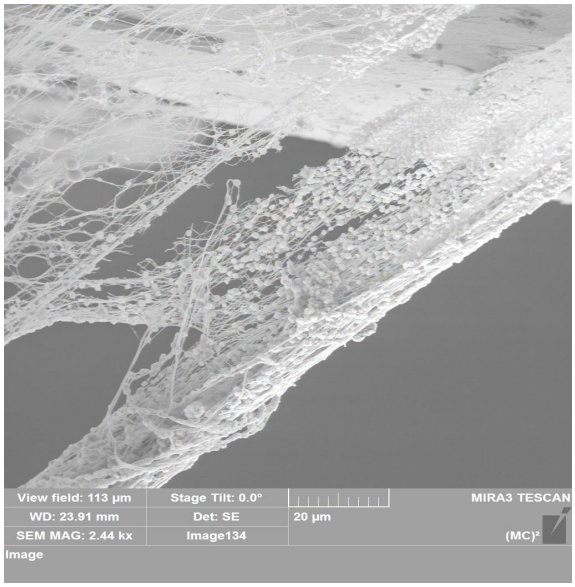

Fig. 20 Biofilm observed on end of metal rod from Cordley Lake.

Microbial colonization on the metal rod from the Huron River deployment appeared to be primarily limited to diatoms on the surface. The metal rod from Cordley Lake showed a complex biofilm consisting of extracellular material linking the rod and endpiece. The dense organic material contained abundant cocci, which may include bacteria due to their observed size. The fibrous material may also include fungal hyphae due to its webbed appearance.

Discussion

SEM imagery revealed distinct species of early diatom colonizers on the substrates deployed in the Huron River compared to the colonizers on the deployment sampled from Cordley Lake, which can be seen through the colonization of cells on a larger surface area at the Huron River versus a clear lack of cells on most SEM images shown from Cordley Lake. This can be observed for the hard plastic cup samples, the biodegradable straw samples, the pipette tips samples, and the glass samples. Comparing these samples from the two sites showed that the Huron River sample exhibited an abundance of cells in which the substrate is fully covered, whereas the Cordley Lake sample was the opposite in which the minimal number of cells observed would congregate in groups, leaving portions of the substrate covered only in extracellular matrix. This supports our hypothesis to a certain extent, as the Huron River samples contained higher abundance, but also contradicted our hypothesis because Cordley Lake samples were observed to have higher diversity.

It is expected to see diatoms as early colonizers since they are known to colonize readily due to their ubiquity in aquatic environments, and their role in "feeding" such environments because of their photosynthetic abilities[15,16]. Due to their ability to feed surrounding environments, diatoms play an important role in their ability to create environments hospitable to subsequent biofilm colonizers. While our sampling period was relatively short (four weeks), the presence of apparent higher species diversity in Cordley Lake supports our hypothesis that a freshwater system with lower anthropogenic input will have a distinct and MCC compared to a more urbanized waterway, the Huron River. Future analysis of our 16S rDNA sequencing results will allow us to quantify how the taxonomic composition of the communities between the two sites differs. We plan to determine both the alpha and beta diversity of our samples and examine the communities for the presence of genera linked to environmental and public health concerns[2,3,6].

Based on observational methods, plastic samples deployed in the Huron River appeared low in species richness but high in abundance. Overall, smooth plastics showed higher colonization than rough plastic of the same chemical composition. By comparison of the SEM images from the Huron River deployment of the three different surface textures of polystyrene, the smooth plastic cup, the small pore styrofoam strip, and the large pore styrofoam ball, there is an evident progression in the abundance of microbial growth, with the hard plastic cup having the most, while the large pore styrofoam had the least. All three substrates had the same chemical composition, therefore the difference in microbial growth can be attributed to surface texture. The SEM images obtained of the twine sample, which was the representative of rough polypropylene, were not sufficient for comparison with the pipette tip (smooth polypropylene). Therefore, it is unclear whether the trend in surface texture and colonization applies for different chemical compositions of plastic other than polystyrene. However, supporting the observation of microbes colonizing smoother substrates, plastics showed higher colonization than natural substrates with the exception of the smooth-surfaced glass substrate. These findings contradict our hypothesis that a rougher surface texture would result in more biofilm growth. Adding complexity to the results, there was greater abundance of microorganisms growing on the curved ridges of the styrofoam substrates than the flat portions, indicating that future studies on surface texture would be necessary to determine the cause of higher colonization of smooth plastic surfaces.

SEM imagery of experimental plastics exposed to natural environmental conditions for four weeks showed evidence of diatoms as an early biofilm colonizer. Relatively few bacterial microbes were found on the surface of these substrates suggesting a longer experiment would be needed to address questions regarding the MCC of microplastics. Another variable that could be accounted for in the future is the presence of coatings on experimental substrates. Because the

items used on the experimental deployments were prefabricated, it is difficult to know if their surfaces were free of any chemicals added during the manufacturing process. If this is the case, resulting communities on the surface of both plastics and natural substrates could incorrectly reflect substrate type.

Suttons Bay biofilms showed more bacteria than the biofilms from the deployment substrates, and those were mainly rod-shaped. These microplastics were not experimentally tested, but instead sampled in the field, and thus consist of samples of unknown age. Since the deployments were submerged for four weeks and showed diatoms as the majority community members, the presence of bacteria in Suttons Bay biofilms can be assumed to indicate an older biofilm. Future studies should incorporate an experimental set-up at the Suttons Bay or Lake Michigan locations.

Overall, the current study results indicate that plastic is more readily colonized than natural occurring substrates–with the exception of glass–during the four-week time frame in which we sampled. We conclude that anthropogenic input does have an impact on microbial communities on waterways based on our visual observations of distinct species in the Huron River deployment compared to the Cordley Lake deployment. The higher species richness of Cordley Lake likely indicates a healthy ecosystem or the presence of environmental factors favoring the observed colonization. Contrary to our predictions, we found higher colonization levels on smooth plastic polystyrene than rough polystyrene, even though the foamed polystyrene has greater surface area. We question if this is because nutrients would be more easily accessible to the surface colonizers on the smooth plastics. Further research is needed to characterize how the MCC on plastic substrates in these freshwater environments changes with a longer sampling period. Future analysis of our 16S rDNA sequencing data will yield insight into differences in the MCC between our substrates and sampling locations at the taxonomic level, and confirm whether plastics may act as a vector for pathogenic and invasive organisms in these environments.

Supplemental Data

Supplemental Table 1. Outline of sampling method at each site.

Sampling Site	**Samples collected**		**Analysis**
Lake Michigan	Environmental microplastics		DNA
			SEM
	Nutrients		Nutrients
Cordley Lake	Experimental substrates		DNA
			SEM
	Environmental microplastics		DNA
			SEM
	Environmental water	PA (3µm filter)	DNA
		FL (0.22µm filter)	
	Nutrients		Nutrients
Huron River	Experimental substrates		DNA
			SEM
	Environmental microplastics		DNA
			SEM
	Environmental water	PA (3µm filter)	DNA
		FL (0.22µm filter)	
	Nutrients		Nutrients

SEM Images - Huron River (HR) Natural Substrates

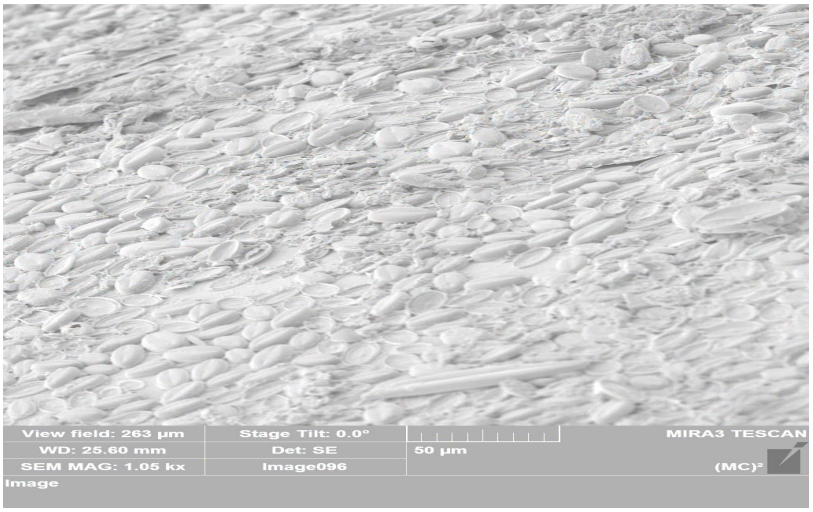

Fig. S1 Glass bead at HR - panoramic.

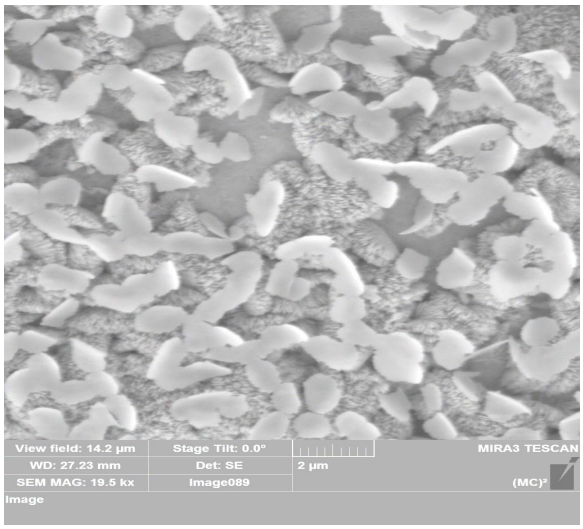

Fig. S2 Metal from HR - panoramic

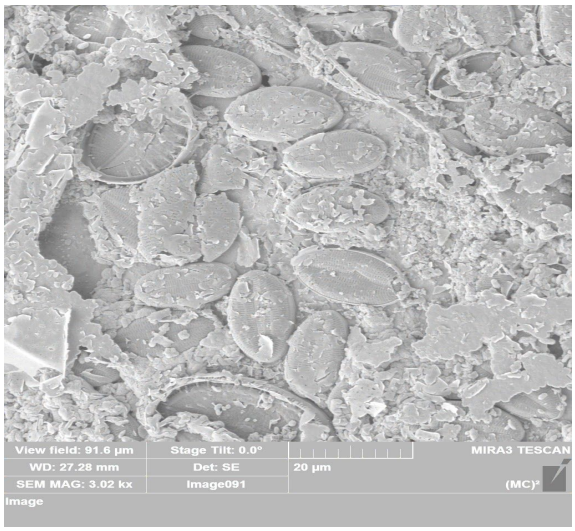

Fig. S3 Metal from HR - side view

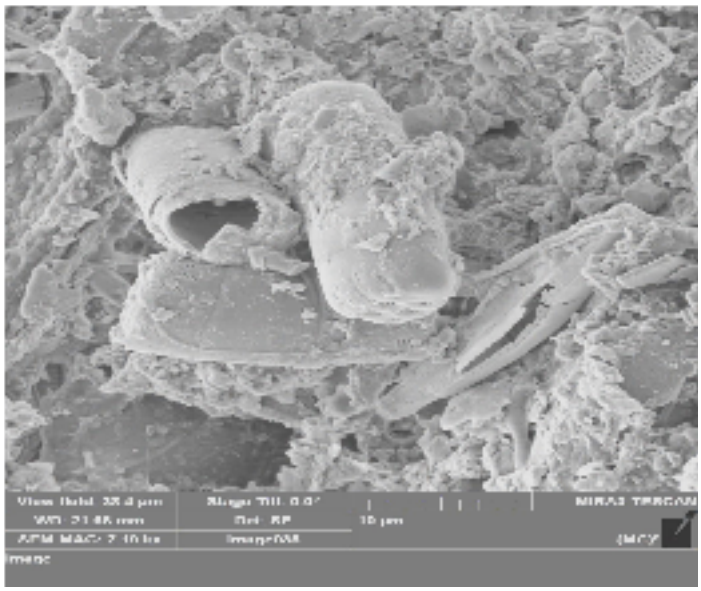

Fig. S4 Wood biofilm close up from HR

SEM Images - Huron River (HR) Plastic Substrates

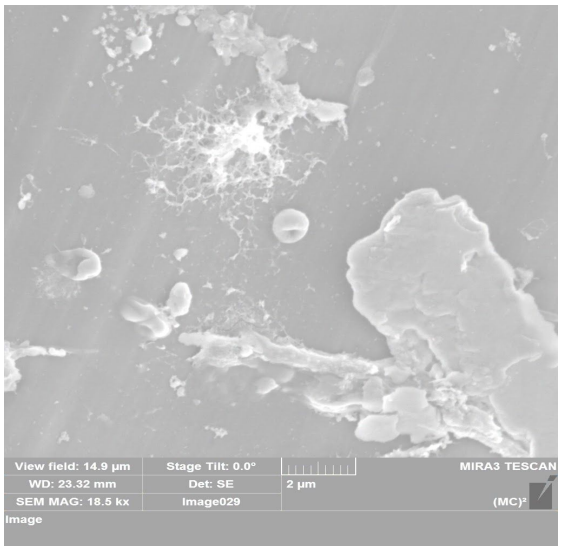

Fig. S5 Plastic twine form HR biofilm

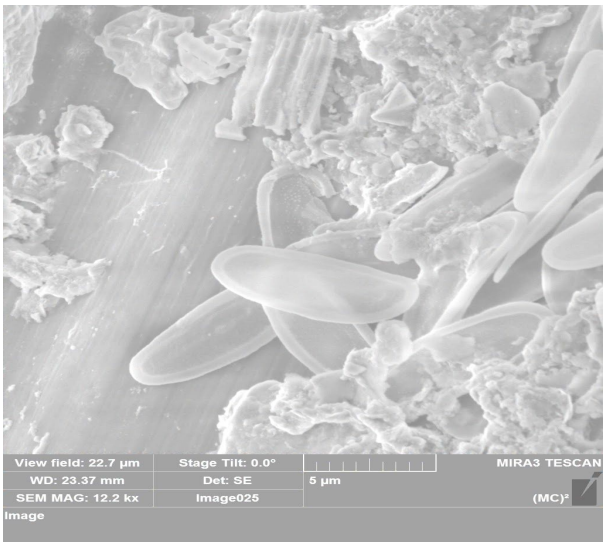

Fig. S6 Plastic twine form HR biofilm

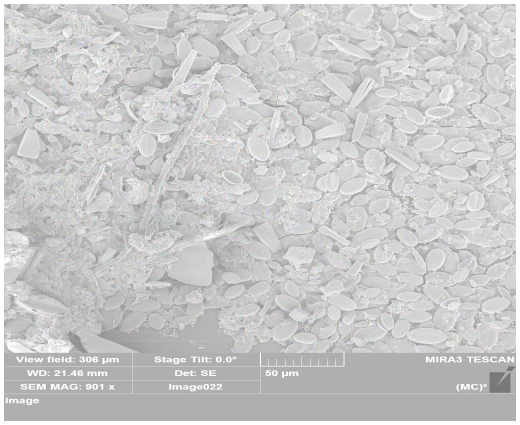

Fig. S7 Pipette from HR biofilm

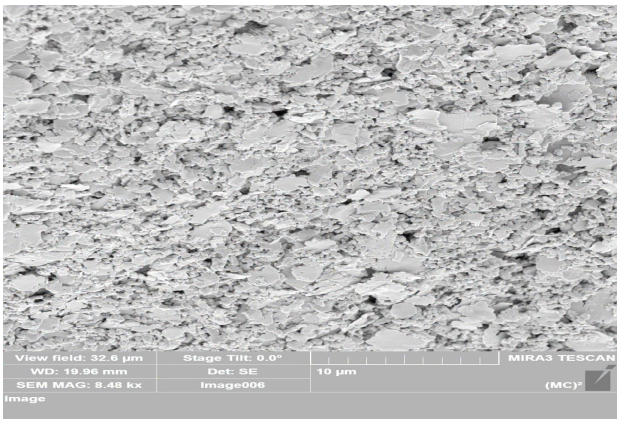

Fig. S8 Panoramic styrofoam from HR

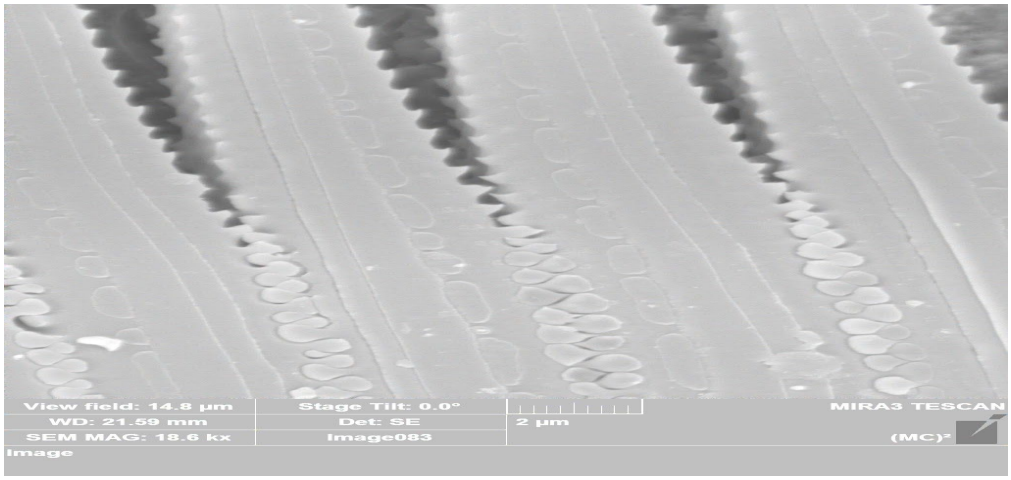

Fig. S9 Nano zipper close up HR styrofoam

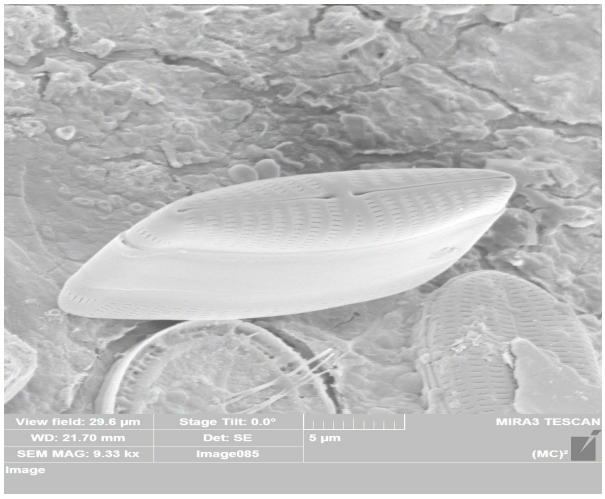

Fig. S10 Diatom found on HR styrofoam

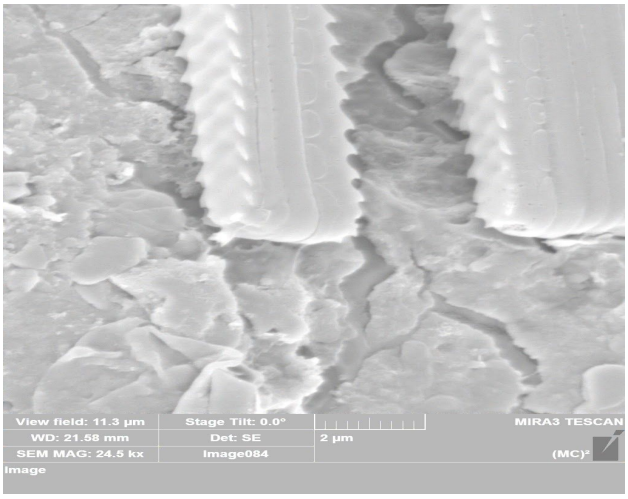

Fig. S11 Nano zipper diatom on HR styrofoam

SEM Images - Cordley Lake (CL) Natural Substrates

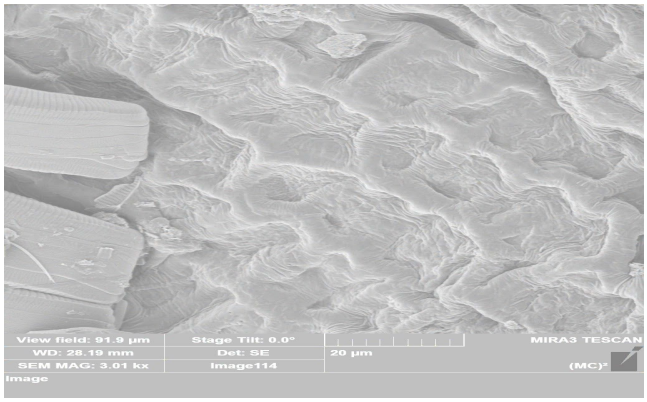

Fig. S12 Algae from CL closeup

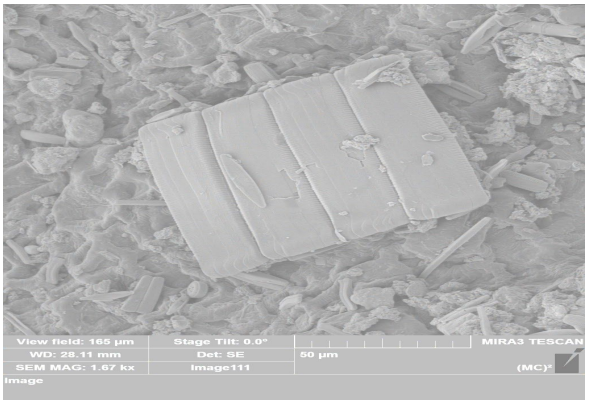

Fig. S13 Diatoms colonizing algae from CL

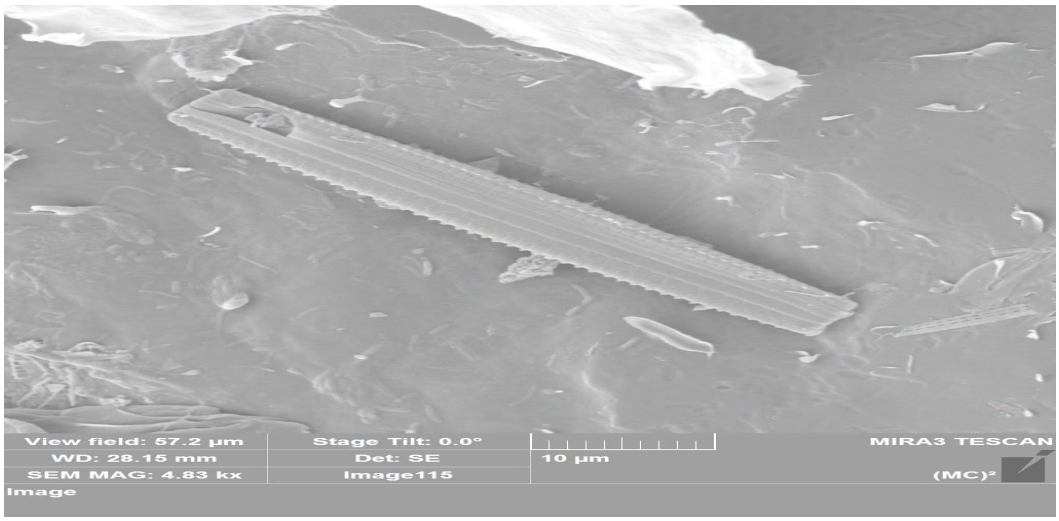

Fig. S14 Nanosaw diatom found on algae CL

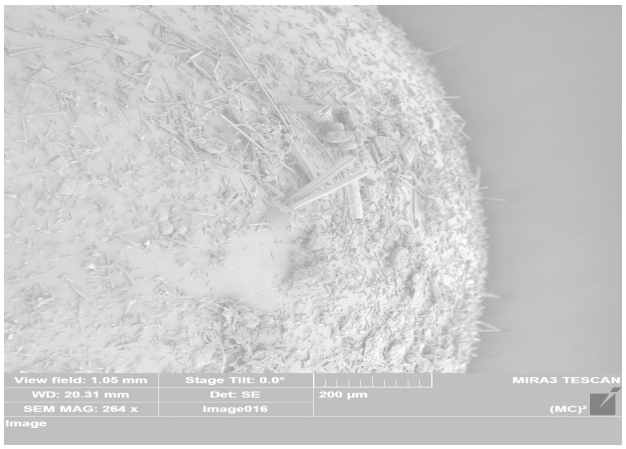

Fig. S15 glass bead biofilm panoramic CL

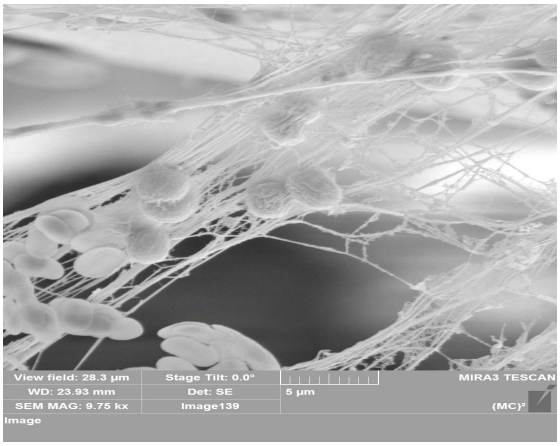

Fig. S16 Metal biofilm formation close up CL

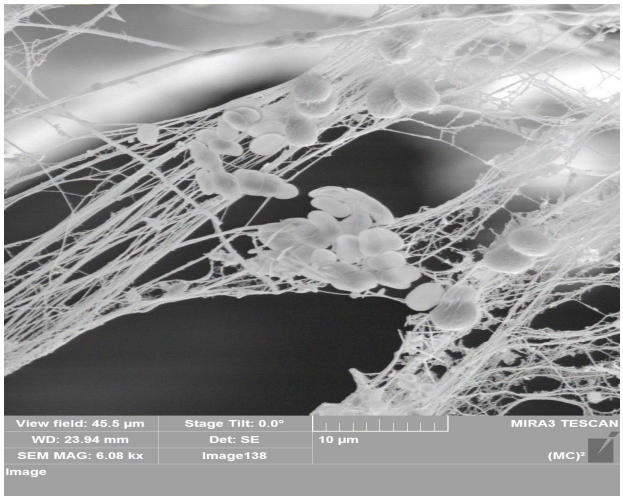

Fig. S17 Metal biofilm formation CL

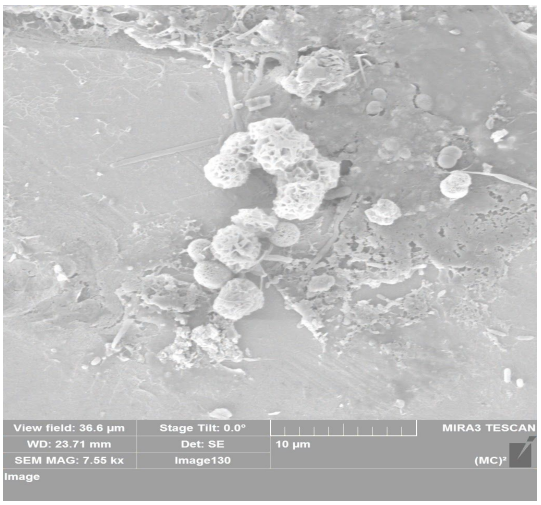

Fig. S18 Metal biofilm formation CL

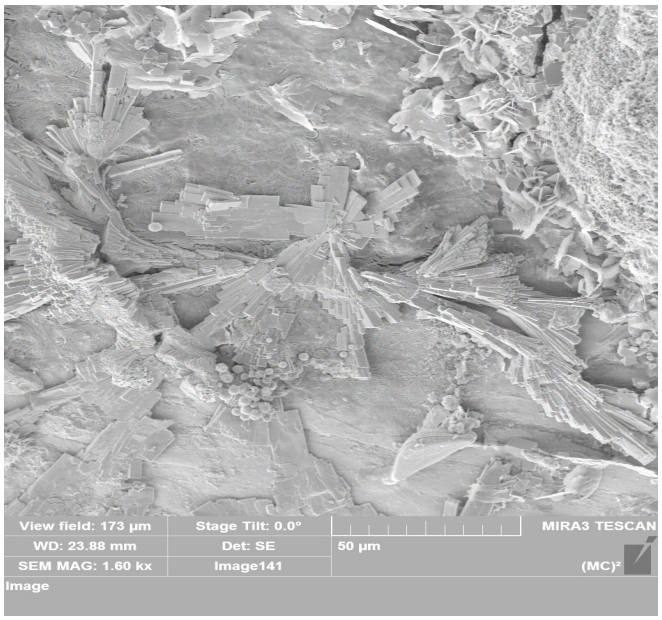

Fig. S19 Zinc flakes from metal CL

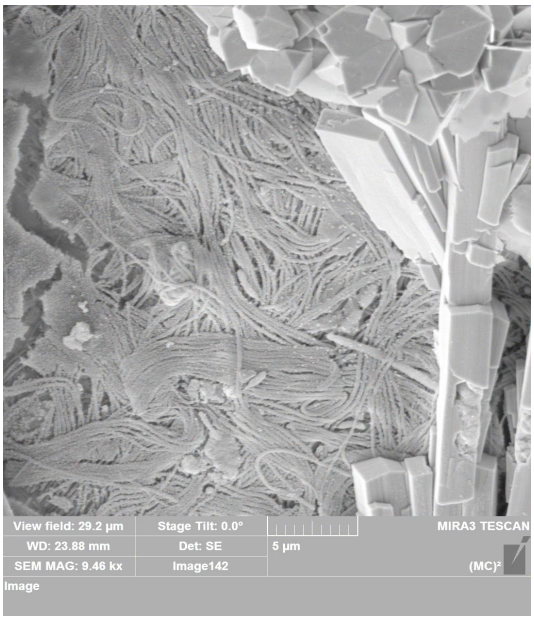

Fig. S20 Close up on metal from CL

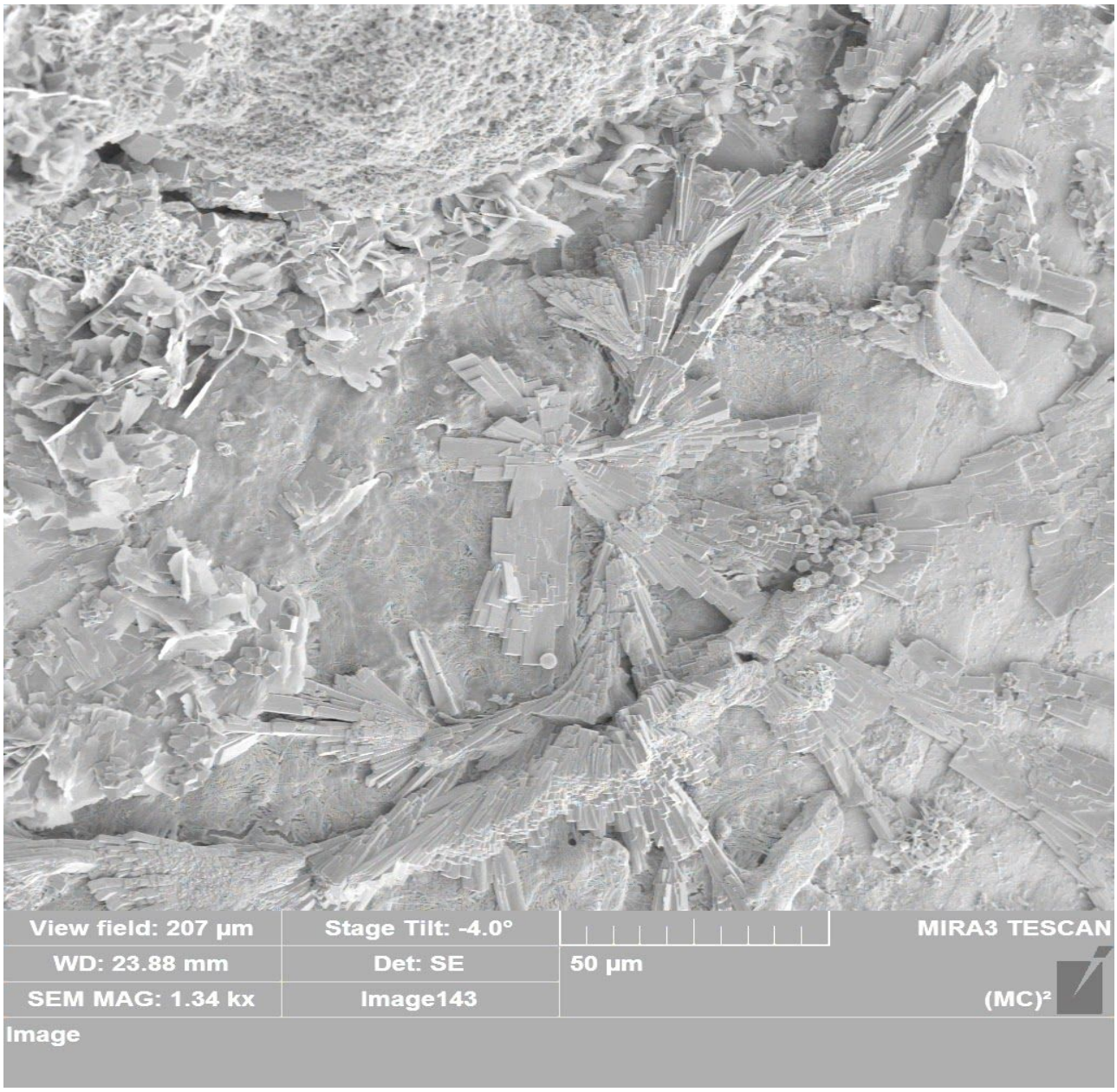

Fig. S21 Landscape form metal from CL

SEM Images - Cordley Lake (CL) Plastic Substrates

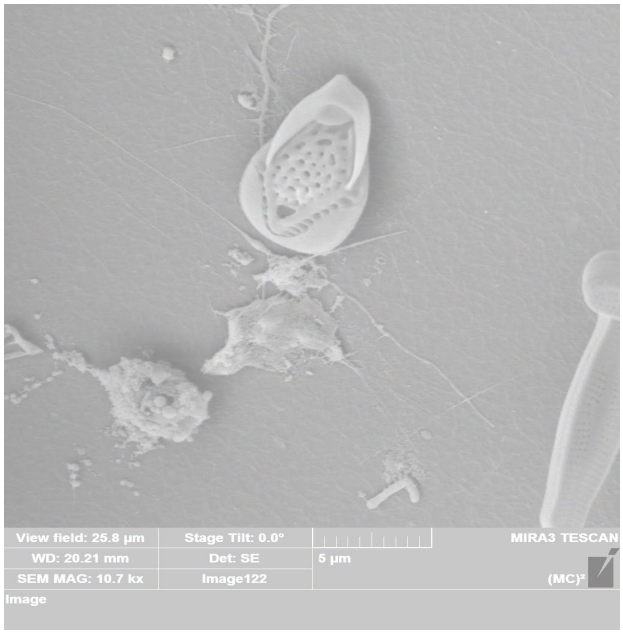

Fig. S22 Diatoms on styrofoam on CL

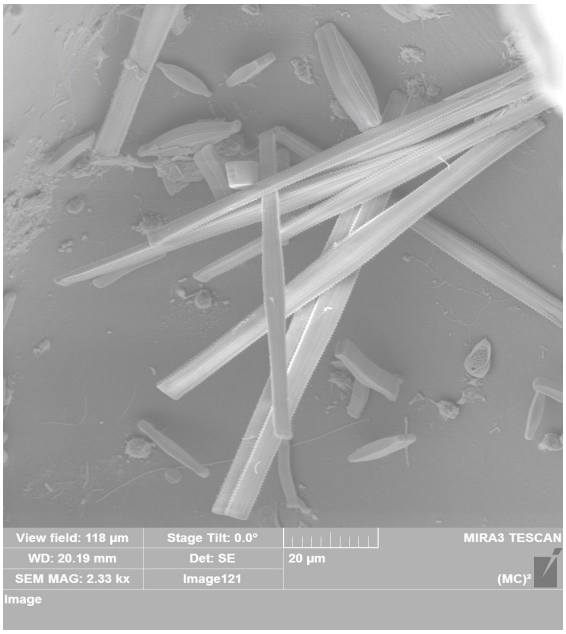

Fig. S23 biofilm formation on styrofoam on CL

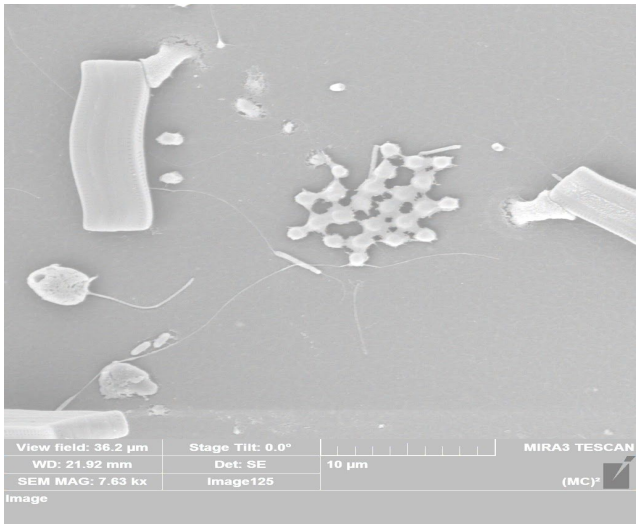

Fig. S24 Biofilm on styrofoam on CL

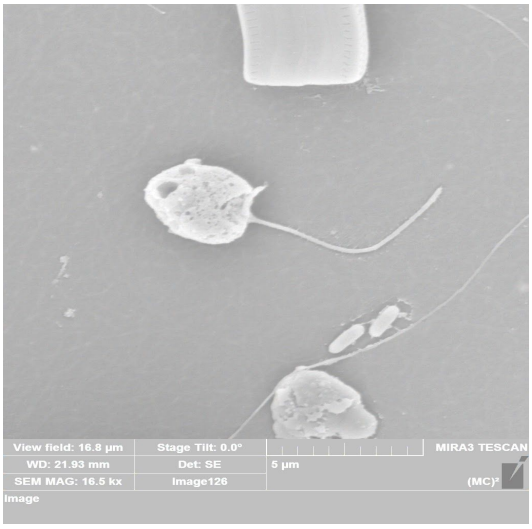

Fig. S25 Close up on styrofoam biofilm on CL

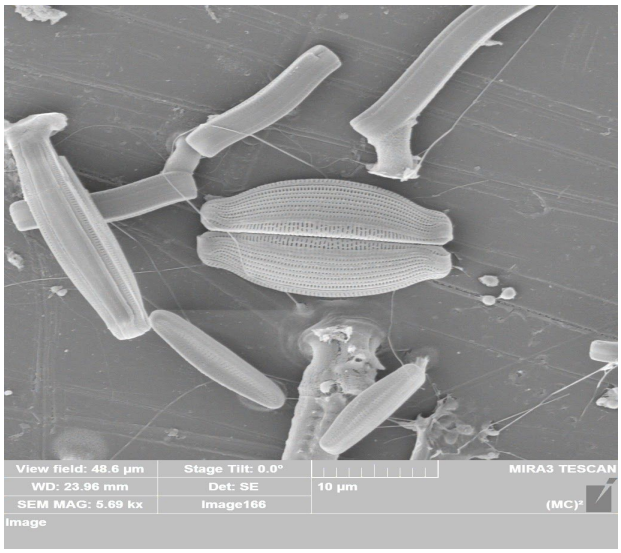

Fig. S26 biofilm formation on hard plastic CL close up

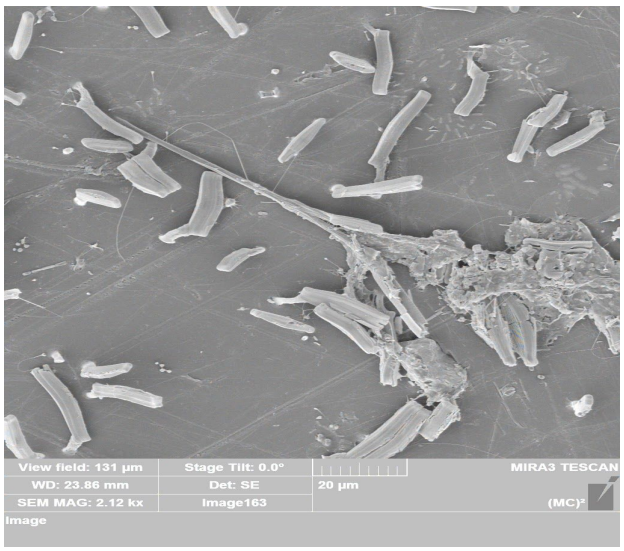

Fig. S27 Biofilm formation on hard plastic CL panoramic

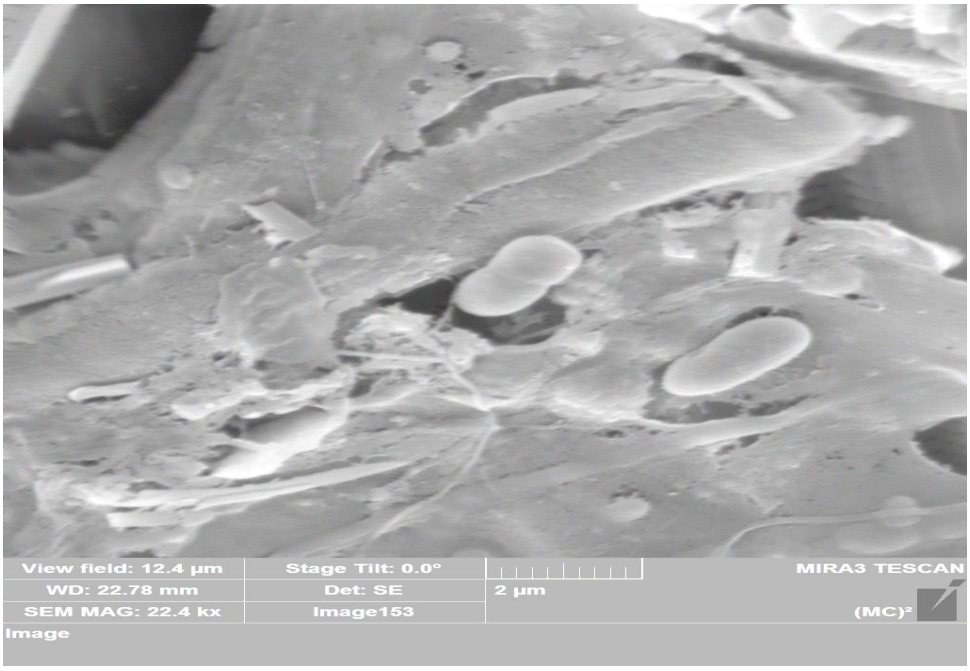

Fig. S28 Pipette tip biofilm close up CL

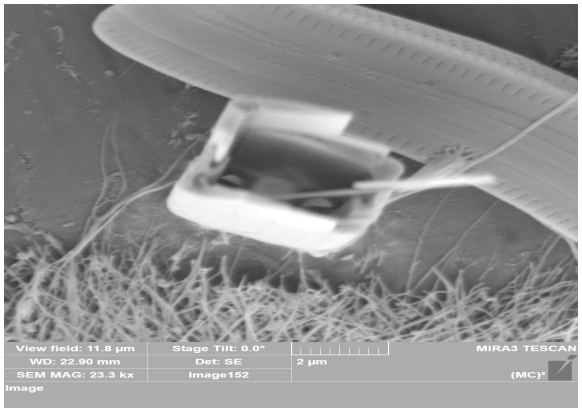

Fig. S29 Diatoms on pipette tip on CL

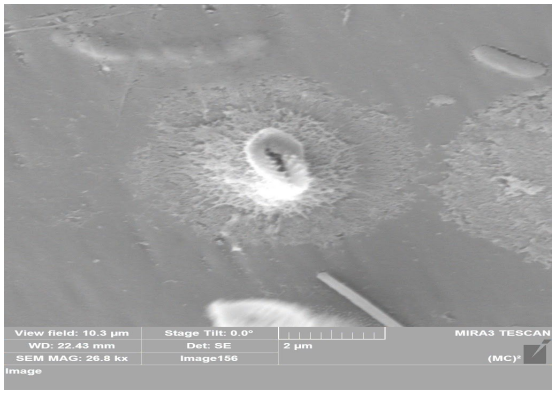

Fig. S30 Insertion point on biofilm on pipette tip CL

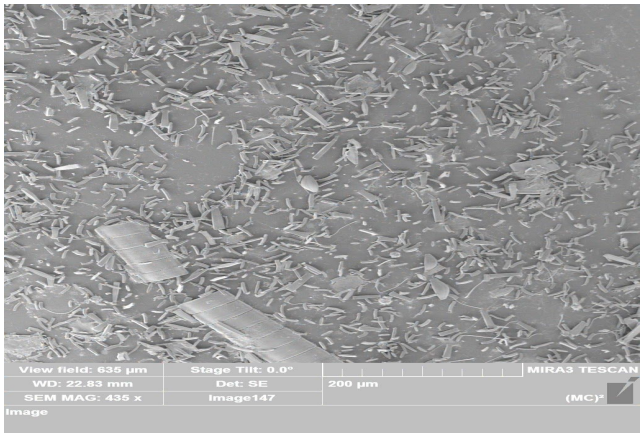

Fig. S31 Biofilm on pipette tip CL panoramic

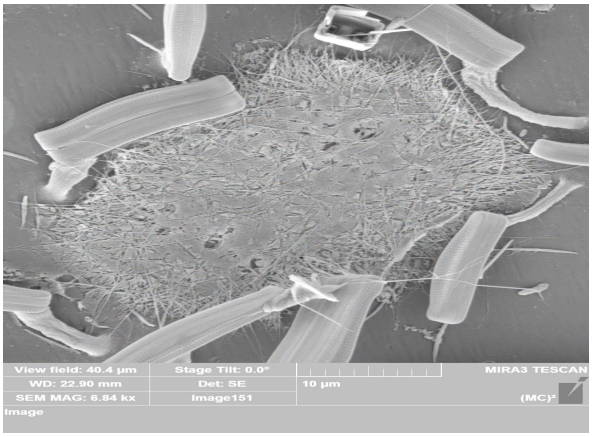

Fig. S32 Biofilm on pipette tip CL close up

SEM Images - Suttons Bay (SB) Microplastics

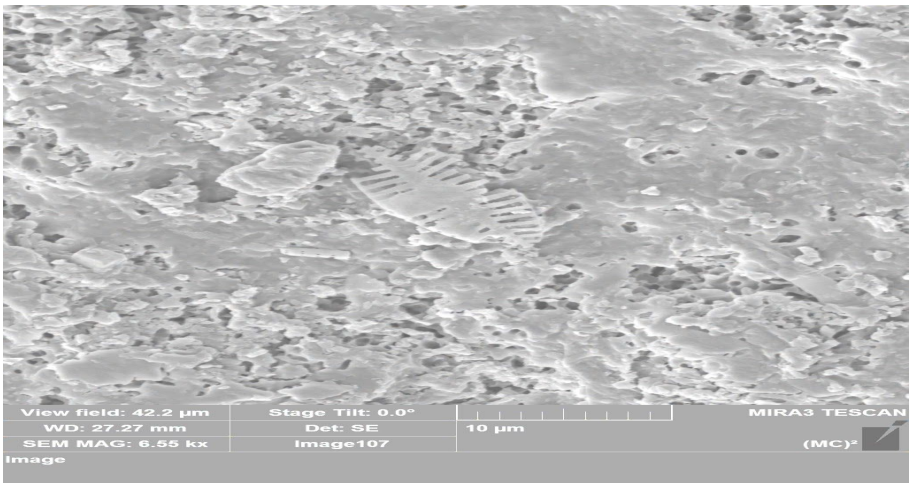

Fig. S33 Landscape on white microplastic from SB

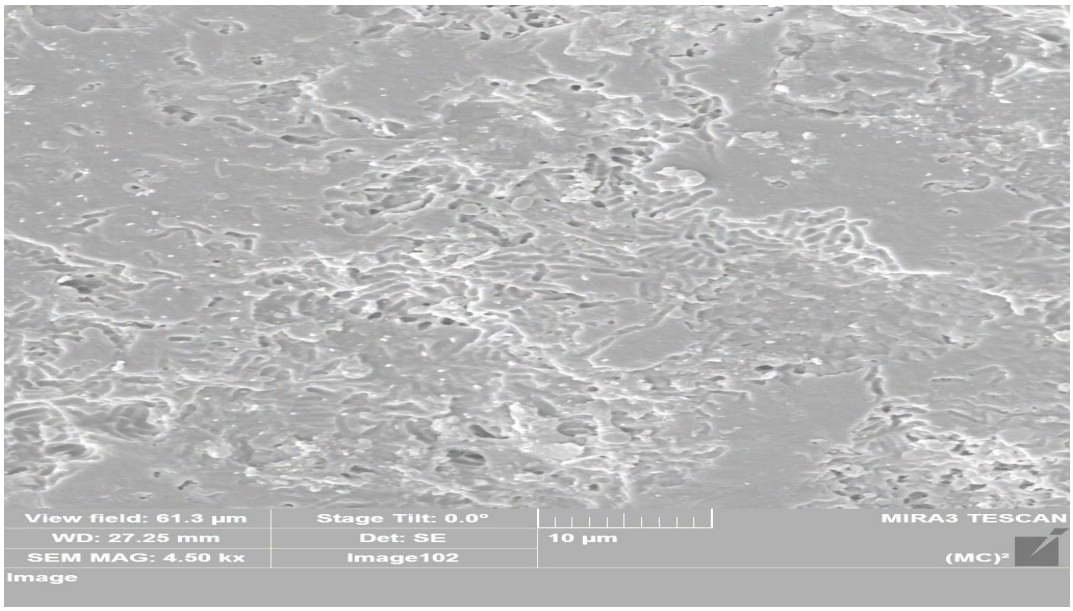

Fig. S34 Biofilm landscape on withe plastic form SB

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