Thuto Magome

Progress report:

Characterization of microbial assemblages on micro-plastics in a South African river system

SAMPLING SITES

- Sampling was conducted at five sites(3X) across the Mooi River, with Site M1 (co-ordinates: S26°29'36.3, E27°07'48.5) being the upstream site.
- River water was filtered *in situ* in search for microplastic

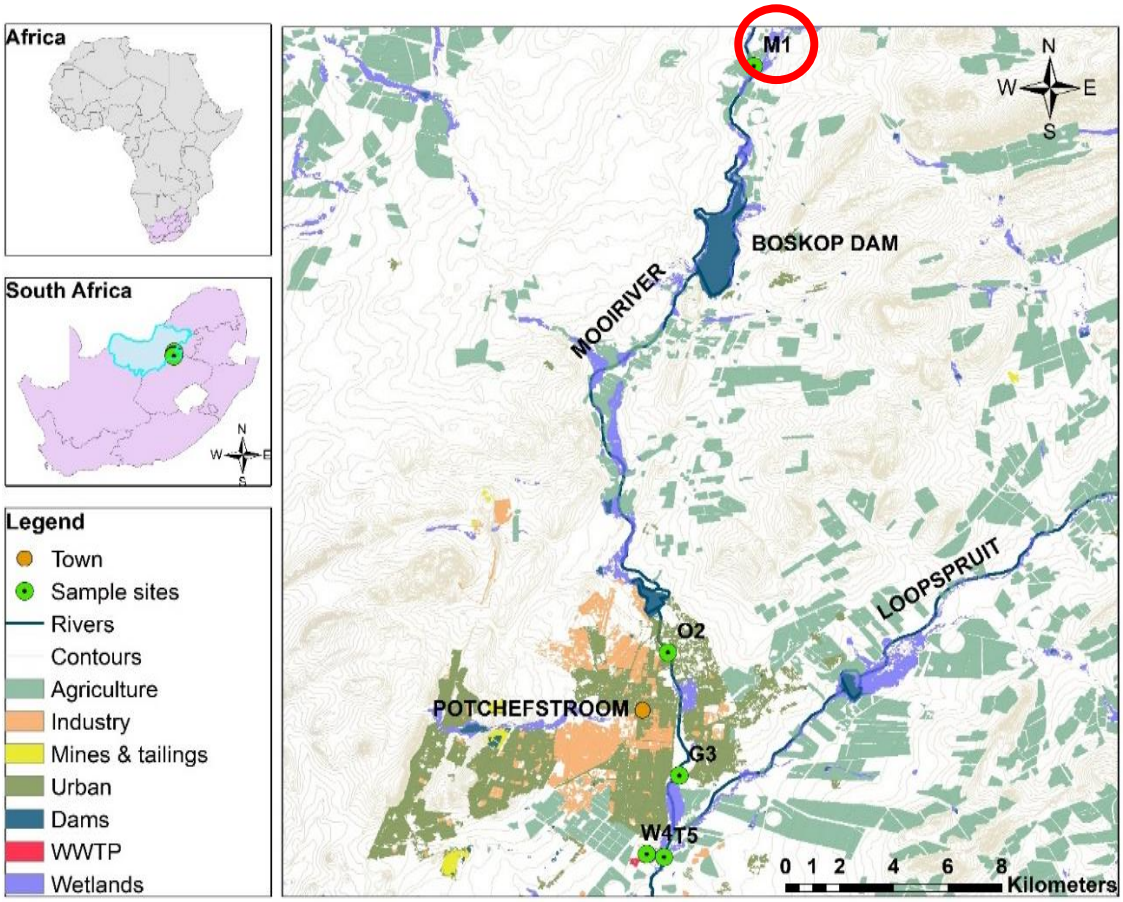

Figure 1: Map showing the sampling sites and land cover activity dominance

- The river water passing through site M1 was dominated by microplastic fibres through out the sampling stages compared to other sites, which were dominated by fibres, films, foams and fragments.
- 206 microplastics were counted and used for further analysis

Figure 2: Microplastics isolated from river water

- Microbial biofilms and unicellular cells were identified on microplastics after visualization with the Scanning Electron microscope

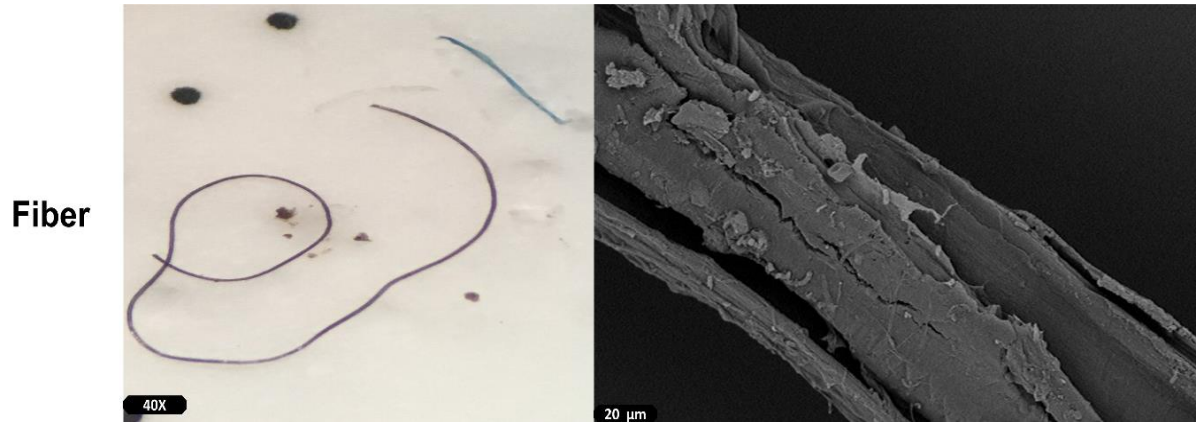

Figure 3: Microplastic fibre viewed under a stereomicroscope at 40X magnification versus under the scanning electron microscope at 20 µm.

Microbial consortia on microplastics versus water

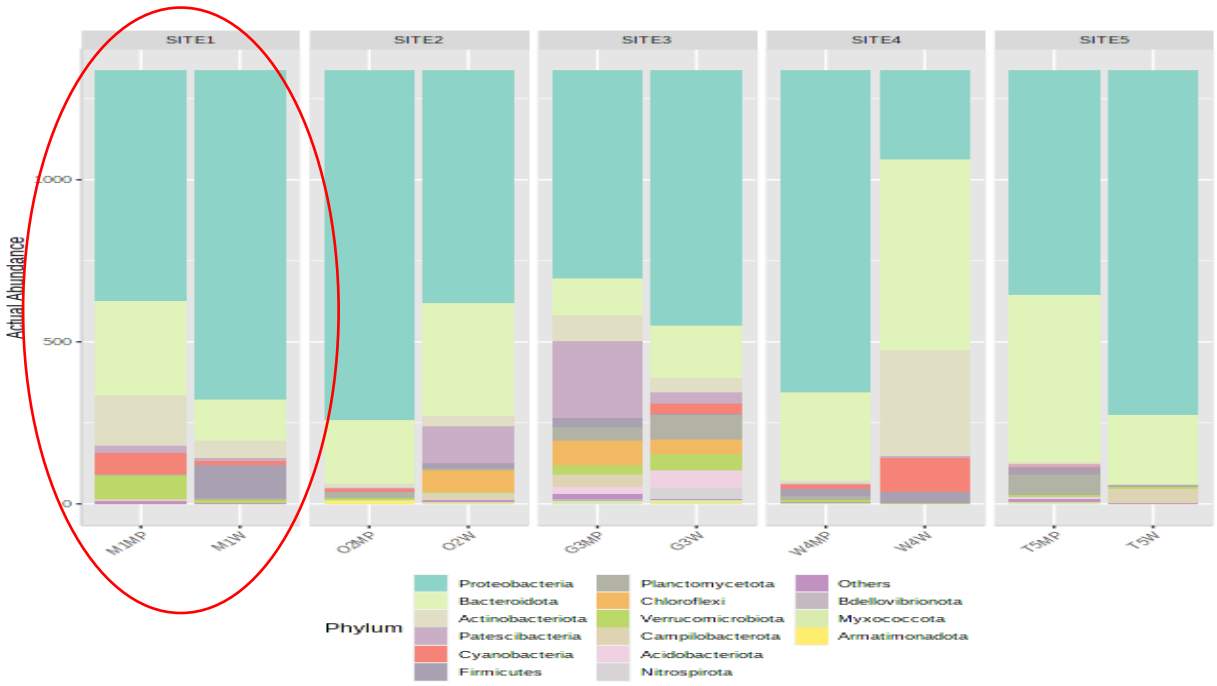

Figure 4: Relative abundance of the top 20 most abundant phyla based on 16S rRNA metabarcoding for taxonomic composition from microplastic (MP) and water (W) samples from each respective site.

- Overall, the microbial composition in water samples was slightly richer and diverse than in microplastics
- In site M1 microplastic richness and diversity was higher in microplastics than the surrounding water community
- In terms of species evenness and dominance, microplastics samples showed a slightly higher Simpson value
- Both microplastics and corresponding filtrate water samples were dominated by the phylum Proteobacteria. Dominance and representation of phyla varied amongst microplastics and water at each site

Table 1: Distribution of the core bacterial taxa (by numbers) at family level on microplastics and water samples.

Family	P-value	microplastics	water	LDA score
Enterobacteriaceae	0.007	81808	1012.6	-4.61
Exiguobacteraceae	0.016	3058.5	221850	5.04
Isosphaeraceae	0.019	5700.2	0	-3.46
Legionellaceae	0.019	7704.7	0	-3.59
Geminicoccaceae	0.019	2258.7	0	-3.05
Tannerellaceae	0.019	7479.3	0	-3.57
Bacteroidaceae	0.019	21712	0	-4.04
Reyranellaceae	0.034	41324	1635.9	-4.3
Hydrogenedensaceae	0.034	7620.3	262.23	-3.57
Rhizobiaceae	0.047	223250	35597	-4.97
Alteromonadaceae	0.047	121010	805690	5.53

Red numbers: Indicate a p-value < 0.050 which shows significance; (-) in Linear Discriminant Analysis (LDA) score indicates the skewness of distribution, the negative sign indicates prevalence on microplastics.

- The results showed that some families were significantly higher (p-value < 0.050) on microplastics than the surrounding water community for example, *Enterobacteriaceae* (p-value = 0.007), *Isosphaeraceae* (p-value = 0.019), *Legionellaceae* (p-value = 0.019) were among the top 11 families that were dominant on microplastics.
- It was also determined that genera (*Eschericia_Shigella*, *Legionella*) belonging to the above families (*Enterobacteriaceae* and *Legionellaceae*) were significantly higher on microplastics than water.
- The results also revealed that some groups were significantly higher in the surrounding water than microplastics samples. These included genus *Exiguobacterium* (p-value = 0.001) which belongs to the *Exiguobacteraceae* family (p-value = 0.016).

Antibiotic resistance genes and microplastics

Table 2: Average copy number of ARGs (AmpC gene groups) quantified from field environmental samples of microplastics and water (units in gene copies/16S rRNA ± standard deviation).

Sample	Site	Source	ACC	DHA	FOX	LAT/CMY/BIL	MIR/ACT	MOX/CMY
M1P	M1	MP	0.28 ± 0.20	-	-	-	0.64 ± 0.12	-
M1F		River	0.01 ± 0.01	-	-	-	0.10 ± 0.07	-
O2P	O2	MP	0.17 ± 0.12	-	-	-	11.60 ± 10.08	-
O2F		River	0.12 ± 0.12	1.20 ± 0.56	8.67 ± 2.38	0.46 ± 0.41	1.09 ± 0.01	0.77 ± 0.10
G3P	G3	MP	0.75 ± 0.53	-	11.92 ± 0.89	1.32 ± 0.24	1.97 ± 4.07	1.17 ± 0.04
G3F		River	0.16 ± 0.12	-	6.01 ± 1.32	0.02 ± 0.01	0.06 ± 0.01	0.09 ± 0.03
W4P	W4	MP-WWTe	15.94 ± 11.27	0.18 ± 0.13	3.33 ± 2.35	24.01 ± 5.42	1.01 ± 1.13	4.35 ± 4.78
W4F		WWTe	71.08 ± 50.26	1.65 ± 0.91	10.97 ± 7.75	248.19 ± 304.99	91.94 ± 103.90	188.95 ± 370.57
T5	T5	MP	0.86 ± 0.61	0.06 ± 0.05	-	-	0.24 ± 0.17	1.25 ± 0.45
TF		River	0.07 ± 0.05	0.01 ± 0.65	7.88 ± 0.04	0.17 ± 0.0	0.65 ± 0.58	3.12 ± 0.21

(-) = undetected; MP (microplastic); WWTe (Wastewater treatment effluent

- The field samples were separated into two groups which are microplastics and water according to each respective site (Table 2).
- Six AmpC β-lactamase gene groups were quantified using real-time qPCR. The AmpC gene groups DHA, FOX, LAT/CMY/BIL, and MOX/CMY were not detected in samples from Site M1 and microplastic samples from Site O2 (Table 2).
- Copy numbers of the ACC gene group ranged from 0.01 ± 0.01 gene copies/16S rRNA to 71.08 ± 50.26 gene copies/16S rRNA. MIR/ACT copy numbers ranged from 0.06 ± 0.01 to 91.94 ± 103.90 gene copies/16S rRNA.
- From Table 2, the results indicated that the ACC gene groups' copy numbers in microplastic samples were greater than copy numbers detected in water samples from Site M1 (0.28 ± 0.20 > 0.01 ± 0.01 gene copies/16S rRNA), Site O2 (0.17 ± 0.12 > 0.12 ± 0.12 gene copies/16S rRNA), Site G3 (0.75 ± 0.53 > 0.16 ± 0.12 gene copies/16S rRNA), and Site T5 (0.86 ± 0.61 > 0.07 ± 0.05 gene copies/16S rRNA).
- In the MIR/ACT gene group, copy numbers in microplastic samples were greater than in water samples from Site M1 (0.64 ± 0.12 > 0.10 ± 0.07 gene copies/16S rRNA), Site O2 (11.60 ± 10.08 > 1.09 ± 0.01 gene copies/16S rRNA) and Site G3 (1.97 ± 4.07 > 0.06 ± 0.01 gene copies/16S rRNA