# SUPPLEMENTAL INFORMATION

## MICROBIOME AND HYDRAULIC PERFOMANCE CHANGES OF DRINKING WATER BIOFILTERS DURING DISRUPTIVE EVENTS- MEDIA REPLACEMENT, LAKE DIATOM BLOOM, AND CHLORINATION

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## SECTION S1- CORE SAMPLING PROCEDURE

The core sampling procedure happened based on "Filter Assessment Manual"3rd Edition- Dec 2003. (Trofatter et al., 2003)

1. Drain and isolate one of the filters.
2. Plan the sampling points within the filter. Choose a sample point in order to obtain a representative composite sample for the filter.
3. Access the filter bed by placing plywood panels on the bed surface.
4. Go to the first sample point. Remove the plug from the end of the coring device (unless you plan to use your hand as the plug). Place the beveled edges of the coring device into the filter bed. Rotate the coring device as it sinks into the media. STOP when resistance is encountered. When the coring device has reached the gravel it will be apparent by the grinding noise made as the device is rotated.
5. Replace the removable plug from the end of the coring device (or simply place your hand over the end to form a seal).
6. Slowly raise the coring device out of the filter media.
7. Place a long strip of aluminum foil along the top of one of the plywood boards.
8. Gently tap the edge of the coring device so that the contents are slowly emptied across the length of the aluminum foil.
9. Separate the media along the anthracite/sand interface.
10. Label bags for sand and anthracite samples.
11. Remove all materials from the test filter.
12. Backwash the filter before placing it on-line.
13. Send the sample bags to a lab for sieve analysis.

Table S1 Sampling schedule through different depth and dates

| | **Baseline** | | | **Old Media** | **Fresh Media** | **Lake Diatom Bloom** | **Chlorination** |
|---|---|---|---|---|---|---|---|
| **Depth (In)** | **March 2016** | **April 2016** | **May 2016** | **February 2018** | **March 2018** | **July 2018** | **August 2018** |
| **0-2'** | + | + | + | + | + | + | + |
| **2-6'** | + | + | + | + | + | + | + |
| **12-18'** | + | + | + | + | + | + | + |
| **18-24'** | + | + | + | + | + | + | + |
| **24-30'** | + | + | + | + | + | + | + |
| **30-36'** | + | + | + | + | + | + | + |

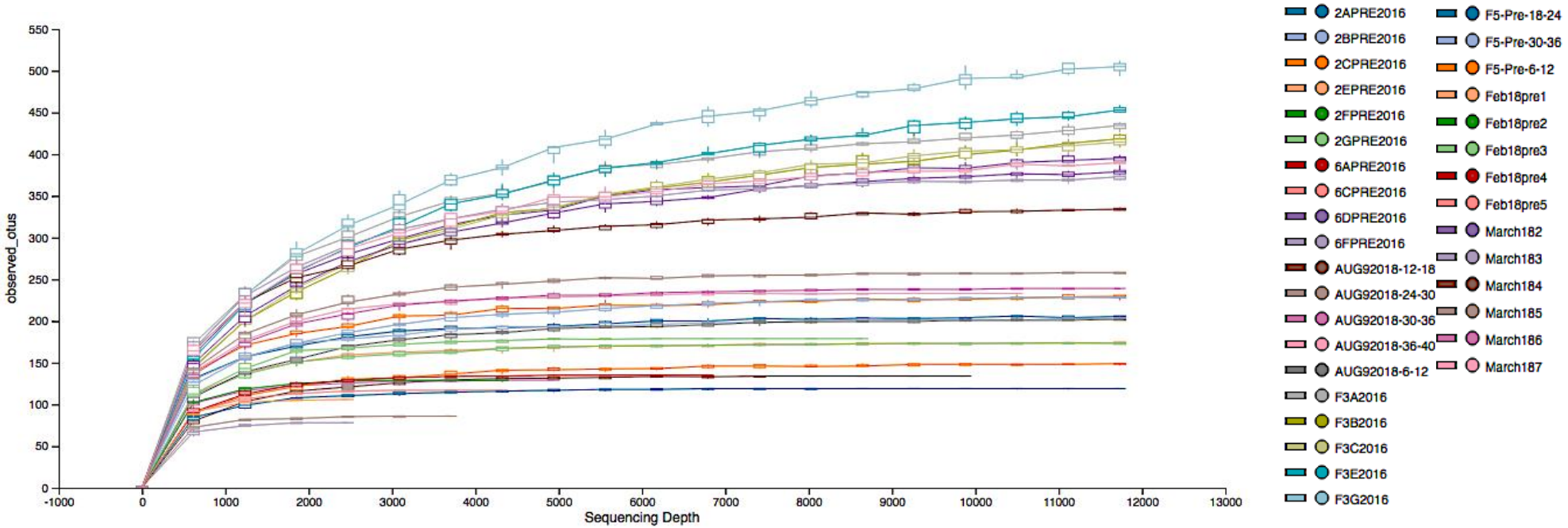


Figure S1- Alpha rarefaction for all samples

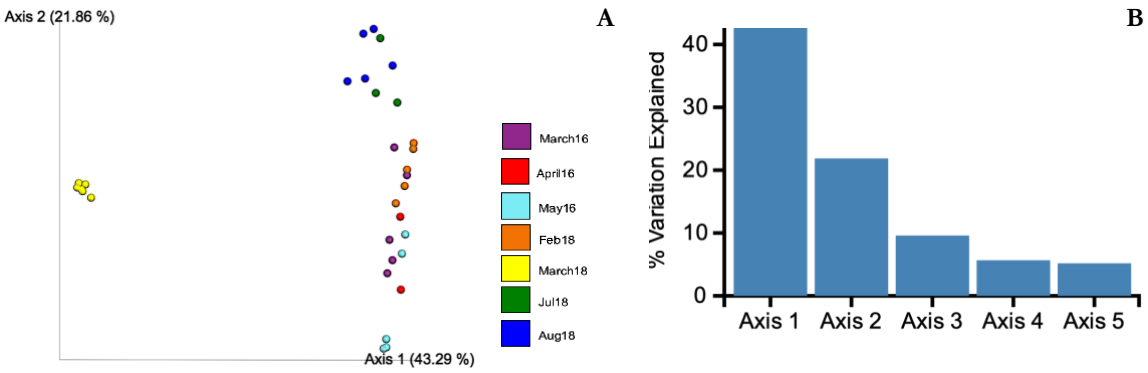


Figure S2- A-Bray-Curtis-PCoA of biofilter microbiome for the regular and challenging events. Each point represents a different filter depth for various sampling dates which is shown in different colors. It is observed that fresh media (shown in yellow circles) microbiome is distinctly different than other sampling event. B- Scree plot for Bray-Curtis beta diversity analysis.

Table S2- Alpha indices for each event.

| Sampling Event | Filter Depth (in) | ASV | Pielou's Evenness | Shannon | Faith' s PD |
|---|---|---|---|---|---|
| **Mar 2016** | 0-2' | 302.0 | 0.8 | 6.7 | 24.9 |
| | 2-6' | 273.0 | 0.7 | 6.2 | 25.0 |
| | 6-12' | 270.0 | 0.7 | 6.1 | 25.3 |
| | 18-24' | 300.0 | 0.8 | 6.4 | 27.6 |
| | 30-36' | 314.0 | 0.8 | 6.5 | 28.4 |
| **May 2016** | 0-2' | 187.0 | 0.8 | 6.2 | 17.4 |
| | 2-6' | 172.0 | 0.8 | 6.2 | 16.3 |
| | 6-12' | 296.0 | 0.8 | 6.3 | 17.4 |
| | 18-24' | 155.0 | 0.8 | 5.6 | 16.7 |
| | 30-36' | 160.0 | 0.8 | 5.6 | 16.9 |
| **Feb 2018** | 0-2' | 106.0 | 0.8 | 5.2 | |
| | 2-6' | 128.0 | 0.8 | 5.6 | 16.0 |
| | 6-12' | 169.0 | 0.8 | 5.7 | 17.7 |
| | 12-18' | 128.0 | 0.8 | 5.3 | 15.6 |
| | 18-24' | 117.0 | 0.8 | 5.4 | 13.4 |
| **Mar 2018** | 2-6' | 265.0 | 0.7 | 6.2 | 23.8 |
| | 6-12' | 296.0 | 0.8 | 6.6 | 24.4 |
| | 12-18' | 259.0 | 0.8 | 6.6 | 23.8 |
| | 18-24' | 217.0 | 0.8 | 6.2 | 20.7 |
| | 24-30' | 213.0 | 0.8 | 6.0 | 21.9 |
| | 30-36' | 285.0 | 0.8 | 6.5 | 25.6 |
| **Jul 2018** | 6-12' | 126.0 | 0.8 | 5.4 | 13.8 |
| | 18-24' | 112.0 | 0.8 | 5.2 | 12.5 |
| | 30-36' | 189.0 | 0.8 | 5.9 | 19.8 |
| **Aug 2018** | 6-12' | 163.0 | 0.7 | 5.1 | 18.7 |
| | 12-18' | 123.0 | 0.7 | 4.8 | 13.7 |
| | 24-30' | 85.0 | 0.7 | 4.8 | 10.5 |
| | 30-36' | 123.0 | 0.8 | 5.6 | 14.1 |

Table S3- PERMANOVA pairwise statistical analysis of microbiome between chlorination and other events

| Group 1 | Group 2 | Sample size | P-value | Q-value |
|---|---|---|---|---|
| Aug 2018 (Chlorination) | Mar 2016 (Baseline) | 10 | 0.012 | 0.025 |
| Aug 2018 (Chlorination) | May 2016 (Baseline) | 10 | 0.011 | 0.025 |
| Aug 2018 (Chlorination) | Apr 2016 (Baseline) | 7 | 0.054 | 0.067 |
| Aug 2018 (Chlorination) | Feb 2018 (Old media) | 10 | 0.012 | 0.025 |
| Aug 2018 (Chlorination) | Mar 2018 (Fresh media) | 11 | 0.002 | 0.016 |
| Aug 2018 (Chlorination) | Jul 2018 (Diatom bloom) | 8 | 0.31 | 0.31 |

Table S4- PERMANOVA pairwise statistical analysis between of microbiome between diatom bloom and other events

| Group 1 | Group 2 | Sample size | P-value | Q-value |
|---|---|---|---|---|
| Jul 2018 (Diatom bloom) | Mar 2016 (Baseline) | 8 | 0.014 | 0.025 |
| Jul 2018 (Diatom bloom) | May 2016 (Baseline) | 8 | 0.019 | 0.031 |
| Jul 2018 (Diatom bloom) | Apr 2016 (Baseline) | 5 | 0.097 | 0.102 |
| Jul 2018 (Diatom bloom) | Feb 2018 (Old media) | 8 | 0.022 | 0.033 |
| Jul 2018 (Diatom bloom) | Mar 2018 (Fresh media) | 9 | 0.013 | 0.025 |
| Jul 2018 (Diatom bloom) | Aug 2018 (Chlorination) | 8 | 0.31 | 0.31 |

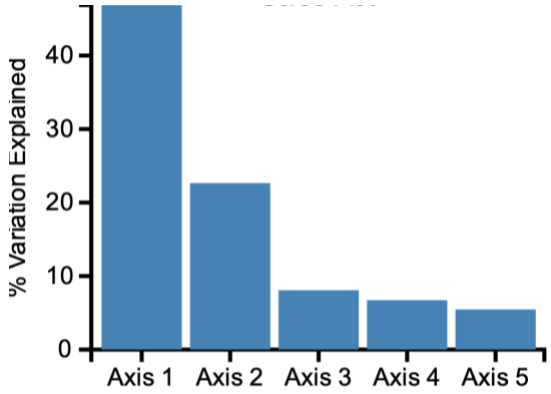


Figure S3- Scree plot for weighted Uni-Frac beta diversity analysis

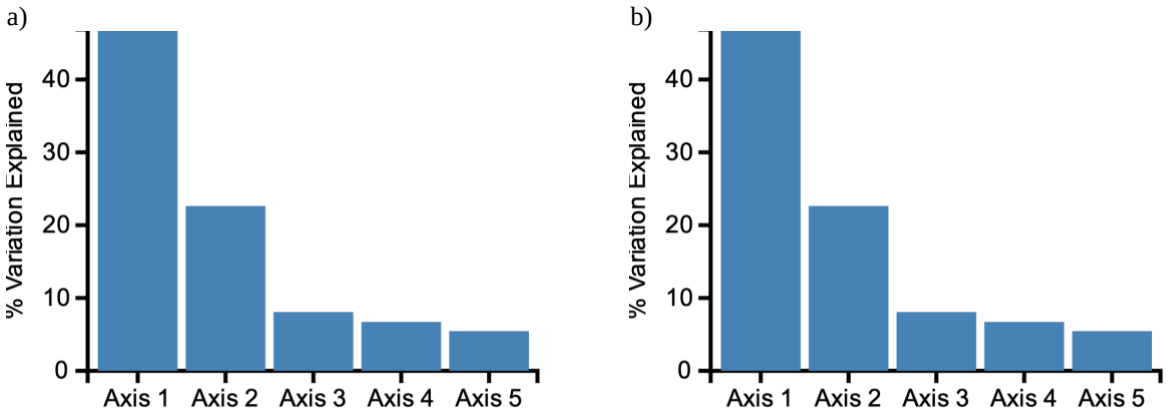


Figure S4- Scree plot for depth analysis of weighted Uni-Frac PCoA- Mar (a) and May (b) 2016 sampling events.

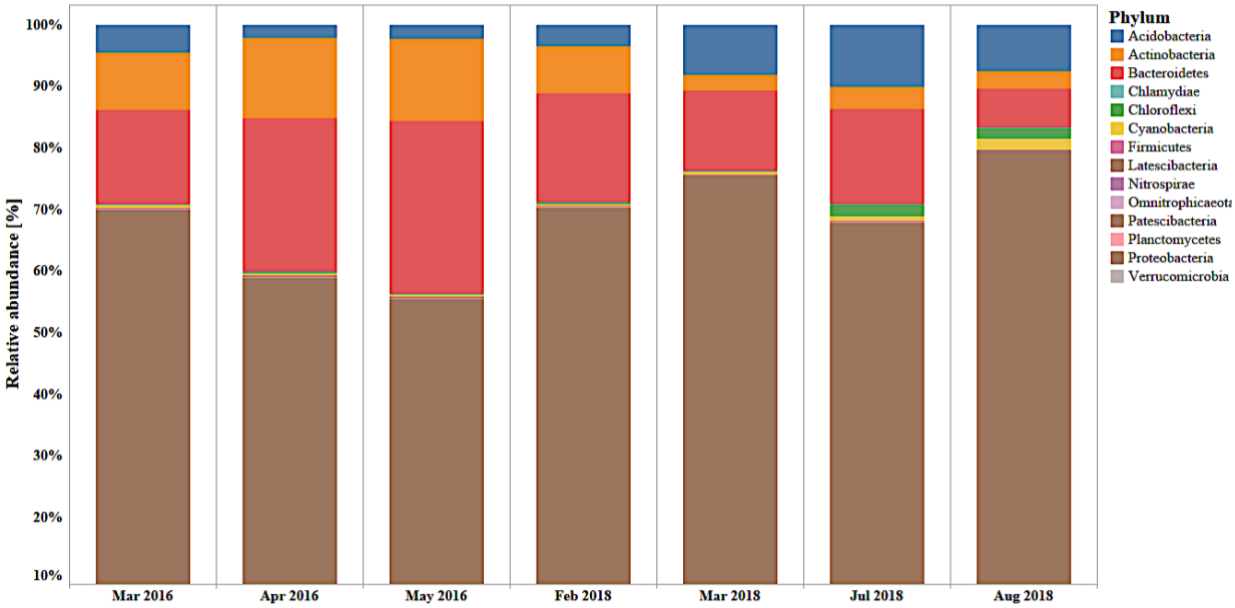


Figure S5- Relative abundance of all the filter events at phylum level. As shown the protobacteria followed by Bacteroidetes are the most abundant phyla in all the biofilters

Table S6- Relative abundance of each family in Mar, Apr and May 2016 (regular operation/baseline)

| **Family** | **Mar 2016** | **Apr 2016** | **May 2016** |
|---|---|---|---|
| *Sphingomonadaceae* | 19.57% | 8.91% | 7.90% |
| *Chitinophagaceae* | 9.51% | 11.37% | 21.39% |
| *Burkholderiaceae* | 11.02% | 15.16% | 15.28% |
| *SAR11 clade III* | 9.21% | 12.04% | 10.25% |
| *Sporichthyaceae* | 6.71% | 10.47% | 11.22% |
| *Acetobacteraceae* | 7.30% | 6.30% | 6.94% |
| *Unknown* | 4.30% | 5.78% | 6.19% |
| *uncultured bacterium* | 4.98% | 1.98% | 1.65% |
| *Saprospiraceae* | 3.01% | 10.30% | 4.10% |
| *Solibacteraceae (Subgroup 3)* | 4.02% | 2.07% | 2.12% |
| *Beijerinckiaceae* | 3.09% | 3.21% | 2.36% |
| *Microscillaceae* | 2.46% | 2.51% | 1.60% |
| *Hyphomicrobiaceae* | 2.57% | 1.09% | 0.57% |
| *Xanthobacteraceae* | 1.97% | 0.42% | 0.55% |
| *Nitrosomonadaceae* | 1.93% | 1.06% | 0.16% |
| *Pedosphaeraceae* | 1.54% | 0.97% | 1.15% |
| *Caulobacteraceae* | 1.30% | 1.05% | 1.43% |
| *Terrimicrobiaceae* | 0.76% | 1.90% | 2.31% |
| *Steroidobacteraceae* | 0.94% | 1.45% | 1.50% |
| *TRA3-20* | 1.11% | 0.42% | 0.17% |
| *Methylophilaceae* | 0.77% | 1.03% | 0.91% |
| *Microbacteriaceae* | 1.06% | 0.34% | 0.11% |
| *0319-6G20* | 0.67% | 0.17% | 0.09% |
| *Rhizobiales Incertae Sedis* | 0.18% | 0.01% | 0.05% |
| *Reyranellaceae* | 0.01% | 0.00% | 0.00% |

Table S7- Relative abundance of each family in Feb 2018 (old media) and Mar 2018 (fresh media)

| Family | Feb 2018 (Old media) | Mar 2018 (Fresh media) |
|---|---|---|
| *Xanthobacteraceae* | 1.99% | 19.23% |
| *Chitinophagaceae* | 5.18% | 11.06% |
| *Rhizobiales Incertae Sedis* | 0.00% | 11.54% |
| *Reyranellaceae* | 0.00% | 10.47% |
| *Sphingomonadaceae* | 15.64% | 6.19% |
| *Solibacteraceae (Subgroup 3)* | 3.02% | 7.09% |
| *Hyphomicrobiaceae* | 3.52% | 5.90% |
| *Acetobacteraceae* | 8.17% | 3.83% |
| *Burkholderiaceae* | 4.43% | 4.31% |
| *uncultured bacterium* | 8.00% | 3.38% |
| *SAR11 clade III* | 16.94% | 1.42% |
| *Beijerinckiaceae* | 3.11% | 4.07% |
| *Unknown bacteriacea* | 2.88% | 3.20% |
| *Sporichthyaceae* | 5.72% | 1.78% |
| *Saprospiraceae* | 10.37% | 0.00% |
| *Microscillaceae* | 1.95% | 1.32% |
| *Nitrosomonadaceae* | 0.22% | 1.22% |
| *Methylophilaceae* | 0.43% | 1.04% |
| *Caulobacteraceae* | 2.64% | 0.35% |
| *Steroidobacteraceae* | 1.68% | 0.48% |
| *Betaproteobacteriales TRA3-20* | 0.46% | 0.71% |
| *Oligoflexales 0319-6G20* | 0.61% | 0.52% |
| *Pedosphaeraceae* | 1.26% | 0.35% |
| *Microbacteriaceae* | 1.24% | 0.31% |
| *Terrimicrobiaceae* | 0.55% | 0.25% |

Table S8- Relative abundance of each family in diatom bloom and chlorination sampling events

| **Family** | **Jul 2018 (Diatom bloom)** | **Aug 2018 (Chlorination)** |
|---|---|---|
| *Sphingomonadaceae* | 11.90% | 22.89% |
| *Acetobacteraceae* | 12.49% | 18.02% |
| *uncultured bacterium* | 13.29% | 7.33% |
| *Xanthobacteraceae* | 9.77% | 9.62% |
| *Solibacteraceae (Subgroup 3)* | 8.17% | 5.20% |
| *Unknown bacteriacea* | 6.06% | 5.96% |
| *Saprospiraceae* | 7.32% | 3.63% |
| *SAR11 clade III* | 6.28% | 4.58% |
| *Hyphomicrobiaceae* | 2.58% | 5.53% |
| *Burkholderiaceae* | 3.36% | 2.47% |
| *Pedosphaeraceae* | 2.62% | 2.24% |
| *Sporichthyaceae* | 2.63% | 1.85% |
| *Microscillaceae* | 2.87% | 1.16% |
| *Chitinophagaceae* | 2.37% | 1.37% |
| *Beijerinckiaceae* | 1.42% | 2.05% |
| *Caulobacteraceae* | 1.78% | 0.94% |
| *Steroidobacteraceae* | 1.03% | 1.28% |
| *0319-6G20* | 1.01% | 1.13% |
| *Nitrosomonadaceae* | 1.01% | 1.06% |
| *Microbacteriaceae* | 0.75% | 0.79% |
| *Betaproteobacteriales TRA3-20* | 0.79% | 0.44% |
| *Terrimicrobiaceae* | 0.33% | 0.32% |
| *Methylophilaceae* | 0.16% | 0.12% |
| *Rhizobiales Incertae Sedis* | 0.00% | 0.01% |
| *Reyranellaceae* | 0.00% | 0.00% |

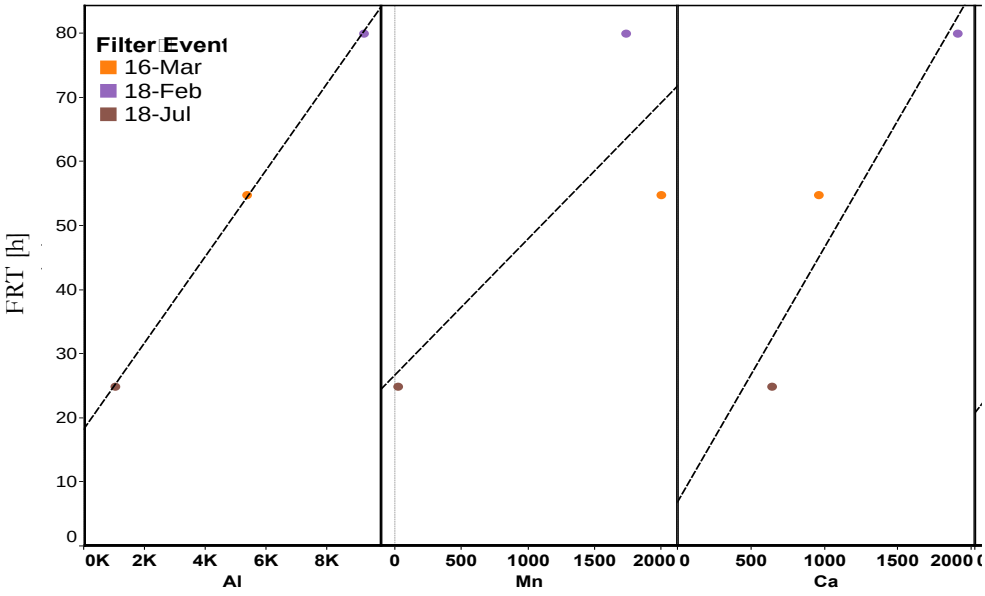


Figure S6- Positive linear correlation between aluminum concentration on top of the biofilter media (all the events except the fresh media) and FRT (P-Value=0.009- R-squared=0.99). The correlation between FRT and Mn and Ca were not significant. This shows that the filter runtime has a strong impact on the concentration of the deposited aluminium on the media, therefore the higher the filter runtime, the higher the metal concentration accumulated on the filter media. The used coagulant in the plant was alum.

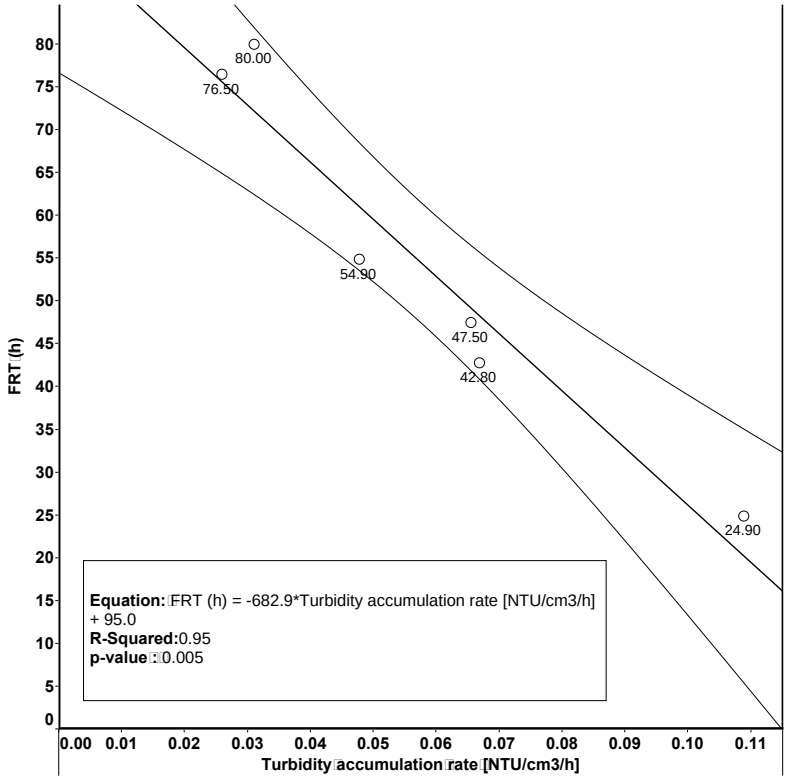


Figure S7- There is a negative linear correlation between the FRT and turbidity accumulation rate (at the top of biofilter media). The results are statistically significant.

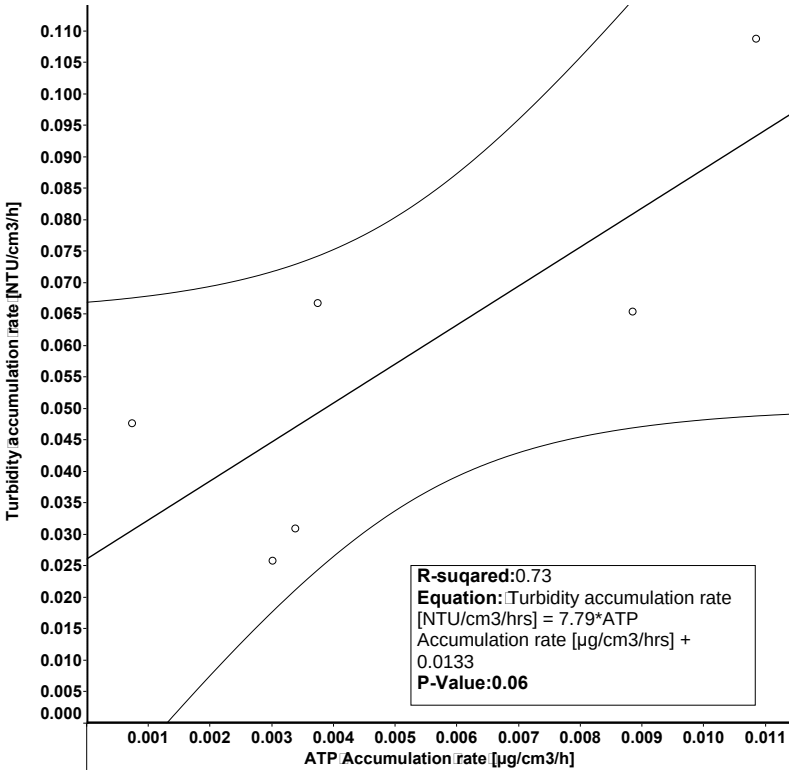


Figure S8- Although there is a positive linear trend between ATP accumulation rate [µg/cm$^3$/h] shown in X-axis and turbidity accumulation rate [NTU/cm$^3$/h] shown in Y-axis, no significant correlation was observed between them. (°) shows the collected data from the top of biofilter media, (–) depicts the linear correlation, the upper and lower bound are 95% confidence intervals. P-value >0.05 shows that the correlation is not statistically significant.