MAD-AS: the MAD AutoSampler

low-cost, easily accessible, open-source, open-hardware, automatic in-stream sampler

Field Trials for Treatment Plant Deployments

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Aim

This study aimed to validate the use of the MAD-AS for sampling of wastewater at sewage treatment plants. The objective was to compare the daily time-weighted mean concentrations of SARS-CoV-2, *E. coli* and enterococci in wastewater sampled using the MAD-AS to that found using traditional automatic samplers (SD900, HACH, Australia).

Methods

Sites. Four sewage treatment plants were selected for the study, all located in the Melbourne metropolitan area: Western Treatment Plan (WTP), Aurora Sewage Treatment Plant (AuSTP), Altona Sewage Treatment Plant (AlSTP) and the Craigieburn Sewage Treatment Plant (CSTP).

Trial dates. Between 13th July and 29th of July 2021, three trials were conducted at each site, resulting in 12 trials where we could compare wastewater collected using traditional samplers to that of the MAD-AS. For each trial, sampling occurred over 24-hour periods, typically starting in the morning and ending the following morning.

Traditional samplers. HACH SD900 automatic samplers were installed at each site and were programmed to take time-weighted subsamples from the wastewater using 15minute increments. At AuSTP & CSTP, 12 discrete samples were collected over each test day, each of which representing a two-hour period, resulting in 12 L of wastewater being collected at these sites on each trial date. At WST and AlSTP, we were restrained to having each 15-minute subsample delivered to a single composite sample, resulting in a 2L composite of wastewater being collected at these sites on each trial date. Regardless of the sampling method, each collected sample was analysed for SARS-CoV-2, *E. coli* and enterococci.

MAD-AS samplers. For details on the MAD-AS, please visit http://www.bosl.com.au/wiki/MAD-AS. On each trial date we deployed a MAD-AS as close as possible to the intake tube for the traditional

sampler; at the Craigieburn site, we deployed dual MAD-AS to explore any between-sampler differences. The start time of the MAD-AS was kept as close as possible to the traditional sampler, but in some cases there was up to a 15minute shift between the start and end times of each sampling method. The MAD-AS sampling interval was set to the same constant time interval as the traditional sampler (i.e. 15mins), although in future deployments the real benefit of the MAD-AS is that it can sample at much higher frequency. The volume of water pumped each 15minutes was set to 2mL, resulting in an expected 192mL of wastewater being collected at each site on each trial date. These time-weighted samples were analysed for SARS-CoV-2, *E. coli* and enterococci.

Sample assays. All discrete and composite wastewater samples were processed for *E. coli* and enterococci using the IDEXX Colilert and Enterolert methods, resulting in a mean probable number of cells per 100mL of wastewater. 50mL of all wastewater samples were filtered through 0.45µm membranes; this was repeated up to four times for each sampling type so that replicate analyses were possible. These membranes were immediately frozen at -80^{o}C until extraction was possible. RNA extraction was conducted and followed by qPCR for the detection SARS-CoV-2 gene copies following that of Schang et al. (2021).

Data analysis. We used all available data to estimate daily time-weighted mean concentrations of *E. coli*, enterococci and SARS-CoV-2 and plotted these values obtained using the MAD-AS collected samples against those obtained by traditional methods.

Results and discussion

Eight of the 15 MAD-AS deployments conducted in this study failed to obtain the expected volume of wastewater after 24 hours of deployment, suggesting that the MAD-AS failed at some point during the trial. Most of these failures occurred at the start of the trial where the sampling pipes were clogged with debris. We fixed this issue near the end of the trial and the final four deployments were a success as they had been modified with a new screen to prevent clogging. This screen will be further tested into the future. As a result of these failures, the comparisons that follow only focus on the seven successful deployments of the MAD-AS, representing five trial dates (noting that at Craigieburn we deployed two MAD-AS on each trial date).

There were statistically significant trends (R^2>0.85, p<0.05) observed between the concentrations of *E. coli*, enterococci and SARS-CoV-2 in the samples collected using the MAD-AS and in those collected by the traditional autosampler (Figure 1). While the number of trials in this comparison is low (n=5), the high degree of agreement between the two sampling methods for all three target microbes is encouraging. Importantly, the MAD-AS can sample SARS-CoV-2 from wastewater to a similar level of accuracy as traditional techniques, meaning that the device could be used in wastewater systems to help early detection of COVID-19 transmission in the community.

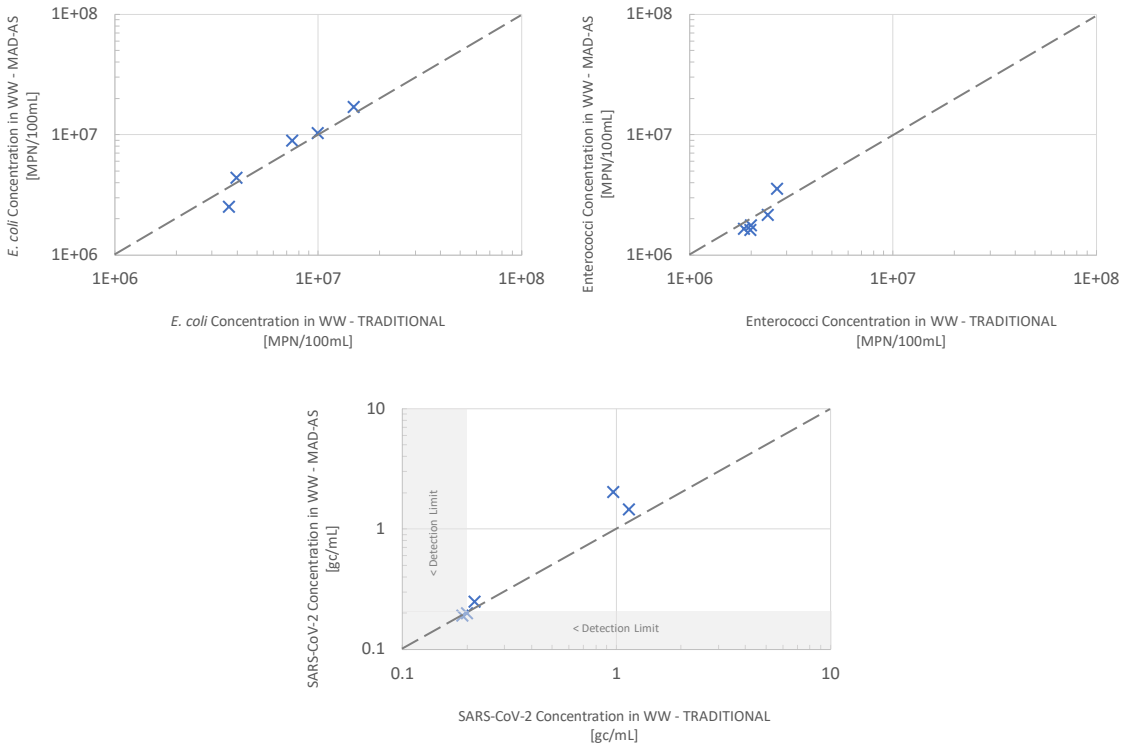

Figure 1. Concentrations of E. coli (top left), enterococci (top right) and SARS-CoV-2 (bottom left) in wastewater samples collected using the MAD-AS (y-axes) versus in those collected using traditional automatic sampler (x-axes).

Conclusions and future work

The results demonstrated the comparative performance of the low-cost MAD-AS as compared to traditional sampling techniques for three target microbes. While the MAD-AS experience some clogging issues, the new screen design prevented clogging and ensured the MAD-AS functioned appropriately. The next trial of the MAD-AS should be within the sewer network (i.e. Barwon sites), where the efficiency of the MAD-AS and the new screen design will be further examined.

References

Schang et al. 2021 - https://pubs.acs.org/doi/10.1021/acs.est.1c01530