MAD-AS: the MAD AutoSampler

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

Field Trials for Treatment Plant Deployments

Round A Reported: 4th August 2021

Round B Reported: 7th December 2021

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1 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).

2 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. Round A. 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.

Round B. Between 29th November and 2nd December 2021, an additional three trials were conducted at both AuSTP and CSTP, resulting in an additional six comparisons that could be made between traditional samplers to that of the MAD-AS. The sampling was one again conducted over 24-hour periods, as above.

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. The traditional sampling was the same for both Round A and Round B trials.

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. The only difference between Round A and Round B trials was the type of sampler and the number of MAD-AS deployed per deployment period: Round A mainly used a single MAD-AS v 0.1, per site, per day but sometimes two MAD-AS per site (resulting in 4 sites x 3 trial dates x 1 deployment per site per day = 12 MAD-AS deployments, + 3 dual deployments = 15) while Round B used three MAD-AS v0.5, per site, per day (resulting in 2 sites x 3 trial dates x 3 deployment per site per day = 18).

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.

3 Results and discussion

3.1 Deployment success

Round A. 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).

Round B. In all 18 deployments, the MAD-AS were deployed and retrieved intact and without damage, and it is firmly believed that the installation ensured all samplers remained submerged for their entire deployment duration. 12 of the 18 deployments were successful, pumping approximately the correct amount of wastewater and able to be reused with minimal cleaning or maintenance. Two of the six unsuccessful deployments were due to poor battery connections, which is something that is easily corrected in the next version of the MAD-AS (thicker 3D printed walls will

fix this issue), while the other four unsuccessful deployments were all due to clogging issues of the inner-tubing of the pump. These clogging issues were caused by an incorrect assembly process which meant that the tubing was compressed in a location that resulted in the build-up of debris. This again has been re-engineered to avoid such clogging in the future (and will be part of the next version of the MAD-AS). As a result, only 12 of the deployments are included in the analysis that follows.

3.2 Traditional sampler vs. MAD-AS

Round A. 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.

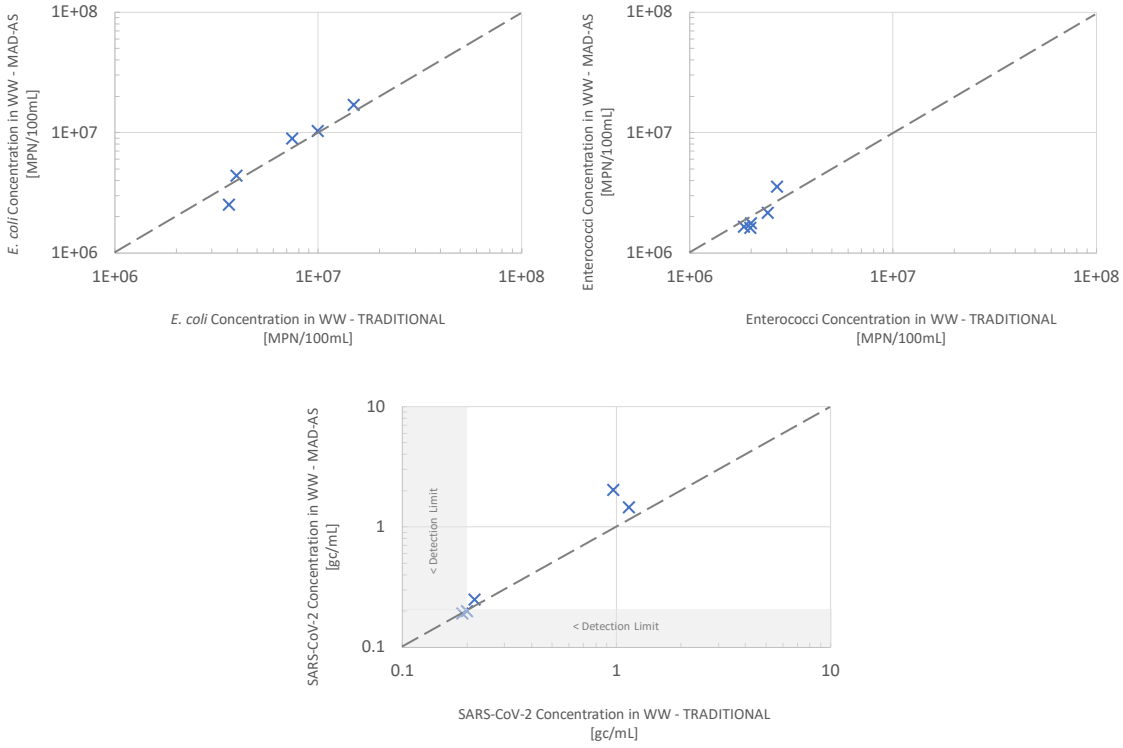

Figure 1. Round A Results: Concentrations of E. coli (top left), enterococci (top right) and SARS-CoV-2 (bottom left) in wastewater samples collected using the MAD-AS (y-axes) versus in those collected using traditional automatic sampler (x-axes).

Round A+B. Including the extra data collected in Round B into the above analysis reinforced the earlier findings from Round A for SARS-CoV-2 with a highly significant correlation (R^2>0.9, p<0.001) between the log concentrations found using the traditional sampler and the MAD-AS (Figure 2). While the link between *E. coli* concentrations from the traditional autosampler and the MAD-AS was still statistically significant (R^2=0.65, p=0.002), the addition of the Round B datasets significantly reduced this correlation for enterococci (R^2 = 0.04, p>0.5). Although the correlation is poor for enterococci, all results are within 0.7 log of each other (i.e. close to the measurement error of the methods) and the variation of the enterococci data is much narrower than the other microbes, both of which make it difficult to detect significant trends. Furthermore, we observed multiple issues with

enterococci readings during this trial including multiple samples being outside of our detection limits.

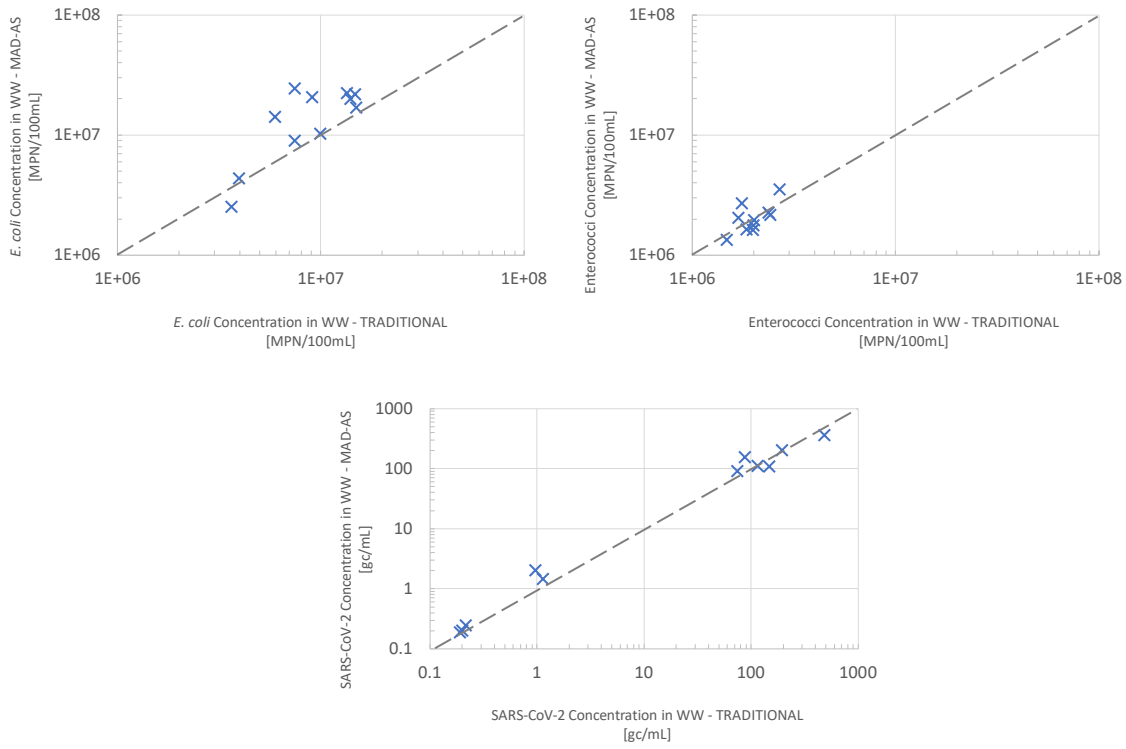

Figure 2. Round A+B Results: Concentrations of E. coli (top left), enterococci (top right) and SARS-CoV-2 (bottom left) in wastewater samples collected using the MAD-AS (y-axes) versus in those collected using traditional automatic sampler (x-axes).

4 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 newest MAD-AS version experiences far fewer clogging issues, careful construction methods will eliminate these issues. 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.

5 References

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