# An innovative, low-cost, small MAD-AS sampler for wastewater sampling in the sewage network

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## Highlights

- The small innovative MAD-AS sampler can be easily installed in sewage networks to take time-weighted composite samples.
- The MAD-AS sampler is an Arduino-based device to which customised programs can be uploaded for different sampling requirements.
- In comparison with traditional autosamplers, the MAD-AS pump produces reliable results in bacteria indicators (E. coli and Enterococci) and specific virus (SARS-CoV-2).

### Introduction

Wastewater sampling is very important to understand the pollutant level in the wastewater for environmental and public health protection (Symonds et al., 2009). To prevent viral outbreaks and protect community hygiene Wastewater Based Epidemiology (WEB) sampling has been emphasised especially after the outbreaks of COVID-19 (Baldovin et al., 2021; Capone et al., 2021; Murakami et al., 2020). However, most of the wastewater sampling activities were taken by autosamplers at wastewater treatment plants, although this sampling regime produces reliable results, it can only indicate the pollutant concentration at the community level and does not provide an understanding of the accurate location of an outbreak or allow source tracking (Schang et al., 2021). In order to obtain the spatial pollutant results, wastewater samples need to be taken from the sewage network this narrows down the hotspot area for easier Near Source Tracking (NST) (Hassard et al., 2021). However, sampling from the sewage network is limited by the installation of the bulky, expensive and power-consuming traditional autosamplers. Therefore, in order to conduct high-spatial-density resolution wastewater network sampling with reliable pollutant level results, new innovative lowcost sampling devices need to be applied in sewage network sampling activities. .

# Methodology

#### Innovative MAD-AS pump development

The MAD-AS (<u>MAD AutoSampler</u>) pump is an upgraded and practical version of the BoSL FAL pump (McCarthy et al., 2021), which is built with 3D printed case and parts, an Arduino based microcontroller (Micro-BoSL board), a geared motor, M8 cable glands, 4mm external and 2mm internal diameter silicon tubing, and two 3.3V batteries.

The pumping interval and the pumping duration can be easily customised by uploading a program into the microcontroller, so the user can design the required sampling regime. The shape of MADAS a tapered cylinder (can be seen in Figure 1) this reduces ragging problems in the sewage. The volume of MADAS is small at only 30cm in height and 4cm in diameter. When installing the MADAS, a 19mm diameter silicon tubing need to be connected to the outlet of the pump for collecting the composite wastewater sample, when sampling, the MADAS should be tied somewhere to make sure the entire system cannot flow away.

The total cost of a MADAS is less than \$50 USD which provides a possibility for high spatial resolution wastewater sampling in the sewage network.



**Figure 1.** Internal parts and key components of MADAS, (a) inlet of MADAS, (b) outlet of MADAS, (c) microcontroller Micro BoSL board, (d) MADAS batteries, (e) body cover case

#### Validation of the MAD-AS performance

To validate the performance of the MAD-AS, comparison tests were conducted between a traditional autosampler (Hach SD900 autosampler) and the MAD-AS. These two samplers were installed in the wastewater treatment plants in Melbourne, and they are located beside each other to make sure the samples they take are spatially identical. The sampling regime is same for both devices: both of them kept sampling for 24 hours and pumped every 15 mins. Autosampler was set for taking discrete samples, 12 bottles should be collected throughout 24 hours of continuous sampling. For the MAD-AS, it pumped every 15 minutes, and all the wastewater samples will be accumulated in the tubing, a 200ml composite wastewater sample was expected to collect from the MAD-AS.



**Figure 2.** MAD-AS validation set up in the wastewater treatment plant. Hach SD900 autosampler (grey machine) sitting in front of the lagoon (left picture); MAD-AS sitting in the lagoon (tubing come out from the white PVC pipes), the tubing is connected with the MAD-AS and fixed on the rail to avoid it flowing away, PVC pipe is used for fixing the tubing at the right place (middle picture); MAD-AS connecting to the tubing which is coved by a PVC pipe (right picture).

The discrete samples collected from the autosampler need to be combined together to make a composite sample and tested for comparison with the MAD-AS sample. Normally, a 50ml wastewater sample was collected from each well-shaked discrete bottles and mixed as a composite sample for testing.

The *E. coli*, Enterococcus and SARS-CoV-2 concentration were tested for in the samples collected by both samplers, the results were compared to see if the MADAS sample could get a similar result with autosampler samples. For *E.coli* and Enterococcus, IDEXX method was applied for testing the concentration, as the pollutant level is high in the wastewater, dilution series method was used to dilute the wastewater samples to minimize the testing uncertainty. The final dilution factor of the wastewater for *E.coli* and Enterococcus testing is 1:25000. For SARS-CoV-2 concentration testing, 50ml water sample from each sampler was filtered by a filter paper, and then PCR test was run to understand the SARS-CoV-2 concentration.

From July 2021 to February 2022, 16 comparison activities had been conducted and 40 MAD-AS samplers were deployed for performance validation. In most of the comparison activities, multiple MAD-AS (at least 2) were deployed for sample collection. Sometimes, the MAD-AS could not collect

enough water sample for analysis due to clogging issue or battery connection problem, therefore, the comparison can only be done with enough sample tests.

## Results and Discussion

Three pathogens were compared between the traditional autosampler samples and the MAD-AS composite samples, the results are shown in Figure 3. In the diagram, each colour shows the sampling activity at the same site, and different points in the same colour mean the results of different MADAS in the same sampling event. The horizontal axis is the pollutant concentration from the tradition autosampler (Hach SD900) and the vertical axis indicates the pollutant concentration from the MAD-AS. The dash line in each diagram is the y=x centre line which means the results from the two samplers are exactly the same.

From the results shown in Figure 3, it can be seen that for the three pollutants, almost all the points are located around the y=x centre line which means the Hach autosampler and MAD-AS are showing similar results. Also, the same colour points are located in different positions meaning even for the same sampling event, different MAD-AS present different results, that variation is caused by the uncertainty which comes from the different sampling locations or testing methods. Although the MAD-AS are deployed beside the autosampler, they are still sitting in different locations which could show different pollutant concentration. Also, each testing method also has its own uncertainty, for example, when testing *E.coli* by IDEXX method, the uncertainty need to be considered (McCarthy et al., 2008).



**Figure 3.** Pollutant concentration comparison between autosampler samples and MADAS samples, *E. coli* concentration comparison (top left), Enterococci concentration comparison (top right), SARS-CoV-2 comparison (bottom left).

Also, to compare with the SARS-CoV-2 results, the *E.coli* and Enterococci results are more scattered around the centre line, that may be caused by the inaccurate dilution factor of the samples. As the wastewater samples have high pathogen level, a slight change of the dilution will cause higher uncertainties in the results.

## Conclusion

An Arduino based low-cost autosampler (MAD-AS) was developed, its small volume and low power consumption make it possible to conduct wastewater sampling in the sewage network. The total cost of the device is less than \$50 USD. This low-cost device fills the gap of lacking low-cost sewage samplers in the market. After the MAD-AS was developed, in-field experiments were run to validate the performance. The discrete samples collected by the traditional autosampler (Hach SD900) were combined as a composite sample and then compared with the MAD-AS sample. Three pollutants (*E.coli*, Enterococci and SARS-CoV-2) were tested and compared. The results indicate that MAD-AS showed similar concentrations to the Hach sampler. The pollutant concentration differences within the same sampling event may be caused by the different sampling locations and the sampling uncertainties. Dilution factor is another possible reason causing the variation of the results.

## References

- Baldovin, T., Amoruso, I., Fonzo, M., Buja, A., Baldo, V., Cocchio, S., & Bertoncello, C. (2021). SARS-CoV-2 RNA detection and persistence in wastewater samples: An experimental network for COVID-19 environmental surveillance in Padua, Veneto Region (NE Italy). Science of The Total Environment, 760, 143329.
- Capone, D., Chigwechokha, P., de los Reyes III, F. L., Holm, R. H., Risk, B. B., Tilley, E., & Brown, J. (2021). Impact of sampling depth on pathogen detection in pit latrines. *PLoS Neglected Tropical Diseases*, *15*(3), e0009176.
- Hassard, F., Lundy, L., Singer, A. C., Grimsley, J., & Di Cesare, M. (2021). Innovation in wastewater near-source tracking for rapid identification of COVID-19 in schools. *The Lancet Microbe*, *2*(1), e4–e5. https://doi.org/10.1016/S2666-5247(20)30193-2
- McCarthy, D. T., Deletic, A., Mitchell, V. G., Fletcher, T. D., & Diaper, C. (2008). Uncertainties in stormwater E. coli levels. *Water Research*, *42*(6–7), 1812–1824. https://doi.org/10.1016/j.watres.2007.11.009
- McCarthy, D. T., Shi, B., Wang, M., & Catsamas, S. (2021). BoSL FAL pump: A small, low-cost, easily constructed, 3D-printed peristaltic pump for sampling of waters. *HardwareX*, *10*, e00214. https://doi.org/10.1016/j.ohx.2021.e00214
- Murakami, M., Hata, A., Honda, R., & Watanabe, T. (2020). wastewater-based epidemiology can overcome representativeness and stigma issues related to COVID-19. *Environmental Science & Technology*, *54*(9), 5311.
- Schang, C., Crosbie, N. D., Nolan, M., Poon, R., Wang, M., Jex, A., John, N., Baker, L., Scales, P.,
  Schmidt, J., Thorley, B. R., Hill, K., Zamyadi, A., Tseng, C. W., Henry, R., Kolotelo, P., Langeveld,
  J., Schilperoort, R., Shi, B., ... McCarthy, D. T. (2021). Passive Sampling of SARS-CoV-2 for
  Wastewater Surveillance. *Environmental Science and Technology*, 55(15), 10432–10441.
  https://doi.org/10.1021/acs.est.1c01530
- Symonds, E. M., Griffin, D. W., & Breitbart, M. (2009). Eukaryotic viruses in wastewater samples from the United States. *Applied and Environmental Microbiology*, *75*(5), 1402–1409.