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Implementation of faecal source tracking as a lagoon leakage management approach

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Abstract: High nutrient levels have been detected in monitoring bores across several South Australian regional wastewater treatment sites. To gain a greater understanding of the source of leakage (human origin or derived from other sources), quarterly faecal source tracking (FST) monitoring was conducted over a 24-month period. The FST analysis undertaken included utilisation of two Bacteroidales markers, BacUni (general vertebrate faecal) and HF183 (human specific faecal), in addition to the quantification of anaerobic spores (total Sulphite Reducing Clostridia spores {SRC} and *Clostridium perfringens*). Early detection of lagoon leakage is not only critical to protect environment and public health but also as a management approach to develop investment planning required to minimise leakage and environmental harm.

Keywords: Faecal source tracking; Lagoon leakage; Monitoring bores

Wastewater treatment lagoons are widely used in regional communities to collect, naturally treat, store (prior to reuse or discharge) or dispose of wastewater (evaporation). These lagoons can vary in age and construction methodology, with most polishing lagoons on SA Water regional sites constructed using a clay liner. A major environmental concern of wastewater lagoons is their potential to leak into the groundwater system if not properly maintained or operated, causing environmental harm (SA EPA 2019). Detection of lagoon leakage can be undertaken using several methods including geoelectrical integrity assessment, leakage detection systems, groundwater monitoring, and water balances, or a combination of methods. Monitoring bore (leak detection) systems are installed on SA Water sites to determine if leakage to groundwater is occurring, and to give an indication of any off-site impact ensuring compliance with the Environment Protection Act 1993. Currently, a bi-annually monitoring program is conducted which includes the standardisation of monitoring bore sample collection methods and standardised sample analysis for a range of physical and chemical parameters.

An additional groundwater monitoring program was initiated for five South Australian regional WWTPs over 24 months (2 × Eyre Peninsula, 3 × Southeast), to evaluate the application of FST analysis as a lagoon leakage management approach. Faecal indicator bacteria (FIBs i.e., coliforms, *Escherichia coli*, and clostridia spores) have been widely used to assess the water quality and treatment performance for decades and their presence points to the possible existence of similar pathogens. However, they fail to discriminate between human and animal sources and there are many complexities related to the ecology of FIBs. These include prolonged environmental persistence and the inability to distinguish between recent and past contamination events, particle association, and the capability of some FIB to not only persist but replicate outside a host. Advances in molecular biology have led to the advancement of molecular based FST techniques which utilise quantitative Polymerase Chain Reaction (qPCR) assays targeting the Bacteroidales 16S rRNA genes in the analysis of water samples. An advantage this approach possesses, is that specific molecular markers exist that can identify contamination of human origin. Furthermore,

Bacteroidales rapidly perish in the environment and therefore their presence is suggestive of recent contamination (WaterRA 2021).

The FST analysis in this work utilised two qPCR markers, BacUni and HF183, to assess the bore water's microbiological quality. The BacUni marker indicates general faecal contamination from warm blooded vertebrates—including humans. The marker quantifies the presence of Bacteroidales (Order Level—group of bacteria associated with warm blooded vertebrates). It signals recent faecal contamination as it breaks down rapidly in the environment (temperature dependent $\log_{10}$ reduction in days). The HF183 marker quantifies the presence of a human specific *Bacteroides* (derived from the organism *Bacteroides dorei*). It also indicates recent faecal contamination as it too breaks down rapidly in the environment.

The incorporation of FST markers (BacUni and HF183) in combination with other parameters (nutrient analysis, anaerobic spores, hydrology assessment), has identified that some of the treatment plant locations have dispersed faecal contamination. Figure 1.1 and Figure 1.2 illustrate the BacUni abundance and *Clostridium perfringens* concentrations at one of the regional treatment sites. While these FST results are overall higher compared to other sites investigated, scrutiny of these results indicate that bore 2 is particularly problematic due to multiple indicators suggesting high levels of contamination. In addition, the program is also being used to monitor the remediation of a treatment site which has had sludge lagoons decommissioned and the installation of new sludge management processes.

This project highlights how industry is applying recent molecular biology techniques to move towards a greater understanding of the risk of aging regional infrastructure with higher lagoon leakage potential. Early detection of lagoon leakage is not only critical to public health due to the importance in preventing disease but also due to the economic repercussions associated with treatment. This project has investigated opportunities to make leakage monitoring at WWTPs more efficient and effective, and ensure we maintain water quality.

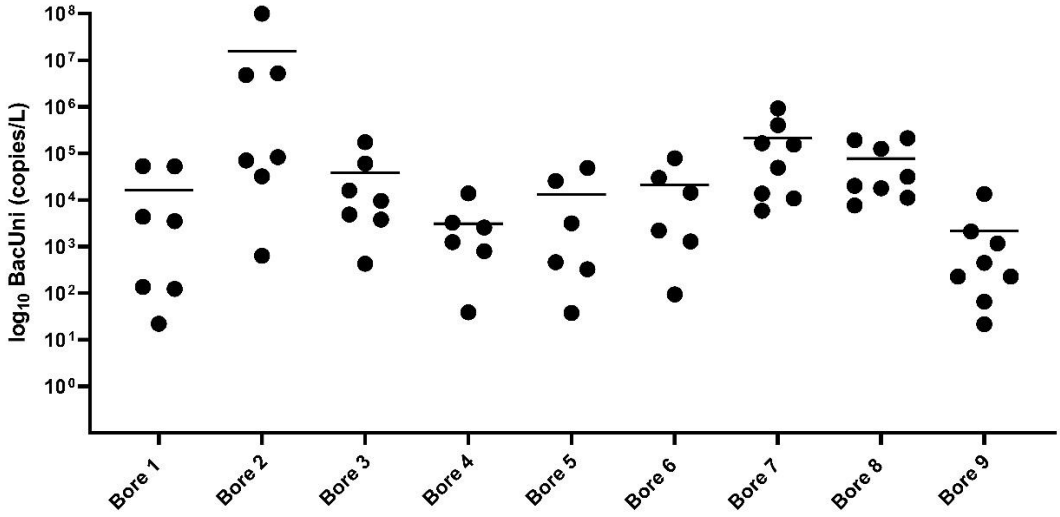

Figure 1.1 Log_{10} BacUni abundance at a regional South Australian WWTP. Line represents mean.

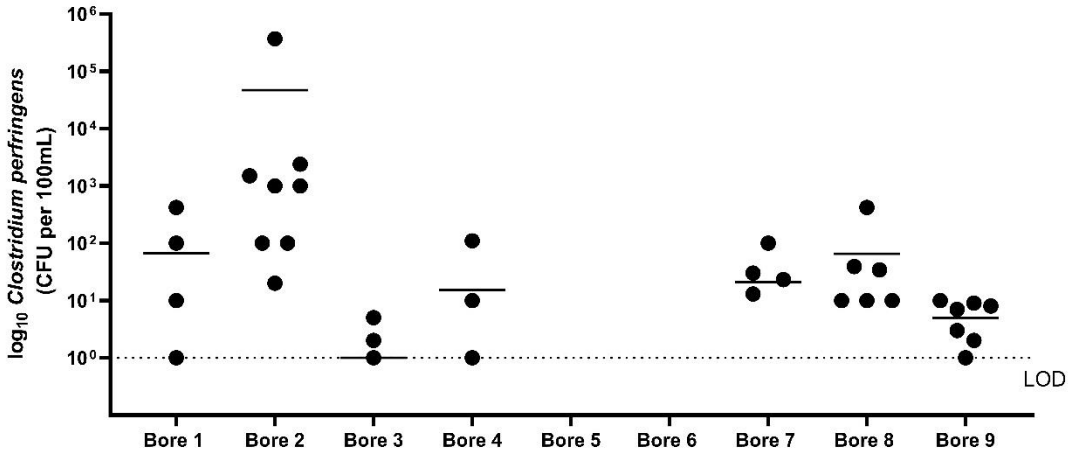

Figure 1.2 Enumeration of *Clostridium perfringens* at a regional South Australian WWTP. Dotted line indicates the limit of detection (LOD). Solid line represents mean.

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