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Development of a rapid approach for the enumeration of *Escherichia coli* in riverbed sediment: case study, the Apies River, South Africa

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Abstract

Purpose: Research in the last few decades has increasingly demonstrated riverbed sediments as reservoirs of pathogenic bacteria, which could be released into water resources when sediments are resuspended. Current methods for enumerating these organisms in sediments could lead to either overestimation or underestimation of the microbial population in sediments. Therefore, an approach was developed based on Archimedes' principle to accurately determine microbial counts in sediments and enable better comparison of water and sediment microbial counts.

Materials and methods: Using the common approach (weighing method), 100 g of sediment was accurately weighed and transferred into a graduated 1-l Durham bottle containing 400 ml of 1× phosphate-buffered saline (PBS). This gave a 20 % dilution (g/v). In the proposed approach (water displacement method), 400 ml of 1× PBS was transferred into a graduated Durham bottle, and sediment was gradually added until the 500-ml mark was reached, giving a 20 % dilution (v/v). The bottles containing sediments from both methods were then manually shaken to dislodge the microorganisms, and an appropriate volume was extracted from each bottle for analysis using the Colilert™ 18/Quantitray-2000 system from IDEXX.

Results and discussion: There was a statistically significant difference between the mean *Escherichia coli* counts when comparing the weighing method to the water displacement approach. The time required for the proposed (water displacement) approach was significantly lower than that required for the weighing approach. This method was found to be highly reproducible. Application of the water displacement method to samples collected at different points of the Apies River, Gauteng, South Africa, gave a greater microbial yield than the weighing method. Calculating the actual volume of sediment with the water displacement method results in a greater yield when determining microbial counts in the sediment. Furthermore, since sediment and water microbial counts are expressed in the same unit (MPN/100 ml), direct comparison of counts is possible.

Conclusions: The water displacement approach to quantifying bacteria in sediment results in a more accurate microbial count is easy to carryout, highly reproducible and time efficient when processing large number of samples. Considering that pathogens such as *Vibrio cholerae*, *Salmonella* sp. and *Shigella* sp. also reside in sediment but in very low infectious

doses, the proposed method could be critical for accurate enumeration when determining what public health risk these organisms might pose.