Misdiagnosing Our Water Quality? A Cumulative Water-Sampling Method Suggests Need for New Standards in the United States

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Introduction

Across the world, it is estimated that 4.5 billion people[1] live near water sources "impaired" for use or contact. Standards for human-interaction are established by international organizations such as the WHO, and legislative bodies from national to local levels with jurisdiction over the quality of our waterways to ensure public & environmental health. Standards are often assessed from "grab-samples" taken from a waterbody at a certain time, with a minimum number analyzed. Water-quality standards in the United States are enforced under the Clean Water Act[2] (CWA) via the Environmental Protection Agency (EPA), applying to "waters of the United States" (WOTUS). After January 2019,[3] "WOTUS" has been interpreted with variation as "traditionally navigable waters", their tributaries, or bodies with a "significant nexus" to them – leaving others outside federal jurisdiction or protection. However, since the CWA requires States[4] to develop "Total Maximum Daily Loads" to provide a framework for when water quality is not healthy for contact, States enforce TMDL-standards under their own jurisdictions. Regardless of whether one could traditionally "mark twain"[5] to navigate a vessel on a stream, or the variations in State jurisdictions, the most legally promoted scientific methodology used to assess TMDL's for surface water quality across the U.S. has not changed: grab-sampling.
This paper describes a water quality study focused in southwest Louisville, Kentucky, compared to two different streams in the Salt River sub-basin to the Ohio River. The study developed cumulative methods for sampling that were accessibly cost-effective. The method profiled fecal contamination before stream-restoration with strong correlations compared to frequent grab-samples, diagnosed contamination causes, and ultimately, suggests improvement over TMDL grab-sampling and a need to form new standards with cumulative methods.

Methodology & Results

In this study, grab-samples used to assess fecal contamination for *E. coli* bacteria were based on U.S. and Commonwealth of Kentucky TMDL contact standards[6] and compared to a cumulative method. The study focused on sites on Mill Creek in southwest Jefferson County (a "study-

[1] Harvey, Fiona. "Water Crisis Widening: 4.5 Billion People Live near 'Impaired Water Sources.'" Mongabay, Conservation News, 28 May 2013, news.mongabay.com/2013/05/water-crisis-widening-4-5-billion-people-live-near-impaired-water-sources/.
[2] 33 U.S.C §§ 1251 *et seq.*
[3] *Rapanos v. United States*, 547 U.S. 715 (2019), applying *Marks v. United States*, 430 U.S. 188 (1977) to plurality.
[4] 33 U.S.C. §§ 1251-1387, 40 C.F.R. 131
[5] "Mark Twain" is an archaic term describing the final of three marks of length (half, quarter, mark) on a line used to measure depth in a river, historically exclaimed by American riverboat captains to signify a depth of two fathoms, or 12 feet, which is regarded as a safe depth for river boat navigation; and is also the pseudonym for the author Samuel Langhorn Clemens.
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stream"), two "poorer-quality" reference sites on a stream "impaired" under TMDL standards for *E.coli* contamination (Beargrass Creek, urban Louisville), and two "higher-quality" sites on a restored & protected stream with little contaminants (Wilson Creek, Bernheim Research Forest and Arboretum, Nelson County, Kentucky). Frequent grab-samples were taken over various periods (including 1-month on Mill Creek with little rain) and a cumulative sample device was also placed and then retrieved for analysis at the end of the period. Two grab-sample methods were also tested for comparison. One is the Aquagenex Compartment Bag Test (CBT) developed at UNC Chapel Hill, promoted for low-cost, WHO standard compatibility, and incubation in the field. It has been used globally, yielding results in a most-probable range. The other test used is a common historic standard of membrane filtration[7] (MFT).

Cumulative sampling methods were studied using sediment bags in the 1990's. These original sediment-bag studies found direct linear-correlation with grab-sampled *E.coli* in a lab setting, and demonstrated sediment bags as more effective at contaminant tracing in the field. Drawbacks to the method included particle-loss to flows, or inability to function in low-flow. Methods from these studies were then more-standardized through a U.S. Geological Survey study[8] in 2005. No further study occurred until the present, with no reports[9] of correlation between frequent grab-samples and cumulative sample methods with sediment.

This study designed a new device with diatomaceous earth (DE)[10] and structural protections to mitigate past issues. This device is patent-pending with the US Geologic Survey. Sediment-based sampling for fecal contaminants works by placing a contained volume of sediment in the stream, where bacteria attach and may be deposited in the sediment from stream-flow. The bacteria form a "biofilm" on the periphery of the sediment column. DE is retrieved and MFT tested, representing a sum count. These levels are dependent on flow-based deposits of bacteria from tributaries, surfaces, and streambeds[11]; however, source and growth-based processes are more important factors[12]. Cumulative-sampling inherently reflects a more-total result from these processes of contamination than grab-samples. Grab-samples provide a single-window with limited ability to make inferences until frequent samples are taken.

Combining the cumulative device with frequent grab-samples allowed for improved diagnosis of contaminants, and the first naturally observed correlation in this study. The table below categorizes Spearman rho correlation strength across all subsets of comparison between cumulative and grab-samples where significant p-value was indicated.

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[7] EPA Method 1604. (Author's note: A water sample is obtained, filtered through a membrane leaving *E.coli* bacteria on a filter paper with nutrient "MI agar", and colonies may be counted on the paper after growth.)
[8] Cinotto, P.J. (2005): Occurrence of fecal-indicator bacteria and protocols for identification of fecal-contamination sources in selected reaches of the West Branch Brandywine Creek, Chester County, Pennsylvania: U.S. Geological Survey Scientific Investigations Report 2005-5039, 91 p.
[9] Y. A. Pachepsky & D. R. Shelton (2011): Escherichia Coli and Fecal Coliforms in Freshwater and Estuarine Sediments, Critical Reviews in Environmental Science and Technology, 41:12, 1067-1110
[10] Diatomaceous earth is a naturally porous sediment-material formed from diatoms, with properties conducive to water absorption.
[11] Fang H. et al. (2020): Numerical Simulation of Bio-sediment Transport. Mechanics of Bio-Sediment Transport.
[12] McCarthy, D. T., Deletic, A., Mitchell, V. G., & Diaper, C. (2013): Predicting Between-Event Variability of *Escherichia coli* in Urban Storm Water. Journal of Environmental Engineering, 139(5), 728–737. ; He, L. M., Lu, J., and Shi, W. (2007): Variability of fecal indicator bacteria in flowing and ponded waters in southern California: Implications for bacterial TMDL development and implementation. Water Research, 41, 3132–3140.

Table: *Qualitative level of significant (p<.05) Spearman's Rho Correlation with cumulative sampling compared to the indicated Grab Sample test and associated TMDL standards, across relevant subsets.*			
CBT Test (Reflecting U.S., and KY Standards), **Subset**: All Samples	**MFT Test** (Reflecting U.S., and KY Standards), **Subset:** All Samples	**MFT Test** (Reflecting U.S. Standards), **Subset:** Mill Creek Study-Site Samples	**MFT Test** (Reflecting U.S. Standards), **Subset:** Low-Flow Samples
Very Strong	Strong to Very Strong	Strongest	Very Strong

[13]

Conclusions & Discussion

The comparison of this cumulative sampling method to grab-sampling questions the ability for grab-sample based TMDL methods to assess environmentally healthy contaminant levels. The frequency of grab-sampling from this study is wholly impractical to expect for any public or environmental health authority to regularly replicate. If data were analyzed by any combination of the State of Kentucky's five sample legal minimum, perhaps similar to other states, no significant p-value would be observed let alone correlation of worth. This also leaves the question if the quality of waterways may be mis-diagnosed with use of grab-sampling. Any lack of correlation or significance in comparison to grab-sample values (averages, geometric means, or theoretical sums from estimated integration of a grab-sample scatterplot) from this study or similar studies may *not* be from an inability of cumulative-sampling to reflect total contamination, but rather the variance from an inherent lack of capability to capture more than single "snapshots" of pollutant levels with TMDL grab-sampling. Observed trends in cumulative-sampling also suggested low values on Mill Creek corresponded to higher than expected grab-samples. These trends diagnosed fecal persistence as a concern, not flow-based pollution as traditional methods may suggest, suggesting that nutrient-abundance and uptake by organics in the stream (not detected from past nutrient grab-samples in the water) must persist the *E.coli* presence.

Grab-samples leave us asking how much contamination has truly occurred, which is a fundamentally similar to if waterbody is healthy or impaired- regardless of navigability. This cost-effective cumulative water sampling is a powerful idea because it allows an answer. That answer provides knowledge on the quality of water in our environment relevant to our own health and informs us if that quality is truly healthy for the environment. To ensure both and harness the power of this idea, new legal standards must be developed for cumulative sampling.

[13] Qualitative scale: 0.5-0.65 (strong), 0.66-0.80 (very strong), > 0.81 (strongest). Arithmetic mean suggested to reflect TMDL grab-sample standards across the U.S. given statutory variation, Geometric Mean to reflect Kentucky standards given statutory assessment of a geometric mean from grab-sample minimum within a 30-day period.

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