Can We Get *E.coli* Results Faster From Tryptophan Fluorometry?

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PROBLEM

The microbial contamination of waterways by fecal microbes, and specifically, pathogenic microbes, presents a major problem in the United States and worldwide. The World Health Organization (WHO) estimates that diarrheal diseases account for 1.8 million of the 3.1 million water-related deaths per year worldwide (Yan et al. 2007). Recreational waters are susceptible to variety of microbial pollution sources containing pathogenic microorganisms that can cause GI, upper respiratory tract, ears, eyes, nasal cavity and skin infections (Seurinck et al. 2006). Existing approaches typically used for measuring fecal indicator bacteria take 18-24 hours, which is too long since water conditions may change rapidly putting swimmers at increased risk. More rapid methods for water quality determination include the use of lake-specific predictive models and expensive molecular methods. An alternative approach hereby considered uses tryptophan fluorescence real-time.

BACKGROUND & SIGNIFICANCE

Globally, it is estimated that each year more than 120 million cases of gastrointestinal disease and 50 million cases of severe respiratory diseases are caused by swimming and bathing in wastewater-polluted coastal waters, (Abdelzaher et.al 2010). Sources of microorganisms include: sewage, storm water, combined sewer outflows & effluent from wastewater treatment plants. The overall goal of the current ambient water quality criteria for bacteria in the United States is to provide public health protection from GI illnesses associated with exposure to waters contaminated by fecal microbes during water-contact recreation. The U.S. EPA guidelines require monitoring either *E. coli* (freshwater) and enterococci (marine waters) since these organisms have shown strong relationships to gastrointestinal illnesses in epidemiological studies (Glassmeyer et al. 2005). The hypothesis here is that tryptophan fluorometery will predict *E.coli* densities in both Lake Reba and Wilgreen Lake.

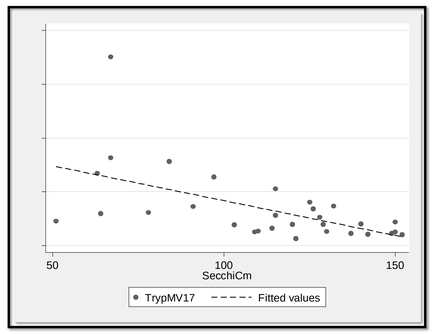
METHODS

- The project involved analysis of water from Lake Reba and Wilgreen Lake located in Madison County, Kentucky.
- Water samples were collected using sterile Whirl-Pak® bags
- Secchi depth (cm) was used to measure the transparency of both lakes
- *E.coli* density was quantified using EPA method 1603 (Modified m-TEC).
- Nitrates analysis was performed in accordance with Hach® DR890 colorimeter, Cadmium Reduction Method
- Total Phosphorus was analyzed with Method 8190, U.S. EPA PhosVer ® with Acid Persulfate Digestion Method with a DR 2800 spectrophotometer
- Turner Designs Cyclops® Submersible Fluorometer was utilized to collect tryptophan data. The Raw Fluorescence Units (RFUs) and Millivolts (MVs) were measured in the surface waters at 17cm and 1m depths.

Figures 1 & 2 (far left and left)**.** Fluorometer in use and Whirl-Pak® sample collection at Lake Reba (Madison Co., Kentucky)

RESULTS & DISCUSSION

- A total of 30 samples collected. The results demonstrate Wilgreen Lake had greater transparency with secchi depth (Table 1). Tryptophan signals were consistently stronger at Lake Reba than Wilgreen Lake at both depths (1m & 17 cm).The *E. coli* and phosphorus levels were typically higher at Lake Reba than Wilgreen Lake. Only nitrate was higher at Wilgreen Lake typically compared to Lake Reba.
- Data were not normally distributed, so Spearman correlations were considered
- Strongest association was between the 1m and 17cm for tryptophan, suggesting that tryptophan levels at both depths are strongly related (Table 2).
- Tryptophan levels correlate with *E. coli* levels when the results are aggregated (Table 2).
- Water transparency and tryptophan were correlated at both 1m and 17cm (Figure 1).
- When evaluating the predictive capability of tryptophan for *E. coli* densities, there is a marginal association when doing linear regression analysis using the data for the two lakes aggregated ($p = 0.052$; $R^2 = 12.7\%$; Figure 2).



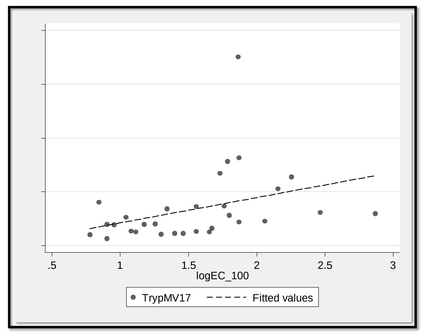


Figure 3 (left). Scatterplot between tryptophan results (mV) at 17cm versus secchi depths (cm) at both lakes. **Figure 4 (right)**. Scatterplot between tryptophan results (mV) at 17cm versus $\log_{10}$ *E. coli* (CFU/100 mL) at both lakes.

Table 1. Descriptive data for water quality parameters by sample location.

Variable	Location	Mean	Median	Range
Secchi Depth (cm)	Reba	96.3	91	51.0-150
	Wilgreen	128	126	103-152
Tryptophan-1m (mV)	Reba	1446	1332	1138-1840
	Wilgreen	1198	1144	1093-1425
Tryptophan-17cm (mV)	Reba	1505	1360	1128-2752
	Wilgreen	1176	1132	1066-1401
Total Nitrate (mg/L)	Reba	1.30	0.70	0.20-2.60
	Wilgreen	1.37	1.50	0.30-2.80
Total Phosphorus (mg/L)	Reba	0.64	0.62	0.47-0.99
	Wilgreen	0.61	0.44	0.20-2.70
***E. coli* (CFUs/100 mL)**	Reba	135	73.00	18.0-742
	Wilgreen	18	13.00	6.00-47

Table 2. Spearman correlation coefficients for the aggregated samples (*bold with italics indicates the correlation is significant at $p < 0.05$).

	tryp_mv17cm	tryp_mv1m	secchi	nitrate	total_P
tryp_mv1m	***0.9318****				
secchi depth	***-0.6033***	***-0.5887***			
nitrate	-0.3338	-0.2467	0.1951		
total_P	0.2330	0.1218	***-0.4526***	***0.3893***	
E. coli	***0.5644***	***0.4752***	***-0.5490***	-0.0800	***0.5203***

IMPLICATIONS/CONCLUSIONS

- Inverse relationship between tryptophan and nitrates; as tryptophan levels decrease, nitrate levels increase.
- Both secchi depth and nitrate were associated with phosphorus, whereby the secchi depth association is inverse.
- Tryptophan levels decrease as the water becomes clearer (higher secchi depth values). As the water is more turbid (less clear), the tryptophan levels are higher.
- No association between tryptophan and *E.coli* when evaluating individual lakes potentially limiting the application of this technology.
- With a larger sample size, multivariable regression analysis can be performed to enable a better assessment of the relationship between secchi depth and tryptophan as well as for developing a multivariable model that can predict *E. coli* levels based upon a measurement of both secchi depth and tryptophan.
- Presently, results are inconclusive at the lake-specific level and additional samples are being collected to increase sample size.
- In the aggregate (combining both lakes), tryptophan levels are indicative of fecal bacteria densities, which potentially could be used by health officials issuing swimming advisories and still presents some promise for potential use for routine monitoring of beach water quality instantaneously.

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