



# Investigating caffeine levels in water sources in Morehead, Kentucky



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## INTRODUCTION

The purpose of this research is to determine the caffeine levels in local water sources and to determine the level of pollutants in the water. This research is valuable due to the growing precedence of pollutants in water samples.<sup>1</sup> These pollutants not only affect the drinking water, but also affect fish and nearby wildlife that utilize these water sources.<sup>2</sup> These negative effects can greatly change the entire ecosystem around polluted bodies of water.

It has been well established that caffeine can be found in natural water samples. Buerge et al.<sup>3</sup> has attributed the high caffeine levels to be a result of wastewater treatment plant discharge. In December 2002, Gardinali et al.<sup>4</sup> reported finding trace levels of caffeine in surface waters around Biscayne Bay, Florida. Nagoda et al.<sup>5</sup> reported finding no correlation between average inches of rainfall and the caffeine concentration on the river surface in the San Diego River region, even though caffeine was present in 56% of the 85 sample locations. Rodriguez del Ray et al.<sup>6</sup> have attributed environmental caffeine to excess human consumption and subsequent urination.

## MATERIALS AND METHODS

All site samples were obtained in one liter sterilized plastic bottles and stored for a maximum of three months at room temperature or below. The site samples were collected from the Morehead City Park Complex, Triplett Creek before and after the water treatment center (WTC), Cave Run Lake, the Spillway, Stoney Cove, and Eagle Lake, an on-campus accessible water source (Figures 1 & 2). Prior to solid-phase extraction, conc. NaOH was added until the site samples turned pH 9.

Caffeine extraction was performed using C18-reverse phase SPE cartridges (Resprep, 6 mL, 500 mg C18, Cat. No. 24052). SPE cartridges were prepared using MeOH (6 mL) and then pH 9 H<sub>2</sub>O (6 mL). The site sample was passed through the cartridge as shown in Figure 3, flow rates were approximately 5 to 10 mL/min. The cartridge was then washed with pH 9 H<sub>2</sub>O (1 mL). Caffeine was recovered using MeOH (2 x 3 mL) and pH 3.7 MeOH (with AcOH, 2 x 2 mL) into a large test tube. The solvent was removed with gentle heating and reconstituted with 2 mL of HPLC eluent for analysis.

HPLC analysis was performed on a Dionex UltiMate 3000 HPLC with an Acclaim® 120 C18 5 mm (4.6 x 150 mm) column at 254 nm wavelength. The eluent was 60:40 H<sub>2</sub>O:MeOH at a 0.7 mL/min flow rate.

Calibration standards were made from caffeine (CCI, 21591-010) in MeOH (pH 3.7 by AcOH), as reported in Table 2.

## RESULTS

Sample Location	Fall 2017	Spring 2018
	Concentration (mg/L)	Concentration (mg/L)
Eagle Lake 1	BDL	5.12
Eagle Lake 2	BDL	0.618
Spillway 1	BDL	Not tested
Spillway 2	BDL	Not tested
Stoney Cove 1	BDL	0.566
Stoney Cove 2	BDL	0.885
Picnic Area 1	0.689	1.66
Picnic Area 2	6.43	0.759
Cave Run 1	BDL	Not tested
Cave Run 2	15.5	Not tested
Triplett Creek Before WTC 1	BDL	0.492
Triplett Creek Before WTC 2	BDL	18.3
Triplett Creek After WTC 1	BDL	5.11
Triplett Creek After WTC 2	BDL	1.75

Table 1. HPLC analysis of each site sample collected.

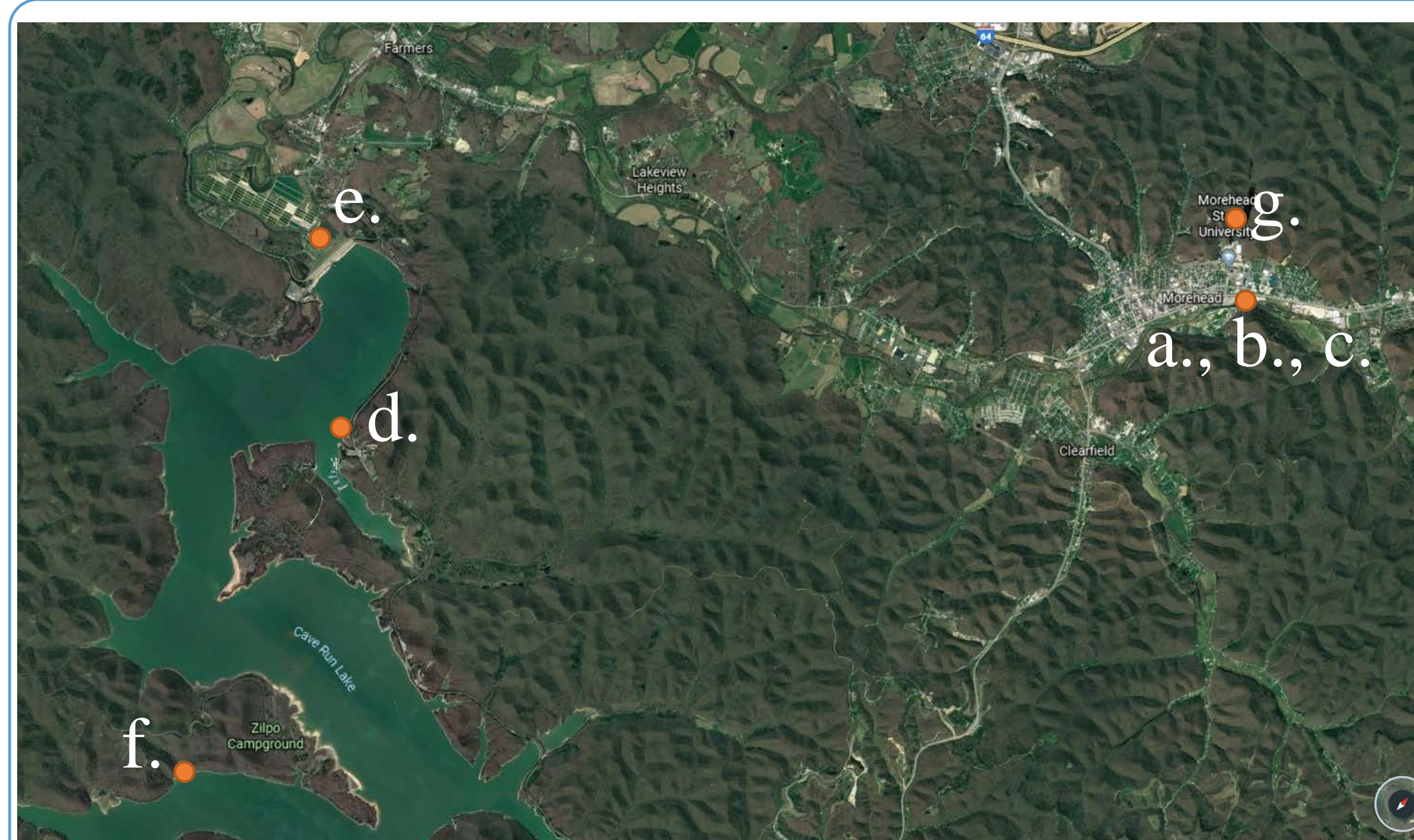


Figure 1. Map locations of (a) Morehead City Park picnic area, (b) Triplett Creek before WTC, (c) Triplett Creek after WTC, (d) Cave Run Lake, (e) Spillway, (f) Stoney Cove, (g) Eagle Lake sample locations.

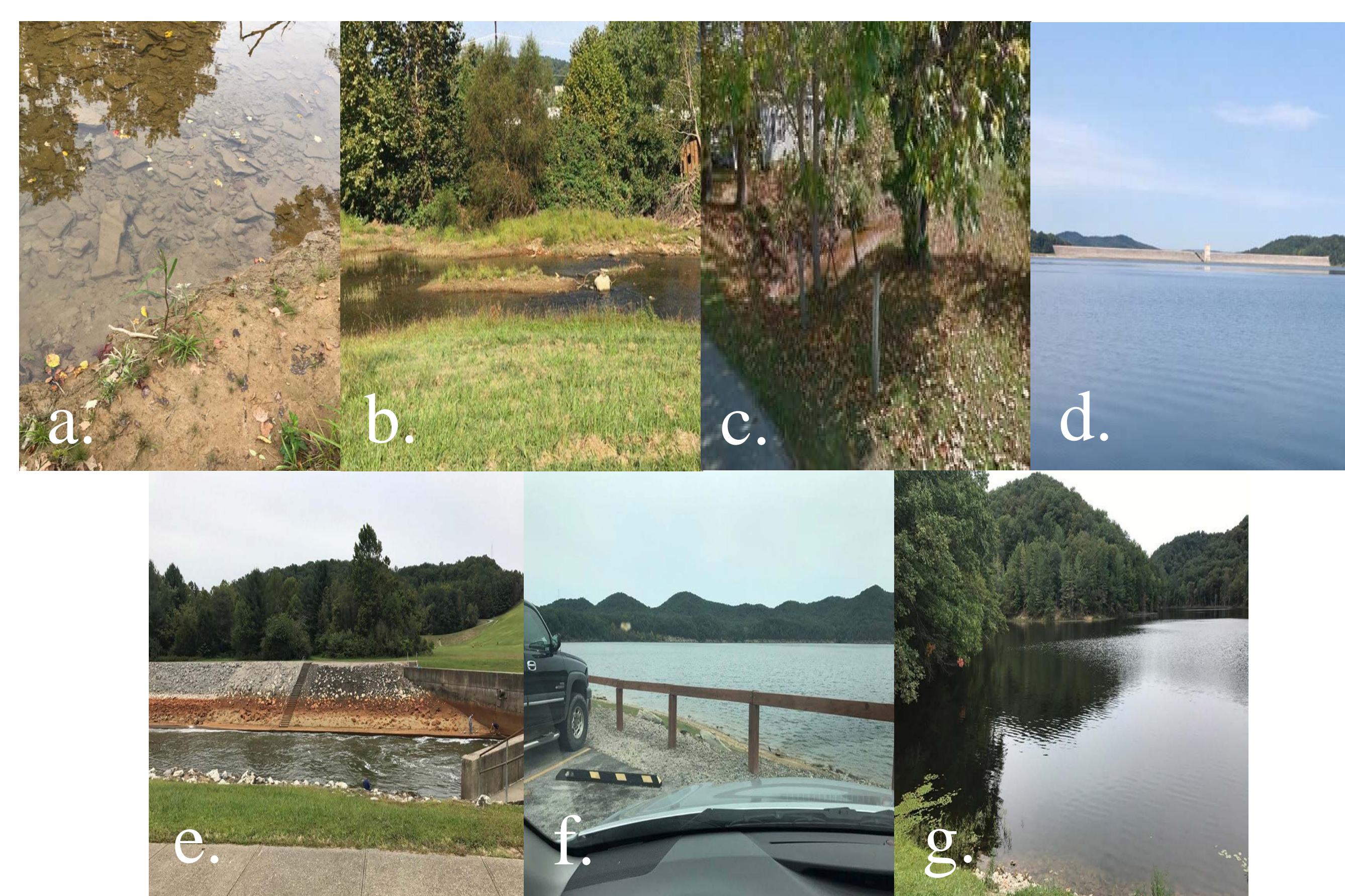


Figure 2. Locations of sample (a) Morehead City Park picnic area, (b) Triplett Creek before WTC, (c) Triplett Creek after WTC, (d) Cave Run Lake, (e) Spillway, (f) Stoney Cove, (g) Eagle Lake sample locations.



Figure 3. Extraction set-up for sample solutions

Concentration (mg/L)	Area (mAU*min)
0.01	0
0.1	0.4081
0.8	0.7745
5	2.1175
10	5.7674
50	21.1956
200	68.4276
1000	377.0077

Table 2. Calibration standards

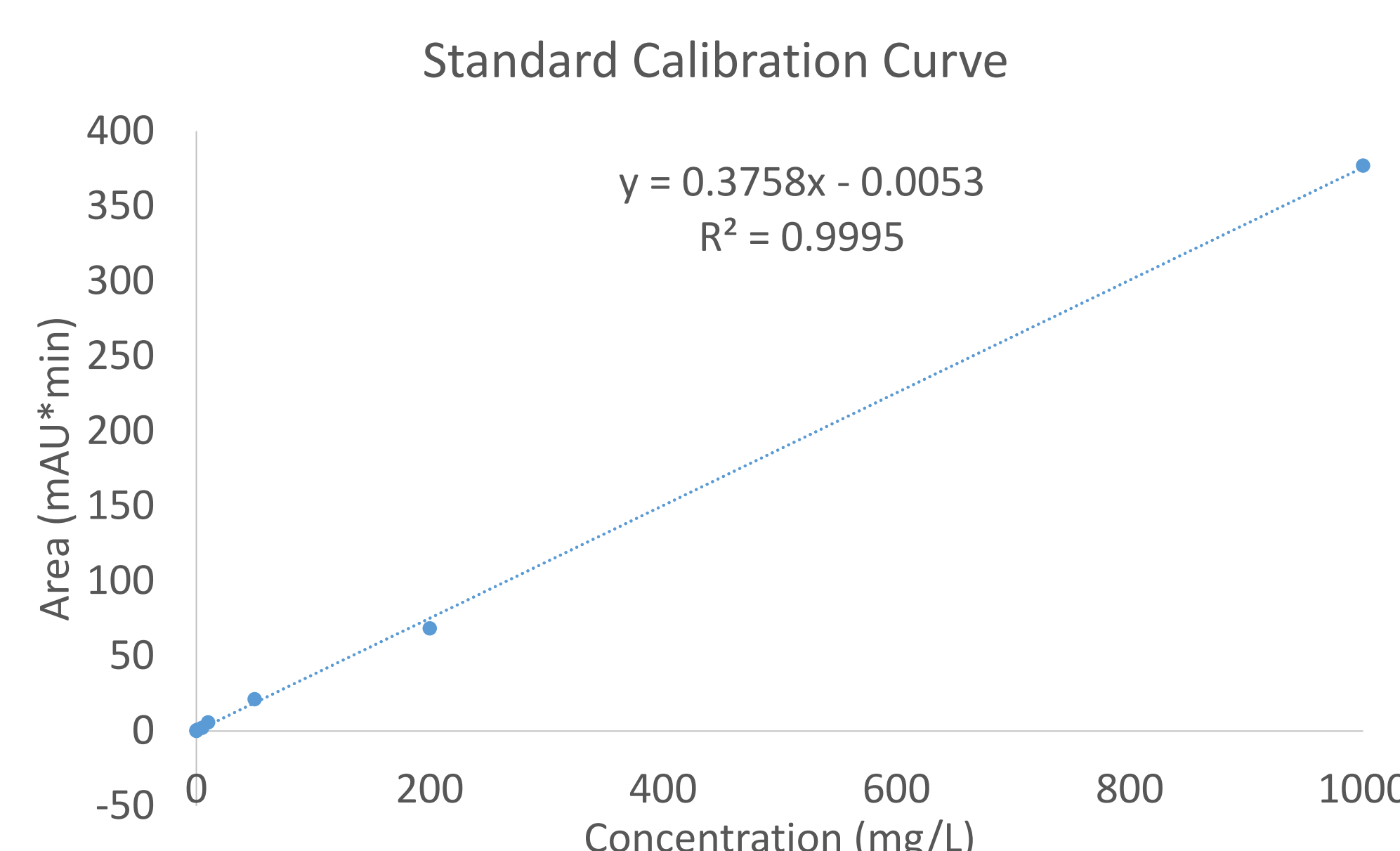


Figure 4. Calibration curve.

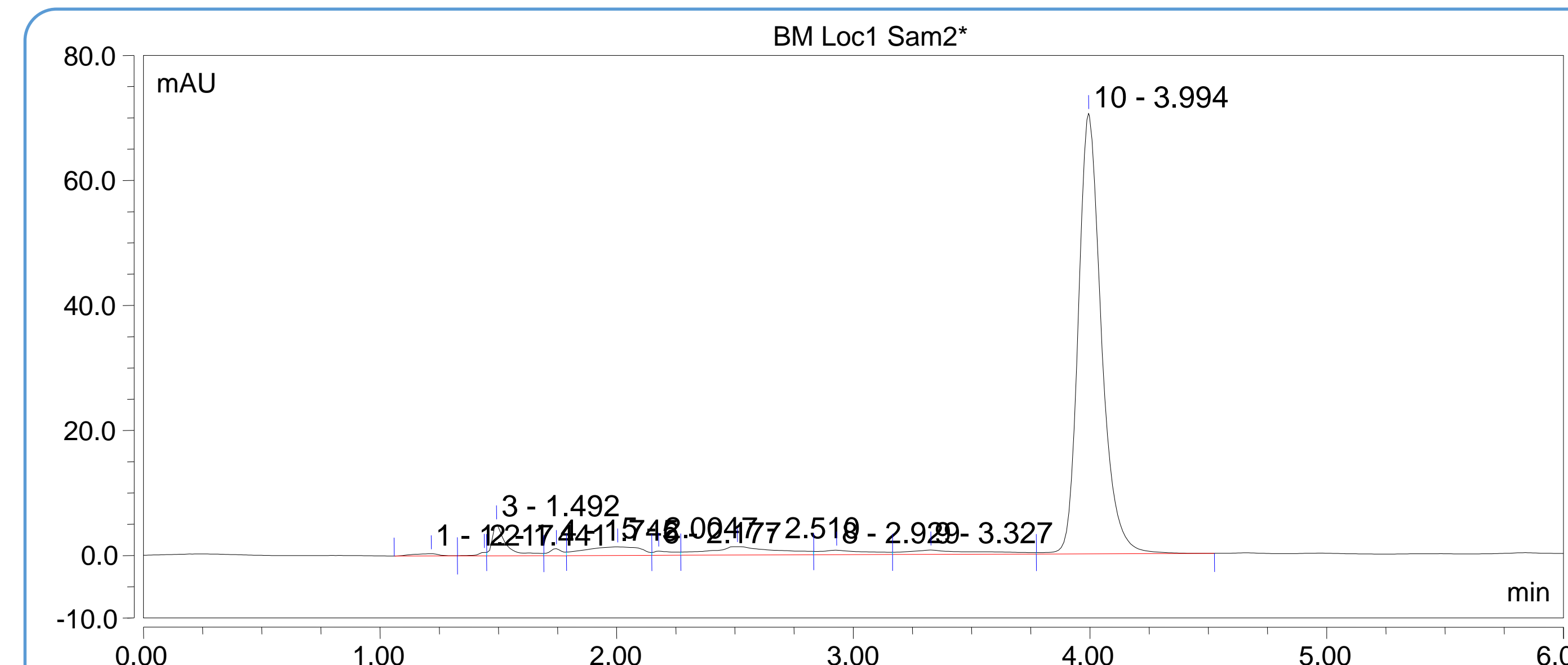


Figure 5. HPLC chromatograph of Cave Run sample 2.

## CONCLUSIONS

Through the evidence gathered in this experiment, there is an indication that not only does caffeine exist in quantifiable concentrations in water sources around Morehead, Kentucky, but there is also other unknown pollutants in these water sources that could potentially include pharmaceuticals. In Fall 2017, only two locations contained caffeine, the Morehead City Park picnic area and Cave Run Lake, which are areas with heavy human traffic. In Spring 2018, caffeine was isolated at every location tested.

The purpose of this testing was to determine if it was possible to detect caffeine in surface water sample, which we have been able to do successfully.

## FUTURE PLANS

We will continue to monitor the caffeine levels at all test sites to determine the extent of caffeine pollution in our local environment. In the future, we want to use a more sensitive instrument to overcome the detection limit of our HPLC and quantify the caffeine levels in sub-milligram ranges.

This research will be further extended in the upcoming semesters to explore other pollutants we found and the results will be potentially evaluated by the United States Department of Agriculture Daniel Boone National Forrest Branch if deemed necessary.

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