Research Article

Domestic Wastewater Discharges into Rural Alabama Streams and Impact on Water Quality

Evsel Atıksuların Alabama Kırsalındaki Akarsulara Deşarjı ve Su Kalitesine Etkisi

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Abstract

In rural areas, direct discharge of untreated domestic wastewaters can cause harmful effects on the region's rivers and streams, so determining and addressing the water quality of these aquatic ecosystems requires identification of the sources of contamination, such as runoff sources from straight pipes, or septic systems. Surface water quality in Hale County in Alabama, USA was evaluated at least once a month at twenty sites in wet and dry seasons. Samples were analyzed for physical (turbidity), chemical (pH, conductivity, chloride, sulfate, calcium, iron, magnesium, potassium, sodium, ammonium, ortho-phosphorus, nitrite, nitrate, dissolved organic carbon, optical indices), and microbiological (*E. coli*) water quality parameters. Excitation-emission matrixes Parallel Factor Analysis was used to identify and classify fluorescence emitting organic substances based on fluorescence peak location. Principal Component Analysis was used to identify analytic signatures associated with sewage contamination. To detect direct discharge wastewater impacts on water quality, three main sites were sampled upstream, midstream and downstream of the town of Newbern, Alabama over the three months of the drought period (i.e., from September to November, 2016). Over 20 water quality parameters were analyzed and compared with the World Health Organization, Environmental Protection Agency and Alabama Department of Environmental Management standards. The results showed that *E. coli* values highly exceeded the water quality standards, particularly after the drought when peak *E. coli* concentrations in the downstream site exceeded 100,000 MPN per 100 mL. Our study is one of the first documents concerning the adverse impacts of direct discharges on water quality in the United States.

Keywords: *water quality, water management, wastewater, watershed, rural Alabama streams*

Öz

Kırsal alanlarda, evlerden doğrudan deşarj edilen arıtılmamış atık sular bölgenin nehirleri ve akarsuları üzerinde zararlı etkilere yol açabildiği için bu sucul ekosistemlerin su kalitesini belirlemek düz borulardan ya da fosseptiklerden gelen atık sular gibi kirlilik kaynaklarının tanımlanmasını gerektirmektedir. Amerika Birleşik Devletlerinin Alabama Eyaletinde bulunan Hale County'deki yüzey suyu kalitesi, en az ayda bir kez olmak üzere, yağışlı ve kuru mevsimlerde yirmi noktada değerlendirilmiştir. Numuneler fiziksel (bulanıklık), kimyasal (pH, iletkenlik, klorür, sülfat, kalsiyum, demir, magnezyum, potasyum, sodyum, amonyum, ortofosfat, nitrit, nitrat, çözünmüş organik karbon, optik indeksler) ve mikrobiyolojik (E. coli) su kalitesi parametreleri olarak analiz edilmiştir. Uyarma-emisyon matrisleri Paralel Faktör Analizi floresan tepe noktasını temel alan floresan yayıcı organik maddeleri tanımlamak ve sınıflandırmak için kullanılmıştır. Ana Bileşen Analizi (PCA), atıksu kirliliği ile ilişkili analitik imzalarını tanımlamak için kullanılmıştır. Doğrudan deşarjların su kalitesi üzerindeki etkilerini tespit etmek için yağışsız dönemde (Eylül-Kasım 2016) üç ay boyunca Newbern, Alabama kasabasının üç ana bölgesinden (nehrin memba, orta ve mansabı) numune alınmıştır. Yirmiden fazla su kalitesi parametresi analiz edilmiş ve Dünya Sağlık Örgütü, Çevre Koruma Ajansı ve Alabama Çevre Yönetimi Bölümü standartları ile karşılaştırılmıştır. Sonuçlar, E. coli değerlerinin su kalite standartlarını oldukça aştığını, özellikle yağışsız dönem sonrası mansapta pik değerlerin 100.000 EMS/100 mLdeğerini aştığını göstermiştir. Bu çalışma, doğrudan deşarjların ABD'deki su kalitesi üzerindeki olumsuz etkilerini belgeleyen ilk çalışmalardan biridir.

Anahtar kelimeler: *su kalitesi, su yönetimi, atıksu, havza, kırsal Alabama akarsuları*

Introduction

About 75% of US population is served with different types of sewer systems which end-up with a domestic wastewater treatment plants. The remaining 25%, 80 million people, should treat their wastewater on site, mostly using conventional septic systems (U.S. EPA, 2013). It is recognized that these systems can fail and, when they do, inadequately treated effluent can have serious environmental effects. Since failing systems pose public health and environmental risks, strategies need to be adopted to control these risks. Additionally, some soil conditions are unsuitable for septic systems. In rural areas with these soil conditions and no access to sewers, many poor residents are left with no affordable, safe and legal options for onsite waste water management.

In the Black Belt region of Alabama USA (Figure 1), many communities struggle with wastewater management. Soil and geology type of Black Belt region is mostly unsuitable for the conventional septic system (He et al., 2011). In addition to failing septic systems, household raw sewage is often discharged directly into wooded areas by using a pipe, so called "straight pipe" (Figure 2) in the rural Black Belt. The prevalence of straight pipes and septic tanks failures in rural areas can cause environmental and human health problems when associated pollutants are discharged to nearby waterways.

White and Jones (2006) reports that in Bibb County, Alabama, 15% of homes not connected with any sewer systems were directly discharging raw sewage. While the impacts of raw sewage discharges to waterways and ecosystems are expected to be substantial, the greatest concern is their threats to human health. Based on these results and Bibb County water use report by Littlepage et al. (2005), we estimate that homes with straight pipes account for over 567 m^3 of raw sewage discharged into the land surface and streams each day in Bibb County alone. Based on average pathogen concentrations in wastewater (Payment and Locas, 2011; Robertson et al., 2006), we estimate that straight pipes in Bibb County potentially discharge billions of infectious pathogens per day. Representative pathogen loads from these straight pipes per day in Bibb County are >3 billion enteric viruses, >5 billion Giardia cysts, and >1 billion Cryptosporidium oocysts. These pathogens have very low infectious doses and survive much longer in the environment than indicator bacteria like E. coli—the absence of E. coli may indicate the absence of viable pathogenic bacteria while these more robust pathogens are still present.

Figure 1. Alabama Black Belt Counties. (URL 1)

Figure 2. A typical straight pipe discharge (EPA Region 4, 2002).

Dissolved organic matter (DOM) is an assemblage of heterogeneous compounds present in all natural waters and is a massive carbon pool in aquatic ecosystems (Purves et al., 2001). DOM is becoming highly recognized for the role it plays in moderating the environmental and ecological processes, such as transforming carbon into the microbial food web, protecting aquatic biota through buffering pH, complexing metals, transporting organic pollutants and absorbing ultraviolet radiation (Martell et al. 1988; Williamson et al. 1994; Findlay 2003; Clements et al., 2008). Although a complex mixture, DOM composition can be characterized by optical properties including using UV-VIS (visible) and fluorescence spectroscopy methods. Relative to molecular methods (e.g., Lu et al., 2015), optical measurements provide a more rapid and economic means for characterizing DOM compositions and distinguishing biological sources. Using UV spectroscopy, a large number of useful proxies can be obtained, such as $SUVA_{254}$ (specific UV absorbance at 254 nm) that indicate aromaticity, E2/E3 ratios (absorbance at 254 nm divided by absorbance at 365 nm) and spectral slopes ratio (S_R) (S_R=S275– 295 nm/S350–400 nm) that measure molecular weight (Helms et al. 2008; Shang et al. 2017). Using fluorescence spectroscopy, fulvic-like, humic-like and protein-like fluorescence components can be detected by gathering emission spectra over a range of excitation wavelengths, generating three-dimensional spectra referred to as excitation-emission matrices (EEM) (Coble, 1996). PARAFAC analysis of EEM creates a model to determine primary components in DOM. This method has been widely applied to DOM studies including evaluating photochemical and microbial modifications (Stedmon and Markager 2005; Lu et al., 2013) and discerning primary sources (Lu et al., 2014; Hu et al., 2016). Using DOM to identify pollution from wastewater in rural streams was one of the focuses of this investigation. The organic matter detected in the stream has a distinct fluorescence signature and distinct characteristics that are different from in stream samples. The sample compositions show small $SUVA_{254}$ value with high DOC concentrations indicating more labile and less aromatic carbon structures. The fluorescence index (FI), calculated as the ratio of intensity at 470 nm/520 nm emission and 370 nm excitation, has been commonly used to indicate the relative contributions of algal versus terrestrial derived DOM. While lower FI is related to more highly processed, terrestrial derived material that has greater aromatic content and higher molecular weight, higher FI is with algal derived material which has lower aromatic content and lower molecular weight (McKnight et al., 2001, Jaffe et al., 2008).

The detection of optical brighteners (OBs), also known as fluorescent whitening agents, is a chemically based microbial source tracking method. OBs comprise of several classes of compounds including water soluble dyes that act as brightening agents by absorbing light in the ultraviolet range and fluorescing in the visible region. They are added not only to the manufacture of paper but also to laundry detergents solid in the United States and other countries (Waye, 2003). OBs may appear in surface waters adjacent to a failing on-site sewage treatment system, since laundry effluents are estimated to be a significant part of total on-site wastewater treatment system (US. Environmental Protection Agency, 1990). The main advantage of OBs as an effluent tracing technique is that sources of OBs are exclusively anthropogenic, which provides indisputable evidence of human impacts on surface waters (Dates, 1999). Besides, OBs are relatively persistent under environmental conditions and resistant to microbial degradation as a tracer (Poiger et al., 1998). Many investigators have reached the same conclusion that these compounds have a high potential as a monitoring wastewater contamination in natural waters (Hagedorn and Weisberg, 2011; Cao et al., 2009; Tavares et al., 2008). These compounds demonstrate a real promise in our data to act as an indicator of human fecal contamination from onsite wastewater discharges into the streams.

This study aims to identify a water quality signature that can be used to identify the presence of wastewater contamination in rural streams in Alabama. It also serves as a baseline study for our ongoing investigation of stream water quality conditions of the Hale County in Alabama. The specific objective of the paper is, we focus on Big Prairie Creek water samples taken from upstream, midstream and downstream before (drought condition) and after precipitation (Post-drought condition), to determine if these samples contain wastewater signals from septic effluent or straight pipes.

Methods

Site Descriptions and Experimental Design

This study was conducted in and around Hale County in the west-central part of Alabama, USA. Samples were collected each month for nine months (March 2016 to November 2016) from twenty sampling points selected along the course of the Hale County (Figure 3) and also three main sites: Upstream, midstream and downstream of the Big Prairie Creek in the town of Newbern were sampled over the three months of the drought period (i.e., from September to November, 2016) to detect straight pipe wastewater impacts on water quality. The stream samples were taken as grab samples, and all were stored in 500 ml polypropylene bottles. These bottles were cleaned by soaking in HCl and then rinsed with tap water and deionized water. The bottles were kept in an ice-packed cooler while they were transported and then kept in the refrigerator for analysis. Some samples were analyzed within five days of their collection and in the case of frozen samples, analyses were made in a month for their date frozen. A portable dissolved oxygen meter and a HACH model portable pH meter were used to make in-situ measurements.

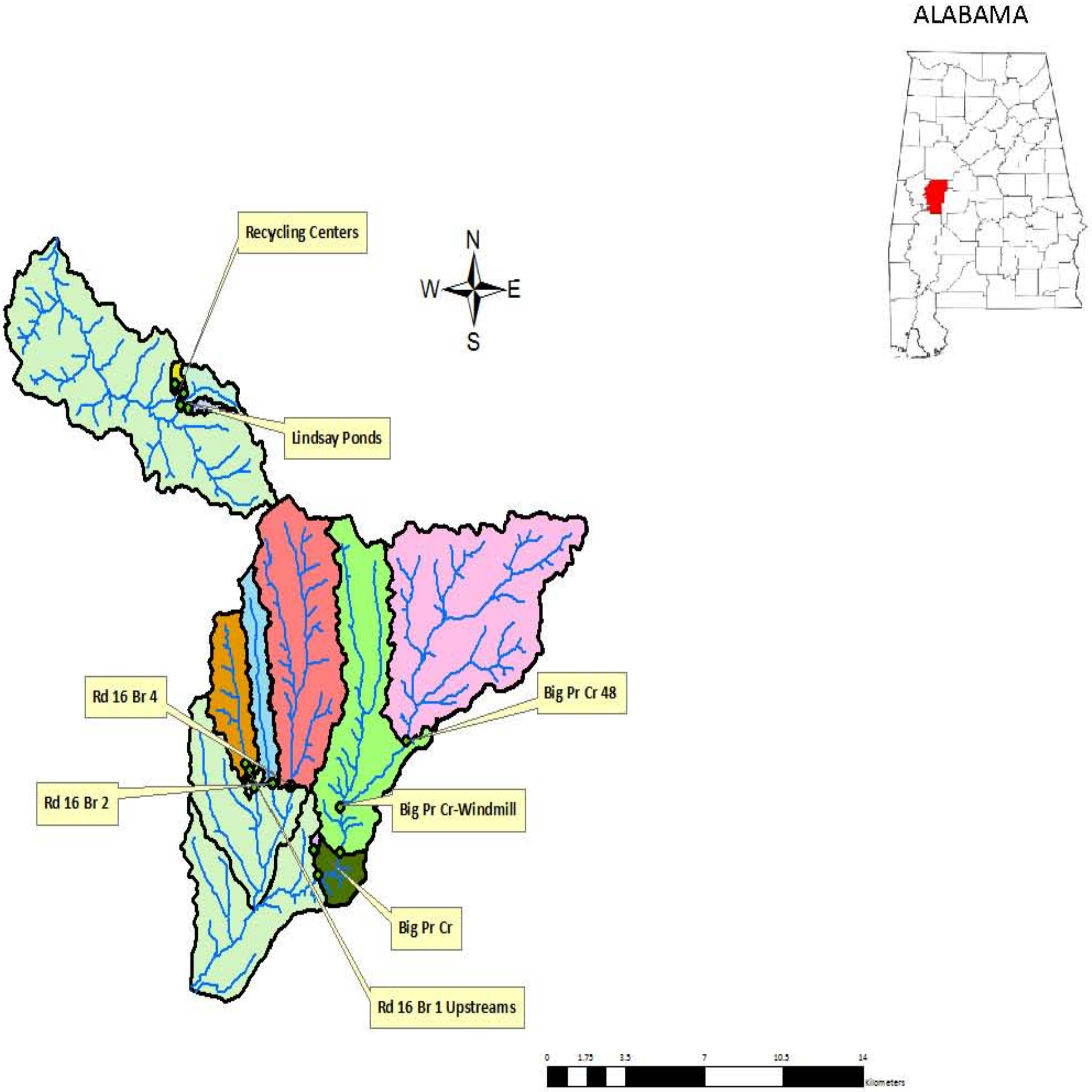

Figure 3. Locations of study sites in Hale County Watershed, Alabama, USA, streams are indicated by blue lines, watershed boundaries are indicated by black lines, and sampling sites are denoted by black dots.

Water Quality Measurements

Water samples were tested for chemical, physical and microbiological parameters. Chemical parameters included anions, cations, DOM, Dissolved Organic Carbon (DOC), nutrients, pH and conductivity. Turbidity was analyzed as a physical measure of particles in the water. E. coli and coliform bacteria were analyzed as microbial indicators.

For microbiological parameters, the IDEXX Quanti-Tray 2000 system with Colilert media (IDEXX Laboratories, Inc., Westbrook, ME) was used for detection of E. coli and coliform bacteria. Dilutions were carried out in sterile phosphate buffered saline (PBS) when high bacterial concentrations were anticipated to ensure a readable quantitative result. The standard analysis procedure was used, as follows: the Colilert media snap pack was opened, and the reagent was added to 100 ml of water sample in a sterile 120 ml vessel and allowed to dissolve. The Quanti-Tray was held upright, the foil tab was pulled without touching the inside of the foil, and the sample was poured into the tray. The Quanti-Tray was sealed with the sealer and incubated 24 and 48 hours at 35 °C. After incubation, wells positive for total coliform turned yellow, and a negative sample looked the same visually as when the sample was collected. Each tray was placed under long-wave, 365 nm ultraviolet (UV) light and wells positive for E. coli fluoresced blue. The most probable number (MPN) of coliforms and E. coli in each 100 mL sample was obtained by counting the positive wells and using appropriate Quanti-Tray table to find the MPN (USEPA 2007).

Turbidity measurements were obtained by the nephelometric method using a HACH 2100Q Portable Turbidimeter with turbidity units (NTUs). The method is based on a comparison of the intensity of light scattered by the sample under defined conditions as compared to the intensity of light scattered by standard reference suspensions under the same circumstances. The standards were used to reference and calibrate the turbidity meter. The higher the intensity of scattered light, the higher the turbidity. Results were reported as nephelometer turbidity units (NTUs).

For chemical analysis, all samples were filtered to 30 mL bottles as anions, cations, nutrients, DOM, DOC separately through a glass-fiber filtration unit in the lab. For the DOC and nutrient concentration analyses were stored at −20°C in the dark. We preserved samples for anion, cation and DOM quality analysis at 4°C in the dark to avoid any potential interferences. Duplicate samples (A & B) were analyzed for 20 locations.

Fluorescence excitation-emission matrix (EEM) measurements were conducted by using a Horiba Jobin Yvon Flouromax-3 spectrofluorometer. To attain fluorescence EEMs, excitation wavelengths increased from 240 to 500 nm at step intervals of 5 nm, yielding 53 excitation wavelength data points (240 nm, 245 nm…. 500 nm). Emission and fluorescence were measured at emission wavelengths of 280 to 538 nm at step intervals of 3 nm resulted in 87 emission wavelength data points. The excitation and emission slits were set to a 5 nm bandpass. A flowthrough water bath was used to keep a constant temperature of 20°C. UV-visible absorbance measurements were conducted with a UV-VIS Spectrophotometer. DOC concentrations were measured as non-purgeable organic carbon and total dissolved N (TDN) using Shimadzu TOC-VCSH analyzer with lower detection limits of 0.4 mg C/L for DOC and 0.1 mg N/L for TDN using high-temperature catalytic oxidation. Glucose was used to construct standard curves, and a consensus seawater reference standard (URL: 2) was used to confirm analytical accuracy. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) was determined by dividing the UV absorbance measured at 254 nm by the DOC concentration and are reported in units of liter per milligram of carbon (Weishaar et al., 2003). E2/E3 ratios were calculated (Dahlen, 1996) and specific UV absorbance at 280 nm (SUVA280) was obtained by normalizing the absorbance at 280 nm to dissolved organic carbon concentration (Chin et al., 1994; Chin et al., 1998). We also conducted S_R for molecular weight, biological indices, humification indices, and percentage contributions of different fluorescence component, etc. (Yang et al., 2015, Lu et al., 2013); we also looked for the optical brighteners used in detergent.

Several post-acquisition steps were used to adjust the EEM data. First, the excitation and emission data were corrected for the instrument-specific response. Second, the EEM response of Milli-Q water was subtracted from sample EEMs. Third, the UV-visible absorption spectra were used to correct the EEM data for inner filter effects (McKnight et al., 2001). And finally, the fluorescence intensities of the EEMs were normalized to the area under the Raman peak, thereby converting the arbitrary units (AU) into Raman units (RU). Following the creation of EEMs, they were then exported into Excel files and MATLAB files for further interpretation and modeling, including removing portions of the EEMs at which there was interference from Raleigh scattering, converting the data into vectors, and selecting characteristic peak signals based on documented key excitation/emission pairings (McKnight et al., 2001, Coble, 1996) and also plotting the data into contour and surface maps. The fluorescence index (FI), which is the ratio of emissions at 470 nm to 520 nm at an excitation of 370 nm, was calculated for all samples.

Analysis of the cations for this research (most notably Ca^{++}, Mg^{++}, K^+, Na^+) in the stream water samples was made by spectrometer PerkinElmer Inc. Optima 4300 DV ICP-OES with PerkinElmer AS-93 Plus Autosampler. This setup uses inductively coupled plasma mass spectrometry for detecting metals and several non-metals. Calibration and Quality Control Standards: 0.25, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/L (prepared from multielement standards (100 mg/L) from CPI International and High Purity Inc.). Concentrations were measured in mg/L. Each cation were measured in three replicates and averaged. Before running analysis, if required, samples were diluted by 2% HNO_3 Blank Solution.

Analysis of the anions for this research (most notably chloride and sulfate) in the stream water samples were made by Ion Chromatography (IC). Ion Chromatograph is used to measure concentrations of inorganic anions in aqueous samples. In ion chromatography, separation of a mixture of compounds into its components was achieved based on their relative interactions with an inert matrix. The detection of ions separated through the ion chromatographic column was done with a conductivity detector.

Concentrations of chloride and sulfate (Cl^-, SO_4^{2-}) in aqueous samples were measured using a Dionex (now acquired by Thermo Scientific) DX 600 Ion Chromatograph (IC) instrument. Aqueous samples collected for the analysis of inorganic anions were filtered through 0.45 micron or lower syringe filter and collected in clean HDPE bottles. At least 20 mL of sample was required for analysis with the IC. Samples were kept frozen and thawed on the day of analysis. When analyses were done, concentrations were calculated by using the Dionex PeakNet software.

Nutrient samples were analyzed for nitrate, nitrite, ammonium (NH_4^+), and orthophosphorus with Lachat Quikchem 8500 series 2 Flow Injection Analyzer. The method used was for Ammonia (Phenolate) in Waters, Lachat method # 10-107-06-1-F.

Parallel Factor Analysis (PARAFAC)

PARAFAC can statistically decompose EEMs into fluorescent groups or components (Stedmon et al., 2003). PARAFAC modeling can be obtained by either creating or validating the model using the complete data set of EEMs or by fitting the EEMs to an already established PARAFAC model. The data consisted of 98 samples, each analyzed for 87 emissions and 53 excitation wavelengths. Resulting excitation and emission matrices were processed for PARAFAC in MATLAB using the DOMFluor toolbox according to (Stedmon and Bro, 2008; URL: 3). The data were evaluated by split-half analysis where it was split randomly into two halves, a calibration and validation array, each consisting of 49 samples. The PARAFAC algorithm was then applied stepwise to both arrays for 2-10 components. The three component model was determined to be the best fit for those datasets after validation using four approaches: residual analysis, examination of spectral properties, split-half analysis and random initialization (Stedmon and Bro 2008). We described fluorescence components in this study as "humic-like" or "protein-like" since these components were likely a mixture of similar fluorophores rather than pure fluorophores.

Wastewater Dilution

Wastewater samples that were too concentrated for analysis were diluted with deionized (DI) water. Dilutions were carried out at the following ratios to enable analysis: 1:2, 1:5, 1:10, and 1:20.

Principal Component Analysis (PCA)

Principal components analyses (PCA) were made with varimax factor rotation on the drought/post-drought, wet/dry and wastewater data sets, individually. PCA helped us to identify promising parameters for inexpensive and robust detection of wastewater contamination. Variables incorporated in the two PCAs were: Ca, Fe, Mg, K, Na, Chloride, Sulfate, DOC, $SUVA_{254}$, S_R, E2/E3, NH4, ortho-phosphorus, Nitrate, Nitrite, E. coli, FI, Optical Brighteners and the percent contribution of each of the three PARAFAC components.

Results and Discussion

Surface water quality results were presented in this section, organized based on precipitation conditions. In figure 4 daily precipitation for the sampling period with precipitation on sampling dates indicated by blue dots and the corresponding precipitation categories (e.g., Wet, Post-Drought) specified for each sampling date were shown. Household wastewater is the pollution source of interest; therefore, wastewater samples were also analyzed. Data from the extended drought in fall 2016 were grouped with other dry weather points; water sample data collected in late-November 2016 were categorized as post-drought and grouped separately from other wet weather sampling data.

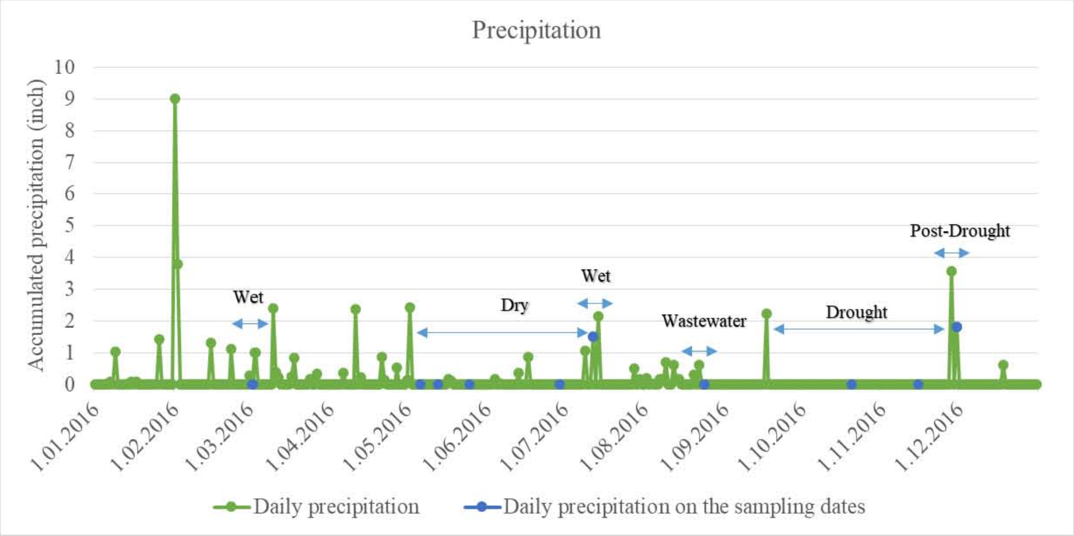

Figure 4. The precipitation data of the sampling areas. Sampling dates are 2 March, 6 May,13 May, 25 May, 29 June, 12 July, 24 August, 20 October, 15 November and 30 November.

The specific objective of the paper was, to focus on stream water samples taken from upstream, midstream and downstream, before (drought condition) and after (Post-drought condition) precipitation to determine whether or not these samples had significant wastewater signals from septic effluent or straight pipes.

We found that there was a positive correlation between E. coli, DOC, and Optical Brightener in the basin. The source of contamination within the surface water in Hale County could be runoff sources from roads, straight pipes, or septic systems as the water is brought to the surface through flushing of the soil.

Clustered column graphs were displayed for the parameters of E. coli, DOC, S_R, $SUVA_{254}$, E2/E3, FI, and Optical Brighteners. These parameters were shown to be above the criteria and recommendations for Alabama streams. The results were intended to be used as an essential mean for differentiating water quality between the three sampling sites. During the drought under baseflow conditions, the sampling sites showed no notable changes in pollutant concentrations from its upstream to downstream. In contrast, during the post-drought condition, we observed large increases in key water quality parameters from its upstream to downstream. In addition, both PCA and EEM-PARAFAC model were used to verify these observations.

DOC and Optical Indices

The minimum DOC measurements were 4.79, 5.54, 2.99 mg/L, the maximum DOC were measured as 4.90, 9.98, 7.13 mg/L and the concentrations were averaged as 4.85 ± 0.05; 2, 7.30 ± 0.83; 6, 5.75 ± 1.38; 3 mg/L (mean ± SE; n) through its upstream, midstream, downstream respectively tunder the site's drought condition. And also, the minimum DOC measurements were 5.96, 16.21, 7.90 mg/L, the maximum DOC measurements were 6.02, 24.95, 49.42 mg/L and the concentrations were averaged as 5.99 ± 0.03; 2, 20.53 ± 2.42; 4, 35.55 ± 13.83; 3 mg/L (mean ± SE; n) through its upstream, midstream, downstream respectively under the site's post-drought condition (Figure 5a).

The minimum $SUVA_{254}$ measurements were 3.88, 2.95, 2.96 L/(mg m), the maximum $SUVA_{254}$ measurements were 3.98, 3.35, 5.42 L/(mg m) and the concentrations were averaged as 3.93 ± 0.05; 2, 3.12 ± 0.07; 6, 3.78 ± 0.82; 3 L/(mg m) (mean ± SE; n) through its upstream, midstream, downstream respectively under the site's drought condition. And also, the minimum $SUVA_{254}$ measurements were 4.17, 2.73, 1.90 L/(mg m), the maximum $SUVA_{254}$ measurements were 4.19, 3.22, 4.59 L/(mg m) and the concentrations were averaged as 4.18 ± 0.01; 2, 2.97 ± 0.13; 4, 2.80 ± 0.89; 3 L/(mg m) (mean ± SE; n) through its upstream, midstream and downstream respectively under the site's post-drought condition (Figure 5b).

The minimum S_R measurements were 0.90, 0.90, 0.92 L/(mg m), the maximum S_R measurements were 0.91, 0.99, 0.95 L/(mg m) and the concentrations were averaged as 0.91 ± 0.01; 2, 0.95 ± 0.02; 6, 0.94 ± 0.01; 3 L/(mg m) (mean ± SE; n) through its upstream, midstream, and downstream respectively under the site's drought condition. And also, the minimum S_R measurements were 0.82, 0.83, 0.77 L/(mg m), the maximum S_R measurements were 0.83, 0.85, 1.03 L/(mg m) and the concentrations were averaged as 0.83 ± 0.00; 2, 0.83 ± 0.00; 4, 0.94 ± 0.09; 3 L/(mg m) (mean ± SE; n) through its upstream, midstream, and downstream respectively under the site's post-drought condition (Figure 5c).

The minimum E2/E3 measurements were 3.96, 4.99, 5.06, the maximum E2/E3 measurements were 4.02, 5.90, 5.48 and the concentrations were averaged as 3.99 ± 0.03; 2, 5.38 ± 0.15; 6, 5.34 ± 0.14; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively under the site's drought condition. And also, the minimum E2/E3 measurements were 3.71, 4.46, 3.97, the maximum E2/E3 measurements were 3.75, 5.08, 4.01 and the concentrations were averaged 3.73 ± 0.02; 2, 4.76 ± 0.16; 4, 3.98 ± 0.01; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively under the site's post-drought condition (Figure 5d).

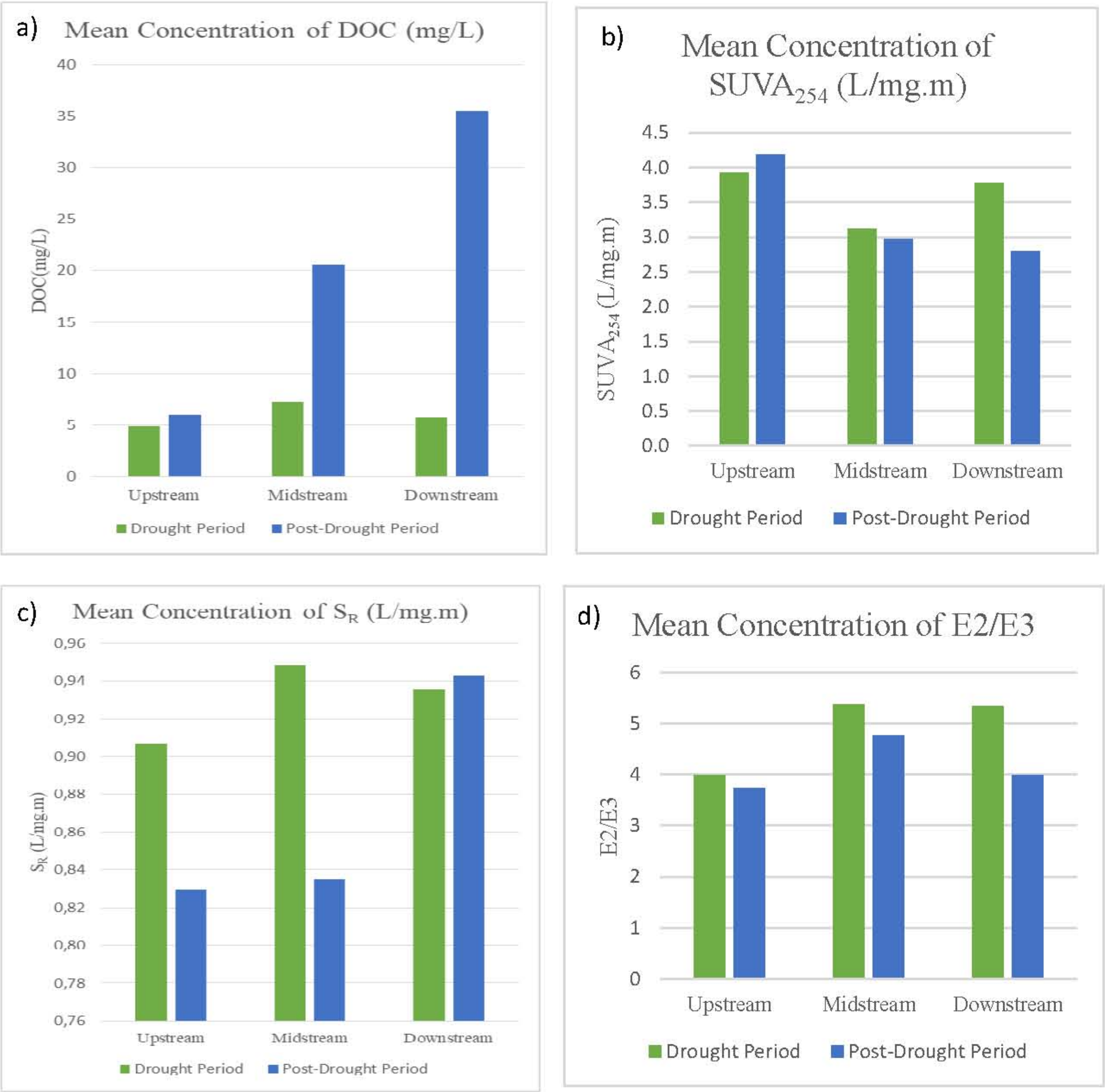

Figure 5. Mean values of DOC, $SUVA_{254}$, S_R and E2/E3 for water quality of the Big Prairie Creek's upstream, midstream and downstream both under the drought and post-drought conditions.

Bacteria

Figure 6 shows that sampling during drought revealed average concentrations of E. coli bacteria around 50-100 CFU per 100 mL; during the storm, at the end of November, the drought E. coli levels increased by about 10-times upstream of the creek in Newbern (this is expected because E. coli is in animal and bird feces and washes into stream. Downstream of Newbern, average E. coli concentrations increased to nearly 100,000 E. coli per 100 mL. This indicates a primary source of E. coli in and around Newbern.

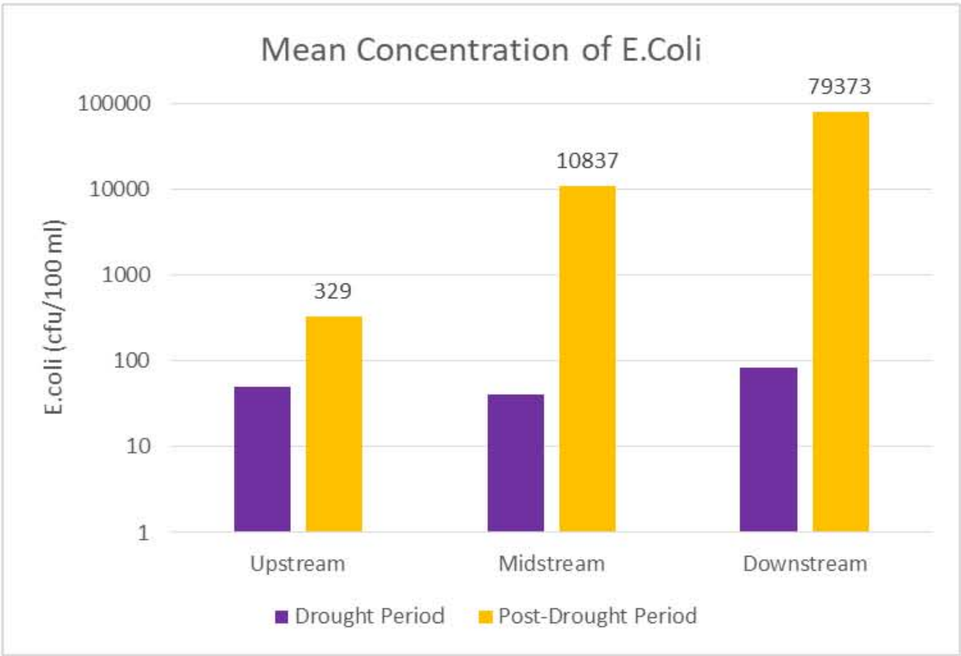

Figure 6. Mean values of *E. coli* for water quality of the Big Prairie Creek's upstream, midstream and downstream.

The minimum values for E. coli were measured as 50.20, 32.60, 51.80 cfu/100 mL, the maximum values for E. coli measurements were 50.20, 46.13, 143.90 cfu/100 mL and the concentrations were averaged as 50.20 ± 0.00; 2, 40.98 ± 2.67; 6, 82.50 ± 30.70; 3 cfu/100 mL (mean ± SE; n) through its upstream, midstream, and downstream respectively under the site's drought condition. And also, the minimum value for E. coli were measured as 329.25, 1139.95, 5800.00 cfu/100 mL, the maximum value of E. coli measurements were 329.35, 20535.00, 116160.00 cfu/100 mL and the concentrations were averaged as 329.30 ± 0.05; 2, 10837.48 ± 5598.87; 4, 79373.33 ± 36786.67; 3 cfu/100 mL (mean ± SE; n) through its upstream, midstream, and downstream respectively under the site's post-drought condition (Figure 6).

Around Newbern, *E. coli* concentrations were getting high and high following the period of precipitation (Figure 6). Additionally, the rate of increase in the downstream of Newbern was greater than the upstream. *E. coli* value increased about 5 times in its upstream, 250 times in its midstream and almost 1000 times in its downstream from its drought period through its post-drought period. The reason of this increase in *E. coli* was likely abundan in the large number of straight pipes in Newbern. Wastewater directly discharge onto the ground surface by straight pipes led to stream contamination as the accumulated wastewater in Newbern was flushed into the nearby creek during precipitation.

In the study, *E. coli* concentrations taking from different sampling sites and the results showed that the *E. coli* counts varied between 40.98 ± 2.67 cfu/100 ml and 79373.33 ± 36786.67 cfu/100 mL (Figure 6). In the study, RD 61 Bridge which had the highest *E. coli* concentration (116160 cfu/100mL), so single sampling location could be used as an example for the comparison of the Alabama Bacteria's Criteria, so E. coli values (colonies/100 ml) categorized respectively; single sample maximum should be equal or less than 235 according to Outstanding

Alabama Water (OAW), single sample maximum should be equal or less than 2507 October through May according to Public Water Supply (PWS), single sample maximum should be equal or less than 235 according to Swimming and Other Whole Body Water Contact Sports (S), single sample maximum should be equal or less than 235 according to Shellfish Harvesting (SH), single sample maximum should be equal or less than 2507 October through May according to Fish and Wildlife (F&W), single sample maximum should be equal or less than 2507 according to Limited Warmwater Fishery (LWF) and single sample maximum should be equal or less than 3200 according to Agricultural and Industrial Water Supply (A&I) for Non-Coastal Waters (ADEM, 2016). The range of variation of E. coli concentrations amongst sampling sites from upstream to downstream indicates a high level of fecal bacteria contamination during drought and post-drought. The maximum E. coli measurements were recorded 50.20, 46.13, 143.90 cfu/100 mL through upstream, midstream, downstream respectively at site Drought condition, while the maximum E. coli measurements were 329.35, 20535.00, 116160.00 cfu/100 mL through upstream, midstream, downstream respectively at site Post-Drought condition. The possible source of higher concentrations during the post-drought condition discharge of septic system and untreated wastewater discharge from the houses located at Newbern.

Indices

The minimum FI measurements were recorded in 1.83, 1.85, 1.85, the maximum FI measurements were recorded in 1.83, 2.24, 1.86 and its average concentrations were recorded in 1.83 ± 0.00; 2, 2.01 ± 0.07; 6, 1.86 ± 0.00; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively at drought conditions of the site. And also, the minimum FI measurements were recorded in 1.79, 1.64, 1.57, the maximum FI measurements were recorded in 1.79, 1.97, 2.22 and its average concentrations were recorded in 1.79 ± 0.00; 2, 1.80 ± 0.09; 4, 2.00 ± 0.22; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively at post-drought conditions of the site (Figure 7a).

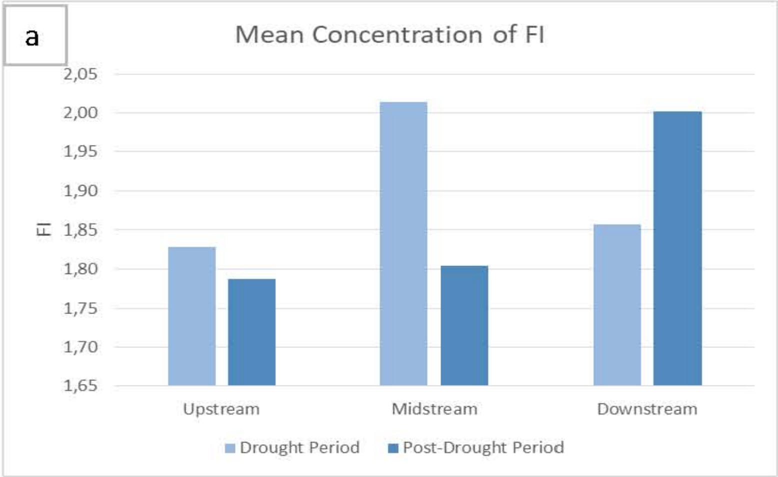

Figure 7a. Mean values of FI for water quality of Big Prairie Creek's upstream, midstream and downstream.

The minimum OPT (Optical Brighteners) measurements were 2.83, 3.20, 3.21, the maximum OPT measurements were 2.84, 6.34, 3.87 and the average concentrations were 2.84 ± 0.00; 2, 4.45 ± 0.60; 6, 3.63 ± 0.21; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively at the site's drought conditions. And also, the minimum OPT measurements were 3.04, 7.67, 3.87, the maximum OPT measurements were 3.03, 10.19, 11.72

and the average concentration were 3.03 ± 0.01; 2, 8.93 ± 0.72; 4, 9.08 ± 2.61; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively at the site's post-drought conditions (Figure 7c).

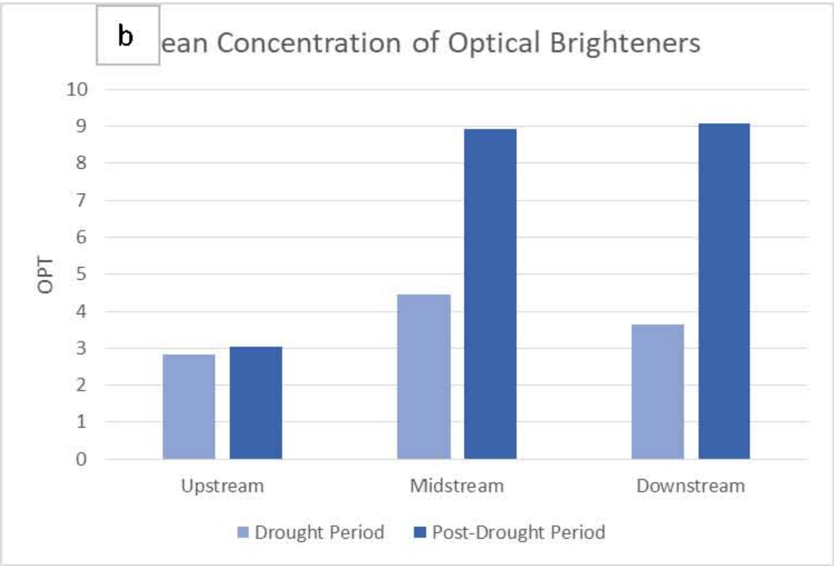

Figure 7b. Mean values of Optical Brightener for water quality of Big Prairie Creek's upstream, midstream and downstream.

In our study, the optical brightener concentrations, found at Newbern, indicated that the source of fecal bacterial contamination was human waste in the form of sewage leaks or spills or septic system leakage. As noted above, Newbern has suffered for many years from problems with onsite wastewater management. We observed that the maximum values of optical brightener ranged from 2.84, 6.34, 3.87 and the average concentrations in the samples were 2.84 ± 0.00; 2, 4.45 ± 0.60; 6, 3.63 ± 0.21; 3 (mean ± SE; n) through upstream, midstream, and downstream of Big Prairie Creek respectively at the site drought conditions. After precipitation, the maximum optical brightener values were ranged from 3.03, 10.19, 11.72 and the average concentrations in the samples were 3.03 ± 0.01; 2, 8.93 ± 0.72; 4, 9.08 ± 2.61; 3 (mean ± SE; n) through its upstream, midstream, and downstream respectively at the site post-drought conditions (Figure 7b). It is evident that the optical brightener values at its post-drought conditions were three times more than the values at its drought conditions. Data at our pristine (no human influence) control site at Mayfield Creek showed that an average optical brightener values ranged from 0.83 and 2.83 through its drought and post-drought conditions respectively. Therefore, we determined that there was a close relationship between the optical brightener values and precipitation at Newbern. This experiment showed that the value of using optical brighteners are promising as a supplement of fecal indicator bacteria, probably enabling differentiation of wastewater from animal fecal contamination in rural areas (Tavarese et al., 2008).

In our study, stream samples all included an FI value bigger than 1.66, classifying the organic matter in those samples as having a more microbial derived structure (Figure 7a). Furthermore, our samples also consisted of higher DOC concentrations with high FI values and $SUVA_{254}$, determinative of less changeable carbon and more aromatic structure (Figure 7b). The downstream samples demonstrated their characteristics in the mid-range for DOC, $SUVA_{254}$ and FI values. This supported the idea that the organic matters at these sites had a highly complex structure with their sources which might have been adequately characterized by the structures of the stream samples.

Principal Component Analysis (PCA)

The principal component analysis, and figures show the high information content inherent to peak C1, C2 and C3 in the excitation-emission matrix to delineate the different sources of the variable organic matter in this study sampling set. The skill to use a model for predictive abilities requires that characteristic properties can be traceable in aquatic samples.

The PCA (varimax rotation) identified three primary components (Eigenvalue >1), accounting for 40.4%, 19.0% and 8.7% of total variance respectively. The wastewater and members have well separated from stream water and post-drought (first flush) water samples by PC1, which are dominated by soluble reactive phosphorus (SRP), dissolved organic carbon concentration (DOC), *E. coli*, Ammonium-N, protein fluorescence, and optical brighteners. All these indicators show that wastewater has a characteristic nutrient & microberich signal that can be used to trace water sources in natural waterways, which are well synthesized by PC1. The post-drought samples had a higher PC1 score than stream water samples, indicating precipitation mobilizes sewage associated chemicals to nearby streams. Using base-flow stream water and wastewater as end members, we estimated that post-drought stream water contains 6.7% of wastewater. PC2 and PC3 are associated more with hydrological flow paths and hence not discussed in detail in this study. The drought period ensured a critical opportunity for multiple sampling trips before and then immediately following the first rain after two months. Newbern, Alabama, a small town with a conservative estimate of 50% straight pipes and very impermeable clay soil, provided an ideal setting to sample runoff containing untreated wastewater. Figure 6 displays how during the drought period under baseflow conditions, sampling sites upstream, adjacent (midstream) and downstream of the creek in Newbern show no change in *E. coli* concentration. In contrast, during the first rainstorm following the drought period, median *E. coli* increased by less than one log unit upstream but by nearly 3 log10 (1000x) downstream. The PCA results verified these observations by showing increasing PC1 scores from upstream to the downstream sites (Figure 8).

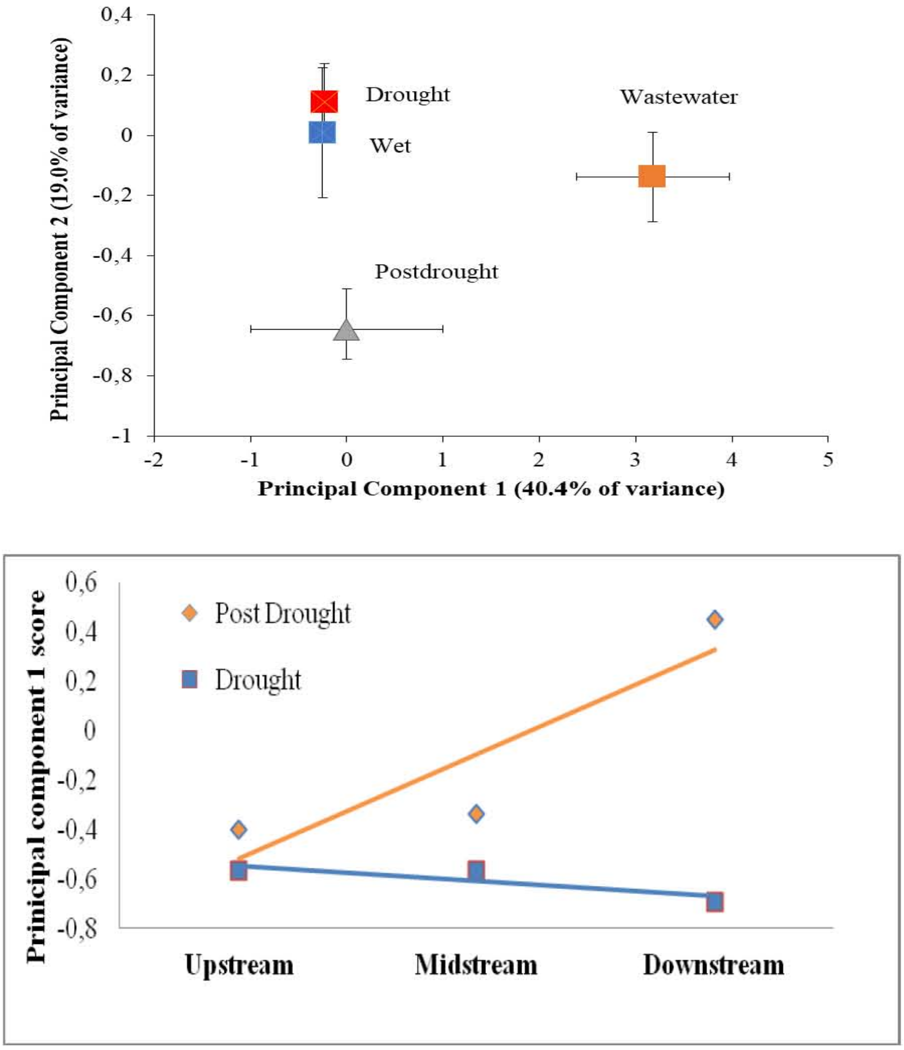

Figure 8. Median loading scores of PC1 (wastewater indicator) increase dramatically from the upstream to downstream sites of the creek in Newbern after a precipitation event, relative to a decrease or little change during drought period.

EEM-PARAFAC Model

Wastewater was lastly detected in our study area in Newbern by using EEM-PARAFAC model. The model identified three fluorescence components: 1) Terrestrial humic-like compounds derived from soils, 2) microbial humic-like compounds and 3) protein-like humic compounds respectively. The fluorescence spectra obviously demonstrated that wastewater samples (Figure 9a) had higher amounts of microbially produced, protein-like compounds than a regular stream water without an input of wastewater (Figure 9b). As it is known that the upstream site of Newbern no input of wastewater to the downstream site collecting data from wide range of straight pipe discharges that lead to shifting what the dominant fluorescence region from indicating humic, soil-derived compounds to indicating microbially-derived compounds.

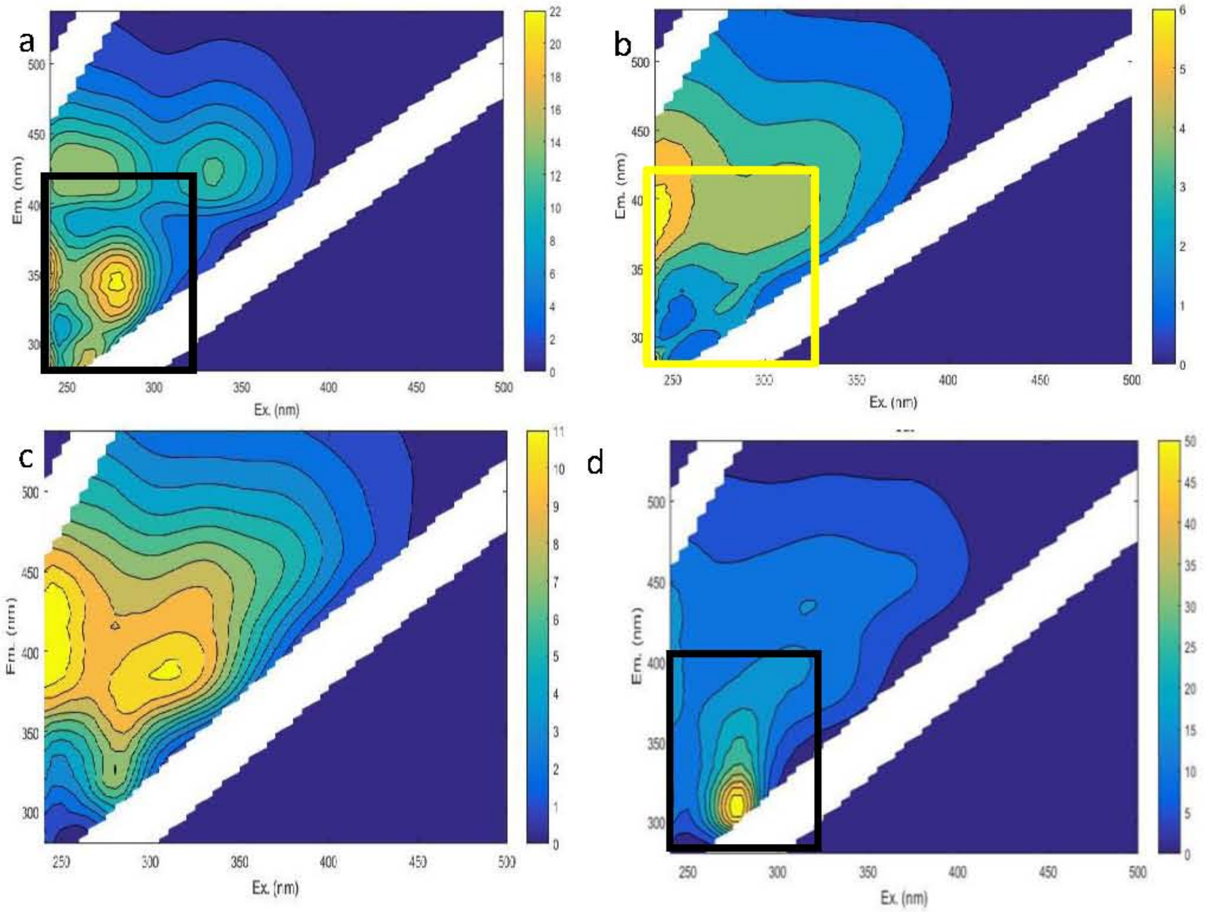

Figure 9. (a) Typical wastewater DOM Fluorescence results for project samples. Intensity is in raman units. The black rectangle outlines the fluorescence region indicative of Microbial-produced Compounds and the yellow rectangle indicates Humic, Terrestrial Fluorescence. DOM Fluorescence results for samples taken upstream sites in the post-drought period (b), adjacent (c) downstream (d) straight pipe discharges.

Conclusion

During the nine months investigation of wastewater signals in Hale County Alabama, a total of 20 water quality samples from twenty sites were collected and analyzed for physical (turbidity), chemical (anions, cations, DOM, DOC, nutrients, pH and conductivity) and microbiological (E. coli and coliform bacteria) parameters. The relationships between the water quality parameters were determined. These observed patterns led to the following conclusions.

This study analyzed the water quality parameters during and immediately following the extended drought period from September to November, 2016. The maximum E. coli measurements were recorded as 50.20, 46.13, 143.90 cfu/100 mL through its upstream, midstream, and downstream respectively during drought conditions, while the maximum E. coli measurements were 329.35, 20535.00, 116160.00 cfu/100 mL through its upstream, midstream, and downstream respectively at the site's post-drought conditions. The minimum E. coli concentrations averaged as 50.20 ± 0.00; 2, 40.98 ± 2.67; 6, 82.50 ± 30.70; 3 cfu/100 mL (mean ± SE; n) through its upstream, midstream, and downstream respectively during the drought conditions. The minimum E. coli concentrations averaged as 329.30 ± 0.05; 2, 10837.48 ± 5598.87; 4, 79373.33 ± 36786.67; 3 cfu/100 mL (mean ± SE; n) through its upstream, midstream, and downstream respectively during the site's post-drought conditions. The most likely sources of higher concentrations during the post-drought condition were the discharges of septic systems and untreated wastewater discharges from the houses, located at Newbern. Although a lot of cows were observed near Big Prairie Creek, the number of cows were up gradient from the midstream and downstream sampling sites. Fecal contamination from cow wastes were washed into the stream following rainfall, three major evidences indicated that E. coli contamination was primarily resulted from sewages: (1) the increase in E. coli was greatest downstream of Newbern, (2) the cows have constant access to Big Prairie Creek and often defecate directly into the stream during both wet and dry conditions and (3) optical brighteners, found in detergents and toilet paper also increased substantially in the downstream of Newbern.

We also found a link between optical brightener values and precipitation at Newbern as well. We observed that the maximum optical brightener values were 2.84, 6.34, 3.87 through its upstream, midstream, and downstream respectively during the site's drought conditions. After precipitation, we found that the maximum optical brightener values were 3.03, 10.19, 11.72 through its upstream, midstream, and downstream respectively during the site's post-drought conditions. This experiment showed that using optical brighteners is promising as a supplement to fecal indicator bacteria, potentially enabling identification of wastewater from straight pipes.

The PCA (varimax rotation) identified three primary components (Eigenvalue >1), accounting for 40.4%, 19.0% and 8.7% of total variance respectively. The wastewater and members have well separated from stream water and post-drought (first flush) water samples by PC1. The post-drought samples had a higher PC1 score than stream water samples, indicating precipitation mobilizes sewage associated with chemicals to nearby streams. Using base-flow stream water and wastewater as end members, we estimated that post-drought stream water contains 6.7% of wastewater.

Finally, the EEM-PARAFAC model identified three fluorescence components: 1) Terrestrial humic-like compounds derived from soils, 2) microbial humic-like compounds and 3) protein-like humic compounds respectively. The fluorescence spectra clearly demonstrate that wastewater samples have higher amounts of microbially-produced, protein-like compounds than a regular stream water sample.

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This article was adapted from the master dissertation titled "*Straight Pipes and Household Wastewater Discharges into the Rural Alabama and Impact on Watershed Water Quality with Wetland Land-Uses" at The University of Alabama, 2017.* I gratefully acknowledge. I would like to many thanks my supervisor Dr. Mark Elliott and committee member Dr. Yuehan Lu for their guidance, wisdom, and patience throughout this research. And finally, thanks to my family and friends who always kept encouraging me to get it done.

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Extended Turkish Abstract
(Genişletilmiş Türkçe Özet)

Evsel Atıksuların Alabama Kırsalındaki Akarsulara Deşarjı ve Su Kalitesine Etkisi

ABD nüfusunun yaklaşık % 75'i, belediyelerin atıksu arıtma hizmetinden faydalanırken geri kalan % 25'i, 80 milyondan fazla kişi, atık sularını arıtmaktan sorumludur vebunların çoğu arıtmada geleneksel septik sistem kullanmaktadır. Septik sistemlerde, atık suları yeraltı sularına sızmakta, filtrasyon ve doğal bozulma süreçleri ile atıksu arıtımı mümkün olmaktadır. Bazı toprak ve jeolojik koşullar atık suların toprağa sızmasına engel olduğundan geleneksel septik sistemleri bu koşullarda kullanma olanağı bulunmamaktadır. Bu durumlarda maliyetli alternatif sistemler kullanılır. Böylece, kanalizasyona erişimi olmayan,, elverişsiz toprak koşullarına sahip yoksul kırsal bölge sakinleri bütçeleriyle karşılayamayacakları atık su arıtma seçeneklerine terk edilmiş oluyorlar. Alabama merkezinin büyük bir bölümünde topraklar: Geçirimsiz kil ve sert kireçtaşı tabakasından oluşmaktadır.

Alabama'nın Kara Kuşak bölgesindeki toprak ve jeoloji tipi, geleneksel septik sistemlerin bu bölge için uygun bir seçenek olmadığından, bu bölgede yaşayanlar atıksularını arıtmak için yoğun mücadele etmektedirler. Kullanıldığı durumlarda ise septik sistemler sıklıkla arızalandığından, evsel atık sular genellikle bir boru sistemiyle kırsalın ağaçlık alanlarına doğrudan boşaltılır, buna "doğrudan deşarj" adı verilir. Kırsal alanlardaki doğrudan deşarj ve fosseptik arızası yaygınlığından dolayı atıksular yakınlardaki su yollarına deşarj edilmekte, bu da çevre ve insan sağlığı üzerinde olumsuz etkilere neden olmaktadır.

Bu çalışma, kırsal kesimlerde atıksu kirliliğini göstermeyi ve kullanılabilir bir su kalitesi yöntemini tanımlamayı amaçlamaktadır. Ayrıca, Alabama Hale County'deki akarsuların su kalitesini araştırmak için temel bir çalışma olması hedeflenmektedir. Çalışma alanındaki mevcut su kaynaklarına, yağıştan önce (kuraklık durumu) ve yağıştan sonra (kuraklıktan sonraki durum), fosseptik atık veya doğrudan deşarjlardan gelen atık suların karışıp karışmadığını belirlemek amacıyla, memba, orta akış ve mansap kaynaklarından örnekler alındı.

Bu çalışmayı, Alabama'nın batı orta kesimindeki Hale İlçesi'nde ve yakınçevresinde gerçekleştirdik. Newbern Kasabası'nda birçok evin işlenmemiş atık suyu ağaçlık alanlara boşaltıldığından, araştırmamız için bu kasabayı seçtik. Örnekleme istasyonlarını Newbern'deki nehirlerin üst kısım, orta kısım ve aşağı kısımların akış yönünde belirledik. Kümelenmiş kolon grafikleri E. coli, DOC, S_R, $SUVA_{254}$, E2 / E3, FI, Optik parlaklaştırıcılar parametreler için gösterdik.. Bu parametrelerin Alabama akarsuları için kriterlerin ve tavsiyelerin üzerinde olduğunu gözlemledik. Sonuçları, üç örnekleme sahası arasında su kalitesi farklarını göstermek için temel bir araç olarak kullandık.

Bu çalışmada, bölgelerde E. coli, DOC, Optik Parlatıcı ve FI arasında pozitif korelasyon bulunduğunu tespit ettik. Hale County'deki yüzey sularındaki kirlilik kaynaklarının, yollardan akan yüzeysuları, doğrudan deşarjlar veya toprağın suyla yıkanmasıyla getirilen atıklar ile fosseptik sistemlerden kaynaklandığını belirledik.

Bu çalışma, 28 Eylül 2016'dan sonraki bir yıllık veri kullanılarak kuraklık koşullarının uzunluğuna göre ve kuraklık döneminden hemen sonra ölçülen su kalitesi parametrelerinin analizleriyle gerçekleştirilmiştir. Bu çalışmada elde edilen sonuçlar şu şekilde özetlenebilir: Minimum E. coli ölçümleri kuraklık sırasında sırasıyla memba, orta akım ve mansap yönünde 50.20, 46.13, 143,90 EMS/100 mL olarak kaydedilmişken, maksimum E. coli ölçümleri, kuraklık sonrası durumda sırasıyla membadan mansaba doğru 329.35, 20535.00, 116160.00 EMS/100 mL'dir. Minimum E. coli konsantrasyonları, kuraklık durumunda membadan mansaba doğru sırasıyla, ortalama 50.20 ± 0.00; 2, 40.98 ± 2.67; 6, 82.50 ± 30.70; EMS/100 mL'dir (ortalama ± SE; n). Minimum E. coli konsantrasyonları, kuraklık sonrası durumda membadan mansaba doğru sırasıyla, ortalama 329,30 ± 0.05; 2, 10837.48 ± 5598.87; 4, 79373.33 ± 36786.67; 3 EMS/100 mL'dir (ortalama ± SE; n) . Kuraklık sonrasında tespit edilen en yüksek konsantrasyonların kaynağı, foseptik sistemin deşarjı ve Newbern'deki evlerden gelen arıtılmamış atıksuların deşarjıdır. Her ne kadar büyük baş hayvanlar memba (Big Prairie Deresi) yakınlarında görülse de, onlardan kaynaklı kirlilik orta akış bölgesinde ve mansaptaki örnekleme sahalarında artış göstermektedir. Yağmur suları dışkılarını akarsulara taşımaktadır.Buna üç ana kanıt E. coli kontaminasyonunun esas olarak kanalizasyondan kaynaklanmaktadır: (1) E. coli'deki artış Newbern'in mansabında en büyük seviyedpolicy 2) Büyük baş hayvanlar Big Prairie Deresi'ne sürekli erişime sahip olduklarından hem yağmurlu hem kuru havalarda çoğu zaman dışkılarını doğrudan akarsuyun içine boşaltırlar ve (3) deterjan ve tuvalet kâğıdında bulunan optik parlatıcılar da Newbern'in mansabında büyük ölçüde artmıştır. Ayrıca, optik parlatıcı değerleri ile

Newbern'deki yağışlar arasında da bir bağlantı bulduk. Kuraklık koşullarında maksimum optik parlatıcı değerlerinin sırasıyla membadan mansaba doğru 2.84, 6.34, 3.87 olduğunu gözlemledik. Yağıştan sonra, kuraklık sonrası koşullar altında maksimum optik parlaklaştırıcı değerleri membadan mansaba doğru sırasıyla 3.03, 10.19, 11.72 olarak bulduk. Bu çalışmanın, doğrudan deşarjlardan gelen atık suların tanımlanmasına potansiyel olarak olanak sağlaması optik parlatıcıların kullanımının fekal indikatör bakterileri tamamlayıcı olarak gelecek vaat edici bir çalışma olduğunu göstermiştir.