


Occurrence and concentrations of traditional and emerging contaminants in onsite wastewater systems and water supply wells in eastern North Carolina, USA

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ABSTRACT

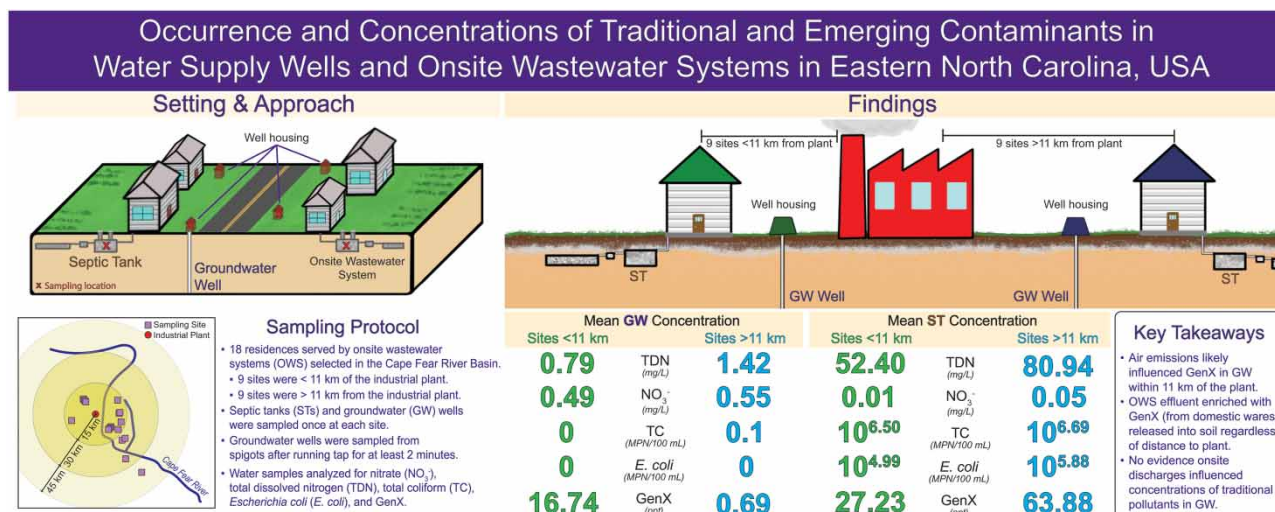
Onsite wastewater treatment systems (OWTSS) and private wells are commonly used in Eastern North Carolina, USA. Water from private wells is not required to be tested after the initial startup, and thus persons using these wells may experience negative health outcomes if their water is contaminated with waste-related pollutants including bacteria, nitrate or synthetic chemicals such as hexafluoropropylene oxide dimer acid and its ammonium salt (GenX). Water samples from 18 sites with OWTSS and groundwater wells were collected for nitrate, *Escherichia coli* (*E. coli*), total coliform, and GenX concentration analyses. Results showed that none of the 18 water supplies were positive for *E. coli*, nitrate concentrations were all below the maximum contaminant level of 10 mg L⁻¹, and one well had 1 MPN 100 mL⁻¹ of total coliform. However, GenX was detected in wastewater collected from all 18 septic tanks and 22% of the water supplies tested had concentrations that exceeded the health advisory levels for GenX. Water supplies with low concentrations of traditionally tested for pollutants (nitrate, *E. coli*) may still pose health risks due to elevated concentrations of emerging contaminants like GenX and thus more comprehensive and routine water testing is suggested for this and similar persistent compounds.

Key words: *E. coli*, GenX, nitrate, private wells, septic systems

HIGHLIGHTS

- Wastewater from septic tanks and the water supply at 18 sites were tested for *E. coli*, nitrogen, and GenX.
- Wastewater at every site had detectable concentrations of GenX and elevated concentrations of nitrogen, *E. coli* and total coliform.
- None of the water supplies had *E. coli* or nitrate concentrations that exceeded the MCL, but four had GenX concentrations above the health advisory level.

GRAPHICAL ABSTRACT



INTRODUCTION

Onsite wastewater treatment systems (OWTSs) are commonly used in rural areas for treatment and dispersal of wastewater. OWTSs include a septic tank which separates solids and liquids and provides an environment for the anaerobic digestion of organic matter, thus reducing the total suspended solids and biochemical oxygen demand of the waste (Lusk *et al.* 2017). Septic tank effluent is piped to drain field trenches where the effluent infiltrates soil. Various physical, chemical, and biological processes may occur in the soil to remove pollutants in the wastewater. However, groundwater contamination may result if OWTSs are not properly designed, installed, and maintained (Lusk *et al.* 2017). After installation, OWTSs are operated and maintained by the home/property owner. Thus, it is the owner's responsibility to evaluate the functionality of their OWS, but many people may not be aware of the maintenance recommendations such as routinely pumping the tank to remove solids, resulting in little maintenance of the systems (Noss & Billa 1988). Also, many communities in North Carolina (NC) that rely on OWTSs also use private groundwater wells for their drinking water supply (Naylor *et al.* 2018). Like with OWTSs, once a well is installed it is the responsibility of the goods owner to operate and maintain the well (Lee & Murphy 2020). Well owners may not be aware of the harmful effects that pollutants in groundwater supplies may cause. Water from private groundwater wells in NC is not tested routinely unless the property owner requests and pays for the sample analysis (Naylor *et al.* 2018). These proactive water quality assessments may be cost-prohibitive for impoverished communities, many of which include a predominance of racial minorities, thus contributing to environmental justice (Gavino-Lopez *et al.* 2022) and health disparities concerns (Stillo & MacDonald 2017).

There are many different pollutants that can be present in groundwater supply wells that may result in negative health outcomes for water consumers. Examples include contaminants associated with wastewater that have traditionally been tested for by public water providers such as pathogens (or pathogen indicators) like *Escherichia coli* (*E. coli*) (Humphrey *et al.* 2011; Schneeberger *et al.* 2015) and compounds such as nitrate (Humphrey *et al.* 2010; Wigginton *et al.* 2018). Testing is important because the consumption of water or food with pathogenic *E. coli* may cause severe gastroenteritis, diarrhea, and sometimes death (Kosek *et al.* 2003; Estrada-Garcia *et al.* 2009). Human and animal wastes are sources of elevated concentrations of *E. coli* and other bacteria (Hynds *et al.* 2014). Studies have shown a significant correlation between fecal indicator bacteria in water resources and OWTS density (Borchardt *et al.* 2003; Humphrey *et al.* 2018). Therefore, effective waste management strategies including policies regarding septic system density and site condition requirements (vertical separation distances) for system installations are important (Humphrey *et al.* 2011, 2018; Cox *et al.* 2019). Also, proper siting, construction and sealing of wells are vital for reducing the likelihood of groundwater contamination with *E. coli* (Lee & Murphy 2020). The maximum contaminant level (MCL) for *E. coli* in water supplies is zero colony-forming units per 100 mL, thus any *E. coli* observed is considered a hazard (US EPA 2023). Total coliform bacteria have also been used as a potential indication of contamination of water supplies (Theng-Theng *et al.* 2007; Rosso *et al.* 2012). In a study of 16 water supply wells in South Bass Island Ohio,

USA, Theng-Theng *et al.* (2007) reported that all the water samples from the wells were positive for total coliform and *E. coli*, while seven wells also tested positive for enterococci and *Arcobacter*. The study was conducted following multiple reports of gastroenteritis by visitors to the area. Theng-Theng *et al.* (2007) attributed the water contamination to malfunctioning wastewater treatment facilities and septic systems following heavy rainfall. Rosso *et al.* (2012) evaluated the presence of total coliform in newer (post 2008) and older (pre-2008) wells in central NC. Rosso *et al.* (2012) reported that 29% (10 of 35 wells) of older wells and 31% (11 of 35 wells) of newer wells sampled for the study were positive for total coliform. Stillo & MacDonald (2017) tested 171 drinking water wells in central NC and found 29% were positive for total coliform and 6.4% were positive for *E. coli*. These findings highlight the need for monitoring of drinking water supplies.

Elevated nitrate-nitrogen concentrations can cause methemoglobinemia or 'blue baby syndrome' in infants (Sadeq *et al.* 2008), and some evidence suggests that chronic consumption of nitrate can increase the risk of various cancers (Ward *et al.* 2005). The MCL for nitrate-nitrogen is 10 mg L⁻¹ (US EPA 2023). There are many different potential sources of nitrate in the environment including human waste, animal waste, fertilizers, automobile emissions, and natural decomposition of organic matter (Havlin *et al.* 1999). Prior research has shown a significant correlation between OWTS density and nitrate concentrations in groundwater (Naylor *et al.* 2018) and surface water (Hoghooghi *et al.* 2016). Humphrey *et al.* (2010) reported that nitrate concentrations in groundwater near OWTS exceeded 10 mg L⁻¹ in more than half of the 16 OWTS evaluated in a study conducted in the NC Coastal Plain. Naylor *et al.* (2018), summarizing nitrate data from private drinking water wells across NC, found that seven of the top 10 counties with the highest mean nitrate concentrations were in the Inner Coastal Plain region. Groundwater from wells in those counties had a mean nitrate concentration of 3.4 mg L⁻¹ and there was an average of over 25,000 OWTS in those 7 counties. Therefore, communities that rely heavily on OWTS and private wells, such as those in Eastern NC, may be at high risk with regard to elevated nitrate concentrations in groundwater.

Other pollutants that have more recently been screened for in water supplies include synthetic chemicals such as perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and hexafluoropropylene oxide dimer acid and its ammonium salt (GenX) which may be considered contaminants of emerging concern (US EPA 2022). The PFOS, PFOA, and GenX chemicals are used in various products including cookware, fire foams, paints and varnishes, detergents, and contact paper (Coperchinin *et al.* 2017). Exposure to high concentrations of PFOA and PFOS may lead to decreased fertility, developmental delays in children, low birth weight, increased risk of some cancers, reduced immune system function, and higher risk of obesity (EPA 2022). Production with PFOS and PFOA at many industrial plants ended more than a decade ago. However, these compounds are persistent in the environment and can accumulate, thus continuing to cause public health threats (Pritchett *et al.* 2019) even long after production ceased. In fact, PFOS and PFOA have recently been found in water, soil, and air in many countries across the world (Pan *et al.* 2018; EPA 2022). Production using GenX chemicals instead of PFOA was initiated to better protect public health as GenX was thought to be less of a health risk (Choi *et al.* 2018). However, animal toxicity studies of GenX exposure have shown detrimental health effects related to the liver, kidney, and immune system, and development of cancers in the test animals (EPA 2022). Recent work has also shown that exposure to GenX may also negatively influence amphibian growth (Barragan *et al.* 2023) and fish immune system and liver functions (Guillette *et al.* 2020). Thus, GenX exposure may pose risks to humans and wildlife. The US EPA (2022) has issued a lifetime health advisory (HA) of 10 parts per trillion (ppt) for GenX chemicals. Communities in Eastern NC near an industrial chemical plant that produces GenX have been exposed to chemicals via legacy and current wastewater discharges and atmospheric emissions (Sun *et al.* 2016; McCord *et al.* 2018; Pritchett *et al.* 2019). For example, Cahoon (2019) reported that the industrial plant discharged effluent to the Cape Fear River for many years that had GenX concentrations that exceeded 500 ppt. Sun *et al.* (2016) and McCord *et al.* (2018) reported GenX concentrations in the finished water of a water treatment plant that exceeded 450 ppt. The raw water intake for the plant was in the Cape Fear River and downstream from an industrial manufacturer of GenX (located in Fayetteville within Cumberland County, NC). Pritchett *et al.* (2019), summarizing a study by NC health officials, reported that 207 drinking water wells within a 12 km radius of the industrial plant in Cumberland County had GenX concentrations that exceeded 140 ppt. Therefore, prior research has shown contamination of groundwater and surface waters near the industrial plant. Emissions of GenX into the atmosphere from the industrial plant have also impacted nearby communities. NC DEQ (2018) estimated that the annual air emissions of GenX from the industrial plant in Fayetteville NC could have sometimes exceeded 1,000 kg and that some rain samples collected within 10 km of the plant had Gen X concentrations between 45 and 60 ppt. Winds typically move in a northeasterly direction in NC due to the Gulf Stream (NOAA 2023), but wind patterns change based on atmospheric conditions. Research by Galloway *et al.* (2020) in Ohio and West Virginia near an industrial plant that produces GenX found that 38% of soil samples collected

downwind of the plant had detectable concentrations of GenX and one sample contained 8.14 ppt. Roostaei *et al.* (2021) used a machine-learned Bayesian network model with groundwater and air quality data and other geographical/geological information from near the industrial plant in Fayetteville, NC and concluded that one of the most important factors regarding risk for GenX contamination in groundwater wells was the historic atmospheric deposition rate of GenX and the distance and orientation from the plant. Brandsma *et al.* (2019) reported concentrations of GenX (1 to 27 ppt) in or on all grass and leaf samples analyzed within 3 km of an industrial manufacturing plant. These studies have shown that atmospheric emissions of GenX from industrial plants may influence air, soil, and water quality in the surrounding areas. Exposure to GenX may also occur due to consumption after migration of the chemical from cookware and food packaging articles (Choi *et al.* 2018). Migration of GenX from household items into the waste stream (e.g., washing cookware, garbage grinders) may result in the discharge of GenX to subsoil and groundwater near OWTS. A study by Semerad *et al.* (2020) in the Czech Republic reported detectable concentrations of GenX in about 20% of sewage sludge samples with concentrations ranging from 0.3 to 1.2 ppt. However, the potential contribution of GenX to subsoil and groundwater via OWTS is unknown.

This project aims to provide field-based data on the presence and concentrations of traditional and emerging contaminants in wastewater from OWTS and groundwater wells in low-income communities of Eastern NC. To the authors' knowledge, this is the first study where wastewater samples from septic tanks and groundwater samples from water supply wells were collected and analyzed for GenX along with *E. coli*, nitrate, and total coliform.

METHODS

Study location

The study sites were in Cumberland, Bladen, and Robeson Counties in the Middle Coastal Plain of NC (Figure 1). The geology of this area can be described as a wedge of sediments comprising layers of sand, gravel, and shell material that slope and thicken to the east forming various groundwater aquifers separated by clayey, low-permeability confining units (Lautier 2006). Groundwater wells installed in the deep Black Creek and Cape Fear confined aquifers have been used historically as the water supply for many communities in Eastern NC because they yielded high-quality water (Lautier 2006). However, excessive withdrawals from these aquifers have led to dewatering, saltwater intrusion, and/or declining water levels

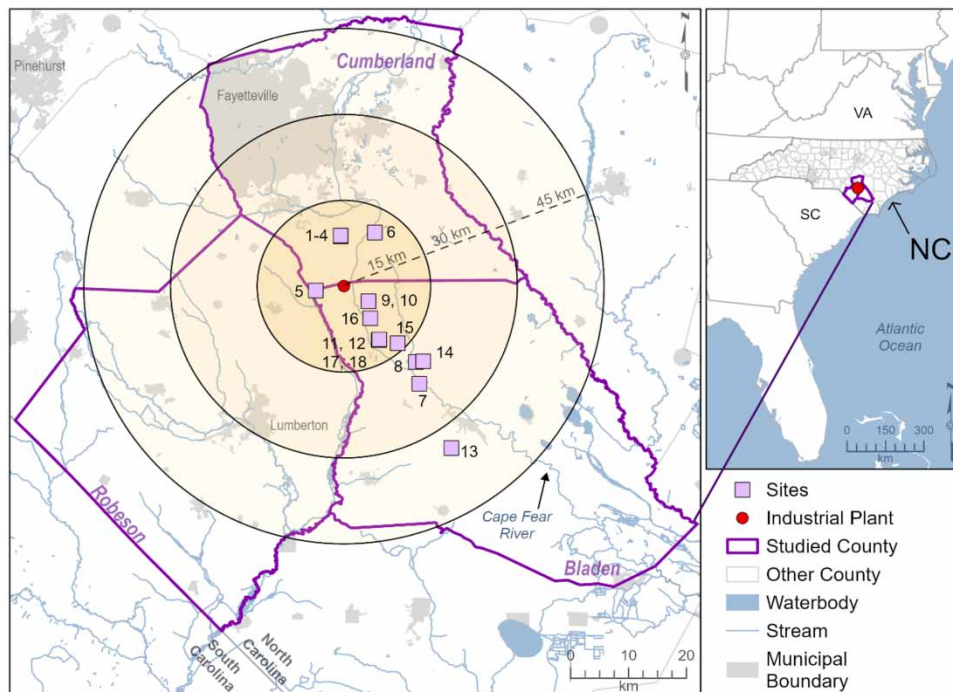


Figure 1 | Study sites were in Cumberland, Bladen, and Robeson Counties which included sites upstream and downstream of the Cape Fear River from the industrial plant.

prompting the State of NC to impose withdrawal restrictions from some aquifers (NC DENR 1998). Many communities in Eastern NC had to switch water sources from deep aquifers to surface water or shallow aquifers that require more intensive treatment to make the water potable (NC DENR 1998). So, while historically, deep aquifers have been used for water supply, there is a regulatory directive to use shallower aquifers which may be more susceptible to contaminants near the land surface and closer to wastewater discharges from OWTS, sewage and industrial treatment plant effluents, and contaminated surface waters. The Cumberland, Bladen, and Robeson region was selected because of the predominance of low-income communities using OWTS and wells, and it is within 40 km of the only chemical manufacturing plant that produces GenX in NC. More specifically, there are over 80,000 active OWTS and more than 4,400 private drinking water wells in the region (Naylor *et al.* 2018; NC DHHS 2024). Volunteer sites with OWTS and private water supply wells that were upstream and downstream of the Cape Fear River where the plant historically discharged effluent were identified after consultation with local Environmental Health Departments and water providers regarding public water and sewer service areas. Researchers visited with people in communities served by OWTS and wells, discussed the objectives of the study and solicited volunteers. Many homeowners declined the opportunity to participate in the study even with the incentive of free water testing and pumping of their septic tank. However, 18 homeowners agreed to be included in the research. Characteristics of the 18 sites are shown in Table 1.

Sample collection and analyses

A septic contractor was hired to uncover the septic tank at each site and remove the lid/manhole to enable the collection of wastewater samples (Figure 2). After samples were collected, the contents of the septic tanks were pumped by the contractors. Water samples were also collected at each site. The well was located, water was allowed to run for a few minutes and then a sample was collected by placing opened bottles underneath the spigot. NC regulations (15A NCAC 18E .0601) require water supply wells to be at least 16 m away from all OWTS. The well at each site was compliant with the setback regulation. For one site, public water had recently been extended to the home, so a sample was collected from a spigot on the outside of the home. Wastewater and drinking water samples were collected on three separate dates within a 60-day window in the Fall of 2022. Specifically, the sampling dates included 26 October, 21 November, and 20 December. During each sampling event, six sites

Table 1 | Characteristics of the 18 sites including the age, repair history, soil series, and distance from the industrial plant

Site	Location	Install date	Repair history	Soil series	Distance (km)
1	Cumberland	1997	N/A	Candor	8.70
2	Cumberland	1999	New drainfield (<2 years)	Candor	8.74
3	Cumberland	1997	N/A	Candor	8.80
4	Cumberland	1994	N/A	Candor	8.85
5	Robeson	2002	Uneven distribution	Wagram	4.92
6	Cumberland	1999	Deep drainfield, shallow soil	Roanoke	10.80
7	Bladen	1998	Unequal distribution, <3" freeboard	Wagram	21.45
8	Bladen	1970	Excessive solid layer thickness	Lakeland	18.17
9	Bladen	1987	N/A	Lakeland	5.02
10	Bladen	1987	N/A	Lakeland	5.00
11	Bladen	1988	N/A	Centenary	11.14
12	Bladen	1988	Wastewater near top of inlet pipe	Leon	11.15
13	Bladen	1992	N/A	Norfolk	33.82
14	Bladen	1972	N/A	Wagram	18.98
15	Bladen	1980	N/A	Lakeland	13.62
16	Bladen	2003	Roots in tank, thick sludge/scum	Lakeland	7.23
17	Bladen	1960	New drainfield trenches (<2 years)	Centenary	11.19
18	Bladen	1961	Tank had hole < 1 ft wastewater	Centenary	11.17

Every system was gravity-flow, conventional style technology.



Figure 2 | Septic tanks were uncovered to allow for collection of wastewater samples (left image) and to pump the septage from the tanks using a pump truck and suction hose (right image).

were visited, and samples were collected between 11 am and 3 pm on those days. Sampling dates were selected based on the scheduling availability of the septic contractors and researchers and recent weather conditions. Sampling events were rescheduled if rainfall exceeding 1 cm was received within 2 days of a planned event. A Hanna Instruments 9,829 multi-meter (Hanna Instruments, Smithfield, RI, USA) was used to determine pH, oxidation reduction potential, temperature, and specific conductance of the water and wastewater samples in the field. Separate sample bottles were filled for nutrient, bacteria, and GenX analyses at each sampling location. The bottles were placed in ice-filled coolers for transport to labs at East Carolina University (ECU) for analysis. More specifically, samples were analyzed for total dissolved nitrogen (TDN), nitrate, ammonium, *E. coli*, total coliform, and GenX. Samples for nutrient analyses were filtered using vacuum filtration and the filtrate was analyzed for TDN using a *Shimadzu* TNM-L (Shimadzu Corp., Kyoto, Japan) and a SmartChem 200 auto-analyzer (KPM Analytics, Westborough, Massachusetts, USA) was used for nitrate and ammonium analyses. Concentrations of *E. coli* and total coliform were enumerated using IDEXX™ *Colilert* media with QuantiTray2000® method (IDEXX Laboratories, Inc., Westbrook, Maine, USA). Dilution factors of up to 10,000 were used for bacteria analyses of the wastewater samples. Samples were analyzed for GenX using EPA method 537.1 using a SciEx 3200 triple quadruple LC-MS/MS equipped with Gemini 3 mm NX-C18 110 LC column. Data were analyzed using SCIEX MultiQuant Software with peak areas and retention times recorded using Microsoft Excel. The SciEx instrument was calibrated using appropriately formulated standard solutions with seven solutions spanning a concentration range of 0–250 $\mu\text{g L}^{-1}$. Calibration standards were injected before every analysis run and at every 10th sample as a calibration continuation check. Analysis of one of the standard solutions was performed to demonstrate precision. Calculation of the concentration of these standards compared to the calibration curve served as an accuracy check. Internal standard peak areas were also checked to ensure accuracy. A conservative estimate of the level of quantification was 2 ppt. Concentrations of nitrate and *E. coli* in water samples were compared to the MCL of 10 mg L^{-1} and 0 MPN 100 mL^{-1} , respectively. While not a health hazard, if any total coliform was present in the water samples, then the concentration was noted. GenX concentrations in water supply samples were compared to their HA concentrations of 10 ppt (US EPA 2022).

Statistical analyses

Concentrations of nitrogen, *E. coli*, total coliform, and GenX in wastewater samples were compared to groundwater samples to determine if wastewater was influencing groundwater. Groundwater concentrations of GenX within 11 km of the industrial plant were compared to concentrations beyond 11 km of the industrial plant to determine if emissions from the plant may be an influencing factor with regard to GenX concentrations. It was anticipated that nitrate, *E. coli*, or total coliform in drinking water wells on the properties would more likely be related to OWTS discharge of effluent rather than industrial plant emissions. Comparisons of *E. coli*, total coliform, and nitrate within and beyond 11 km of the industrial plant were also made in case groundwater contamination at sites was influenced by faulty construction or maintenance of the wells that could facilitate the transport of all the contaminants (GenX, *E. coli*, total coliform, and nitrate) to water supply aquifers. Using parcel data in ArcGIS Pro, centroid points were created for the industrial plant and the study sites. The 'Measure' tool was used to determine the distance between centroid points of the home sites and plant. An Anderson-Darling test of normality was performed on each data set. When data exhibited a normal distribution, t-tests were used to determine if differences in concentrations between comparison groups were significantly different ($p < 0.05$). If data were not normally distributed, Mann Whitney non-parametric tests were used to determine significance. Spearman correlations were performed to determine if there were statistically significant associations between GenX concentrations in water supplies and distance from the industrial plant. Minitab 20 statistical software was used for the tests.

OWTS evaluation

The condition of the OWTS was evaluated at each site and later compared to water quality testing results to determine if poorly functioning or poorly maintained OWTS were influencing the water supply. Signs of potential septic system malfunction such as surfacing effluent, wastewater backup into the freeboard of the septic tank, and dead or discolored grass over the drain field trenches were assessed at each site and recorded if present. If a property owner mentioned they had problems with their septic system, then that too was documented. The drain field trenches of each OWTS were located using a tile-probe. Within a few meters of the drain field, an auger was used to create boreholes, and soil was laid onto the ground in the sequence in which it was excavated. Pictures of the soil profiles were taken to document the characteristics. [Figure 3](#)



Figure 3 | Septic drainfield trenches were located using a tile-probe (left image) and soil borings near the system were laid onto the ground and characterized (right image).

shows an example of a soil profile picture near the drainfield of a volunteered site. If unsuitable soil characteristics with regard to the installation of a new OWTS were observed such as expansive clay, shallow groundwater, or restrictive horizons, then those issues were noted. The soil series for each property as shown on the Web Soil Survey (USDA 2023) was recorded and the characteristics of soils observed were compared to characteristics of the soil series.

RESULTS AND DISCUSSION

Bacteria concentrations

None of the water supplies sampled at the 18 sites were positive for *E. coli* (Table 2), and only one water supply sample (5.5%) was positive for total coliform. The water supply at Site 8 had 1 MPN 100 mL⁻¹ of total coliform (Table 2). Based on these data, the risks for negative health outcomes related to indicator bacteria in the water supplies were low. The percent positive samples observed in this research were lower in comparison to separate findings by Rosso *et al.* (2012) and Stillo & MacDonald (2017) that each reported 29% of wells sampled in central NC contained total coliform. Wastewater sampled from the septic tanks had between 2,000 and 2,176,000 MPN 100 mL⁻¹ of *E. coli* and between 111,200 and 10,000,000 MPN 100 mL⁻¹ of total coliform (Table 2, Figure 4) and thus were variable but within the range of concentrations reported in other studies including Schneeberger *et al.* (2015) (2,400 to 98,000 CFU 100 mL⁻¹ of *E. coli*), Humphrey *et al.* (2011) (420,000 to 14,000,000 CFU 100 mL⁻¹ of *E. coli*) and Lusk *et al.* (2017) (2,000,000 MPN 100 mL⁻¹ of total coliform and 100,000 to 100,000,000 MPN 100 mL⁻¹ of *E. coli*). Differences in concentrations of indicator bacteria in wastewater and groundwater were statistically significant ($p < 0.001$). Concentrations of total coliform and *E. coli* in wastewater within and beyond 11 km of the industrial plant were highly variable (Figure 4) and not expected to be influenced by emissions from the plant. Despite the elevated concentrations of total coliform and *E. coli* discharged by the septic tanks to the drain field trenches and soil, only one water supply tested positive for total coliform. These data suggest that indicator bacteria in the wastewater discharged to the subsurface via the OWTS were not influencing groundwater supply wells which were likely drawing water from deep, confined aquifers (Lautier 2006). Prior studies have shown that fecal bacteria can be successfully filtered in the soil if >0.6 m of vadose zone is present beneath the drain field (Stall *et al.* 2014). During water sample collection, groundwater was not encountered within 0.6 m of the drain field trench bottoms at any of the sites. Research (Schneeberger *et al.* 2015) has shown that shallow groundwater perched above a confining unit and near OWTS may contain elevated concentrations of fecal indicator bacteria, but groundwater movement is more horizontal across the confining unit rather than vertical, thus helping reduce the likelihood of groundwater contamination in deeper aquifers. These conditions may have also been present at the 18 sites evaluated in this study.

Nitrate concentrations

The concentrations of nitrate in the water supplies ranged from less than 0.01 mg L⁻¹ at several sites (8–12) to 3.2 mg L⁻¹ at Site 15 (Table 2). The overall mean concentration of nitrate in the water supplies observed at the 18 sites was 0.52 mg L⁻¹ (Figure 5) and within the range of mean nitrate concentrations (0.50–3.9 mg L⁻¹) reported by Naylor *et al.* (2018) for counties in NC. None of the samples collected from the water supplies exceeded the MCL for nitrate of 10 mg L⁻¹. The mean concentration of nitrate within 11 km of the industrial plant (0.49 mg L⁻¹) was not significantly different ($p = 0.332$) relative to concentrations beyond 11 km (0.55 mg L⁻¹) (Figure 5). Emissions from the industrial plant were not expected to influence groundwater nitrate concentrations. Wastewater sampled from the septic tanks at the sites had less than 0.4 mg L⁻¹ nitrate. This was expected because of the reducing conditions in septic tanks which favor ammonia/ammonium forms of nitrogen rather than nitrate (Humphrey *et al.* 2010; Lusk *et al.* 2017; Wigginton *et al.* 2018). The oxidation reduction potential of wastewater was between -107 mV at Site 18 to -313 mV at Site 5 (Table 3) and therefore there were reducing conditions in the wastewater. The mean concentration of TDN in wastewater observed at the 18 sites (66.7 mg L⁻¹) was within 1 standard deviation of the mean (57.7 ± 17.1 mg L⁻¹) of 43 septic tank samples included in a literature review by Lusk *et al.* (2017), thus wastewater strength was typical of domestic effluent. Ammonium concentrations in wastewater ranged from 28.11 mg L⁻¹ at Site 4 to 166.59 mg L⁻¹ at Site 15 (Table 1). All water supply samples had ammonium concentrations below 0.75 mg L⁻¹ except for at Site 15 (4.4 mg L⁻¹). There were no visible signs that the OWTS at Site 15 was malfunctioning, and soils on the site (Table 1) (Lakeland series) typically have water tables deeper than 2.5 m below the surface (USDA 2023). However, wastewater and groundwater at Site 15 had the highest concentrations of TDN, thus it may be possible that the OWTS was influencing groundwater TDN concentrations. It was also noted that the well at Site 15 was within 300 m of a confined animal feeding operation (CAFO) lagoon. Swine waste has concentrations of nitrogen that are elevated relative to human waste and studies have shown that groundwater and/or surface waters near CAFOs may also have elevated nitrogen

Table 2 | Concentrations of total dissolved nitrogen (TDN), nitrate (NO₃), ammonium (NH₄), *E. coli*, total coliform (TC), and GenX in wastewater (tank) and water supplies at the 18 sites

Location	TDN (mg/L)	NO ₃ -N (mg/L)	NH ₄ (mg/L)	<i>E. coli</i> (MPN/100 mL)	Total colif. (MPN/100 mL)	GenX (ppt)
Site 1 – Tank	67.76	<0.01	67.76	32,200	2,599,400	2.3
Site 1 – Water	0.61	0.46	0.04	0	0	0.35
Site 2 – Tank	56.07	<0.01	56.07	39,400	4,840,000	33.7
Site 2 – Water	1.36	1.22	0.14	0	0	2
Site 3 – Tank	34.56	<0.01	34.56	244,600	111,200	11.3
Site 3 – Water	0.72	0.52	0.2	0	0	0
Site 4 – Tank	28.12	0.01	28.11	2,000	4,840,000	8.5
Site 4 – Water	1.87	1.81	0.06	0	0	0
Site 5 – Tank	64.48	0.03	64.45	2,000	383,600	25
Site 5 – Water	0.44	0.04	0.4	0	0	31.7
Site 6 – Tank	32.52	0.01	32.51	120,400	1,226,200	13.8
Site 6 – Water	0.54	0.3	0.07	0	0	29.6
Site 7 – Tank	80.74	0.01	80.73	1,804,500	9,931,500	96.3
Site 7 – Water	0.44	0.01	0.06	0	0	0
Site 8 – Tank	45.26	0.02	45.24	487,000	3,873,000	9.5
Site 8 – Water	0.44	<0.01	0.24	0	1	0
Site 9 – Tank	46.58	0.01	46.57	26,000	2,897,000	120.1
Site 9 – Water	0.51	<0.01	0.25	0	0	44
Site 10 – Tank	72.56	0.01	72.55	15,000	1,361,500	20.8
Site 10 – Water	0.34	<0.01	0.34	0	0	43
Site 11 – Tank	121.7	0.02	121.68	61,000	4,604,000	2.6
Site 11 – Water	0.55	<0.01	0.34	0	0	0
Site 12 – Tank	31.57	0.01	31.56	1,732,800	4,840,000	17.3
Site 12 – Water	0.59	<0.01	0.59	0	0	0
Site 13-Tank	84.92	<0.01	84.92	67,000	1,179,500	221.2
Site 13 – Water	0.44	0.01	0.12	0	0	0
Site 14 – Tank	102.2	0.01	102.19	37,000	540,500	155
Site 14 – Water	0.44	0.01	0.23	0	0	0
Site 15 – Tank	166.6	0.01	166.59	308,000	6,498,500	71.3
Site 15 – Water	7.62	3.21	4.41	0	0	6.2
Site 16 – Tank	68.58	0.02	68.56	133,000	10,000,000	9.6
Site 16 – Water	0.71	0	0.71	0	0	0
Site 17 – Tank	56.63	0.01	56.62	113,000	9,931,500	0.12
Site 17 – Water	0.44	0	0.21	0	0	0
Site 18 – Tank	38.82	0.37	38.45	2,176,000	2,442,000	1.6
Site 18 – Water	1.78	1.64	0.14	0	0	0

Bolded values indicate exceedance of health advisory levels.

concentrations (Naylor *et al.* 2018; Sousan *et al.* 2021). Prior research has documented that nitrogen is mobile in the subsurface and plumes with elevated concentrations of nitrogen can extend for long distances away from a source (Lusk *et al.* 2017).

Concentrations of GenX

Concentrations of GenX were detectable in 7 of 18 (38.8%) water supplies and 4 of those sites (5, 6, 9, 10) had GenX concentrations that exceeded the HA of 10 ppt. The water supply had a higher concentration of GenX than the wastewater at

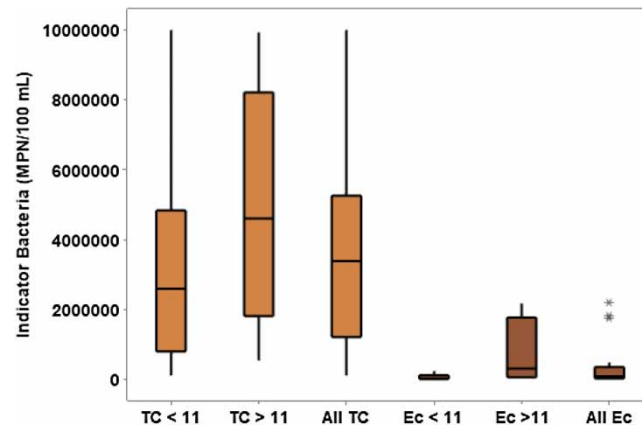


Figure 4 | Concentrations of total coliform (TC) and *E. coli* (Ec) in wastewater sampled from septic tanks within 11 km (<11) and beyond 11 km (>11) of the industrial plant. No *E. coli* were detected in the water supplies and 1 MPN 100 mL⁻¹ was detected at one of the wells.

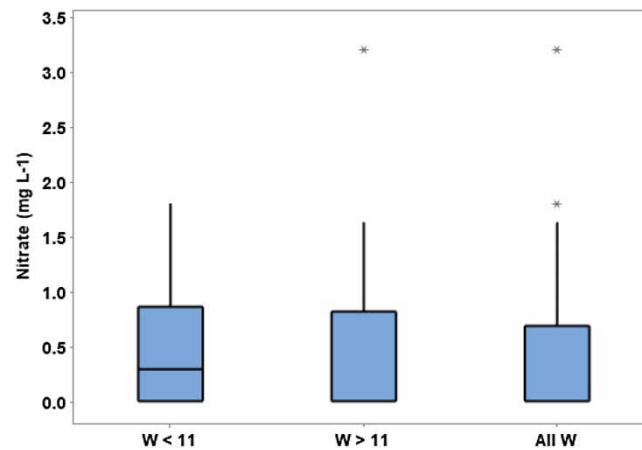


Figure 5 | Nitrate concentrations in wells located within 11 km (W < 11) and beyond 11 km (W > 11) from the industrial plant, and all wells (All W) combined. All samples were below the 10 mg L⁻¹ standard.

Sites 5, 6, and 10 thus contaminated groundwater may have been the main source of GenX detected in the wastewater at 3 of 4 sites which exceeded the HA. GenX was detected in the wastewater at all 18 sites and had a range in concentrations of 0.12 (Site 17) to 221.2 ppt (Site 13) (Table 2). GenX concentrations in wastewater samples at 11 of 18 (61.1%) sites exceeded the HA. While some processing including dilution is expected to occur in soil beneath the drainfield trenches, these OWTS are contributing GenX to the environment via subsurface wastewater discharges. There were 15 sites where the main source of GenX in the wastewater was likely from origins other than the water supply, such as from migration from cookware or food packaging (Choi *et al.* 2018) used in the homes and discharged via wastewater into the OWTS. With regards to the potential for negative human health outcomes related to drinking water quality, 22.2% of sites exhibited some risks due to exceeding the HA for GenX (Table 2). The sites with the highest potential for negative health outcomes related to water supply (5, 6, 9, and 10) were all within 11 km of the industrial plant. Wastewater sampled from septic tanks within 11 km of the industrial plant had concentrations of GenX (median = 13.8 ppt) that were not significantly different ($p = 0.93$) than concentrations in tanks further away (median = 17.3 ppt) (Figure 6). However, with regards to water supply, a significant ($p = 0.006$) inverse correlation ($r = -0.623$) was observed between distance from the industrial plant and GenX concentration in the water supply samples.

Also, GenX concentrations were significantly greater ($p = 0.021$) in water supplies located within 11 km (mean = 16.74 ppt) of the industrial plant relative to water supplies further away (mean = 0.69 ppt) (Figure 6). These findings agree with

Table 3 | Physicochemical characteristics of water supply and wastewater (tank) at the 18 sites including temperature, specific conductance, pH, turbidity, and oxidation reduction potential (ORP)

Location	Temp (°C)	Conductivity (µS/cm)	pH	Turbidity (FNU)	ORP (mV)
Site 1 – Tank	21.4	981	6.5	98.0	–280
Site 1 – Water	19.3	69	3.9	5.5	216
Site 2 – Tank	20.3	882	6.3	115.0	–263
Site 2 – Water	19.0	56	3.9	6.2	148
Site 3 – Tank	21.3	538	5.9	122.0	–250
Site 3 – Water	19.3	83	3.4	5.2	181
Site 4 – Tank	24.5	496	5.5	156.0	–224
Site 4 – Water	20.2	68	4.1	5.0	131
Site 5 – Tank	23.1	1,317	7.2	155.0	–313
Site 5 – Water	20.8	173	6.4	3.0	571
Site 6 – Tank	22.2	614	6.3	41.1	–209
Site 6 – Water	19.1	112	4.6	3.2	141
Site 7 – Tank	17.3	1,126	6.8	224.0	–243
Site 7 – Water	12.6	162	6.3	0.4	525
Site 8 – Tank	19.7	753	6.2	462.0	–230
Site 8 – Water	20.1	21	4.7	2.6	38
Site 9 – Tank	24.5	1,279	6.4	92.6	–265
Site 9 – Water	20.1	50	5.7	1.0	–71
Site 10 – Tank	19.9	850	6.4	1,000.0	–221
Site 10 – Water	14.8	53	4.9	6.0	–11
Site 11 – Tank	19.7	1,403	7.2	114.0	–226
Site 11 – Water	20.1	54	6.2	0.0	–15
Site 12 – Tank	17.6	588	6.9	30.2	–162
Site 12 – Water	20.6	78	5.9	0.0	–6
Site 13-Tank	16.4	1,144	6.5	136.0	–224
Site 13 – Water	13.3	134	6.2	2.8	673
Site 14 – Tank	13.6	1,349	7.0	135.0	–232
Site 14 – Water	17.4	711	8.0	1.0	525
Site 15 – Tank	11.8	1,452	7.0	420.0	–253
Site 15 – Water	17.2	127	5.0	0.7	–72
Site 16 – Tank	13.3	630	5.6	1,000.0	–183
Site 16 – Water	15.7	44	5.2	0.2	–116
Site 17 – Tank	14.2	653	6.6	137.0	–216
Site 17 – Water	19.3	51	6.3	1.5	–20
Site 18 – Tank	10.3	634	7.5	295.0	–107
Site 18 – Water	10.6	59	5.9	6.2	–21

a recent study by Roostaei *et al.* (2021) that concluded that elevated concentrations of GenX in water supply wells were greatly influenced by atmospheric deposition rates of GenX and distance and orientation (relative to prevailing winds) relative to the industrial plant. Roostaei *et al.* (2021) found that wells sampled southwest or northeast of the industrial plant and within 11 km had a higher risk of exceedance of the HA for GenX. Pritchett *et al.* (2019) reported that 25% of groundwater wells sampled within a 12 km radius of the industrial plant in Fayetteville NC exceeded the HA for GenX. These results imply that water supplies especially within 11 km of the industrial plant pose the most risk of contamination with GenX likely

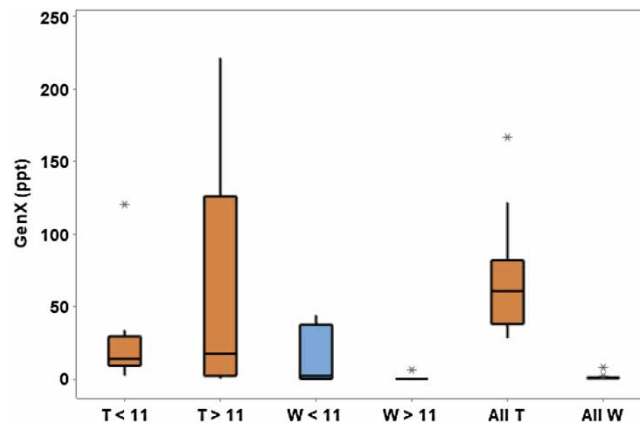


Figure 6 | Concentrations of GenX in wastewater sampled from septic tanks (T) and drinking water supplies (W) at sites within 11 km (<11) and beyond 11 km (>11) from the industrial plant.

because of historic atmospheric emissions sources. The industrial plant installed a packed bed scrubber and thermal oxidizer to improve air emissions in late 2019 which should reduce future atmospheric emissions (NC DEQ 2024).

Physicochemical characteristics of water and wastewater

Wastewater sampled from the septic tanks at the 18 sites had significantly higher ($p < 0.001$) concentrations of TDN and ammonium relative to groundwater (Table 2). This was expected as wastewater is enriched with organic matter from fecal material and food wastes generated and discharged from the homes (Lusk *et al.* 2017). The specific conductance and turbidity of wastewater were higher relative to the water supply at each site (Table 3). More specifically, wastewater samples had a mean turbidity and specific conductance of 263 FNU and $927 \mu\text{S cm}^{-1}$ respectively, while water supplies were significantly ($p < 0.05$) lower at 2.8 FNU and $117 \mu\text{S cm}^{-1}$. Wastewater has high concentrations of ions and solids which influence the conductance and clarity (Lusk *et al.* 2017; Humphrey *et al.* 2018). The pH of wastewater at the sites ranged from 5.5 at Site 4 to 7.47 at Site 18 (Table 3). The mean pH of wastewater (6.54) was elevated relative to the mean pH of the water supplies (5.36). The mean temperature of wastewater was also slightly elevated (18.4°C) relative to the water supplies (17.7°C) (Table 3).

Onsite system functionality

Each of the septic tanks sampled at the volunteer sites were pumped to remove the solids and information regarding suggested routine maintenance of the OWTS was communicated to the owners. The soil types for the 18 sites were mostly dominated by sandy-textured, well-drained soils (Table 1). However, the soils at Sites 6 (Roanoke) and 12 (Leon) had indications of seasonal high wetness conditions (grey mottles) within 0.3 m of the OWTS trench bottoms and would not be suitable for a conventional style system using today's regulations in NC, unless water table monitoring revealed groundwater was deeper than soil morphological indicators suggested. During the time of the site visits though, groundwater was not within 0.6 m of the trenches at any of the sites. Four of the 18 OWTSs (22%) evaluated were exhibiting active signs of malfunction. The malfunctions included a leaking septic tank at Site 18 as indicated by a wastewater level approximately 0.3 m below the inlet pipe of the tank; encroachment of freeboard volume in septic tanks at Sites 12 and 7; and roots growing in a septic tank at Site 4 (Table 1). However, the 4 malfunctioning OWTSs did not seem to have a negative influence on well water quality as none of the water supplies at those sites contained detectable concentrations of GenX, *E. coli*, or total coliform, and the nitrate concentration was below 2 mg L^{-1} at each one. The OWTSs at Sites 7 and 5 were exhibiting evidence of unequal distribution of septic tank effluent as the color and growth of grass were noticeably different over one trench relative to the others. The GenX concentration in the water supply at Site 5 was 31.7 ppt and thus exceeded the HA while wastewater at the site had a lower concentration of 25 ppt. Given the lower concentration of GenX in wastewater relative to groundwater, it could not be determined that wastewater from the OWTS was a source of GenX in groundwater at that location. Wastewater at Site 7 had a GenX concentration of 99.6 ppt, but GenX was not detected in groundwater, so GenX contributions from the OWTS to groundwater supply could not be confirmed at the site. Two of the OWTSs (Site 2 and 17) had recently received

new drain field trenches due to hydraulic malfunctions within the past few years, but GenX was not observed in the water supply at those sites. Excessive solids layers were observed in the septic tanks at Sites 8 and 16, demonstrating a lack of routine maintenance, but GenX was not detected in the water supplies at those sites either. Therefore, most of the water quality data suggest that OWTs were not influencing the water supply wells with regard to contaminants. Water supply wells are typically installed in deep, confined aquifers in Eastern NC (Lautier 2006) which may have reduced the likelihood of contamination from OWTs discharges to the shallow subsurface.

Implications

NC did not have a state-wide well program until 2008, thus water testing was not required even during the initial startup in many communities. Well water is now tested prior to first use for common contaminants such as nitrate, *E. coli*, and coliform bacteria. However, this research has shown that well water that has lower than the MCL concentrations of nitrate and indicator bacteria may still be a health hazard due to contaminants of emerging concern, such as synthetic chemicals like GenX. It is important that private drinking water wells and community water supplies in this region near the industrial plant are routinely monitored for these chemicals and filtration systems and/or alternative sources of water provided where needed to protect human health. The industrial plant in the community evaluated in this study has agreed to test water supplies in the area and based on the results, has provided alternative sources of water and/or provided advanced water treatment systems to some affected residences (NC DEQ 2024). The industrial plant now sends wastewater to a hazardous waste landfill rather than discharging effluent to the Cape Fear River (Cahoon 2019), and to reduce atmospheric emissions, they have installed a packed bed scrubber and thermal oxidizer (NC DEQ 2024). These efforts should help reduce future loadings of GenX to the environment, but contamination of groundwater is still present.

Despite four of 18 OWTs showing active signs of malfunction, groundwater from the water supply wells did not seem to be influenced with regards to *E. coli* or nitrate at those sites. Removal mechanisms for *E. coli* including filtration, sorption, and die off and for nitrate including denitrification, anammox, and immobilization (Lusk *et al.* 2017; Wigginton *et al.* 2018) may have been active in the soil beneath the studied OWTs, thus resulting in little to no negative health impacts for the occupants of the homes with regards to these pollutants. Persistent contaminants such as GenX were observed in wastewater at each of the 18 homes likely due to migration of GenX from cookware and food packaging. Therefore, the discharge of wastewater to the subsurface from homes contributes GenX to soil and potentially groundwater near those OWTs. The encouragement by the State of NC to use shallower aquifers and surface waters for a water supply to reduce the trends of dewatering, saltwater intrusion, and declining water levels for the deeper aquifers (NC DENR 1998) may increase the risk of GenX exposure if shallow groundwater contains high concentrations of the chemical.

CONCLUSIONS

The goal of this study was to gain a better understanding of the presence and concentrations of traditional and emerging contaminants in OWTs and water supply wells in some economically distressed communities of Eastern NC. Based on the results from sampling wastewater and water supplies from 18 sites, it was shown that 22% of water supply samples contained concentrations of GenX that exceeded the HA. None of the water supplies sampled had *E. coli*, while one of the 18 sites was positive for total coliform (1 MPN 100 mL⁻¹). Nitrate concentrations in the water supplies were all below 10 mg L⁻¹ and most were below 0.8 mg L⁻¹. The main threat to human health with regards to contamination of the water supplies was with the synthetic chemical GenX, which was elevated above the HA for most evaluated sites within 11 km of the industrial plant. GenX discharges via OWTs effluent to the subsoil were observed at all 18 sites. More research is suggested to assess the occurrence and concentration of GenX in soil and shallow groundwater near OWTs. Monitoring for persistent compounds in groundwater is suggested especially for communities using OWTs and that rely on surficial or shallow groundwater for a water supply.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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