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ACETIC ACID REMEDIATION OF ANTHROPOGENIC CONTAMINATION OF WATER AT THE GBNERR IN MISSISSIPPI

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Abstract

Grand Bay National Estuarine Research Reserve (GBNERR) is an important ecosystem in the Mississippi Gulf Coast. The GBNERR may be a potential source for contamination with anthropogenic bacterial pathogens that may play a significant role in the causation of waterborne human diseases. The objective of this study was to evaluate the interaction of physicochemical and microbiological water quality parameters at the GBNERR to determine quantitative levels and establish the potential for remediation of post-contamination of water and seafood by human fecal pollution from anthropogenic sources at the reserve. Water samples were collected aseptically from Bayous Heron, Cumbest, Point Aux Chenes Bay and Bangs Lake (Pine-O-Pine). Physicochemical parameters were determined using standard protocols. Eight bacterial species including Campylobacter were concentrated from water samples by membrane filtration. Water samples were tested for the presence of traditional indicator microorganisms including: heterotrophic (HPC), total coliforms (TC), fecal coliforms (FC), and enterococcus (ENT) in CFU/ml concentrations. Mean values of temperature, specific conductivity, dissolved oxygen, and pH were within acceptable levels in comparison to MDEQ, USEPA, and the USGS standards during the time of investigation. However, the values of turbidity in Grand Bay water exceeded USEPA recommended levels in several occasions during the investigation. Data from this study indicates significant variability ($p < 0.0001$) in mean bacteria concentrations between sites. The data also indicates significant impact of acetic acid treatment in the remediation of post contamination and survival of pathogens from the GBNERR Bayous Heron, Cumbest, and Pine-O-Pine when compared with control findings. The interaction of physicochemical and microbiological parameters of water through external chemical manipulation by acetic acid may provide utility in the remediation of post-contamination with anthropogenic pathogens such as *E. coli*, Enterococci, Campylobacter, Vibrio, Giardia, and Cryptosporidium. Presence of high numbers of indicator bacteria suggests public health concerns for oyster and shellfish consumers as well as other water contact activities. Hence, control strategies should be developed and implemented to prevent or remediate any future contamination of the GBNERR waters citing the economic impact of such contamination on shellfish fishing activities on the reserve.

Keywords

Acetic acid; anthropogenic; water and foodborne disease; natural remediation; shellfish

INTRODUCTION

Water quality monitoring is the foundation for first-hand data on environmental water management. A reasonable water quality monitoring network should not only meet the needs for long-term data accumulation, water quality assessment, and trend analysis but also reflect in a timely manner, the dynamic status of the water environment, and water pollution. Water quality monitoring provides scientific guidance for water resource management and water environment protection [1]. There has been great emphasis on the importance of assessing the microbiological quality of water in Grand Bay National Estuarine Research Reserve (GBNERR) in Mississippi due to the increased impact of pathogenic bacteria from anthropogenic sources. Improving access to safe water sources can result in significant benefits to both health and quality of life [2, 3].

An estimated 1.1 billion people worldwide rely on water supplies that are at high risk of fecal contamination [4]. The deterioration of aesthetic aspects of drinking water, such as taste, odor, and color represents up to 80% of consumer complaints to water utilities [5]. Diarrheal disease is a major cause of death and disease, especially among young children in low-income countries where the most common causes are fecal-contaminated water and food, or poor hygiene practices. The bacterial, viral, and protozoan pathogens causing diarrheal disease are primarily transmitted via the fecal-oral route, through the consumption of fecal-contaminated food and water. Among the most important of these are rotavirus, *Cryptosporidium spp., Escherichia coli, Salmonella spp., Shigella spp., Campylobacter jejuni, Vibrio cholerae, norovirus, Giardia lamblia*, and *Entamoeba histolytica*, though the relative importance of these varies among settings, seasons, and population groups [4, 6]

Water quality monitoring prevented Vibrio infection in Mobile Bay, Alabama. In 1992, *Vibrio cholerae* infected the shellfish supplies due to contaminated ballast water in cargo ships. The United States Food and Drug Administration (FDA) recommended the U.S. Coast Guard to have ships dump and change ballast water at high seas before entering ports; overall, no infections were documented. In 1989, Cabool, Missouri lacked disinfection of the city's water supply and during heavy rains runoff containing *E. coli* O157:H7 from cattle manure washed into the well system. This infection resulted in 243 cases, 32 hospitalizations, and four deaths [7–11].

Oxidation processes, such as chlorination and ozonation or UV/H_2O_2 advanced oxidation, are commonly applied as primary disinfection strategies for bacterial inactivation. However, oxidative treatments often result in modification of the substrate composition. Primary disinfection processes with high ozone or free chlorine dosage also typically result in inactivation of the entire bacterial community, thus leaving room for new microorganisms to colonize the treated water and consume the altered nutrient pool. These oxidative processes create highly unstable water due to combined effects of increased nutrients availability and absence and/or inactivation of bacterial cells, thus, creating a new niche for bacterial growth [5, 12].

Recreational water quality programs and drinking water distributors worldwide collect water samples, test for indicator bacteria, and post, close or otherwise restrict access to

recreational waters based on the concentrations of indicator bacteria present [4, 12]. Common indicator bacteria including heterotrophic (HT), total coliforms (TC), fecal coliforms (FC), enterococci (ENT), *E. coli*, Shigella, and Salmonella are observed in this study to contaminate GBNERR surface waters according to United States Environmental Protection Agency (USEPA) [13–18] and Centers for Disease Control and Prevention (CDC) Maximum Contaminant Level Goal (MCLG) [7–11].

The goal of this research was to establish an effective natural and inexpensive methodology to remediate against anthropogenic seafood contamination. We hypothesized that the chemical modulation of physical-chemical parameters of water will provide remediation of anthropogenic contaminants with the resultant increase in the achievement of seafood safety end points. To achieve our goal, we set three specific objectives. 1. Assess the bacteriological quality of water by evaluating variations of heterotrophic and indicator microorganisms in specific habitats of the reserve; indicator bacteria are found abundantly in wastes where pathogenic microorganisms exist. They are typically non-pathogenic, but their occurrence can predict disease outbreaks. 2. Assess the characteristics of water quality in terms of temperature, pH, salinity, conductivity, dissolved oxygen, total dissolved solids, and nutrients as well as determine their potential influence on bacterial densities in comparison to state and federal public health guidelines, and 3. Perform water remediation/treatment using natural chemical modulators (acetic acid; [19–25] to assess effectiveness in inhibiting bacterial growth and survival in laboratory environments and within anthropogenic contaminated water sources by altering physical and chemical parameters.

METHODS

Sampling Stations and Study Sites: The boating dock from Bayou Heron, Cumbest, and Point-O-Pines of Grand Bay were chosen as sampling sites for this investigation. Individual sites along the boating docks were chosen randomly at each bayou [2]. **Sample Collection and in situ Analysis of Physicochemical Parameters**: Water samples were collected aseptically between 2016 and 2017. The HANNA Instrument 9828 model multi parameter meter (YSI) was used to measure the physical and chemical characteristics of water temperature, pH/acidity, dissolved oxygen (DO), turbidity, and conductivity; all samples were collected in situ. At each boating dock, water samples were collected in duplicate in sterile 250mL screw-caped plastic bottles. Samples were processed at the Environmental Microbiology Research Laboratory at Jackson State University. **Materials:** The funnel assembly used for membrane filtration was obtained from Micro Filtration Systems (MFS); Lenntech B.V., Delft, Netherlands; EMD Millipore Microbiological Analysis Membrane Filters 0.45 μm, and the 47 mm carrying units were obtained from Millipore (EMD Millipore, Milton, Abingdon, England; Barnant Company Vacuum Pressure Station 115V 60hz 1.5 A was from (Barnant Company, Barrington, IL 60010. A precision Coliform water bath incubator was used for *E. coli* and fecal coliform growth on EMB agar (Precision Scientific Inc., Chicago, IL 60647). Isotemp Incubator (Thermo Fisher Scientific, Fair Lawn, New Jersey 07410). Fisher Scientific Digital Vortex Mixer, 115 VAC, 150 Watts, 50/60 Hz (Thermo Fisher Scientific, Fair Lawn, New Jersey 07410). Various flask sizes including, 75 CM^2 corning flask, canted neck, tissue culture treated nonpyrogenic flasks, polystyrene, sterile corning flask; 25 CM^2 were used in this study. **Culture Media Sources and**

Preparation: m-HPC agar and enterococcus agar were from (Becton, Dickinson and Company, Sparks, Maryland 21152). The m-FC agar: Sigma-Aldrich m-FC agar (Sigma-Aldrich, St. Louis, Missouri, 63103), The m-Endo agar: Oxoid m-Endo agar LES and Remel (Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar were from Thermo Fisher Scientific, Remel Products Lenexa, KS 66215. Campylobacter agar: Oxoid Blood-Free Campylobacter agar (Oxoid LTD., Basingstoke, Hampshire, England). EMB agar: Oxoid Levine Eosin Methylene Blue (EMB) Agar (Oxoid LTD., Basingstoke, Hampshire, England). After the preparation of each agar according to manufacturer's recommendations, 4ml of medium were poured into 50 mm × 9 mm pre-sterilized Lab craft petri dishes (Curtin Matheson, Labcraft, Morris Plains, NJ 07950). The petri dishes were then allowed to solidify at room temperature. The prepared media were stored at 4°C for a maximum of two weeks.

Bacteriological Assessment of Water Quality and Chemical Remediation: Water samples were processed within eight hours of collection or frozen for evaluation of microbiological water quality. Samples were used to determine the concentrations of Vibrio spp. (TCBS), Campylobacter spp., fecal coliforms (FC), enterococci (ENT), heterotrophic bacteria (HPC) and total coliforms (TC). The samples were processed using membrane filtration technique; APHA protocol 9215D, 9222B, 9222D and USEPA Method 1600 for testing HPC, TC, FC, Vibrio (TCBS), and ENT respectively. Briefly, 10 to 100 mL of water samples was used to enumerate bacteria chosen for isolation in this study. Samples were treated with 1–20% concentrations of acetic acid (50% w/w) for 2 and 4hrs prior to plating. Ten milliliter of the sample was passed through a 0.45 μm membrane filter that trapped bacteria on its surface [20]. Colonies were counted, counts corrected for dilution factors and used for comparisons of treatment vs. control samples in CFU/ml. To differentiate between bacteriostatic and bactericidal effect, colonies were recounted after 144 hrs to confirm count consistency or differences.

RESULTS

Heterotrophs (HT) are a group of microorganisms (yeast, molds, and bacteria) that use organic carbon as a food source, and are found in all types of water. Detecting heterotrophs using a heterotrophic plate count (HPC) is a standard way to measure the bacteriological content of water, and is used for both public and private waterways. The presence of HT in water does not necessarily pose a health risk to humans, but the information from the HPC can be used as indicator for conditions favorable for bacterial growth. The higher the HT more favorable the water is for microorganism growth. The HT counts from three public water sources on the Gulf Coast in Mississippi are shown in Tables 1–3. The initial HT counts been 140 ± 25 CFU/mL at Bayou Cumbest, 120 ± 50 CFU at Bayou Point of Pines, and 150 ± 12 CFU/mL at Bayou Heron, which are under the 500 CFU/mL limits considered safe limits set by the U.S EPA. These limits are not a health based standard. HT counts higher than 500 CFU/mL interferes with proper detection of the total coliform and E. coli measurements. Even though the HT is well within the EPA limits they are significantly above the 10 CFU/mL limits established for drinking water.

Clean drinking water is a concern for many areas of the world and need for safe, economical, and effective disinfectants is greatly needed. Acetic acid has been shown to be an effective mycobacterial agent. Heterotrophs thrive on organic carbon and acetic acid

treatment may actually enhance the growth of heterotrophs. Acetic acid was used in concentration from 1% to 20% and these concentrations were incubated with the water for longer periods of time (2 and 4 hours) to determine its effects on the survival of common pathogenic bacterial species and HT counts. Tables 1–3 show the Total Coliforms (bacteria found in water or soil that is influenced by surface water, and animal or human waste), Fecal Coliforms (bacteria present in the gut and feces of warm-blood animals), Campylobacter, and Enterococcus were sensitive to concentrations of acetic acid as low as 1% and were completely inactivated at 1% and higher concentrations and longer incubation. *E. coli* and Vibrio were less affected by longer incubation and increasing concentrations of acetic acid ranging from 1% to 10%; these organisms were inactivated at 20% concentration in combination with 4hrs of incubation. Increasing the incubation time as well as the acetic acid concentration did not enhance the clearance of these specific bacteria; showing their resistance to such treatments. Shigella /Salmonella showed modest sensitivity to, and inactivation responses as concentrations of acetic acid increased over 10% with longer incubation times.

Table 1 shows the survival trends of eight microbial species upon exposure to acetic acid in water samples from Bayou Cumbest. As can be seen all organisms grew at 0% (control). Heterotrophic organisms showed survival that is responding negatively to increased acetic acid concentration. Total coliforms, fecal coliforms, Campylobacter, and Enterococcus were all inactivated the 1% acetic acid concentration. Vibrio, *E. coli*, and Salmonella/Shigella showed various resistances to the acetic acid treatment; Salmonella/Shigella were inactivated completely at 10%, while both Vibrio and *E. coli* were inactivated at 20% acetic acid.

Table 2 shows the survival trends of eight microbial species upon exposure to acetic acid in water samples from Bayou Point O Pines. As can be seen all organisms grew at 0% (control). Heterotrophic organisms showed survival that is responding negatively to increased acetic acid concentration. Total coliforms, fecal coliforms, Campylobacter, and Enterococcus were all inactivated the 1% acetic acid concentration. Vibrio, *E. coli*, and Salmonella/Shigella showed various resistances to the acetic acid treatment; Salmonella/Shigella were inactivated completely at 10%, while both Vibrio and *E. coli* were inactivated at 20% acetic acid.

Table 3 shows the survival trends of eight microbial species upon exposure to acetic acid in water samples from Bayou Heron. As can be seen all organisms grew at 0% (control). Heterotrophic organisms showed survival that is responding negatively to increased acetic acid concentration. Total coliforms, fecal coliforms, Campylobacter, and Enterococcus were all inactivated the 1% acetic acid concentration. Vibrio, *E. coli*, and Salmonella/Shigella showed various resistances to the acetic acid treatment; Salmonella/Shigella were inactivated completely at 10%, while both Vibrio and *E. coli* were inactivated at 20% acetic acid and longer incubation.

DISCUSSION

The GBNERR presents as a potential source for contamination with anthropogenic bacterial pathogens that may play a significant role in the causation of waterborne human diseases.

The objective of this study was to evaluate the interaction of physicochemical and microbiological water quality parameters at the Grand Bay NERR, determine quantitative levels and establish the potential for remediation of post-contamination of water and seafood by human fecal pollution from anthropogenic sources at the reserve through the treatment of post contaminated water with different levels of acetic acid. Our study provided evidence for the utility of acetic acid in the remediation of anthropogenic contamination of three Bayous at the GBNERR. The findings show the total inactivation of eight pathogenic bacterial species that constitute public health threat to the fishing activities at the GBNERR with special reference to shellfish production.

The literature shows that 5% acetic acid for 20 minutes reduced the viability of some strains of *E. coli* and often resulted in a more bacteriostatic effect. Cortesia and colleagues (2014) showed the bactericidal activity of acetic acid was due to it carboxylic acid function and because of the drop in pH. The other interesting component was the HT counts, which never reached levels considered safe for drinking water. In fact, two out of three cases resulted in an increase in HT measured by HPC. The results of this study are consistent with results published by others [21–25], which showed that increased acetic acid concentration resulted in a regrowth of HT and accumulation of biofilms in drinking water pipes. Overall the results show that some species of bacteria can be treated with low levels of acetic acid at concentrations which would not necessarily affect the taste of the water. Nonetheless, some bacteria that are associated with food borne illnesses and can result in life threatening illness in both competent and immunocompromised individuals did show some resistance to acetic acid, however, they were cleared by higher concentrations and longer incubation and consistently proven to be bactericidal in treated water samples from three bayous at the GBNERR reserve in MS.

Cortesia et al. [21] found that acetic acid (vinegar) efficiently kills *M. tuberculosis* after 30 min of exposure to a 6% acetic acid solution. The activity is not due to pH alone, and propionic acid also appears to be bactericidal. *M. bolletii* and *M. massiliense* nontuberculous mycobacteria were more resistant, although a 30-min exposure to 10% acetic acid resulted in at least a 6-log10 reduction of viable bacteria. Acetic acid (vinegar) is an effective mycobactericidal disinfectant that should also be active against most other bacteria [19–25].

Acetic acid has been shown to have good antibacterial activity against various planktonic organisms, however data is limited on efficacy, and few studies have been performed on biofilms. Acetic acid was antibacterial against planktonic growth, with a minimum inhibitory concentration of 0.16–0.31% for all isolates and was also able to prevent formation of biofilms (at 0.31%). Eradication of mature biofilms was observed for all isolates after three hours of exposure. Clearly, acid resistance and the development of acid tolerance by food-borne pathogenic bacteria may be significant at several points along the farm-to-table continuum of food production. It is important that we understand how previous environment and processing conditions can affect the acid tolerance status of food-borne *E. coli* O157:H7 in order to devise strategies for better control of the occurrence, growth, or survival of this organism in foods [26–34].

White vinegar is nontoxic, cost-effective, easy to access, and appropriate for household use. However, this agent may not be suitable for medical use. The low pH and corrosive nature of the acid can result in deterioration and destruction of metal and plastic materials.

CONCLUSION

This study provided evidence for the utility of acetic acid in the remediation of anthropogenic contamination of three Bayous at the GBNERR. The findings showed the total inactivation of eight pathogenic microbial species that constitute public health threat to the fishing activities at the GBNERR. The interaction of physicochemical and microbiological parameters of water through external chemical manipulation by acetic acid may provide utility in the remediation of post-contamination with anthropogenic pathogens and indicator bacteria such as coliforms, fecal coliforms, *E. coli*, salmonella and Campylobacter and enterococci. Presence of high HT indicates an environment conducive for bacterial growth. Hence, control strategies should be further developed and implemented to prevent or remediate any future contamination of the GBNERR waters.

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References

1. Wang H, Wu M, Deng Y, Tang YC, Yang R. Surface Water Quality Monitoring Site Optimization for Poyang Lake, the Largest Freshwater Lake in China. International journal of environmental research and public health. 11(11):11833–11845.2014; [PubMed: 25407419]
2. Kishinhi SS, Tchounwou PB, Farah IO. Molecular approach to microbiological examination of water quality in the Grand Bay National Estuarine Research Reserve (NERR) in Mississippi, USA. Environmental health insights. 7:33.2013; [PubMed: 23761974]
3. World Health Organization. Water Sanitation Hygiene. 2014. Retrieved from http://www.who.int/water_sanitation_health/diseases-risks/en/
4. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal contamination of drinking-water in low-and middle-income countries: a systematic review and meta-analysis. PLoS Med. 11(5):e1001644.2014; [PubMed: 24800926]
5. Prest EI, Hammes F, van Loosdrecht MC MC, Vrouwenvelder JS. Biological Stability of Drinking Water: Controlling Factors, Methods, and Challenges. Frontiers in microbiology. 72016;
6. Clasen TF, Roberts IG, Rabie T, Schmidt WP, Cairncross S. Interventions to improve water quality for preventing diarrhoea. The Cochrane Library. 2006
7. Centers for Disease Control and Prevention (CDC). General Information: Cryptosporidium: Parasites. Oct 7, 2015 Retrieved from http://www.cdc.gov.parasities/crypto/general.html
8. Centers for Disease Control and Prevention. General Information: E coli. Mar 2, 2017a Retrieved from https://www.cdc.gov/ecoli/general/index.html
9. Centers for Disease Control and Prevention CDC. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. Mar 2, 2017b http://www.cdc.gov.hicpac/Disinfection_Sterilization/3_3inactivBio/
10. Centers for Disease Control and Prevention (CDC). Health Practices on Cruise Ships: Training for Employees Transcript: Waterborne Illnesses, 2016. Apr 2, 2017 https://www.cdc.gov/nceh/vsp/training/videos/transcripts/water.pdf

11. Centers for Disease Control and Prevention (CDC). The Elderly and Waterborne Cryptosporidium Infection: Gastroenteritis Hospitalizations before and during the 1993 Milwaukee Outbreak. Oct 7, 2015 Retrieved from http://wwwnc.cdc.gov/eld/article/9/4/02-0260 article
12. Weisberg SB, Noble RT, Moore DF, McGee CD, Leecaster MK. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. Pergamon Water Research. 37:1637–1643.2003;
13. Environmental Protection Agency. Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm In Source Water and Drinking Water. Mar 25, 2015 Retrieved from http://www.epa.gov/…/m_415_3Revl_l.pdf
14. Environmental Protection Agency. Dissolved Oxygen and Biochemical Oxygen Demand. Mar 25, 2015 Retrieved from http://water.epa.gov/type/rsl/monitoring/vms52.cfm
15. Environmental Protection Agency. National Aquatic Resource Surveys. Indicators: Conductivity. Mar 1, 2017 Retrieved from https://www.epa.gov/national-aquatic-resource-surveys/indicators-conductivity
16. Environmental Protection Agency. National Aquatic Resource Surveys. Indicators: Salinity. Mar 2, 2017 Retrieved from https://www.epa.gov/national-aquatic-resource-surveys/indicators-salinity
17. Environmental Protection Agency. Temperature. Mar 1, 2017 Retrieved from https://www.epa.gov/sites/production/files/2015-09/documents/temperature.pdf
18. Environmental Protection Agency. Total Solids. Mar 25, 2015 Retrieved from http://water.epa.gov/type/rsl/monitoring/vms58.cfm
19. Johnston CS, Gaas C. Vinegar: Medicinal Uses and Antiglycemic Effect. Med Gen Med. May. 8(2)2006
20. Kahler AM, Johnson TB, Hahn D, Narayanan J, Derado G, Hill VR. Evaluation of an ultrafiltration-based procedure for simultaneous recovery of diverse microbes in source waters. Water. 7(3):1202–1216.2015; [PubMed: 26530003]
21. Cortesia C, Vilchèze C, Bernut A, Contreras W, Gómez K, de Waard J, Takiff H H. Acetic acid, the active component of vinegar, is an effective tuberculocidal disinfectant. MBio. 5(2):e00013–14.2014; [PubMed: 24570366]
22. Halstead FD1, Rauf M, Moiemen M, Bamford A, Wearn CM, Fraise AP, Lund PA, Oppenheim BA, Webber MA. The Antibacterial Activity of Acetic Acid against Biofilm-Producing Pathogens of Relevance to Burns Patients. PLoS One. 2015 Sep 9.10(9):e0136190.doi: 10.1371/journal.pone.0136190 [PubMed: 26352256]
23. Xiaonong, Zhou; Xukun, Zhao; Lihua, Xiao; Lin, Wang; Na, Li; Wei, Jin; Yaoyu, Feng; Jiaxu, Chen. Occurrence, Source, and Human Infection Potential of Cryptosporidium and Giardia spp. Source and Tap Water in Shanghai, China. 2015. http://www.ncbi.hlm.nih.gov/pmc/articles/PMC3127622
24. Webber M, Wearn C, Rauf M, Moiemen N, Lund PA, Oppenheim B, Halstead FD, Fraise AP, Bamford A. The Antibacterial Activity of Acetic Acid against Biofilm-Producing Pathogens of Relevance to Burn Patients. PLoS One. Sep; 10(9):1–12.2015
25. Taylor RK, Almagro-Moreno S. Cholera: Environmental Reservoirs and Impact on Disease Transmission. Microbiology Sepctr. 2013 Dec; 1(2):0003–2013.
26. Swerdlow DL, Mahon BE, Iwamoto M, Ayers T. Epidemiology of Seafood-Associated Infections in the United States. Clinical Microbiology Reviews. 23(2):399–411.2010; [PubMed: 20375359]
27. Reganold John, P, Chiras Daniel, D. Natural Resource Conservation: New International Edition: Management for a Sustainable Future. Tenth. South Asia: Pearson Education Limited; 2013.
28. Reno W, Rao R, Panja A, Obenhuber D, Koelle M, Cohly HH, Angel MF. Evidence for Alteration in Chemical and Physical Properties of Water and Modulation of its Biological Functions by Sunlight Transmitted through Color Ranges of the Visible Spectrum-A Novel Study. International Journal of Environmental Research and Public Health. 2(2):219–227.2005; [PubMed: 16705821]
29. Robb CK. Assessing the impact of human activities on British Columbia's estuaries. PloS one. 9(6):e99578.2014; [PubMed: 24937486]
30. Palamuleni L, Akoth M. Physio-Chemical and Microbial Analysis of Selected Borehole Water in Mahikeng, South Africa. International Journal of Environmental Research and Public Health. 12(8):8619–8630.2015; [PubMed: 26213950]

31. Putignani L, Menichella D. Global distribution, public health and clinical impact of the protozoan pathogen Cryptosporidium. Interdisciplinary perspectives on infectious diseases. 2010
32. Iwamoto M, Ayers T, Mahon BE, Swerdlow DL. Epidemiology of seafood-associated infections in the United States. Clinical microbiology reviews. 23(2):399–411.2010; [PubMed: 20375359]
33. Mull B, Hill VR. Recovery of diverse microbes in high turbidity surface water samples using dead-end ultrafiltration. J Microbiology Methods. 91(3):429–33.2012;
34. Berry ED, Cutter CN. Effects of acid adaptation of Escherichia coli O157: H7 on efficacy of acetic acid spray washes to decontaminate beef carcass tissue. Applied and environmental microbiology. 66(4):1493–1498.2000; [PubMed: 10742232]
35. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. Clinical microbiology reviews. 26(1):115–134.2013; [PubMed: 23297262]
36. Cabral JPS. Water Microbiology. Bacterial Pathogens and Water. Int J Environ Res Public Health. Oct; 7(10):3657–3703.2010 [PubMed: 21139855]
37. Chapman B, Ross T. Escherichia coli and Salmonella enterica are protected against acetic acid, but not hydrochloric acid, by hypertonicity. Applied and environmental microbiology. 75(11):3605–3610.2009; [PubMed: 19346344]
38. Delpla L, Monteith DT, Freeman C, Haftka J, Hermens J, Jones TG TG, Thomas O. A decision support system for drinking water production integrating health risks assessment. International journal of environmental research and public health. 11(7):7354–7375.2014; [PubMed: 25046634]
39. Fisher JC, Eren AM, Green HC, Shanks OC, Morrison HG, Vineis JH, McLellan SL. Comparison of sewage and animal fecal microbiomes by using oligotyping reveals potential human fecal indicators in multiple taxonomic groups. Applied and environmental microbiology. 81(20):7023–7033.2015; [PubMed: 26231648]
40. Goudie, A. The Human Impact on the Natural Environment. 4. The MIT Press; 1994.
41. Hardy Diagnostics. Instructions for Use. Campylobacter Blood-Free Agar. 2016. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CampyBldFreeAgar.htm

Table 1

Distribution of eight microbial species isolated from Bayou Cumbest upon exposure to acetic acid; numbers are in CFU/ml. Data represent mean±SD.

Organisms	0%	1%	5%	10% 2h	10% 4h	20% 2h	20% 4h
Heterotrophic	140.0±25.0	150.0±53.0	130.0±48.0	120.0±45.0	60.0±27.0	30.0±18.0	0.0
Total Coliforms	170.0±25.0	0.0	0.0	0.0	0.0	0.0	0.0
Fecal Coliforms	160.0±25.0	0.0	0.0	0.0	0.0	0.0	0.0
Campylobacter	110.0±25.0	0.0	0.0	0.0	0.0	0.0	0.0
Vibrio	180.0±25.0	20.0±5.0	30.0±10.0	20.0±4.0	20.0±.7.0	10.0±1.0	0.0
Enterococcus	170.0±25.0	0.0	0.0	0.0	0.0	0.0	0.0
E. coli	140.0±25.0	70.0±.36.0	80.0±48.0	70.0±45.0	60.0±27.0	50.0±18.0	0.0
Shigella/Salmonella	150.0±25.0	60.0±26.0	50.0±18.0	0.0	0.0	0.0	0.0

Table 2

Distribution of eight microbial species isolated from Bayou Point O Pines upon exposure to acetic acid; numbers are in CFU/ml. Data represent mean ±SD.

Organisms	**0%**	**1%**	**5%**	**10% 2h**	**10% 4h**	**20% 2h**	**20% 4h**
Heterotrophic	120.0±50.0	70.0±30.0	50.0±22.0	148.0±50.0	388.0±34.0	280.0±98.0	43.0±15.0
Total Coliforms	210.0±50.0	0.0	0.0	0.0	0.0	0.0	0.0
Fecal Coliforms	150.0±50.0	0.0	0.0	0.0	0.0	0.0	0.0
Campylobacter	210.0±50.0	0.0	0.0	0.0	0.0	0.0	0.0
Vibrio	140.0±50.0	40.0±30.0	30.0±22.0	20.0±8.0	20.0±13.0	20.0±9.0	10.0±5.0
Enterococcus	18.00±50.0	0.0	0.0	0.0	0.0	0.0	0.0
E coli	130.0±50.0	60.0±30.0	50.0±22.0	40.0±.8.0	40.0±14.0	0.0	0.0
Shigella/Salmonella	110.0±50.0	50.0±30.0	30.0±2.02	10.0±5.0	0.0	0.0	0.0

Table 3

Distribution of eight microbial species isolated from Bayou Heron upon exposure to acetic acid; numbers are in CFU/ml. Data represent mean±SD.

Organisms	**0%**	**1%**	**5%**	**10% 2h**	**10% 4h**	**20% 2h**	**20% 4h**
Heterotrophic	150.0±12.0	5.00±26.0	70.0±24.0	360.0±25.0	54.0±19.0	147.0±50.0	161.0±56.0
Total Coliforms	160.0±12.0	0.0	0.0	0.0	0.0	0.0	0.0
Fecal Coliforms	160.0±12.0	0.0	0.0	0.0	0.0	0.0	0.0
Campylobacter	170.0±1.02	0.0	0.0	0.0	0.0	0.0	0.0
Vibrio	180.0±12.0	60.0±26.0	40.0±12.0	20±12	20±9	10±6	0.0
Enterococcus	160.0±12.0	0.0	0.0	0	0.0	0.0	0.0
Ecoli	150.0±12.0	40.0±26.0	30.0±.8.0	30.0±15.0	20.0±9.0	0.0	0.0
Shigella/Salmonella	140.0±12.0	40.0±26.0	20.0±4.0	0.0	0.0	0.0	0.0