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CALCIUM OXIDE 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, 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 bacteria/parasitic species including Cryptosporidium 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 Calcium oxide 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 Calcium oxide 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 suggest 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 shell fish fishing activities on the reserve.

Keywords: Calcium Oxide, anthropogenic, water and foodborne disease, natural remediation, shellfish

INTRODUCTION

The United States Centers for Disease Control and Prevention has routinely conducted waterborne disease surveillance since 1971. Incidence of waterborne disease, related to outbreaks and non-outbreaks are significantly underreported because of the generally mild associated symptoms, short duration of illnesses, and lack of patient reporting to a physician, among other factors. Waterborne illness outbreaks are not common in the U.S., but they do still occur and can lead to serious acute, chronic, or sometimes fatal health consequences, particularly in sensitive and immunocompromised populations. From 1971 to 2002, there were 764 documented waterborne outbreaks associated with drinking water, resulting in 575,457 cases of illness and 79 deaths; however, the true impact of disease is estimated to be much higher. Current protocols in municipal water treatment are effective if applied properly, but it was noted that frequent waterborne disease outbreaks occur due to inadequate, interrupted, and intermittent treatment. Contamination of water resources is not uniformly distributed, but their influences are rather affected by the number of bacteria to which humans are exposed. Contaminated water plays an important role in the transmission of bacteria to humans from humans, animals, and sewage sources leading to the ingestion of water contaminated with zoonotic agents [1-8].

Water quality is affected by a combination of natural factors (e.g. precipitation, temperature, bedrock, soil, terrain) and anthropogenic factors (e.g. agricultural practices, domestic wastewater/industrial influent). Anthropogenic and natural factors affect water quality and the changes in temporal and spatial relationship will determine improvements in water quality management efforts. From a microbiological perspective, the quality of treated water can deteriorate as a result of excessive

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bacterial growth, which can lead to problems such as a sensory deterioration of water quality (e.g. taste, odor, turbidity, discoloration) as well as pathogen proliferation [9].

Water quality parameters could include, but are not limited to BOD, COD, total phosphorus, ortho-phosphate, total Kjeldhal nitrogen, and volatile suspended solids. Heterotrophic microorganisms transform incoming organic compounds into biomass and CO_2 through metabolic processes, therefore, microbial ecologists must characterize the composition of incoming wastewater with a high precision to understand the community structure and meaningfully explore the structure-functions relationships of heterotrophs [10-19].

The goal of this research is 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 seafood safety implications. To achieve our goal, we have set three specific objectives. 1. We will access the bacteriological quality of water by evaluating variations of heterotrophic, indicator, and pathogenic 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. We will access the characteristics of water quality in terms of temperature, pH, salinity, conductivity, dissolved oxygen, total dissolved solids, nutrients, and determine their potential influence on bacterial densities in comparison to state and federal public health guidelines; and 3. We will perform water remediation/treatment using natural chemical modulators (Calcium oxide) to access effectiveness in inhibiting bacterial growth and survival in laboratory environments and within anthropogenic contaminated water sources by altering physical and chemical parameters [19-25].

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, 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). Precision Coliform water bath incubator was used to incubate 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). Corning flask, 75 cm2 Cell Culture Flask, Canted Neck, Tissue Culture Treated Nonpyrogenic, Polystyrene, Sterile. Corning Flask, 25 cm2. [38]. **Media 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 was then poured into 50 mm x 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 in the refrigerator 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

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filtration technique; APHA protocol 9215D, 9222B, 9222D and USEPA Method 1600 for testing HPC, TC, FC, Vibrio (TCBS), and ENT respectively. In these techniques, 10 to 100 mL of water samples was used to enumerate all bacteria chosen for isolation in this study. Ten milliliter of the sample was passed through a 0.45µm membrane filter that trapped bacteria on its surface [20]. Samples were treated with 0.25, 0.5 and 1% concentrations of Calcium oxide (W/V) and were incubated for 0, 2 and 4hrs. Colonies were counted, 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 consistency or differences in counts.

RESULTS

Figure 1: displays the physicochemical characteristic of water from three Bayous at the GBNERR. As can be seen, the data showed that there is consistency in the physicochemical trend within the three Bayous with some differences in DO% as well as temporal profile.

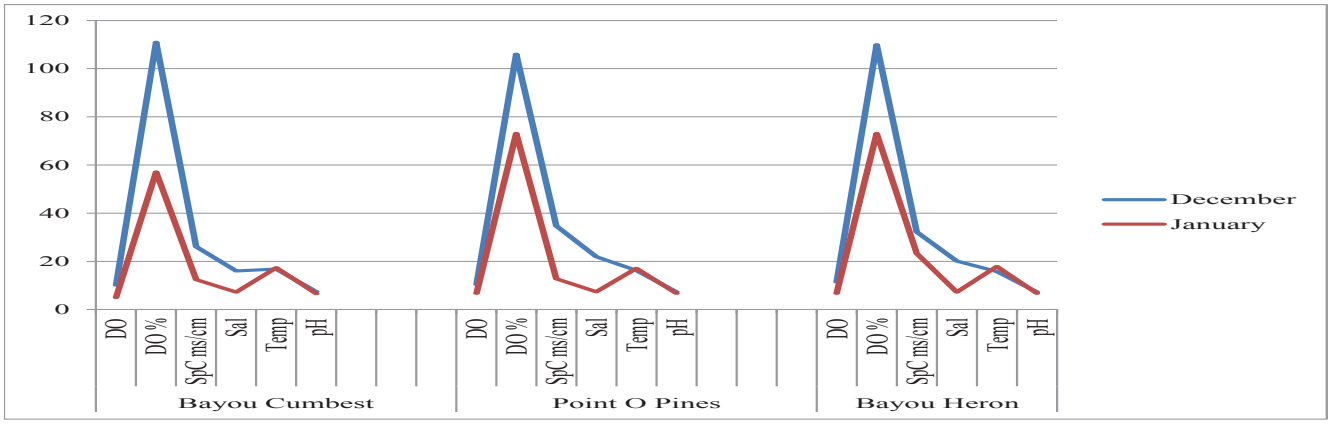

Figure 1: Comparison of Physicochemical parameters of three Bayous within the GBNERR.

Table 1: shows the survival trends of eight microbial species upon exposure to Calcium oxide in water samples from Bayou Cumbest. As can be seen all organisms grew at 0% (control). *Heterotrophic*, *Total coliforms, Fecal coliforms, Campylobacter, Enterococcus*. *Vibrio, E. coli,* and *Salmonella/Shigella* were all totally inactivated at the lowest concentration (0.25%) of Calcium oxide.

Table 1: Distribution of eight microbial species isolated from Bayou Cumbest upon exposure to Calcium oxide; numbers are in CFU/ml.

Organism	0%	Calcium oxide 0.25%	Calcium oxide 0.5%	Calcium oxide 1%
Heterotrophic	180±34.23	0.00	0.00	0.00
Total Coliforms	150±34.23	0.00	0.00	0.00
Fecal Coliforms	160±34.23	0.00	0.00	0.00
Campylobacter	210±34.23	0.00	0.00	0.00
Vibrio	150±34.23	0.00	0.00	0.00
Enterococcus	180±34.23	0.00	0.00	0.00
Ecoli	200±34.23	0.00	0.00	0.00
Shigella/Salmonella	250±34.23	0.00	0.00	0.00

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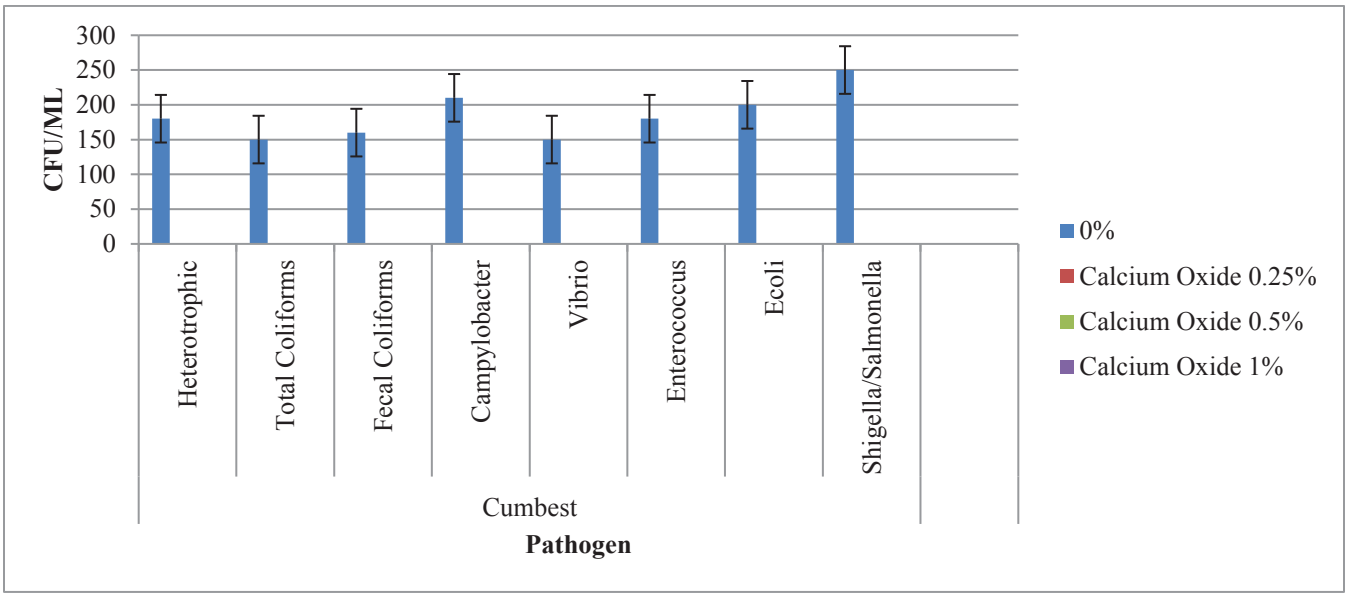

Figure 1: Microbial enumeration after Calcium Oxide exposure in samples from Bayou Cumbest. As can be seen, this figure displays the inactivation and survival trends of eight microbial species upon exposure to Calcium oxide. *Heterotrophic, Total Coliforms, Fecal coliforms, Campylobacter, Vibrio, Enterococcus,. E. coli* and *Salmonella/Shigella* were inactivated at the lowest concentration (0.25%) of Calcium oxide. No species showed resistance to the effects of Calcium oxide.

Table 2: Distribution of eight microbial species isolated from Bayou Point O Pines upon exposure to Calcium oxide; numbers are in CFU/ml.

Table 2: shows the survival trends of eight microbial species upon exposure to Calcium oxide in water samples from Bayou Point O Pines. As can be seen all organisms grew at 0% (control). *Heterotrophic, Total coliforms, Fecal coliforms, Campylobacter* and *Enterococcus*; *Salmonella/Shigella, Vibrio* and *E. coli* were completely inactivated at 0.25% Calcium oxide.

Organism	0%	Calcium oxide 0.25%	Calcium oxide 0.5%	Calcium oxide 1%
Heterotrophic	**130±33.70**	**0.00**	**0.00**	**0.00**
Total Coliforms	**110±33.70**	**0.00**	**0.00**	**0.00**
Fecal Coliforms	**160±33.70**	**0.00**	**0.00**	**0.00**
Campylobacter	**110±33.70**	**0.00**	**0.00**	**0.00**
Vibrio	**120±33.70**	**0.00**	**0.00**	**0.00**
Enterococcus	**210±33.70**	**0.00**	**0.00**	**0.00**
E. coli	**160±33.70**	**0.00**	**0.00**	**0.00**
Shigella/Salmonella	**140±33.70**	**0.00**	**0.00**	**0.00**

Table 3: Distribution of eight microbial species isolated from Bayou Heron upon exposure to Calcium Oxide; numbers are in CFU/ml.

Organism	0%	Calcium oxide 0.25%	Calcium oxide 0.5%	Calcium oxide 1%
Heterotrophic	**210±33.60**	**0.00**	**0.00**	**0.00**
Total Coliforms	**180±33.60**	**0.00**	**0.00**	**0.00**
Fecal Coliforms	**120±33.60**	**0.00**	**0.00**	**0.00**

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Campylobacter	**130±33.60**	**0.00**	**0.00**	**0.00**
Vibrio	**110±33.60**	**0.00**	**0.00**	**0.00**
Enterococcus	**160±33.60**	**0.00**	**0.00**	**0.00**
E. coli	**150±33.60**	**0.00**	**0.00**	**0.00**
Shigella/Salmonella	**130±33.60**	**0.00**	**0.00**	**0.00**

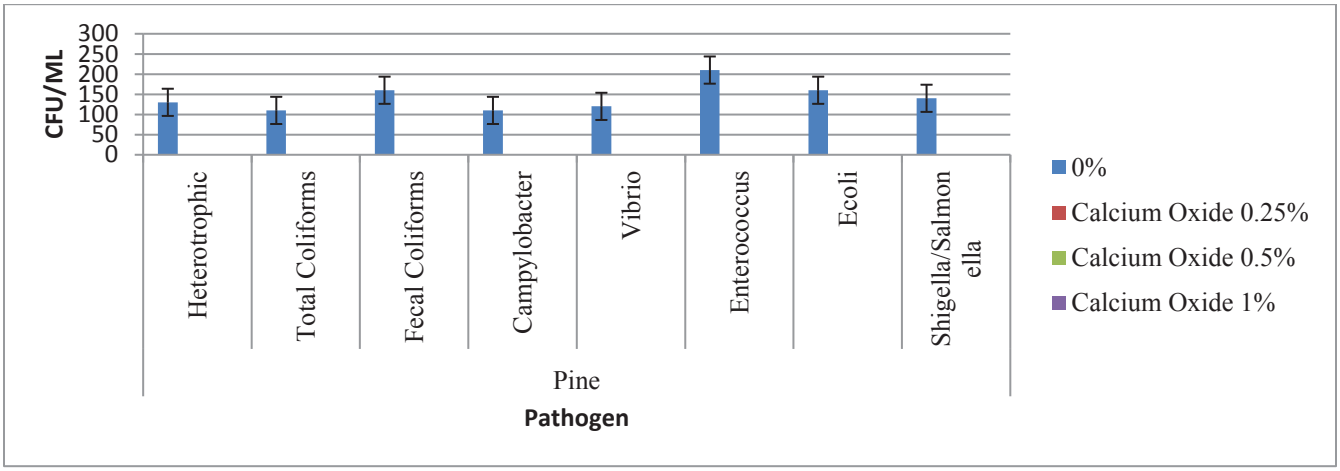

Figure 2: Microbial enumeration after Calcium oxide exposure in samples from Bayou Point of Pines**.** As can be seen, this figure displays the inactivation and survival trends of eight microbial species upon exposure to Calcium oxide. *Heterotrophic, Vibrio, E. coli, Salmonella/Shigella, Total coliforms, Fecal coliform, Campylobacter* and *Enterococcus* were totally inactivated at the lowest Calcium oxide concentration of 0.25%.

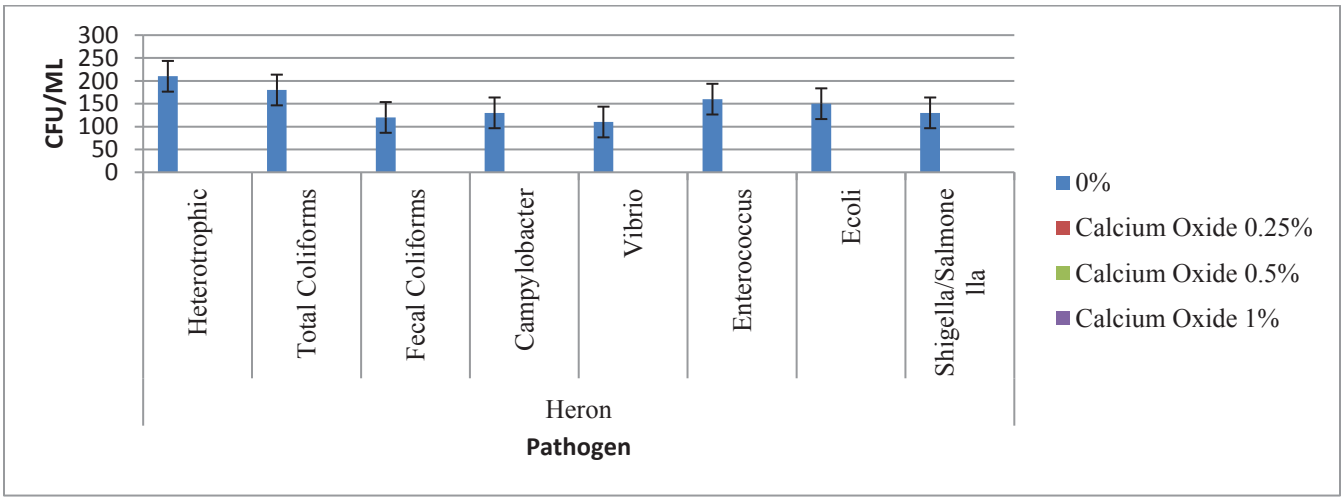

Figure 3: Distribution of eight microbial species isolated from Bayou Heron upon exposure to Calcium oxide; numbers are in CFU/ml. As can be seen, this figure displays the inactivation and survival trends of eight microbial species upon exposure to Calcium oxide. *Heterotrophic, Vibrio, E. coli, Salmonella/Shigella, Total coliforms, Fecal coliform, Campylobacter* and *Enterococcus* were completely inactivated at the lowest Calcium oxide concentration of 0.25%.

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DISCUSSION

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 calcium oxide. Our study provided evidence for the utility of Calcium oxide 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. Solid data in the literature supports our findings on the utility of Calcium oxide in reducing food and water contamination (tables 1-3 and fig 1-4).

According to the National Oceanic and Atmospheric Administration, calcium oxide crumbles with exposure to moist air. Calcium oxide reacts with water to form corrosive calcium hydroxide, with evolution of much heat. Temperatures as high as 800° C have been reached with addition of water (moisture in air or soil). The substance may react with water (some violently), releasing corrosive and/or toxic gases and runoff. Contact with metals may evolve flammable hydrogen gas. Containers may explode when heated or if contaminated with water. Calcium oxide is classified as a base and an oxidizing agent. It neutralizes acids with the generation of heat. The use of lime to reduce or eliminate pathogenic organisms resulting from anthropogenic sources represents a relatively cost effective method of treatment. Calcium hydroxide ($Ca(OH)_2$) or Calcium oxide (CaO) can be added to elevate the pH of the matrix for a defined period of time creating lime stabilization in aqueous solutions. To meet class B requirements for both the pathogen and vector attraction reduction the pH must be elevated to 12 for two hours and be maintained at 11.5 for a minimum of twenty-two hours. Lime has been historically used for the disinfection and odor suppression of solid wastes. Lime treatment reduces the number of microorganisms in discharged effluent when calcium hydroxide binds to the solid material, facilitating flocculation in sedimentation or flotation processes while the hydroxide alkalinity of the lime has an antimicrobial effect. Calcium oxide is nonflammable, but will support combustion by liberation of oxygen, especially in the presence of organic materials. This supports the observed bubbles after calcium oxide was added for treatment. It reacts very violently with liquid hydrofluoric acid and it reacts extremely violently with phosphorus pentaoxide when reaction is initiated by local heating [18-25].

Qualitative and quantitative research has demonstrated the bactericidal properties of lime through inactivation of *Escherichia coli* and *Salmonella* and *Staphylococcus*. Previous research to evaluate the persistence of enteric virus under lime stabilization conditions has demonstrated that a high pH is effective at reducing or eliminating Poliovirus type 1 from sludge, however, there is little information to indicate the removal and inactivation of other human pathogenic viruses, such as rotavirus and adenovirus, which were not cultivatable when original liming studies were conducted. Installation of a lime stabilization treatment system is relatively rapid, with minimal capital costs, compared to alternative treatment technologies, and generates a relatively safe and sustainable product. Conductimetric assay measures the change in electrical conductivity of the growth medium caused by bacterial metabolism. Studies conducted by Sawai [20] concluded that CaO was most effective against *Staphylococcus aureus* followed by metallic oxides, MgO and ZnO. The parameters provide some useful indicators for antimicrobial agents, such as the dependence of antibacterial activity on agent concentration, and the affinity between the agent and the bacterial cells [26-32].

In regards to the bactericidal effects of CaO (*scallop-shell powder*) on foodborne pathogenic bacteria, CaO is a substitute for synthetic chemical substances used as a disinfectant and sanitization agent of foods and food processing equipment. In this study, Calcium oxide treatment was observed against three common foodborne pathogenic bacteria: Escherichia coli, Listeria monocytogenes, and Salmonella typhimurium. Solutions at concentrations of 0.01 and 0.03% did not greatly affect bacterial survival; however, concentrations of 0.05% CaO solution for 10 min were greatest. As in our study, the bactericidal action of CaO was maintained for at least 24 h of storage to observe maximum toxicity. Studies conducted by [22] revealed that, after 40-min treatment with either Ca^{2+} or Mg^{2+}, *S. aureus* cells exhibit viability loss to varying extent in a dose-dependent manner, with a maximal viability loss of ~60% observed at Mg^{2+} concentration of 40 mM, the highest dose tested. It is noteworthy that a relative loss of 60% in viability ratio corresponds to an absolute number density of $\sim 3 \times 10^5$ CFU/mL (colony-forming units per milliliter) in bacterial cells killed. The two alkaline-earth-metal ions, Ca^{2+} and Mg^{2+}, are physiologically essential for diverse living organisms, both disrupt model *S. aureus* membranes and kill stationary-phase *S. aureus* cells, indicative of membrane-activity [21,22, 33-39]. These studies offer solid support to our objectives and findings in this study.

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CONCLUSION

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 Calcium oxide. Our study provided evidence for the utility of Calcium oxide in the remediation of anthropogenic contamination of three Bayous at the GBNERR. Our findings showed total inactivation of eight pathogenic microbial species that constitute public health threat to the fishing activities at the GBNERR with special reference to shellfish production. The data also indicates significant impact of Calcium oxide treatment in the remediation of post contamination by anthropogenic pathogens from the GBNERR Bayous Heron, Cumbest and Pine-O-Pine when compared to control findings. The interaction of physicochemical and microbiological parameters of water through external chemical manipulation by Calcium oxide 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 suggest 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.

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REFERENCES

1. H. Wang, M. Wu, Y Deng, Y. C. Tang, & R. Yang. 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.
2. SS. Kishinhi, PB. Tchounwou, & IO. Farah. 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.
3. World Health Organization (WHO). Water Sanitation Hygiene. Retrieved from http://www.who.int/water_sanitation_health/diseases-risks/en/. 2014.
4. R. Bain, R., Cronk, J. Wright, H., Yang, T. Slaymaker, & J. Bartram. Fecal contamination of drinking-water in low-and middle-income countries: a systematic review and meta-analysis. PLoS Med, 11(5), e1001644. 2014.
5. EI. Prest, F., Hammes, MC. van Loosdrecht, M. C., & JS. Vrouwenvelder. Biological Stability of Drinking Water: Controlling Factors, Methods, and Challenges. Frontiers in microbiology, 7. 2016.
6. TF. Clasen, IG. Roberts, T. Rabie, WP. Schmidt & S. Cairncross. Interventions to improve water quality for preventing diarrhoea. The Cochrane Library. 2006.
7. Momtaz, Hassan, Farhad Safarpoor Dehkordi, Ebrahim Rahimi, and Amin Asgarifar. "Detection of Escherichia coli, Salmonella species, and *Vibrio cholerae* in tap water and bottled drinking water in Isfahan, Iran." *BMC public health* 13, no. 1 (2013): 556.
8. Lee, Sherline H., Deborah A. Levy, Gunther F. Craun, Michael J. Beach, and Rebecca L. Calderon. "Surveillance for waterborne-disease outbreaks--United States, 1999-2000."*Morbidity and mortality weekly report. Surveillance summaries (Washington, DC: 2002)* 51, no. 8 (2002): 1-47.
9. Nescerecka, Alina, Janis Rubulis, Marius Vital, Talis Juhna, and Frederik Hammes. "Biological instability in a chlorinated drinking water distribution network." *PloS one* 9, no. 5 (2014): e96354.
10. Isazadeh, Siavash, Shameem Jauffur, and Dominic Frigon. "Bacterial community assembly in activated sludge: mapping beta diversity across environmental variables." MicrobiologyOpen 5, no. 6 (2016): 1050-1060.
11. SB. Weisberg, RT. Noble, DF. Moore, CD., McGee, & MK. Leecaster. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing, Pergamon Water Research; 37: 1637-1643. 2003.
12. Environmental Protection Agency. Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm In Source Water and Drinking Water. Retrieved from http://www.epa.gov/.../m_415_3Rev1_1.pdf 25 March 2015
13. Environmental Protection Agency. Dissolved Oxygen and Biochemical Oxygen Demand. Retrieved from http://water.epa.gov/type/rsl/monitoring/vms52.cfm 25 March 2015
14. Environmental Protection Agency. National Aquatic Resource Surveys. Indicators: Conductivity. Retrieved from https://www.epa.gov/national-aquatic-resource-surveys/indicators-conductivity 1 March 2017
15. Environmental Protection Agency. National Aquatic Resource Surveys. Indicators: Salinity. Retrieved from https://www.epa.gov/national-aquatic-resource-surveys/indicators-salinity 2 March 2017

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16. Environmental Protection Agency. Temperature. Retrieved from https://www.epa.gov/sites/production/files/2015-09/documents/temperature.pdf 1 March 2017
17. Environmental Protection Agency. Total Solids. Retrieved from http://water.epa.gov/type/rsl/monitoring/vms58.cfm 25 March 2015.
18. Hansen, Jacqueline J., Paul S. Warden, and Aaron B. Margolin. "Inactivation of adenovirus type 5, rotavirus WA and male specific coliphage (MS2) in biosolids by lime stabilization." *International journal of environmental research and public health* 4, no. 1 (2007): 61-67.
19. NOAA; National Oceanic and Atmospheric Administration. Cameo Chemicals: Calcium Oxide. https://cameochemicals.noaa.gov/chemical/311, 2003.
20. Sawai, J. "Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay." *Journal of microbiological methods* 54, no. 2 (2003): 177-182.
21. Bae, Dong-Ho, Ji-Hye Yeon, Shin-Young Park, Dong-Ha Lee, and Sang-Do Ha. "Bactericidal effects of CaO (scallop-shell powder) on foodborne pathogenic bacteria." *Archives of pharmacal research* 29, no. 4 (2006): 298-301.
22. Xie, Yuntao, and Lihua Yang. "Calcium and magnesium ions are membrane-active against stationary-phase staphylococcus aureus with high specificity." *Scientific reports*6 (2016): 20628.
23. R.K. Taylor, S. Almagro-Moreno. Cholera: Environmental Reservoirs and Impact on Disease Transmission, Microbiology Sepctr, 2013 December;1(2): 0003-2013.
24. D.L. Swerdlow, BE. Mahon, M. Iwamoto, T. Ayers. Epidemiology of Seafood-Associated Infections in the United States, Clinical Microbiology Reviews; 23(2): 399-411. 2010.
25. P. Reganold John, & D. Chiras, Daniel. Natural Resource Conservation: New International Edition: Management for a Sustainable Future. Tenth Ed. South Asia: Pearson Education Limited. 2013.
26. W. Reno, R. Rao, A. Panja, D. Obenhuber, M. Koelle, HH.. Cohly, MF. Angel. 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.
27. CK. Robb. Assessing the impact of human activities on British Columbia's estuaries. PloS one, 9(6), e99578.2014.
28. L. Palamuleni, M. Akoth. 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.
29. L. Putignani & D. Menichella. Global distribution, public health and clinical impact of the protozoan pathogen cryptosporidium. Interdisciplinary perspectives on infectious diseases, 2010.
30. M. Iwamoto, T. Ayers, BE. Mahon & DL. Swerdlow. Epidemiology of seafood-associated infections in the United States. Clinical microbiology reviews, 23(2), 399-411. 2010.
31. B. Mull, & VR. Hill. Recovery of diverse microbes in high turbidity surface water samples using dead-end ultrafiltration, J Microbiology Methods; 91(3): 429-33. 2012.
32. ED. Berry & CN. Cutter. 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.
33. M. Bouzid, PR. Hunter, R.M. Chalmers & KM. Tyler. Cryptosporidium pathogenicity and virulence. Clinical microbiology reviews, 26(1), 115-134. 2013.
34. JPS. Cabral. Water Microbiology. Bacterial Pathogens and Water, Int J Environ Res Public Health 2010 Oct;7(10): 3657-3703. 2010.
35. B. Chapman & T. Ross. 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.
36. L. Delpla, DT. Monteith, C. Freeman, J. Haftka, J., Hermens, TG.., Jones, T. G., & O. Thomas. 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.
37. JC. Fisher, AM. Eren, HC. Green, OC. Shanks, HG. Morrison, JH. Vineis & SL. McLellan. 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.
38. A. Goudie. The Human Impact on the Natural Environment. 4th Edition. The MIT Press. ISBN 0-262-07156-8. 1994.
39. Hardy Diagnostics. Instructions for Use. Campylobacter Blood-Free Agar. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CampyBldFreeAgar.htm. 2016.