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Bacteriological Investigation of Pond Water Quality from Ogoniland, Nigeria

Douglas, Salome I.*. And Isor, Faith N.
Department of Applied & Environmental Biology, Rivers State University of Science & Technology, P. M. B. 5080, Port Harcourt Rivers State Nigeria.

Abstract: *A bacteriological study of three ponds in Uegwere Bo-ue community, Khana Local Government Area, of Rivers State, Nigeria were investigated to evaluate the water quality of the ponds. Surface water samples were collected monthly for eight months covering both seasons and evaluated. Bacteriological analyses carried out includes: total heterotrophic bacteria counts(THBC), Total coliform counts(TCC), feacal coliform counts(FCC) and other water borne pathogens. The results of this study reveal high THBC, high TCC and FCC(Escherichia coli). The THBC which ranged from 1.7 x 10^5 to 6.5 X 10^7 CFU/ml for pond A, Pond B 1.3 X 10^5 to 2.5 X 10^7 CFU/ml while pond C had 2.2 X 10^4 to 4.0 X 10^6CFU/ml. Total coliform bacteria for pond A ranged between 120 and 1560MPN per 100ml of sample , 87 and 900MPN per100ml for pond B and pond C ranged 702 to 1200MPN per100ml. Faecal coliform ranged between 350 to 662MPN, 124 to 420MPN 120 to 280MPN per 100ml. Bacteria of public health importance like E. coli, Salmonella sp and Shigella sp were also detected, which suggests possible faecal contamination. Higher bacterial loads were observed in the rainy than the dry seasons. The frequency of occurrence is as follows: E. coli 17%, Salmonella 14.5%, Streptococcus 4.4%, Bacillus 9.4%, Pseudomonas 8.7%, Micrococcus 5.8%, Shigella 7. 3%, Enterobacter 11%, Klebsiella 5.8%, Proteus 3% and Staphylococcus 13%. With pond A having the highest bacterial population, while pond C had the least both in the rainy and dry seasons. Statistically, there are no significant differences ($P<0.05$) between all studied locations. From the results of this investigation, there's need to monitor the water quality from time to time to detect the actual source of contamination and also to pass the water through a form of treatment to prevent epidemic outbreak, since the values obtained are far above the WHO and SON(Standard Organisation of Nigeria) guidelines for water intended for domestic use. There's need for pre- treatment before use for domestic purposes.*

Keywords: *pondwater, coliform, contamination, indicator organisms.*

I. Introduction

Water used for drinking and other domestic purposes in most communities are obtained from natural sources such, as rivers, streams, lakes, ponds and springs, or artificial sources such as wells and boreholes. Most times these water supplies, particularly, streams, rivers, lakes, ponds and wells are likely to be polluted with wastes. And the microorganisms in these natural sources, are numerous in both numbers and diversity[1]. Microbial populations of surface waters are composed of indigenous and transient populations, since surface water are open to contamination from various sources.

A pond is referred to as a man-made or natural water body which is between1m^2 and 20,000m^2 in area (2 ha or -5 acres) in area, which holds water for at least four months of the year or all year around depending on geographic locations[2]. Is also a body of standing water, either natural or manmade that is usually smaller than a lake.They may arise naturally in floodplains as part of a river system, or they may somewhat be isolated depressions. The type of life in pond is generally determined by a combination of factors including water level regime(particularly depth and duration of flooding) and nutrient levels, but other factors may also be important, including presence or absence of shading by trees, presence or absence of streams, effects of grazing animals, and salinity[3]. Pond waters are also facing pollution just like other water bodies are getting polluted due to discharge of effluents from various industries, domestic waste, land and agricultural drainage resulting in the degradation of water quality of these water resources [4].

In human health, water plays a very important role and quality of the water supplied is important in determining the health of individuals and the whole communities. Safe water quality is a major concern with reference to public health importance as health and well being of the human race is closely tied up with the quality of water used [4]. Recently, epidemics of cholera have been reported from different parts of the World, including India, Nigeria (Anambra State) and Zimbabwe. The outbreak was caused by Vibrio cholera 01 isolated from municipal taps and wells [5]. Outbreaks of typhoid fevers and dysentery were linked to unsanitary mixing of some water supplies and sewage. WHO [6] has also reported that, 80% of sicknesses and deaths among children in the world are caused by unsafe drinking water. On the average, every 8 seconds in the world, a child dies of water related diseases (from contaminated water).

Bacteriological monitoring is based on knowledge of the sanitary condition of the water supply, which is based on the detection of coliform bacteria and the specific indicator of human faecal contamination, Escherichia coli [7]. The term “indicator organisms” are used in water analysis, which refers to micro-organisms whose presence in water shows that the water is polluted with faecal material from humans or other warm-blooded animals [8]. This kind of pollution means that the opportunity exists for the various pathogenic organisms, which periodically occur in intestinal tract, to enter the water, such water is described as non-potable water, and it is not safe for drinking. The use of intestinal organisms as indicators of faecal contamination is a universally acceptable process for monitoring and assessing the microbiological safety of water supply before distribution. Coliform bacteria are a group of intestinal bacteria used as indicators to determine if treated water is acceptable for human consumption. Coliforms will not likely cause illnesses. However, the presence of coliforms in drinking indicates the presence of disease-causing organisms [9]. The Coliform includes the members of the family Enterobacteriaceae, e.g. Escherichia coli, Enterobacter aerogenes, Salmonella and Klebsiella. This present study is aimed to evaluate the water quality by analyzing the viable coliforms along with other water borne bacteria pathogens of the three community ponds which are used for human and animals, bathing, washing of clothes and also for drinking. The data of this study may provide some important information about public health risks associated with the use of pond water in the area.

Khana, Uegwere Boue Community is densely populated and due to electricity problems the bore hole built by the government is not always in use due to power failure. This makes most people especially, farmers(farming is the major occupation of the people from the area) now depend on water from the pond for their daily domestic and agricultural needs. This therefore, increases the number of people exposed to water borne pathogens, if the pond water is polluted.

II. Materials And Methods

Study area and sample collection procedure

Freshwater samples were collected from three ponds namely, Kpoku pond, Gbenekiri, and Napaa pond that serves as a source of drinking water in Uegwere Bo-ue community in Khana Local Government Area of Rivers State. The three ponds were designated pond A, pond B, and pond C respectively. Water samples were collected once monthly for eight months covering both dry (Dec. 2013, Jan, Feb. and March, 2014) and rainy(May, Jun, Jul and Aug. 2014) seasons, aseptically from the surface to about a depth of 20cm using sterile bottles. From each pond, three composite samples were collected within a 3m radius and pooled together. Samples were obtained at the areas of the ponds from where the local inhabitants usually fetched their water. This meant that those were the points where humans made direct contact with the water sources. Appropriately labelled and transported to the laboratory immediately for investigation.

Pond water sample from Kpoku pond was labelled sample A, with coordinates of N 04^0 38.202, E $007^0$21.329, Pond water sample from Gbenekiri pond was labelled sample B, with coordinates N 04^0 38.366, E 007^0 21.410, while pond water sample from Napaa pond was labelled sample C, and with coordinates: N 040 38.578, E 007^0 21.325.

The community has a population of about 4,000 people. All ponds that were understudied are privately owned by individual families and are open to the general public. Fetching water from these ponds is done by the use of plastic containers, basins and calabash. The pond, especially Gbenekiri pond is the oldest and over a hundred years followed by Kpoku and Napaa pond, it was dug by ancestral fathers who first lived in the community, these ponds are sometimes emptied during the dry season when there is low rainfall and the water level becomes low.

Isolation of total heterotrophic bacteria

This was done using a tenfold serial dilution(as described by[11] of the pond water using physiological saline up to 10^{-5}, then 0.1ml aliquot of each dilution on nutrient agar using the spread plate method. The plates were incubated in an inverted position for 24hours at 37^0C. Colonies that emerged were counted and colony forming unit per ml calculated. Colonies were subcultured to obtain a pure cultures which were stored in bottles for further test.

Enumeration of total coliforms/faecal coliforms

The multiple tube fermentation technique also called the Most Probable Number (MPN) was used to estimate the total coliforms and faecal coliform. Three sets of test tubes containing lactose broth and the right sample volumes were used, tubes showing acid and gas production indicates positive for the organisms, the number of organisms present was determined statistically using the MPN table. This technique consists of three major steps, the presumptive, confirmatory and completed tests. The presence of faecal coliforms was further characterised by streaking positive tubes from the previous procedures, on EMBA plates. All distinct colony types were transferred from EMBA to Trypticase soy agar (TSA) plates, colonies from this plates were Gram stained and oxidase test carried out (12). Total coliform and thermotolerant (fecal) coliform (FC) indicator tests are common public health tests of the safety of water and wastewater which might be contaminated with sewage or feacal material (13).

Isolation of Salmonella/Shigella

This was done using the Salmonella/ Shigella agar which was prepared according to manufacturer's instruction, then 0.1ml aliquot of the sample was spread on the surface of the SSA plates which were then incubated for 24 to 48hours at 37^0C. After incubation, the colonies were subcultured on fresh SSA plates to obtain pure cultures used for further identification.

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Identification and Characterization of the isolates

This was done based on the cultural, morphological and biochemical characteristics of the various isolates obtained were compared with the criteria in Bergey's manual of Determinative Bacteriology (14). The biochemical tests carried out for the identification and characterization of the isolates include: gram staining, motility, indole production, methyl red-voges Proskauer, citrate utilization, oxidase, catalase, coagulase and sugar fermentation tests.

Statistical Analysis

The one way Analysis of variance was used to analyse the result, to compare the values of Total heterotrophic bacterial (THB), total coliform and faecal coliform count between ponds. From the results of the statistical analysis, if the p value is <0.05, the test is not significant but if p value is >0.05, then there is a significant different between the variables compared. In determining seasonal variation, the mean data for all the months of both seasons at the sampling locations were calculated, which is used to draw a bar chart of bacterial load versus sampling location.

III. Results

The total heterotrophic bacteria counts ranged from 1.7×10^5 to 6.5×10^7 CFU/ml for pond A, Pond B 1.3×10^5 to 2.5×10^7 CFU/ml while pond C had 2.2×10^4 to 4.0×10^6CFU/ml. It was observed that the bacterial counts were higher in the rainy season than the dry season. Total coliform bacteria for pond A ranged between 120 and 1560MPN per 100ml of sample , 87 and 900MPN per100ml for pond B and pond C ranged 202 to 1200MPN per100ml. Faecal coliform ranged between 350 to 662MPN, 124 to 420MPN 120 to 280MPN per 100ml. A total of eleven bacteria genera were isolated from the three ponds during the period of study, which includes; *Salmonella*, *Escherichia*, *Streptococcus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Shigella*, *Enterobacter*, *Klebsiella*, *Proteus* and *Staphylococcus*. Of these isolates *E. Coli* had the highest frequency of occurrence of 17%, while *Proteus* sp had the least of 3%. Table 1, displays the frequency of occurrence. A total of eleven bacteria genera were isolated from the three ponds with the following percentage of occurrence; *E. coli* 17%, *Salmonella* sp 15%, *Streptococcus* sp 4%, *Bacillus* sp 9%, *Pseudomonas* sp 9%, *Micrococcus* sp 6%, *Shigella* sp 7%, *Enterobacter* sp 11%, *Klebsiella* sp 6%, *Proteus* sp 3% and *Staphylococcus* sp 13%.

Table 1: Genera of Bacteria isolated and their Percentage Occurrence

Isolates	Occurrence(n=138)	Frequency of Occurrence(%)
Escherichia	24	17
Salmonella	20	14.5
Staphylococcus	18	13
Enterobacter	15	10.8
Bacillus	13	9.4
Pseudomonas	12	8.7
Shigella	10	7.3
Micrococcus	8	5.8
Klebsiella	8	5.8
Streptococcus	6	4.4
Proteus	4	3

IV. Discussion

Under normal circumstances, water intended for human consumption should not contain chemicals or any microorganism known to be pathogenic or any bacteria whose presence indicates faecal pollution. Water can be perfectly clear, odourless and tasteless and yet unsafe for drinking (15, 16, 17). Based on the WHO and SON guidelines, the pond water is not fit for human consumption. In the same way, water used for washing and bathing should not contain more than 50 coliform bacteria per 100ml of water (18). The results of this investigation revealed high THBC and coliforms counts. However there were variations with locations, which were found not to be significant. Being a natural water body, high bacterial load were observed in all the ponds under study. The THBC test has little value as an index of pathogen presence but can be useful in operational monitoring as a treatment and disinfectant indicator, where the objective is to keep numbers as low as possible. In addition, THBC measurement can be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms(19). Based on the guidelines of US EPA, water samples containing coliform should be considered unacceptable for drinking water as they are regarded as the main indicators of water pollution. The WHO standards for total and faecal coliforms are 1 to 10/100 ml and 0/100 ml, respectively (20, 21, 22, 23). The results in Figures 1- 3 revealed that all the water samples from the ponds had very high counts of total heterotrophic bacteria counts, total and faecal coliforms. These high bacterial load which was observed to be higher in the rainy than the dry season may be as a result of surface runoffs from land during the rainy season, that washes off animal excreta, improperly disposed sewage and disposal of other domestic waste materials, likely closeness of pit latrines and other agricultural waste into the ponds. The higher bioload is probably supported by increased nutrient load as a result of flood and runoffs into the ponds. [24], also observed a similar trend in their studies involving streams where they observed higher bioload in the rainy season.

In the work of (18) it was reported that, the presence of bushes, shrubs or plants makes it likely that smaller mammals may have been coming around these water bodies to drink water, thereby passing out faeces into the water. In addition to, human activities like washing of clothes and bathing near these sites, has exposed it to more pollutant sources. E. coli is the most widely adopted indicator of faecal pollution. E.coli has frequently been reported to be the causative agent of traveller's diarrhoea, urinary tract infection, hemorrhagic colitis, and haemolytic uraemic syndrome (18). Streptococcus faecalis with E. coli are good indicators of gastrointestinal diseases. The presence of such bacteria indicates the possible presence of faecal material (17, 5). Pseudomonas aeruginosa has been isolated in pond water samples. Pseudomonas are opportunistic pathogens and can, in rare circumstances, cause community acquired pneumonias as well as ventilator associated pneumonias, being one of the most common agents isolated in several studies (18). Karfistan and Arik-Colagolu (25) enter Pseudomonas bacteria as indicators of microbiological water quality during their study on Manyas lake. Staphylococcus aureus regarded as important indicators of the whole aquatic ecosystem health, including fish, and birds via the food web and mostly released by human contact(18). Most of these isolated bacteria species from pond water have also been isolated in different water bodies in other studies (25, 5).

A total of eleven bacteria genera were isolated from the three ponds with the following percentage of occurrence; *E. coli* 17%, *Salmonella* 15%, *Streptococcus* 4%, *Bacillus* 9%, *Pseudomonas* 9%, *Micrococcus* 6%, *Shigella* 7%, *Enterobacter* 11%, *Klebsiella* 6%, *Proteus* 3% and *Staphylococcus* 13%. These results were in agreement with the work of Borah *et al;* 2010 who reported that 78% of the ponds they worked with had E. coli contamination and the THBC ranged from 10^4 to 10^5 per ml of water sample. From this study the presence of high numbers of *Salmonella* and *Shigella* (whose ingestion of as low as 10 – 100 organisms can lead to infection). Less than the infective dose of most enteric bacteria pathogen (17), These Isolates with high frequency of occurrence are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, typhoid fever, dysentery, cholera, urinary tract infection, etc(5)

According to WHO guidelines (26), the occurrence of pathogens or indicator organisms in ground or surface water mainly depends on the range of human activities and animal sources that release pathogens to the environment. Nonetheless, the inadequate availability of water, ill maintenance of pond water, unsafe disposing of human, animal and household wastes, unawareness about good sanitation and personal hygienic practices etc. are some key factors responsible for poor drinking water quality in rural communities. (2)

Statistically, there was significant differences between the seasons monitored at $P<0.05$, while among the parameters analysed there was no significant difference.

V. Conclusion

From the results of this investigation, there's need to monitor the water quality from time to time to detect the actual source of contamination and also to pass the water through a form of treatment to prevent epidemic outbreak, since the values obtained are far above the WHO and SON guidelines for water intended for domestic use. There's need for pre- treatment before use for domestic purposes.

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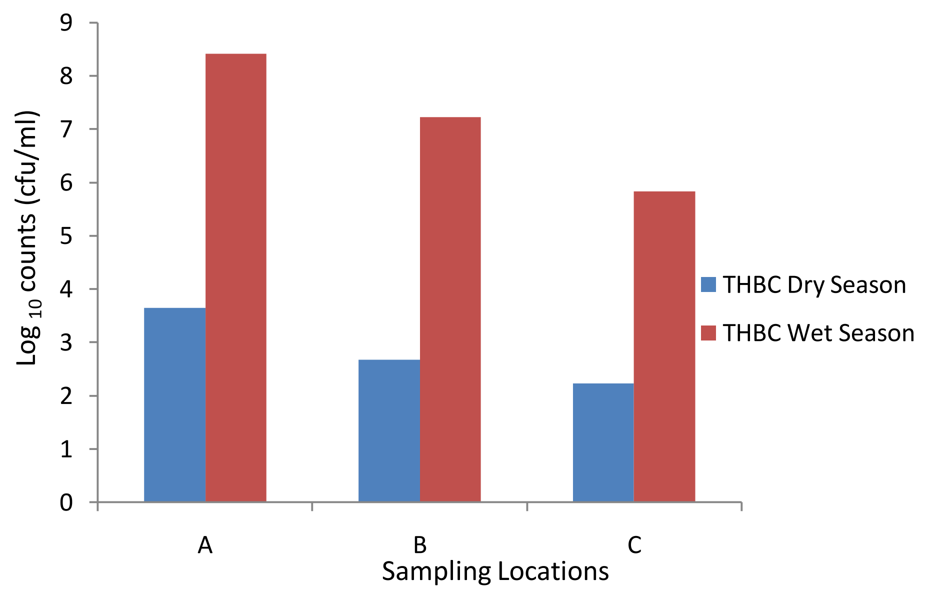

Fig. 1: Seasonal Changes in the level of THBC

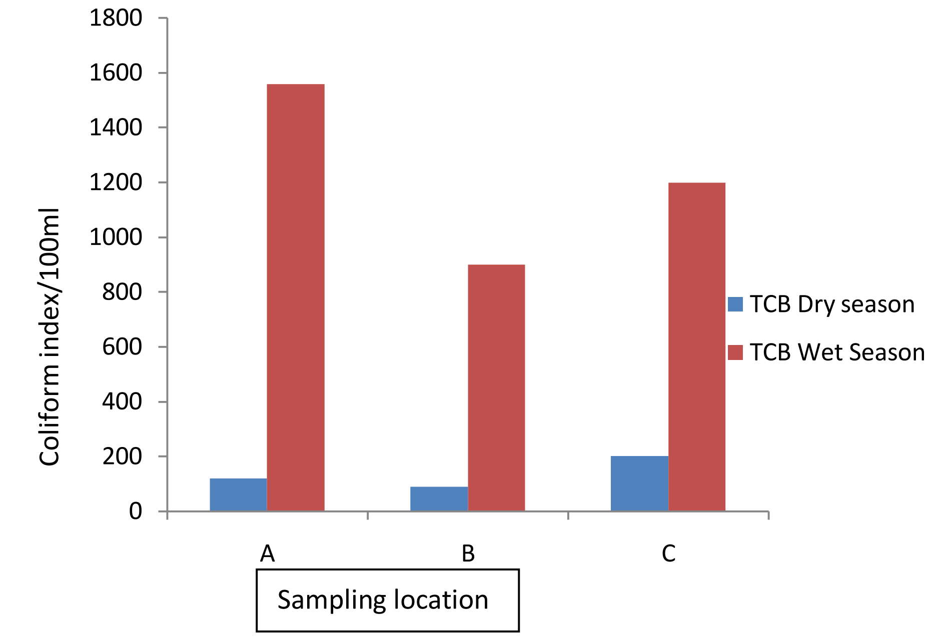

Fig. 2: Seasonal changes in the level of TCB

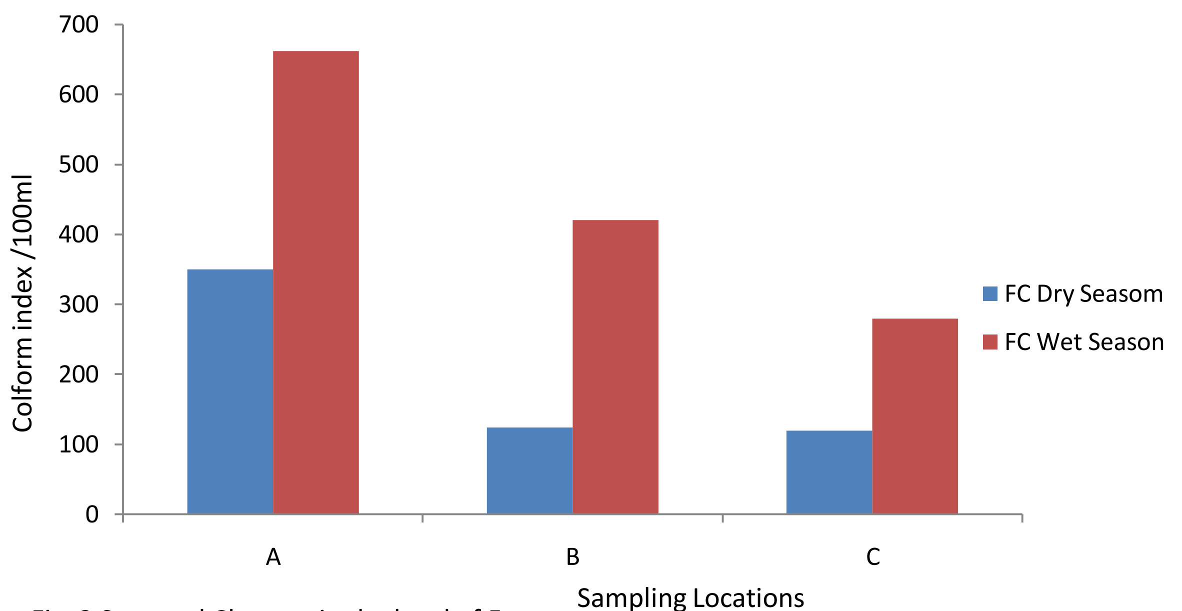

Fig. 3 Seasonal Changes in the level of Fc