CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUME 2, NUMBER 2, 2015, PP. 246-258

Microbiology of Underground Water (Dug Wells) in Abua Central Area of Rivers State in Nigeria

OMOKARO OBIRE & ABIGIRL ALALI
Rivers State University of Science and Technology, Nigeria

ABSTRACT An investigation was carried out on drinking well water samples from four communities to determine the water quality. The microbiological and physiochemical properties were determined using standard plate count, most probable number (MPN) technique and standard analytical techniques. The total heterotrophic bacteria count ranged from 1.70×10^6cfuml^{-1} to 1.96×10^7cfuml^{-1}, faecal coliform bacteria ranged from 9 to 1600 MPN/100ml while total fungal count ranged from 4.0×10^5sfuml^{-1} to 8.0×10^6sfuml^{-1}. Analysis of variance (ANOVA) on the counts of total heterotrophic bacteria showed that, there is significant difference between the well waters at $p= 0.05$ while the reverse is the case for counts of fungi. Generally, the bacteria isolates and frequency from well water in Abua Central communities were *Acinetobacter sp.* (10%), *Bacillus sp.* (10%), *Chromobacter sp.* (13.3%), *Corynebacterium sp.* (3.3%), *Enterobacter sp.* (6.7%), *E. coli* (6.7%), *Proteus sp.* (3.3%), *Salmonella sp* (13.3%), *Shigella sp.* (10%), *Staphyloccoccus sp*. (6.7%) and *Streptococcus sp.* (16.7%). All the potential pathogenic bacteria except *Proteus* were isolated from Emilaghan and all but *Proteus*, *Staphyloccoccus* and *Streptococcus* were isolated from Amalem. *Salmonella* species occurred in well water of all the communities. The fungal isolates were *Aspergillus sp.* (25%), *Chrysosporium sp*. (8.3%), *Fusarium sp.* (12.5%), *Microsporium sp.* (8.3%), *Mucor* (8.3%), *Penicillum sp* (16.6%), *Rhizopus sp.* (12.5%) and *Saccharomyces cerevisiae* (12.5%). The temperature of the well waters ranged from 27.3 to 29°C, pH from 6.7 to 7.1, salinity from 42.9 to 86.14mg/l, Biochemical Oxygen Demand (BOD) from 0.63 to 23.3mg/l, Dissolved Oxygen from 4.1 to 6.3mg/l, Electrical conductivity ranged from 44 to 133μs/cm, total hardness from 12.0 to 12.2mg/l, Total Dissolved Solids from 80.7 to 162mg/l, Total Suspended Solid within the range 20mg/l, Total Hydrocarbon; nil, Potassium; nil, Chemical Oxygen Demand (COD) 3.2 to 27mg/l and Nitrate ranged from 0.43 to 3.51mg/l. The high levels of BOD and Total dissolved solids of the well water especially in Omokwa indicated that the drinking water is highly polluted. The presence of bacteria and fungi especially enteric organisms such as *E. coli*, *Enterobac-*

ter and *Salmonella* are indication of faecal contamination of the water which is a serious public health concern. This suggests that the well waters of these communities are not suitable for human consumption.

Keywords: Well water, faecal coliform, *E. coli*, *Salmonella,* fungi, BOD, nitrate

Introduction

Water is vital to our existence in life and its importance in our daily life makes it imperative that it should have the quality characteristics of its intended use. The public health significance of water quality cannot be over emphasized. Water supplies used for human consumption (potable water) must be free from organisms and from concentration of chemical substances that may be hazardous to health (Castro, 1998). Also supplies of drinking water should be pleasant to drink as circumstances permit, it should be free of colour, taste and odor which are important for public water sources used for drinking (WHO, 1971). However, according to the World health organization (WHO, 2004), 1.1 billion people did not have access to an improved water supply in 2002, and 2.3 billion people suffered from diseases caused by contaminated water. Each year 1.8 million people die from diarrheal diseases, and 90% of these deaths are of children under 5 (WHO, 2004). Besides causing death, water-related diseases also prevent people from working and leading active lives. Rain water percolating into ground constitutes ground water. Ground water is the cheapest and most practical means of providing water to small communities. Ground water is likely to be less subject to contamination than surface water and free from pathogenic agents. It usually requires no treatment and the supply is likely to be certain even during dry season. Dug water wells are open bodies of ground water, normally 2 to 3 feet in diameter. Traditionally, hand dug wells are important source of water supply. In many communities and most rural areas wells are routinely dug and used today. Therefore, hand dug well provides a cheap and low-tech solution to accessing ground water in rural locations from developing countries and may be built with high degree of community participation or by local entrepreneurs who specialize in hand dug wells (Driscoli, 1986).

Human activities create vast amount of various wastes and pollutants, which are released into the environment sometimes causing serious health problems (Obire and Aguda, 2002). A major impact of human activities on surface water is pollution by chemicals and microbes through wastes of different nature (Castro, 1998). The most common and widespread danger associated with drinking water is contamination, either directly or indirectly by sewage or other wastes of human or animal origin (Khupe *et al.,* 1996).

Faecal pollution of drinking water may introduce various forms of intestinal pathogens which may cause mild diseases like mild gastroenteritis to severe and sometimes fatal dysentery diarrhea, cholera, typhoid and hepatitis A. Other organisms may occur naturally as opportunist, but might become pathogenic due to increased attacks. There are several ways a well can be contaminated. Toxic mineral spilled or dumped near a well can leach into the aquifer and contaminate the ground water drown from a well. Polluted water leak through the walls of poorly maintained or shoddily constructed wells. Well can get contaminated from septic tanks placed too close or abandoned wells in the area. Flood events can also impact the quality of well water.

In Nigeria, majority of the rural populace do not have access to potable water and therefore, depend on well, stream and river water for domestic use. The bacteria qualities of groundwater, pipe-borne water and other natural water supplies in Nigeria have been reported to be unsatisfactory, with coliform counts far exceeding the level recommendation by WHO (Edema *et al*., 2001, Obire *et al*., 2010). Abua is a local government in Rivers State and comprises of ten (10) communities in Abua Central in particular and each of these communities has a population of over 20,000 people who had depended and are still depending on the well water supply for drinking, domestic and recreational uses and are situated in every compound in the community. Many infectious diseases are transmitted by water through the faecal-oral route. Following the episode of water-borne diseases, and the likelihood that some recurrent illnesses in the Abua Central area of Rivers State could be associated with water it became necessary to conduct a study for the assessment of the suitability of well waters which are the source of drinking water of the communities.

The aims or objectives of this study therefore, were to examine dug well water by enumerating the population of microorganisms (bacteria and fungi) of well water in the communities, to identify fungal and bacteria isolates from the wells, to carry out bacteriological analysis of these sources of drinking water for "indicator organisms" (that mainly indicate the possibilities of contamination of the water body by pathogenic agent) as to verify their quality and suitability for consumption, to evaluate the general physico-chemical parameters of the sources of water used for drinking and other domestic purposes, and to compare the above findings with requirement of WHO standards for drinking water.

Materials and Methods

Description of the study Area and vicinity of the dug wells

Various drinking well water samples were collected from four (4) different communities namely; Emilaghan, Otari, Omokwa, and Amalem, all located

in Abua Central; the headquarters of Abua/Odual Local Government Area of Rivers State, Nigeria. The study area was chosen because of the large population density and poor infrastructural amenities. The well water so referred to in this study does not receive any form of treatment before consumption by inhabitants of these communities. Location A is a well in Emilaghan Community situated in a nearby bush very close to a private Nursery and Primary school. The distance between the school and the well is 19 meters. Location B is a well in Otari community situated inside Alali's compound 3m from the house to the well location. Location C is a well in Omokwa community situated 15m from occupied houses and Location D is a well in Amalem community situated just about 3m from a plantain and coco-yam plantation where inhabitants use as an open toilet site and about 7m from occupied houses.

Collection of Water Samples

Water samples were collected from wells at weekly interval for a period of three weeks. One litre (1.0 L) capacity sterile plastic bottle was used to collect the water at a depth of about 1-2meter of the well water using a metal bucket. Sterile sample bottle was rinsed with the well water before being filled with water and capped tightly, appropriately labeled and placed in an ice packed cool box. Samples were immediately transported to the laboratory and processed immediately for analysis within one hour after arrival.

Microbiological Analysis of Well Water Samples

Cultivation, Enumeration and Isolation of Bacteria and Fungi in Water Samples

Serial dilution was carried out on each water sample. The dilution factor for the isolation of bacteria and fungi was 10^{-4}. This was done so as to obtain discrete colonics when plated on the medium. One milliliter (1.0ml) of each water sample was added to separate 9.0ml of normal saline (diluent) and further dilution was made up to 10^{-5}. An aliquot (0.1ml) of the appropriately diluted sample was then inoculated onto nutrient agar and Sabouraud dextrose agar plates for the isolation of bacteria and fungi respectively. The spread plate method was done using sterile bent glass spreader to spread the sample evenly on the agar plates. Cultures were prepared in duplicates. Cultured nutrient agar plates were incubated at 37°C for 24 hours while the cultured SDA plates were incubated on the laboratory bench for 5 to 7 days. Discrete colonies that developed on nutrient agar plates were counted, the average calculated and recorded as total heterotrophic counts of bacteria. Discrete colonies were cultured overnight and stored in nutrient agar slants

in the fridge as stocks cultures for further biochemical tests. Colonies which developed on SDA plates were counted and the average count of duplicate cultures was recorded as total viable fungi of each sample. The colour and colonial morphologies or characteristics were also recorded. Discrete colonies were subcultured onto freshly prepared SDA to obtain pure cultures. A total of thirty (30) pure cultures of bacteria and twenty-four (24) pure cultures of fungi were stored and utilized for further characterization and identification tests.

Characterization and Identification of Bacteria and Fungi in Water Samples

Pure cultures of bacteria were subjected to the following characterization tests performed in duplicates. Gram staining, catalase test, coagulase test, urease test sugar fermentation test, methyl red test, indole test and acid gas test were carried out as described by Cappuccino and Macfaddin (2005) and Kirk *et al*., (2005). The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al.,* (1975) and with reference to Holt (1977). Pure cultures of fungi were subjected to the following standard characterization tests performed by macroscopic examination of the colony morphology-diameter, colour (pigmentation), texture and surface appearance. While the microscopic examination of fungi was done by needle mount or wet mount method and observing sexual and asexual reproductive structures. A drop of sterile distilled water was aseptically dropped on a grease free clean slide. A piece of fungal hyphae under test was teased into it using two sterile needles. The teasing was done carefully and slowly so as to make good spread of the fungal hyphae. Each prepared slide was gently covered with a cover slip to avoid air bubble. The slides were observed under low and high power objective, and observation recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1998).

Estimation of Coliform and Faecal Coliform Bacteria

Estimation of the coliform bacteria was conductede using the most probable number (MPN) technique. Reaction to MPN technique and thermotolerant coliform bacteria MPN index/100ml of each water sample was done using double strength MacConkey broth for 10ml of sample and single strength MacConkey broth for 0.1ml and 1ml of the sample. The test for the estimation of coliforms involves the following steps: presumptive, confirmatory and completed test. It was performed as described by Verma *et al*., (1999).

Physico-chemical Analysis of Well Water Samples

The temperature reading of each water sample was read off with the aid of Mercury in glass thermometer and recorded at the sampling site. The pH of each water sample was measured by use of automatic digital pH meter (Model METTLE DELTA-340) made in England. While salinity was determined using Horiba Water Checker (Model U-10) after calibrating the instrument with standard Horiba solution. The dissolved oxygen (DO) was determined by the Modified Winkler's method (APHA 1985) and the Biochemical Oxygen Demand (BOD_5) was calculated after the determination of the DO of the fifth day. Other physico-chemical parameters determined were Chemical Oxygen Demand (COD), electrical conductivity, total Dissolved Solids, total suspended solids, total hydrocarbon, Potassium, Nitrate, and total hardness (APHA, 1995).

Results

The results of total heterotrophic bacteria count (THBC) ranged from 1.7×10^6cfuml^{-1} to 7.2×10^6cfuml^{-1} with a mean of 3.96 x 10^6cfu/ml for Emilaghan community well water, from 2.53×10^6cfuml^{-1} to 8.8×10^6cfuml^{-1} with a mean of 4.77 x 10^6cfu/ml for Otari, from 9.2×10^6cfuml^{-1} to 1.96×10^7cfuml^{-1} with a mean of 1.5 x 10^7cfu/ml for Omokwa, and 4.5×10^6cfuml^{-1} to 7.6×10^6cfuml^{-1} with a mean of 5.53 x 10^6cfu/ml for Amalem.

The count of fungi ranged from 4.0×10^5sfuml^{-1} to 1.5×10^6sfuml^{-1} with a mean of 7.7 x 10^5sfu/ml for Emilaghan water, from 1.1×10^6sfuml^{-1} to 7.0×10^6sfuml^{-1} with a mean of 3.2 x 10^6sfu/ml for Otari, from 1.2×10^6sfuml^{-1} to 8.0×10^6sfuml^{-1} with a mean of 3.5 x 10^6sfu/ml for Omokwa, and from 1.0×10^6sfuml^{-1} to 1.9×10^6sfuml^{-1} with a mean of 1.53 x 10^6sfu/ml for Amalem. Thermotolerant coliform bacteria/Faecal coliform bacteria (TtCB/FCB) ranged from 33 to 1600 MPN Index/100ml in Emilaghan water, from 9 to 33 MPN Index/100ml in Otari, from 14 to 34 MPN Index/100ml in Omokwa and consistent 1600 MPN Index/100ml in Amalem.

Generally, the total heterotrophic bacteria count ranged from 1.24×10^9cfuml^{-1} to 5.53×10^9cfuml^{-1}, faecal coliform bacteria ranged from 9 to 1600 MPN/100ml while total fungal count ranged from 2.3×10^6sfuml^{-1} to 4.6×10^6sfuml^{-1}. Analysis of variance (ANOVA) on the counts of total heterotrophic bacteria showed that, there is significant difference between the well waters at $p= 0.05$ while there was no significant difference in the counts of fungi. The result of bacteria isolates and frequency in well water in the communities is shown in Figure 1.

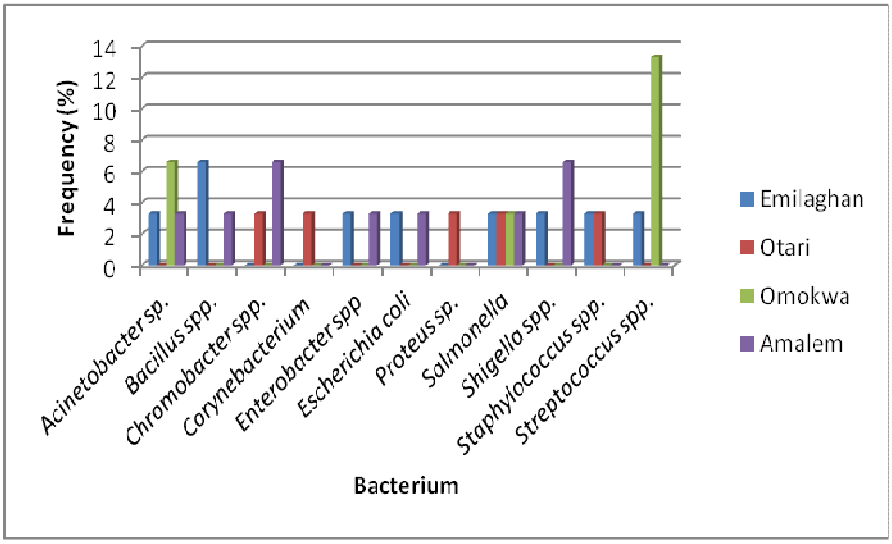

Fig. 1: Frequency (%) of bacteria in well water from the different communities

The result of fungi isolates and frequency in well water in the communities is shown in Figure 2.

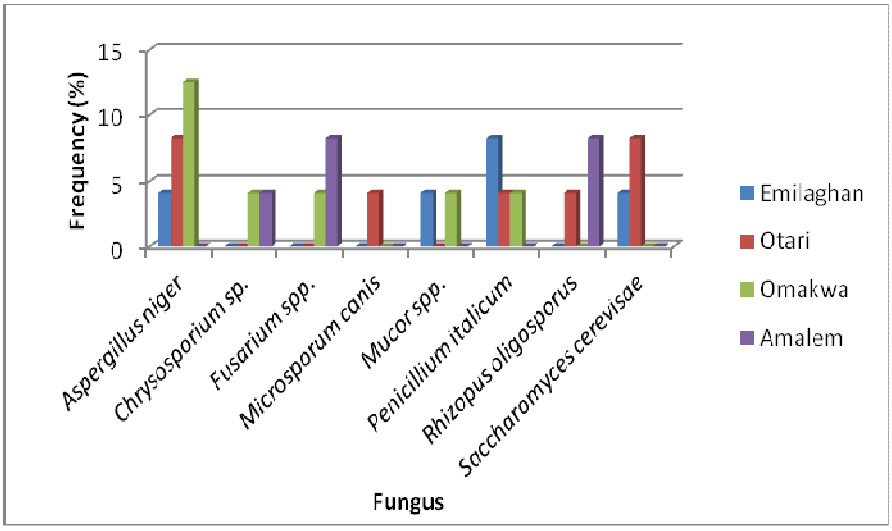

Fig. 2: Frequency (%) of fungi in well water from the different communities

The result of the mean values of the physico-chemical constituents of well water in Abua Central communities is shown in Figure 3.

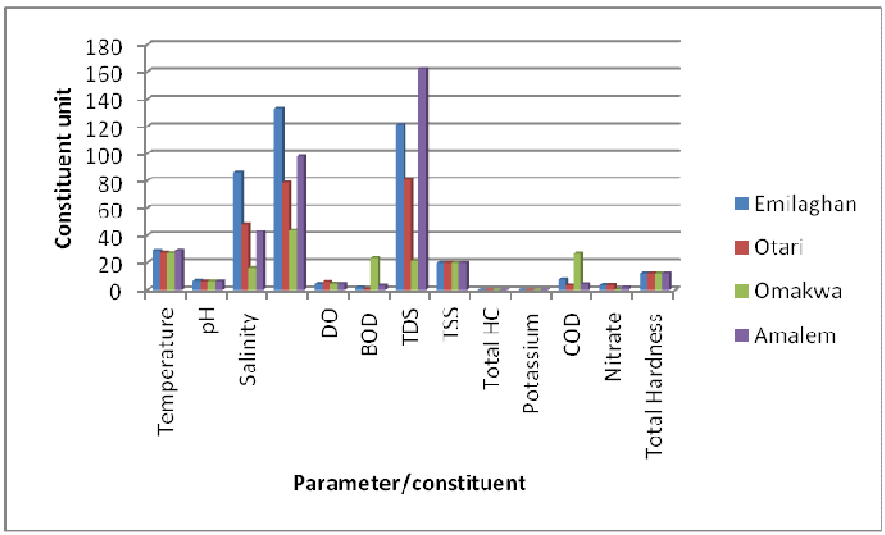

Fig. 3: Mean values of physicochemical constituents of well water from Abua Central communities

The temperature values ranged from 25°C to 29.4°C. The pH of the water samples, ranged from 6.4 to 8.0. The salinity levels range between 16.1 to 86.6mg/l with sample from Omokwa recording the least and Emilaghan the highest. The Electric conductivity levels range between 40 to 135 µs/cm with sample from Omokwa being the least and Emilaghan the highest.

The Dissolved oxygen concentration of water samples ranged from 4.0 to 6.4 dissolved oxygen concentration values was high in the Otari and low in Emilaghan. The Biochemical oxygen demand (BOD) of the various water samples ranged from 0.64 to 23.6mg/l. BOD values was highest in Omokwa and was lowest in Otari.

Total Dissolve Solid (TDS) of the various water samples ranged from 20 to 160mg/l. TDS values were high in Amalem and low in Omokwa. Total Suspended Solid (TSS) had the same value (20mg/l) in all the well waters. Total hardness also had the same value in all the well waters (12.0mg/l).

Total Hydrocarbon and potassium were not detected in any of the water samples. The Chemical Oxygen Demand (COD) levels ranged from 3.1 to 27.3mg/l with sample from Otari recording the least and Omokwa the highest. Nitrate levels ranged from 0.44 to 3.52mg/l with Omokwa samples recording the least and Emilaghan and Otari the highest.

Discussion

This present study has revealed the microbiological and physicochemical constituents of some well water in Abua Central area of Rivers State of Ni-

geria. The total bacterial counts and total fungi count of all the water samples were generally high exceeding the limit of 1.0 x 10^2cfu/ml and 1.0 x 10^2sfu/ml which are the standard limits for drinking water (EPA, 2002). Analysis of variance (ANOVA) using F-test on the data obtained showed that there were no significant differences at $p \geq 0.05$ between the counts of both total heterotrophic bacteria and between the counts of fungi of the different well water. The high total heterotrophic count is indicative of the presence of high organic matter and dissolved salts in the water. The primary sources of these bacteria and fungi in water are animal and human wastes. These sources of bacteria and fungi contamination include surface runoff, and other land areas where animal wastes are deposited. The microbial count was higher in Amalem and Emilaghan communities because these wells were very close to toilet sites than those of Omokwa and Otari, where the microbial counts are lower. Environmental Protection Agency (EPA) establishes heterotrophic plate count as a primary standard, which are based on health considerations. Water quality is the degree of potability which is determined by the amount and kind of suspended and dissolved substances in the water, the degree of alkalinity (pH), temperature and presence of non desirable microorganisms (Obire *et al*., 2008). The various sources of drinking water seem to contain a high microbial load as revealed in this study. The international standards for drinking water states that potable water should not contain 100cells of heterotrophic bacteria per 100ml of water, but unfortunately, the bacteria counts obtained in this study superceded the standard. Therefore, water used directly for drinking in these Communities in Abua Central area of Abua/Odual poses threat to public health.

Generally, the bacteria isolates identified and frequency in well water were *Acinetobacter sp.*, *Bacillus sp., Chromobacter sp.*, *Corynebacterium sp.*, *Enterobacter sp*., *E. coli*, *Proteus sp.*, *Salmonella sp*, *Shigella sp.*, *Staphyloccoccus sp*., and *Streptococcus sp. Chromobacter* and *Salmonella* species recorded the highest frequency of 13.3%. It was observed during this study that the sanitary conditions and standard of living of people in the various communities in decreasing order was Otari > Omokwa > Emilagham > Amalem. Many of the pathogens isolated in this study which are of public health concern were isolated from locations D and A (Amalem and Emilaghan communities). All the potential pathogenic bacteria isolated during this study except *Proteus* were isolated from Emilaghan and all but *Proteus*, *Staphyloccoccus* and *Streptococcus* were isolated from Amalem. *Salmonella* species occurred in well water of all the communities. The fungal isolates were *Aspergillus sp.*, *Chrysosporium sp*., *Fusarium sp.*, *Microsporum sp.*, *Mucor*, *Penicillium sp*, *Rhizopus sp*., and *Saccharomyces cerevisiae*. *Penicillium* species recorded the highest frequency of (16.6%). *Microsporum canis*, a dermatophyte of cats and dogs was isolated from Otari well water.

Proteus spp. is also of public health significance. *Staphylococcus aureus*

is known to produce enterotoxin (Obire *et al*., 2008). *Proteus spp.* belongs to the intestinal flora but is also widely distributed in soil and water. Environmental bacteria such as *Acinetobacter* and *Bacillus* sp., which are mostly saprophytic in origin, were isolated from well waters. It was also found that 80% of the various water samples were positive for coliform MPN showing high contamination and risk to public health. The counts of faecal coliform obtained in these drinking well waters were high and far above recommended standards of zero total coliform per 100ml of water (EPA, 2003). The detection of faecal coliform indicates feacal pollution of the drinking water. The presence of faecal indicators such as *E. coli*, *Shigella* sp and enteric pathogens such as *Chromobacterium*, *Enterobacter*, indicated that the various water sources are polluted with faecal matter. None of these well water samples use for drinking in Abua Central complied with EPA standard for coliform in water due to the presence of faecal coliform especially *E. coli* (WHO, 1971; EPA, 2003). The order of decreasing water quality in the various communities was C > B > D > A (Omokwa > Otari > Amalem > Emilagham).

The pH of all the water samples were in agreement with pH assigned by EPA as the standard pH of water which ranges from 6.5 – 8.5 (EPA, 2002). The total dissolved solid of all water samples are in agreement with the environmental protection agency standard of 500mg/l. The total dissolved oxygen demand of some water samples are not in agreement with the environmental protection agency standard (EPA, 2002). The total hardness of all water samples are not in agreement with the environmental protection agency standard (EPA, 2002).

Diseases associated with the bacteria isolated from the well water in Abua/Odual communities are; *Bacillus spp.* Diarrhea and food poisoning, *Shigella spp.* shigellosis (bacterial dysentery), *Streptococcus spp.* streptococcal pneumonia and sore throat, *Staphylococcus spp.* various staphylococcal disease e.g. boils, *E. coli* gastroenteritis, *Enterobacter spp.* sepsis and septic shock, *Salmonella typhi* typhoid fever, salmonellosis (salmonella gastroenteritis), *Proteus spp.* urinary tract infection. Diseases associated with the fungi isolated from the well water in Abua/Odual communities are; *Aspergillus spp.* aspergillosis and onchomycosis, *Rhizopus spp.* tellutis, *Fusarium solani*- pneumonia and onchomycosis (Robert *et al*., 1981).

Thus, the direct consumption of raw water from wells of the communities studied could contribute to the spread of many infectious diseases and may be the cause of serious epidemic in Abua/Odual Local Government Area. Sterilization by heating of the water for use during cooking and for other purposes leads to denaturation of some microbial species and hence preventing the spread of gastrointestinal infection. The results suggest that efficient and proper sanitary check in drinking water supplies has to be executed regularly in view of its great public health significance and at the same time good

observation of personal and household hygiene has to be emphasized.

In well water supplies, sometimes up to 10 coliforms/100ml are allowed for WHO standards for tropical countries (WHO, 1971; 1996) but this should not occur repeatedly. If occurrence is frequent and sanitary condition cannot be improved, an alternative source must be found if possible. In order to protect public health and to ensure that water is safe for public use, any water intended for drinking, treated or untreated, piped or un-piped must meet certain microbiological standards. A violation of set standards as shown in the results of the various drinking water samples in Abua Central Area warrants treatment of the present source or the need for an alternative water supply.

Conclusion

The bacteriological quality of various source of water in 'Abua Central' area showed failure to meet the zero faecal and non-faecal coliform EPA and WHO standards. The greatest threat posed to water resources arises from microbiological contamination which has long been a concern to public health. Water contamination with potential pathogenic microorganisms represents an obvious health risks for inhabitants of Amalem and Emilaghan communities. Inhabitants with low personal and household hygiene are greatly affected by a wide range of microbial contamination. Contamination of these water sources will continue unless effort is put into pollution prevention. Pollution control strategies should include; Public health training, awareness of methods of transmission of pathogens and diseases and organized waste disposal system. In addition, practical steps at Community and Government levels in addressing the issue must not be ignored. Each community needs an adequate and safe supply of water. Easy-to-handle water treatment techniques should be introduced to the communities to ensure drastic reduction in the magnitude of occurrence of faecal coliforms and enteric pathogens in drinking water. There should be proper well location and construction. There should be control of human activities in the vicinity of wells to prevent sewage from entering water body as to avoid bacterial contamination of drinking water. Unless the situation is rectified on this basis with particular reference to adequate adoption of sanitary measures for provision of potable water, the problem will not be over in the near future as desired.

Correspondence
Omokaro Obire
Department of Applied and Environmental Biology
Rivers State University of Science and Technology
P.M.B 5080, Port Harcourt, Nigeria
E-mail: omokaro515@yahoo.com

References

APHA (1985). American Public Health Association: *Standard Methods for the Examination of water and waste water. 16th Edn*. APHA, Washington D. C. USA. 55 - 1979.

APHA (1995). *Standard Methods for the Examination of Water and Wastewater. 19th Edn*. America Public Health Association, America Water Works Association and Water Environment Federation. Washington, D.C.

Barnett, J. and Hunter. B. (1998) *Illustrated Genera of Imperfect Fungi.* Aps Press. *1*: 32-80.
Cappuccino J and Macfaddin J.F. (2005). *Biochemical tests for the identification of medical bacteria. 2nd Edn*. Baltimore, MD. Williams and Wilkins.

Castro P. (1998). Marine Biology McGraw-Hill Publishing Company. New York. 87 - 88.

Cruickshank, R, Duguid, J.P, Marmion B.P and Swain, R.H.A. (1975). *Medical Microbiology*, *12th Edn*. *Vol. 2*. Church III Livingstone, PP. 77-122,137-180.

Driscoli, F. (1986). Ground Water and Wells Johnson Filtration Systems, Inc., St Paul, Minnesota. 1021.

Edema, M.O. and Fapetu, O.M (2001). Microbiological and Physiochemical Analysis of Different Sources of Drinking Water. *Nigerian Journal of Microbiology. 15*: 57 – 61.

EPA (2000). Report of Task Force on Guide Standard and Protocol for Testing Microbiological Water Purifies. *United States Environmental Protection Agency. 1*: 1 - 50.

EPA (2003). US Environmental Protection Agency Safe Drinking Water Act. Washington, D.C.

Holt, J.G. (1997). *The Shorter Bergey's Manual of Determinative Bacteriology. 8th Edn.* Williams and Wilkins Co: Baltimore.

Khupe, J. S. N. (1996). Water supply, sewage and waste management for Gaborone, Botswana. *Ambio*. *25*: 134 - 137.

Kirk, C.J.C, Peel, N.R, James, K.R and Kershaw, Y.K. (2005). *Basic medical laboratory technology*. Pitman medical Pub. Co. Ltd., London.

Obire, O. and Aguda, M (2002). Bacteria community of leahate from a waste dump and an adjacent stream in Nigeria. *J. Applied Science and Environmental management. 6 (2)*: 71 - 75.

Obire, O., Aguda, M., Puteti, R.R. (2008). Impact of human activities on drinking water quality. *Journal of Basic and* Applied *Biology. 2 (3&4)*: 52 – 58.

Obire, O, Aguda, M and Putheti, R.R. (2010). Impact of palm-oil milling activities on the water quality of a creek. *International Journal of Chemical and Analytical Science. 1(7)*: 154 - 157.

Verma, J. K., Greene, K. D., Relter, M. E., Trother, J. and Nowickiki, S. F. (1999). An outbreak of *Escherichia coli* infection following exposure to contaminated food. *JANA*: 290- 2178.

World Health Organization (1996). *Guidelines for Drinking Water Quality. 2nd Edn*. Vol. 2, Health Criteria and other supporting information. 940 - 949. WHO Geneva.

World Health Organization (1971). *International standard for drinking water world health organization publication, 5th Edn*. WHO, Geneva. 21 – 22.

World Health Organization (2004). *Water Sanitation and Health Programme. Managing water in the home: accelerated health gains from improved water sources*. World Health Organization. www.who.int.