Journal of Toxicology and Environmental Health Sciences Vol. 3(13), pp. 342-346, 14 November, 2011
Available online at http://www.academicjournals.org/JTEHS
DOI: 10.5897/JTEHS11.068
ISSN 2006-9820 ©2011 Academic Journals

Full Length Research Paper

Microbiological assessment and some physico-chemical properties of water sources in Akungba-Akoko, Nigeria

A. O. Ajayi and T. O. Adejumo*

Department of Microbiology, Adekunle Ajasin University, P.M.B. 01, Akungba-Akoko, Ondo state, Nigeria.

Accepted 18 October, 2011

A comparative study was carried out to determine the quality of three water sources: borehole, well and stream in Akungba-Akoko, Ondo State. The water sources were assessed for microbiological quality and physico-chemical properties (temperature, odor, color and pH). The stream had the highest plate count of 40 × 10^5 cfu/ml, while those of borehole and well water had the least of 12 × 10^5 cfu/ml and 14 × 10^5 cfu/ml respectively. The isolated organisms were identified as *Escherichia coli, Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Enterobacter* sp., *Proteus* sp. and *Pseudomonas* sp. The most probable number (MPN) of the water sample sources ranged from 8 to 120 coliforms per 100 ml, which signified undesirable level of water pollution in the area covered. Water samples from the boreholes had a coliform range of 32 to 38 × 10^0 cfu/ml, and were adjudged to be less prone to contamination and potable than the well and stream sources, which recorded relatively higher coliform load of 44 to 70 × 10^0 cfu/ml.

Key words: Bacteriology, borehole, stream, well, water assessment.

INTRODUCTION

Water is one of the most abundant resources on which life on earth depends; in some places, availability of water is critical, limited and renewable. Shortage of water could lead to disease outbreak and economic loss, hence water is a necessity, it is a unique liquid and without it life is impossible. Water plays a vital role in the proper functioning of the earth's ecosystem. Man uses water for various purposes which include drinking, transportation, industrial and domestic use, irrigation in agriculture recreation, fisheries, and waste disposal among others (Shittu et al., 2008; Ajayi and Akonai, 2005). Water that is of a good drinking quality is important to human physiology, and man's continued existence depends so much on its availability (Lamikanra, 1999; FAO, 1997). The quality of water for drinking deteriorates due to inadequacy of treatment plants, direct discharge of untreated sewage into rivers and stream, and inefficient management of piped water distribution system (UNEP, 2001). The contaminated water therefore has critical impact on all biotic components of the ecosystem and this could affect its use for other purposes.

Water receives its bacteria spores from air, sewage, organic waste, dead plants and animal, at times almost all microorganisms may be found in water, but bacteria appeared to be the major water pollutants. Majority of the bacteria found in nature live on dead decaying organic matter as saprophytes (Peter and George., 1989). Bacteria also helps in the digestion of poisons from food and water. Presence of other species could cause various diseases to man and other animals. Water obtained from wells, boreholes, streams and river are never chemically pure, even rain water contains dissolved materials from the air as well as suspended dust intermixed with microorganisms (Prescott et al., 2008). Impurities in water may be floating as suspended matter consisting of insoluble materials of greater density than water which could be removed by sedimentation and in the form of bacteria. The bacteriological examination of water is performed routinely by microbiologists, and this will ensure a safe supply of water for drinking, bathing,

*Corresponding author. E-mail: toadejumo@yahoo.com.

Table 1. Sample sources and their physical parameters.

Sample source	pH	Temperature (°C)	Odor	Color
Ilale Well	7.50	28.3	Odorless	Colorless
Ilale Borehole	6.52	28.7	Odorless	Colorless
Akua Well	7.10	28.5	Odorless	Colorless
Akua Borehole	6.40	28.5	Odorless	Colorless
Okusa Well	7.80	28.3	Odorless	Colorless
Okusa Borehole	6.90	28.3	Odorless	Colorless
Ibaka Well	7.44	28.6	Odorless	Colorless
Ibaka Borehole	7.00	28.9	Odorless	Colorless
Okeoko Stream 1	8.59	28.0	Odorless	Colorless
Okeoko Stream 2	8.10	28.2	Odorless	Colorless

swimming and other domestic and industrial uses. Microbiological examination is usually intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either through improperly treated sewage or improperly functioning sewage treatment system. Chemical analysis can however determine whether water is polluted and provides other useful information (APHA, 1998).

In order to determine whether water is contaminated or contain any microorganism known to be pathogenic or indicative of faecal pollution, it is necessary to carry out a bacteriological examination (analysis) on it. This study therefore aims to investigate the quality of water from boreholes, wells and streams in Akungba-Akoko with a view to determining the presence and levels of pathogenic microorganisms that are indicative of faecal pollution. Similarly, the physico-chemical parameters of the water sources were also determined because it also serves as water quality determinant factor.

MATERIALS AND METHODS

The facilities used for this study like petri-dishes, pipettes, inoculation loop and culture media were adequately sterilized using appropriate laboratory methods (DIFCO, 1984).

Study site and sample collection

Water samples were collected from well, borehole, and stream at different sites from Akungba-Akoko (Table 1). The samples were obtained as follows according to the practice of the inhabitants**:** For the boreholes, the water was allowed to flow out for 2 min before the samples were collected. The stream water was collected by dipping the sample bottle into 2 m depth of the water body, while well water samples were obtained by using a clean fetching bowl to draw out water. The first three bowls fetched were discarded, while the fourth sample was aseptically poured into a sterile sample bottle.

Assessment of physico-chemical parameters

(i) *pH readings*

The pH of the samples was determined using the Fisher Accument pH meter (Model 600 fisher scientific co, U.S.A). 10ml of each of the samples was poured into a sterile beaker and the anode of the pH meter was deep into it and readings were obtained when it was stable.

(ii) *Temperature*

A simple thermometer in centigrade scale (500, 0.5 divisions) was used to measure the water temperature of each sample. The thermometer was inserted into the water sources to determine the approximate temperature.

(iii) *Odor and color*

The odor and the color of the water samples were observed after collecting the samples by physical observation.

Microbiological tests

Media used and their preparation

The media used for these analyses were Nutrient agar (NA), Eosine Methylene Blue (EMB), and MacConkey broth which is a differential medium for isolation of gram negative bacteria and screens them (isolates) for lactose fermentation. Sugars used for fermentation test were Glucose, Mannitol, Lactose, Fructose and Galactose. Similarly, Motility indole ornithine fluid media was used for motility and indole test. All the media were prepared according to the manufacturer's instruction and adequately sterilized in an autoclave at 121°C for 15 min.

Enumeration and detection of bacteria

Pour plate techniques: The aliquot of the specimens to be cultured was placed in the bottom of an empty, sterile Petri dish and melted. A cooled agar was poured over it, the plate was swapped to allow proper mixing. The agar was allowed to gel (solidified) after which the plate was incubated in an incubator at 37ºC for 24 h.

Sub-culturing of isolates and stock cultures Nutrient Agar (NA) was poured aseptically into plates and allowed to solidify.

Table 2. Total bacterial and coliform counts of water samples.

Sample source	Total bacterial count		Coliform count	
	cfu $\times 10^{-3}$ ml^{-1}	cfu $\times 10^{-5}$ ml^{-1}	cfu $\times 10^{0}$ ml^{-1}	cfu $\times 10^{2}$ ml^{-1}
Ilale Borehole	50	16	38	10
Akua Well	54	14	44	12
Akua Borehole	52	18	38	10
Okusa Well	70	20	30	10
Okusa Borehole	45	12	40	11
Ibaka Well	62	17	40	15
Ibaka Borehole	53	14	32	8
Okeoko Stream 1	20	40	70	30
Okeoko Stream 2	02	32	68	24
Control	0	0	0	0

Key: NA = Nutrient Agar and EMB= Eosine Methylene Blue Agar.

Specific colonies on the samples obtained were sub-cultured by streaking on the NA plates incubated at 37°C for 24 h. When primary isolation of the plates has been properly streaked, individual colonies was picked and incubated on fresh NA. Subsequent sub-culturing was carried out until pure cultures of the different isolates were obtained. These pure isolates were transferred onto agar slants in McCartney bottles and kept in the refrigerator as stock culture for subsequent tests during identification.

Total plate counts: The heterotrophic plate count (HPC)/ total count was carried out to provide an estimate of the total number of bacteria in each of the samples that would develop into colonies during the period of incubation on Nutrient agar and Eosine methylene blue Agar plates. This test detects a broad group of bacteria including the pathogens, non pathogenic and opportunistic pathogens. The laboratory procedure involves making serial dilution of the sample in sterile distilled water and cultivating 10^{-3} and 10^{-5} then 10° and 10^{-2} dilution factor into the center of Petri dish. The prepared media were allowed to cool to about 45°C before they were added to the dilution factors. The plates were incubated at 37°C for 24 h in inverted position to prevent condensation from the lid to the agar, after which the number of the colonies formed was counted. The acceptable value of the total number of Colony Forming Units (CFU) during the plate count for potable water was a total of less than 102 per ml.

Coliform count: Most probable number (MPN) method: This was done as recommended by standard method (APHA, 1998). The materials and media used for the analysis consisted of the followings. Fermentation tubes with aluminum caps, Durham tubes, MacConkey Broth (Single and double strength) inoculating loop, Bunsen burner, syringes (10, 5 and 2 ml). The most probable number tube fermentation technique is performed in three stages: Presumptive test, confirmative test and completed test.

Presumptive tests: With sterile pipettes, 1 ml of water samples was dispensed into two sets of 5 tubes containing 5 ml of sterile single strength MacConkey broth: 10 ml of each sample was also dispensed into a set of test tubes containing 5 ml of sterile double strength broth. Each fermentation tube contains an inverted Durham tube. A tube containing single strength broth was inoculated with 1 ml of the sterile distilled water to serve as control. The procedures were carried out aseptically and the tubes were recorded by for 48 h. After incubation, the results were recorded by looking for the presence of trapped gas bubble inside the Durham indicates positive results. The MPN of coliforms in 100 mL of the water sample was estimated by the numbers of positive tubes and the results were checked on the MPN tables.

Confirmative tests: A loop full of the sample from positive tubes were transferred into a plate containing Eosine Methylene blue agar (EMB) by streaked method and incubated at 37°C for 24 to 28 h. The agar inhibits Gram positive organisms and allows the Gram negative coliforms to grow. Coliforms produce colonies with dark centers, green metallic sheen and large pinkish colonies.

The completed tests: The organisms that grew on the confirmed test media were inoculated into nutrient agar slants and tubes of MacConkey broth. After incubation at 37°C for 24 h, the broth was checked for production of gas and a Gram's stain was made from organisms on the nutrient agar slant. A positive test indicates that coliforms were present in the water sample when the tests showed Gram negative, non spore- forming rod with gas production on MacConkey Broth.

Biochemical tests and identification of microbial isolates:

Morphological and biochemical characteristics of the microbial isolates were used for the identification of the isolates according to Baron et al., 1990, Benson (1990) and Bitton (1994). The Bergey's Manual of determinative bacteriology by Buchanan and Gibbons (1974) was used to compare the characteristics with the results obtained.

RESULTS AND DISCUSSION

The temperature of the water samples ranged between 28.0 and 28.9°C, while the pH ranged from 6.40 to 8.59. All the samples were colorless and odorless as shown in Table 1. The water samples from the boreholes had a coliform range of 32 to 38 × 10° cfu/ml were adjudged to be less prone to contamination and potable than the well and stream sources which recorded relatively higher coliform load of 44 to 70 × 10°cfu/ml (Table 2).

This study showed a comparative nature of the bacteria isolates obtained from different water sources in Akungba-Akoko. The assessment of microbiological quality of water from different sources was essential for

Table 3. Morphological and biochemical characteristics of isolates.

Morphological characteristics of isolates on solid media	Gram's staining	Cell morphology	Catalase	Motility	Indole	Starchy hydrolysis	Sugar fermentation					Identification
							Glucose	Lactose	Fructose	Galactose	Mannitol	
Circular Creamy Flat Entire Moderate Smooth	–	Short Rod	+	+	+	+	AG	AG	_	_	+	*Escherichia coli*
Irregular White Flat Entire Moderate Smooth	–	Cocci in chains	–	–	–	+	AG	+	+	+	AG	*Streptococcus* sp.
Circular Creamy Low convex Smooth Moderate Smooth	–	Rod	+	+	_	+	AG	+	_	+	+	*Enterobacter* sp.
Circular Creamy hite Raised Rhizoid Moderate Dull	-	Motile rod	_	+	_	+	AG	AG	+	_	_	*Pseudomonas* sp.
Irregular Pale White Flat Smooth Moderate Smooth	+	Cocci in clusters	+	–	–	+	+	–	–	–	+	*Staphylococcus* sp.
Circular Yellow Flat Undulated Moderate Dull	–	Rod	_	_	_	_	AG	AG	AG	+	+	*Flavobacterium* sp.
Circular Creamy hite Raised Rhizoid Moderate Dull	–	Rod	+	+	_	_	AG	_	_	+	+	*Pseudomonas* sp.
Irregular Creamy Flat Undulated Moderate Rough	+	Motile -rod	_	+	AG	+	_	AG	_			*Proteus* sp.
Circular Opaque Raised Enti re Moderate Smooth	–	Rod	_	_	_	_	AG	AG	_	+	+	*Klebsiella* sp.
W10- Circular White Flat Entire Moderate Smooth	+	Rod	_	+	_	+	AG	_	+	+	+	*Bacillus* sp.

Key: AG= Production of gas and acid, + = Positive tests, - = Negative tests.

detecting the presence or absence of organisms that might constitute health hazards in water, which could be used as a guide to monitor and protect the water sources. The total bacteria counts for all the samples were generally high, exceeding the limit of 1.0×10^2 cfu/ml which was the standard limit of heterotrophic count for drinking water (EPA, 2002).

The high total plate counts observed in stream water indicated the presence of high organic matters and related nutrient sources. The primary sources of bacterial contamination might include the surface runoff, sewage treatment facilities, natural soil/plants bacteria and improper management activities of the inhabitants like washing, refuse dumpage, faecal droppings, dipping of different materials inside the water sources. The stream water also had the highest number of coliform. Various groups of microorganisms were isolated and identified during the study. They include *Escherichia coli, Streptococcus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., and *Bacillus* sp. (Table 3).

The presence of some of these organisms is indicative of water contamination.

Bacteriological tests are extremely sensitive and specifically designed to reveal the evidence of water pollution. A great majority of quality problem

with community drinking water are related to fecal contamination. Although, a significant number of serious probes may occur as a result of chemical contamination from variety of natural and man- made sources.

Water from the wells, boreholes, streams and other sources may look clean and have no undesirable odor or taste. However, it is unfortunate that the pathogens found in these water sources can be harmful by causing serious illnesses. In this study, the boreholes had the lowest bacteria count which may be due to its depth. In some cases, improper construction of boreholes, its pipes, proximity to toilet facilities and various human activities around the borehole could contaminate the water. Well water could also be contaminated due to shallowness, animal waste, closeness of refuse dump sites, proximity to toilet facilities (closeness to latrines), improper placement of well covers, using different drawing bowls and plunging of bowls/bucket directly from the soil into the well as well as human activities around it (Bitton, 1994).

The total coliform for samples examined during this study were exceedingly high as against the EPA maximum contamination level (MCL) for coliform bacteria in drinking water of zero total coliform per 100ml of water (EPA, 2002). The high coliform count obtained in the samples may be an indication that the water sources were faecally contaminated (EPA, 2002; Osunide and Enuezie, 1999). None of the water sources in this study complied with the EPA standard for coliforms in water. The microorganisms generally isolated in this study include *Escherichia coli, Streptococcus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., and *Bacillus* sp. (Table 3). The presence of some this organisms signifies contamination of water from some domestic sources. The *Staphylococcus* species is known to produce enterotoxin (Okonko et al., 2008). *Proteus* species is an intestinal flora, but also widely distributed in soils and water (Schlegel, 2002). P*seudomonas aeruginosa* is an example of non-faecal coliforms, while *E. coli* are a fecal coliform. Other organisms encountered include *Pseudomonas* species, *Klebsiella* species*, Streptoccocus* species and *Bacillus* species.

Conclusion

This study showed that most domestic water sources are potential health risks for consumers. Among the strategies to adopt to combat water pollution problem is the promotion of household water storage, and the need to improve on personal behavior and hygiene practices to reduce microbial load in water supply. The key to avoiding bacterial contamination of water include proper well and borehole location and construction, control of human activities to prevent sewage from entering the water bodies. It is evident that water-borne diseases are due to improper disposal of refuse, contamination of water by sewage and surface runoff. Appropriate programmes must be put in place to educate the general populace on the need to purify water to make it fit for drinking and other domestic purposes.

REFERENCES

Ajayi AO, Akonai KA (2005). Distribution pattern of enteric organisms in the Lagos Lagoon. Afr. J. Biomed. Res., 8(3): 163-168.

American Public Health Associated (APHA) (1998). Standard method for examination of water and waste water, 20th Ed. American Public Health Associated Inc, New York. American Water Work Associated (AWWA). Research Foundation, 1993. Bacterial examination of water. West Quincy Avenue Denver, pp. 81-85.

Baron EJ, Sydney MF (1990). Bailey and Scott's Diagnostic Microbiology. The C.V. Mosby Co., St. Louis, p. 861.

Benson HJ (1990). Microbiological Applications: A Laboratory Manual in General Microbiology. Wm. C. Brown Publishers, Dubuque, p. 459.

Bitton G (1994). Waste Water Microbiology. Gainesville, New York Wiley – Liss, p. 118.

Buchanan RE, Gibbons NE (eds) (1974). Bergey's Manual of Determinative Bacteriology, 8th Edition. The Williams and Wilkins Co., Baltimore, p. 124.

DIFCO (1984). Dehydrated Culture Media and reagents for Microbiology. Tenth Edition. DIFCO Laboratories Detroit Michigan 48232 U.S.A.

Environmental Protection Agency US (EPA) (2002). Safe Drinking Water Act Amendment, http://www.epa. gov/safe/mcl/Html.

Food and Agriculture Organization (FAO) (1997). Chemical Analysis Manual for Food and Water. 5th Ed. FAO, ROME, 1: 20-26.

Lamikanra A (1999). Essential Microbiology for Students and Practitioners of Pharmacy, Medicine and Microbiology. 2nd Edition. Amkra books, p. 406.

Okonko IO, Adejoye OD, Ogunusi TA, Fajobi EA, Shittu OB (2008). Microbiological and Physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. Afr. J. Biotechnol., 7(5): 617-621.

Osunide MI, Eneuzie NP (1999). "Bacteriological analysis of ground water" Nig. J. Microbiol., 13: 47-54.

Peter HR, George BJ (1989). General Microbiology 2nd Edition. Water Treatment. George Brooks, Time mirror/Mosby College publishing. St Louis, USA, p. 231.

Prescott LM, Harley JP, Kleins DA (2008). Microbiology 11th Edition. Treatment of Water McGram Hill Company New York. P. 1088

Schlegel HG (2002). General Microbiology. 7th Edition Cambridge University Press. P 480.

Shittu OB, Olaotan JO, Amusa TS (2008). Physicochemical and Bacteriological Analysis of water used for Drinking and Swimming Purposes in Abeokuta, Nigeria. Afr. J. Biomed. Res., 2: 285-290.

UNEP (2001). State of Water Sanitation and Health Programme Nepal. United Nation Environment Programme (UNEP).