Assessment of the Physico-Chemical and Microbiological Properties of Borehole Water Samples from Akungba - Akoko, Ondo State, Nigeria

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ABSTRACT

Purpose: Water is very important to the existence of humans, though could also serve as vehicle of pathogenic organisms and dangerous organic and inorganic matters. The physicochemical and microbiological analysis of seven borehole water samples used by Akungba- Akoko residents were carried out with the aim of ascertaining their suitability or otherwise for human consumption.

Methods: The total hardness, pH, alkalinity, some common elements and presence of toxic metals were determined. The Most Probable Number (MPN) was used for the detection and isolation of the contaminating microorganisms.

Results: In the physicochemical analysis of the borehole water samples, the lowest pH (6.54) was recorded in IBK1 sample while AKA water sample gave highest calcium concentration (86.97mg/L). The total hardness ranged between 171.76 and 327.33mg/L. Elements such as manganese, zinc, copper, cadmium were below detectable levels in the water samples. Seven bacteria species, *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella paratyphi and Proteus vulgaris* were isolated. AKA sample gave the highest bacteria count of 1.6 X 10^5 cfu/ml. *Staphylococcus aureus* was the most frequently isolated among the bacteria, having been isolated in three of out of the seven samples examined. The antibiotics susceptibility test showed that *Proteus vulgaris* and *Klebsiella pneumoniae* were susceptible to most antibiotics.

Conclusion: The physicochemical properties and the bacteria load of most of the water samples were within WHO standard for drinking water hence, the water samples can be declared fit for drinking.

Key words: Borehole water, physico-chemical parameters, Most Probable Number, Akungba-Akoko, dissolved oxygen.

Running Title: *'Quality of borehole water samples in Akungba-Akoko'*

INTRODUCTION

Water is one of the most important and most precious natural resources. It is essential in the life of all living organisms from the simplest plant and microorganisms to the most complex living system known as human body [1]. Good drinking water is not a luxury but one of the most essential requirements of life itself. Water is fundamentally important to all plants, animals and man [2]. It is significant due to its unique chemical and physical properties [3]. Water is a combination of hydrogen and oxygen atoms, with a chemical formula, H_2O and known to be the most abundant compound (70%) on earth surface [4].

Some microorganisms are commonly associated with water pollution, these include *Pseudomonas aeruginosa, Salmonella* sp, *Mycobacterium* sp, *Escherichia coli, Proteus* sp, *Shigella sonnei and Cyanobacteria* sp [5]. During passage through the ground, water dissolves minerals in rocks, collect suspended particulate matter, particularly those from organic sources as well as pathogenic microorganisms from faecal matters [6]. Chemical and physical parameters includes heavy metals, trace metals, total suspended solid (TSS) and turbidity. These trace elements present in virtually potable water, some of which play a role in metabolism. Major ions in drinking water are correlated with palatable mineralization that affects the quality of drinking water [7]. Certain mineral are also toxic such as the heavy metals. Although, some of the heavy metals such as zinc, manganese, nickel and copper act as micro-nutrients at low concentration. Health risk due to heavy metals contamination of water through soil has been reported [8]. Other elements, such as Arsenic, Bismuth, Cadmium, Mercury, Lead and Titanium, have no apparent metabolic function and are termed non-essential. Some are very toxic such as

lead, Chromium and Carbon [9]. An estimated 1.1 billion persons (one sixth of the world's population) lack access to clean water and 2.6 billion to adequate sanitation [10]. Microorganisms play a major role in determining water quality. The most dangerous form of water pollution are caused when faecal contaminants like *Escherichia coli* enters the water supply [11], pathogen such as *Salmonella spp, Shigella spp, Vibrio cholera and E.coli* that are shed into water body through faecal contamination perpetuate many diseases [8, 11]. These cause typhoid fever and dysentery. Other agents of water borne diseases are protozoan that cause diarrhoea; *Entamoeba histolytica, Giardia lamblia, Balantidium coli* and *Cryptococcus pervum* [12]. Studies have shown that bacteria remain the most important causal agent of enteric disease in Nigeria. Other causal organisms are viruses and helminths [13]. Drinking water has always been a major issue in many countries like Nigeria [14]. Majority of the rural populace in Nigeria do not have access to potable water [15]. Increase in human population has exerted an enormous pressure in human provision of safe drinking water in developing countries [16]. Water borne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated water is consumed [17]. This study therefore aimed at assessing the chemical and physical properties as well as the microbial load of the borehole water samples used in Akungba-Akoko.

MATERIALS AND METHODS

Study Site and Sample Collection

The borehole water samples were aseptically collected from the sources using sterile glass bottles after pumping water sample to waste for one or two minutes. The nozzles of the bore hole were swabbed with cotton wool soaked in 70% ethanol. The water samples were kept between the temperature of 4-10◦C and transported to the laboratory less than two hours of collection and analysed within 24 hours. A total of seven water samples were collected from Akungba-Akoko, Ondo State between the hours of 8.00 a.m. and 10.00 a. m, when the sampling points were free of students and indigenes.

Sample Code

The samples were labelled according to the sight of collection and coded as; ILA= for samples collected from Ilale; AKM= samples from Akunmi; IBK1= samples from Ibaka (1); IBK2= samples from Ibaka (2); ARM= samples from Araromi; OKS= samples from Okusa; AKA= samples from Akua

Preparation of Double Strength MacConkey Broth

Double strength MacConkey broth was prepared according to manufacturer's instruction (multiplying the manufacturer's required weight for normal preparation by 2) then dissolved in distilled water and it was mixed thoroughly and gently heated on the hot plate to obtain a homogenous mixture. The mixture was then sterilized at 121^0C for 15minutes after dispensing into McCartney bottles containing inverted Durham's tubes. They were allowed to cool before inoculating with water samples.

Eosin Methylene Blue (EMB) Agar

This medium was used for the confirmation of the organisms in positive tubes obtained from the presumptive test. It was prepared according to manufacturer's instruction.

Enumeration of Total Heterotrophic Bacteria

Total heterotrophic bacteria in the borehole water samples were enumerated using pour plate method. A five - fold serial dilution (10^{-1} to 10^{-5}) of the samples were prepared using sterile distilled water. MacConkey and Nutrient agar media were prepared in duplicate. 1ml of each dilution was introduced into sterile petri dishes into which 19 mls of the prepared molten media were added. The cultured plates were allowed to cool and solidify then, they were incubated at 37^0C for 24 hours and Petri dishes containing discrete colonies were counted.

Isolation and Identification of contaminating Bacteria

Cultural, microscopic examination, biochemical tests including sugar fermentation tests were done to identify the pure isolate as described by Cheesbrough [18].

Antibiotic Susceptibility test of the Isolates

Peptone water was prepared according to manufacturer's instruction and inoculated with each of the test isolates and incubated at 37^0C for 24hours. Mueller Hinton agar was prepared and poured into sterile Petri dish and allowed to solidify. Each isolate was inoculated into the solidified Mueller Hinton agar using sterile swab sticks. Antibiotic discs were gently placed unto the inoculated plate using sterile forceps. These were incubated for 24hours at 37^0C and observations recorded.

Procedure for Chemical Analysis of Borehole Water

pH/ Conductivity Determination

Electronic pH meter (Digital) Model Exner GMBH, D4040 NEUSSI with a combined electrode was used. Known buffer solutions of pH 4, and pH 9 were prepared and used to standardize the equipment. Immediately

the pH readings had been taken and recorded, samples conductivity was measured using a conductivity meter (Radio-meter Copen-Hagen CDM 83).

ALKALINITY DETERMINATION

50ml of the water sample were pipetted into clean 150ml capacity conical flask. Three drops of phenolphthalein indicator were added. There was a little change in the samples; thus, indicating the presence of hydroxide and carbonate. The samples were titrated with 0.05M H_2SO4, until the colour disappeared and the titre values were recorded as F value. To the colourless solution, 3 drops of methyl orange indicator were added and titrated further until the colour change from yellow to permanent reddish or orange red colour and recorded as M. The readings were then computed..

CHLORIDE DETERMINATION

Using the 50ml sample for Alkalinity HCO^-_3 and CO^{2-}_3 determination, 1ml of potassium chromate indicator were added into the samples, and were titrated with silver nitrate solution, until a brick red colour appeared. The blank titration was also carried out.

SULPHATE DETERMINATION

Gravimetric method was used to determine sulphate using $Bacl_2$ as precipitant. 50ml of the sample were measured into a 250ml beaker, and diluted to 150ml with distilled water. 1ml concentrated (HCL), and 4 drops of methyl orange indicator were added. The samples were placed on hot plate. 10ml, of 10% Barium chloride solution were measured into them, and then boiled for 5minutes. The samples were then left overnight for filtration.

Phosphate Determination

2.5ml water was pipetted into 50ml standard flask, 8ml of ascorbic acid solution were added and made up to mark with distilled water, then, allowed to stand for 30minutes for colour development.

Sodium and Potassium Determination

Serial dilutions were made from the stock solutions (1000mg/L) of Sodium and Potassium and analysed by a Flame photometer model PF P7 Jenway. The operation procedure of the manufacturer was followed. After, the dilution of the samples, the fuel and flame adjustment control were set, compressor and equipment were put 'ON'. Appropriate filter was placed in position and the nebulizer tube was inserted into a beaker with distilled water, and aspirated for 15minutes. The control knob was use to adjust the blank to zero on the meter. The highest concentrations of the working standards were aspirated and the adjustments were done repeatedly for others, until a stable and agreeable emission results were recorded.

Toxic Metal Determination

Atomic absorption spectrophotometer (AAS) was used. The procedure for Atomic absorption spectrophotometer (AAS) is similar to flame emission spectrophotometer (FES). Standard solutions were prepared for each metal using suitable metals of each element to be determined. The instrument was switched 'ON' and the required lamp for each metal was fixed. The samples and the standards of each metal were aspirated simultaneously. The absorbance readings were then recorded under the same condition.

RESULTS

The physical appearances of the water samples were determined, all water samples were colourless and clear except ARM and IBK 2 water samples which were turbid and dirty as shown in Table 1.

Water from ILA and OKS had the lowest total bacterial count and most probable count of 0.2 $X10^5$cfu/ml and 3MPN/100ml respectively while AKA water sample had the highest total bacterial count and MPN value of 1.6 $X10^5$ cfu/ml and 10MPN/100ml respectively as indicated in Table 2.

Staphylococcus aureus was the most frequently isolated bacterium, closely followed by *Bacillus spp* as shown in Table 3. *Bacillus cereus* and *Staphylococcus aureus* showed high resistance to all antibiotics tested while *Proteus vulgaris* and *Salmonella paratyphi* were susceptible to most of the antibiotics in the antibiotic susceptibility test shown in Table 4. pH of water samples ranged between 6.54 and 7.26 which were close to neutrality, total hardness ranged between 171.96 and 327.33. The water sample from IBK 2 and AKA had high alkalinity of 189.1 and 207.14 respectively in the physicochemical properties of water presented in Table 5

Table 1: Physical Parameters of the Water Samples

Sample	Turbidity	Colour	Taste	Temperature ^{0}C	Odour
ILA	Clear	Colourless	Not determined	Ambient	Odourless
AKM	Clear	Colourless	Not determined	Ambient	Odourless
OKS	Clear	Colourless	Not determined	Ambient	Odourless
IBK (1)	Clear	Colourless	Not determined	Ambient	Odourless
AKA	Clear	Colourless	Not determined	Ambient	Odourless
ARM	Turbid	Dirty	Not determined	Ambient	Odourless
IBK (2)	Turbid	Colourless	Not determined	Ambient	Odourless

Key:

ILA= Ilale, AKM= Akunmi, OKS= Okusa, IBK(1)= Ibaka1, AKA= Akua, IBK(2)= Ibaka 2

Table 2: Total Viable Count and Most Probable Number (MPN) of Coliform per 100ml of Water Sample

Sample Code	Total Viable Count Cfu/ml	MPN Per 100ml
ILA	$0.2X10^5$	3
AKM	$0.4X10^5$	5
OKS	$0.2X10^5$	3
IBK (1)	$0.9X10^5$	9
AKA	$I.6X10^5$	10
ARM	ND	3
IBK (2)	ND	9

Key:

ILA= Ilale, AKM= Akunmi, OKS= Okusa, IBK(1)= Ibaka1, AKA= Akua, ND: not determined

Table 3: Frequencies of Occurrence of Bacteria Isolates in Water Samples

SAMPLE CODE	*Klebsiella pneumoniae*	*Bacillus subtilis*	*Salmonella paratyphi*	*Bacillus Cereus*	*Proteus vulgaris*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
ILA	1	1	0	0	0	1	0
AKM	2	0	2	0	0	0	0
OKS	0	0	0	2	1	0	0
IBK (1)	0	1	0	1	0	1	0
AKA	0	0	0	0	0	1	1

Key:

ILA= Ilale, AKM= Akunmi, OKS= Okusa, IBK(1)= Ibaka1, AKA= Akua

Table 4: The Antibiotic Susceptibility Pattern of Bacteria Isolates from

Borehole Water Samples

Antibiotics	Conc.(µg)	1	2	3	4	5	6	7	8
Streptomycin	30	_	_	10(mm)	_	_	_	_	_
Ciprofloxacin	10	11(mm)	_	12(mm)	_	_	_	_	_
Pefloxacin	30	_	11(mm)	9(mm)	_	_	_	_	_
Gentamycin	10	11(mm)	12(mm)	8(mm)	_	8(mm)	_	_	_
Amoxicillin	30	29(mm)	_	9(mm)	_	12(mm)	_	_	12(mm)
Chloramphenicol	30	_	_	_	_	_	_	_	_
Cefriaxone	30	_	_	_	_	_	_	_	_
Erythromycin	5	_	_	_	_	_	_	_	_
Co trimoxazole	25	_	_	_	_	_	_	_	_

Key:

1-*Klebsiella pneumonia*; 2-*Salmonella paratyphi;* 3- *Proteus vulgaris ;* 4- *Bacillus cereus;* 5-*Bacillus subtilis;* 6- *Pseudomonas aeruginosa;* 7-*Staphylococcus aureus*

Table 5: Chemical Characteristics of Borehole Water Samples

Chemical parametals	IBK(1)	IBK (2)	AKA	ILA	AKM	OKS
pH	6.54	7.32	7.52	7.26	6.88	6.88
Conductivity µs/cm	7.30	23.8	38.6	25.7	0.3	5.9
Turbidity (ntu)	0.003	0.002	0.004	0.001	43	44
Dissolved oxygen (mg/l)	47	45	46	42	0.003	0.003
Calcium (mg/l)	45.29	34.07	86.97	85.37	68.94	64.13
Calcium hardness $caco_3$ (mg/l)	113	85	217	213	172	160
Total hardness (mg/l)	171.96	140.70	327.33	317.81	299.21	262.52
Magnesium (mg/l)	13.67	21.63	23.33	19.44	58.32	38.39
Carbonate (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D

Bicarbonate (mg/l)	97.6	189.1	207.4	85.4	61	103.7
Hydroxide (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D
Total alkalinity ($caco_3$) (mg/l)	97.6	189.1	207.14	85.4	61	103.7
Carbonate alkalinity (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D
Chloride(mg/l)	152.44	109.90	155.98	127.62	180.80	145.35
Phosphate (mg/l)	0.78	0.55	0.48	₃.25	₃.05	0.96
Sulphate (mg/l)	0.23	0.24	0.27	0.21	0.24	0.22
Nitrate (mg/l)	0.02	0.01	0.04	0.06	0.06	0.15
Potassium (mg/l)	4.4	4.6	8.0	8.9	20.0	8.1
Sodium (mg/l)	20	26	42	38	38	30
Chromium (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D
Iron (mg/l)	0.51	0.49	0.48	0.45	0.48	0.58
Lead (mg/l)	B/D	0.01	0.01	B/D	B/D	0.01
Manganese (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D
Zinc (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D
Copper (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D
Cadmium (mg/l)	B/D	B/D	B/D	B/D	B/D	B/D

Key: B/D= Below Detection Limit

DISCUSSION

The *physicochemical* properties of water samples analysed showed that pH is close to neutrality and would allow the growth of most bacterial species. Most of the water samples had turbidity value within the WHO permissible value of 5NTU. Turbidity may be due suspended particles in water samples. All the conductivity values were below WHO permissible limit of 1000 µs/cm and agrees with the work of [19]. Dissolved oxygen was found to be within the range of 0.003 to 47 mg/L, this is far higher than what Barvin *et al* [19] reported. Very high levels of dissolve oxygen may exacerbate corrosion of metal pipes [20, 21]. Total hardness (Calcium and Magnesium) measured in the water samples were all below WHO limit of 500mg/L therefore the water are considered to be safe for drinking. The alkalinity of water samples ranges from 61 to 100.14mg/L, (except water samples from IBK (2) and AKA with 189.1 and 207.14mg/L respectively), which is still below 120mg/L value prescribed by WHO [21]. Water from IBK (2) and AKA may not be fit for drinking except some measures are urgently put in place. The level of nitrate, phosphate and sulphate in water sample was generally low, no amount of phosphate in water is believed to have effect on human health [20, 22]. Level of sulphate has no effect on adult but young children who are very sensitive to the element. People that are not used to drinking water with high level of sulphate can experience diarrhoea. Iron in the sampled water ranged between 0.48 and 0.58mg/L which were above WHO limit of 0.3mg/L. Though iron is required for healthy grow, high level of iron may be objectionable to consumer and hazardous to human health [6]. Chloride concentration ranges from 109.90 to

180.80mg/L which are below WHO recommended limit of 250mg/L, high level of chloride make water not palatable for drinking by imparting salty taste and may harm metallic pipe [9]. Sodium and potassium range from 20 to 42mg/L and 4.4 to 20mg/L respectively which were below the European Union recommendation of 200mg/L. [22]

The level of lead in all water samples were below detection limit except water from IBK (2), AKA and OKS which had 0.01mg/L each which is slightly higher than required standard. Further increase should be prevented as high level of lead in water may cause heath problem such as cancer.

The total viable count obtained from the samples examined were less than was obtained by Ogbulie *et al.,* [23] which was between 30 and 300 colony forming units. The most probable number (MPN) for the presumptive total coliform count of the water samples are acceptable for drinking according to Cheesbrough, [18] guideline for drinking water, but require regular surveillance as a control measure.

A total of seven bacterial species were isolated from the borehole water samples, some of which have been incriminated in various disease conditions. *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus* have been implicated in gastrointestinal disorder and associated symptoms [18]. *Salmonella paratyphi*, is the causative agent for paratyphoid fever, a mild form of enteric fever [24, 25]. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumonia* are common causes of urinary tract and wound infections where they cause acute or chronic infections [26]. Organisms like *Klebsiella pneumoniae* and *Proteus vulgaris* were also identified to account for up to 55% of nosocomial infections in some parts of Nigeria [3]. The presences of coliforms, which are used as indicator to the likely presence of entero pathogens in water, though at an acceptable level, pose some worries to the researchers who therefore suggest regular surveillance of these water sources.

There was general resistance to chloramphenicol, Cotrimoxazole , erythromycin and ceftriazone. This calls for more serious concern because these are drugs (except cotrimoxazole) that are rarely abused especially ceftriazone which is an injectable drug. However, similar resistant pattern has been reported by Bello et al, 2013. Multiple resistance was equally demonstrated by *Pseudomonas aeruginosa, Bacillus subtilis and Bacillus cereus,* these equally agrees with the works of Iyasele *et al*.,[27] Adekoyeni and Salako [25] . The variation in susceptibility and resistance of the isolates to different antibiotics could be attributed to the difference in location of the sample sources and drug resistance transfer among the microorganisms within the communities where the boreholes are located [28].

Conclusion.

The study suggested that as at the time of study, the water samples can be considered safe for human consumption, however, regular assessment of all the parameters mentioned in this study is advocated.

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