Evaluation of the Effectiveness of the Treatment of Bonny pipe borne water.

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ABSTRACT

The research was aimed at evaluating the microbiological, physical and chemical quality of Bonny Pipe borne water. The pipe borne water from Bonny Island in Rivers State was screened to evaluate the effectiveness of its treatment by Bonny water Board Company. The microbiological status of the water was determined by using basic growth media to isolate microorganisms present in the water and identifying the organisms using its morphological characteristics. Antibiotic susceptibility was then carried out to ascertain the resistance and sensitivity of the isolates. Swabs of the water outlet taps were also taken at each point of collection from source to delivery. The results were compared with the microbiological status of Omoku's pipe borne water. Chemical analysis was also carried out on the Bonny pipe borne water to determine the level of chemical contamination. The Bonny pipe borne water complied with the microbiological regulations of WHO as there was no bacterial and fungal growth on any of the media used. However, the total heterotrophic bacteria count of the Omoku's water was 2.10 × 10^2 thus exceeding the limit of 1.0 × 10^1 cfu/ml of water, the MPN count for total coliforms was 5MPN/100ml, there was no Faecal coliform present and isolated organisms were *Vibrio* sp., Micrococcus sp., *Salmonella* sp., and *Bacillus* sp.,. The Physicochemical composition analysis revealed the following result for untreated and treated Bonny pipe borne water samples respectively: pH (7.0 and 7.5), Conductivity (60 mg/L and 268mg/L),Total Dissolved Solids (30mg/L and 34mg/L), total hardness (12.012mg/L and 120.12mg/L), chloride (10mg/L and 12mg/L) and Chlorine (Nil and 7.1mg/L) amongst others. The chlorine level for the treated water was above the regulatory standard of WHO (5mg/L), this resulted in increased hardness, taste and odour of the water, which were also above WHO's regulatory limits. Therefore, the Bonny water is not within the regulatory standard of potable drinking water.

Keywords: Pipeborne Water, Bonny Island, Physicochemical parameters, Most Probable Number.

INTRODUCTION

Water plays a significant role in nourishing the body, making it very essential for the human body. In Bonny Island, situated at the southern part of Rivers State in Nigeria, the main source of drinking water is pipe borne water supplied to its indigenes by the Bonny Utility Company (BUC). Hence, knowledge on the microbiological, physical and chemical parameters of the drinking water is essential, as when impure and unsafe water is ingested can cause mild to severe water borne diseases. Thus, it is essential to determine if the indigenes of Bonny Island are supplied with safe and good quality pipe borne drinking water.

The quality of the water gotten from the source, is a major factor in the quality of drinking water. The treatment process also plays a major role in the quality of drinking water as well as the tanks, water distribution system and household filters used for storage. (Peiyue L. & Jianhua W. 2019).

The scope of the work encompasses the microbiological, physical and chemical analysis of Bonny pipe borne water, which passes three basic treatment process. The water is transported from the borehole to the filtration tank (pressure filter) through the connecting lines (high pressure pipes), thereafter air is introduced to it through the aeration line to enable the separation of irons. The water is then filtered in the filtration tank that contains gravel, fine sand and nevtraco ($CaCO_3$), which helps to trap the irons until it's time to wash the tank. Chlorine is then added to the water in the overhead tank which is the last step in the treatment process.

Water samples were collected at four different outlets, swabs of the water outlet taps were also taken at each point of collection from source to delivery. Antibiotic susceptibility testing was then carried out to ascertain the resistance and susceptibility of the test isolates. Physicochemical analysis was also carried out on Bonny pipe borne water. The aim of the study is to evaluate if the Bonny water board's treatment process of the pipe borne drinking water is right, and determine if the treatment process is within required standards.

MATERIALS AND METHODS

Description of Study Area

This study was carried in Bonny Local Government Area of Rivers State, Nigeria. This study area was chosen in order to ascertain the quality of pipe borne drinking water supplied to the indigenes of the area.

Sample Collection

Samples were collected at four different water outlets in Bonny. The first water source was the untreated water outlet from the company's reservoir, the second source was from the treated water outlet from the company's reservoir, the third water source was from a household in the island and the fourth water source was from a community tap. A total of four water samples were taken.

MICROBIOLOGICAL ANALYSIS

Water

Nutrient agar, potato dextrose agar, Centrimide agar, Eosin Methylene Blue agar, Thiosulfate-citrate-bile salts-sucrose agar (TCBS) agar and Salmonella Shigella agar were prepared according to manufacturer's instructions for each of the water sample. Sterile Petri dishes were arranged on the work bench and labeled accordingly. 1ml of the sample was poured directly without diluting into the sterile Petri dish and the growth media was poured and allowed to solidify. The same procedure was repeated for each sample and growth media. The inoculated Petri dishes were incubated for 24 hours a**t** 37^{o}C.

Swab

After the prepared sterile agar plates were properly dried, the sterile swab sticks which were used to swab the water tap at each distribution point were used to make a smear on the prepared sterile, Nutrient agar, Potato Dextrose agar, Centrimide agar, Eosin Methylene Blue agar, TCBS agar and Salmonella Shigella agar plates, and then a wire loop was aseptically used to streak from the point the smear was made. This same procedure was repeated for the rest of the samples, labelled respectively and incubated for 24 hours at 37^{o}C.

Most Probable Number (MPN)

Presumptive Test for MPN of coliforms

This is used to enumerate and identify the presence of Coliform organisms in the water sample. Lactose broth was prepared in three McCartney's bottles respectively, Durham tubes were inserted in an inverted position and the broth was autoclaved and allowed to cool. 0.1ml (Single Strength), 1ml (Single strength) and 10mls (double strength) of water sample were inoculated into three of bottles respectively, labeled properly and incubated for 24 hours at 37^{o}C.

Characterization and Isolation of the Tested Organisms

Distinct colonies were picked from the incubated plates after 24hrs and characterized morphologically. The distinct colonies were then sub-cultured onto prepared nutrient agar plates and incubated for 24hours at 37^{o}C, to obtain pure cultures of the organisms (Taylor, 2008).

Preservation of pure culture

The pure cultures were stored in nutrient agar slants within McCartney's bottles at -4^{o}C in a freezer.

Identification of the Bacterial Isolates

Identification of the bacterial isolates was carried out through biochemical test such as Catalase, Indole test, oxidase, Citrate Utilization test, Methyl red, Voges Prokauer test, while sugar fermentation test was used to confirm the test organisms (Cheesbrough, 2006).

Antibiotics Susceptibility Testing

The recovered organisms were subjected to antimicrobial susceptibility testing using several antibiotics according to their gram's reaction. For the Gram negative isolates; Tarivid (OFX) 10mcg, Reflacin (PEF) 10mcg, Ciproflox (CPX) 10 mcg, AUG mention (AU) 30mcg, Gentamycin (CN) 10mcg, Streptomycin (S) 30mcg, Ceporex (CEP) 10mcg, Nalidixic acid (NA) 30mcg, Septrin (SXT) 30mcg and Amplicin (PN) 30mcg. For the Gram postive isolates; Ciproflox (CPX) 10 mcg, Norfloxacin (NB) 10mcg, Gentamycin (CN) 10mcg, Amoxil (AML), Streptomycin (S) 30mcg, Rifampicin (RD) 20mcg, Erythromycin (E) 30mcg, Chloramphenicol (CH) 30mcg, Ampiclox (APX) 20mcg, Levofloxacin (LEV) 20mcg. A sterile swab stick was used to collect fresh inoculum from a 24 hours culture corresponding to 0.5 McFarland turbidity standard and swabbed evenly across the plates. The antibiotic disc was placed on the surface of the inoculated Nutrient agar plates with the use of sterile forceps. The plates were reversed within 30 minutes of applying the disc and incubated for 24hours at 37^{o}C, the zones of inhibition were read and classified as susceptible, intermediate, or resistant.

PHYSICOCHEMICAL ANALYSIS

Different analytical methods were used to ascertain the presence and amount of certain chemicals in the water samples. Physical parameters like taste, odour, appearance and clarity of the water samples were determined by Physical observation while Chlorine, Chloride, total hardness and nitrate levels were determined by titrimetric methods.

For heavy metals analysis, Atomic Adsorption Spectrophotometer was used to determine the presence of lead, iron and zinc.

RESULTS AND DISCUSSION

Physicochemical Properties of Pipe-borne Water Samples

The physiochemical analysis carried out on pipe-borne water sourced from Bonny for both treated and untreated water revealed that untreated water was more turbid than the treated water. However, the pH, Conductivity, Total Dissolved Solids (TDS), total hardness and chlorine levels for treated water was more than the untreated as the pH levels for untreated and treated water samples were (7.0 and 7.5), Conductivity (60 mg/L and 268mg/L),Total Dissolved Solids (30mg/L and 34mg/L), total hardness (12.012mg/L and 120.12mg/L), chloride (10mg/L and 12mg/L) and chlorine (Nil and 7.1mg/L) respectively. The chlorine level in the treated water was higher than the untreated water. Results for other physicochemical parameters analysed are reported in table 1, in which there are untreated and treated water values versus the World Health Organization (WHO) standards in 3rd column

Table 1: Physicochemical Properties of pipe borne Water Samples

Parameter	Untreated	Treated	WHO/FM.Env.
Apperance	Turbid	Clear	**Clear**
Odour	Odourless	Present	**Odourless**
Colour	Brown	Colourless	**Colourless**
Taste	Present	Present	**Tasteless**
Particles	Present	Absent	**Absent**
pH	7.0	7.5	**6.5 – 8**
Total Dissolved Solids (TDS), (mg/L)	30	134	**600**
Conductivity(mg/L)	60	268	**300**

Alkalinity(mg/L)	13.38	4.48	**100**
Salinity (PPT)	0.03	12.85	**100**
Turbidity(NTU)	8.00	3.00	**5**
Total Hardness (mg/L)	12.012	120.12	**100**
Chlorine (mg/L)	Nil	7.1	**5**
Chloride (mg/L)	10	12	**250**
Sulphate (mg/L)	4.92	0.27	**N**
Nitrate (mg/L)	0.85	0.75	**50**
Phosphate (mg/L)	2	1.11	**N**
Zinc (mg/L)	1	-2.15	**N**
Iron (mg/L)	24.55	4.4	**N**
Lead (mg/L)	Nil	Nil	**10**

Key: N = No guideline value WHO(2006).

Results gotten for the total heterotrophic bacterial count revealed that both treated, untreated, community and household water samples sourced from Bonny had no growth on general purpose media. However, sample Sourced from Omouku had a Total Heterotrophic Count (THBC) of $2.0x10^3$ cfu/ml.

Table 2: Total Heterotrophic Bacteria Counts Of Pipe-Borne Water Samples.

Sample cite	Cfu/ml	Log (Cfu/ml)	WHO Standard
H	-	0	$<1.0x10^1$
C	-	0	
U	-	0	
T	-	0	
O	$2.0x10^3$	3.301	

Key= H= Household water; C = Community water; U= Untreated water; T = Treated water; O= Omoku water

Pseudomonas sp. was not present in any of the samples studied.

Counts obtained on Salmonella Shigella agar was 6.5x101 for the sample sourced from Omouku while all the samples from Bonny were negative for *Salmonella* sp.

Culture done on Thiosulfate-citrate-bile salts-sucrose (TCBS) showed negative for all the samples from Bonny but the sample sourced from Omouku had *vibrio* sp. Count of 6.5x10.No E. coli was found in any of the samples studied. Only the sample sourced from Omouku had a count of $1.35x10^2$ on PDA. All other samples were negative.

The most probable number for determining total coliform revealed that all samples from Bonny had less than 2 MPN/100ml while the Omouku sample had 5 MPN/100ml. None of the samples was positive for fecal coliform.

Swabs done on point of contact (tap handle) showed that the household and community taps had no heterotrophic bacteria, pseudomonas, salmonella, E. coli and fungi. However, untreated and treated samples had counts of $1.2x10^3$ and $1.54x10^3$ cfu/$5cm^2$ for THBC. Only the treated swab sample had $1.0x10^2$ and $3.0x10^1$ cfu/$5cm^2$ for pseudomonas and salmonella. Swabs gotten from untreated and treated had fungal count of $1.2x10^2$ amd $6.0x10^1$ cfu/$5cm^2$. In Figure 1 are reported a comparison of organisms at each site.

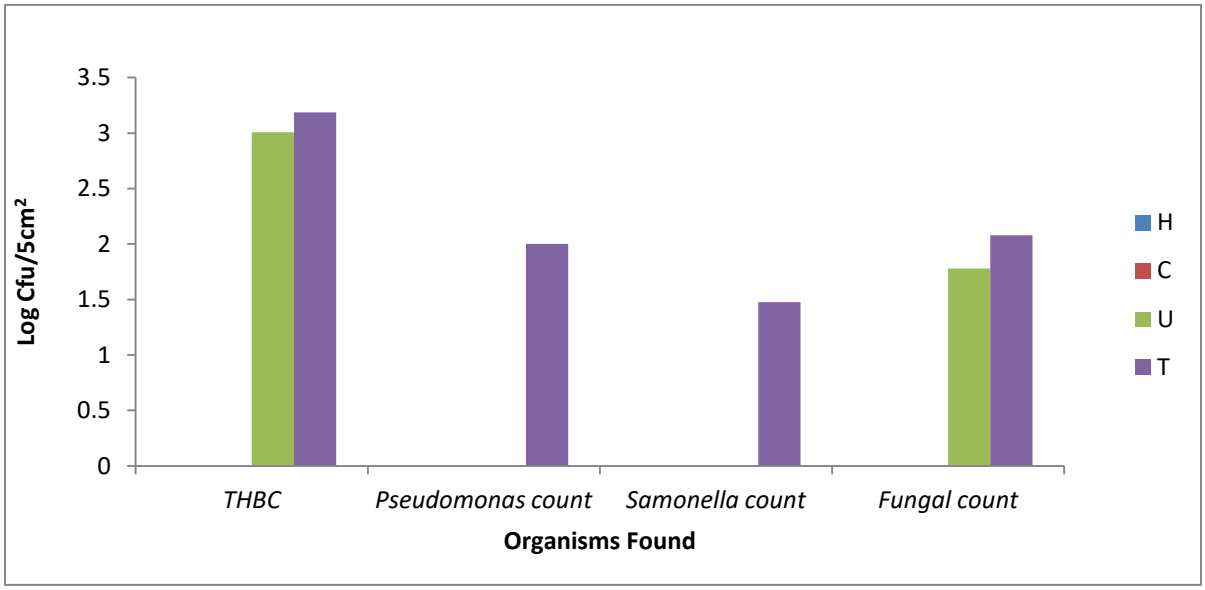

Fig. 1: Microbial Counts Obtained from Point of contamination Swabs in Bonny samples.

Isolated bacterial species and their morphological characteristics are shown in Table 3. From the results obtained, it was observed that the swabs taken at the untreated and treated water sources were contaminated by common microorganisms (*Proteus sp. and Micrococcus sp.*). There was

no growth of microorganisms form swabs taken at the community and household water outlets, this could be due to constant flow and pressure of the water from the outlets. and there was a prevalence of Gram positive organisms in Omoku's water.

	Isolate code	**Colour**	**Size (mm)**	**Shape**	**Margin**	**Opacity**	**Elevation**	**Surface**	**Texture**	
1	SU1	Yellow	0.5	Round	Entire	Opaque	Convex	Shiny	Smooth	*Staphylococcus sp.*

Table 3: Morphological Characteristic Isolates Obtained from Pipe-borne Water Swabs.

2	SU2	Cream	2.0	Round	Irregular	Opaque	Convex	Dull	Mucoid	*Proteus sp.*
3	SU3	Yellow	3.0	Round	Entire	Opaque	Flat	Dull	smooth	*Micrococcus* sp.
4	ST1	Cream	0.5	Round	Irregular	Opaque	Convex	Shiny	Smooth	*Proteus sp.*
5	ST2	Yellow	0.5	Round	Irregular	Opaque	Flat	Shiny	Smooth	*Micrococcus* sp.
6	ST3	Cream	0.8	Round	Irregular	Opaque	Convex	Dull	Mucoid	*Salmonella* sp.
7	OW1	Cream	0.5	Round	Entire	Opaque	Puncti form	Shiny	Smooth	*Micrococcus sp.*
8	OW2	White	2	Round	Entire	Opaque	Convex	Shiny	Smooth	*Bacillus* sp.
9	OW3	Cream	0.5	Round	Entire	Opaque	Flat	Shiny	Smooth	*Salmonella* sp.
10	OW4	Cream	0.5	Round	Irregular	Opaque	Flat	Shiny	Smooth	*Vibrio sp.*
11	OW5	Yellow	1	Round	Convex	Opaque	Puncti form	Dull	smooth	*Micrococcus* sp.

Key= SU1 – SU2 = Swab Untreated, ST1-ST2= Swab of Treated, OW1-OW2= Omoku water

The isolates showed different occurrence frequencies in all samples as shown in Fig 2. *Micrococcus sp* which are non-motile, were more predominant in samples gotten from swabs at

the water outlets,while Salmonella sp. which are mostly motile were more predominant from water sample gotten from Omoku's water.

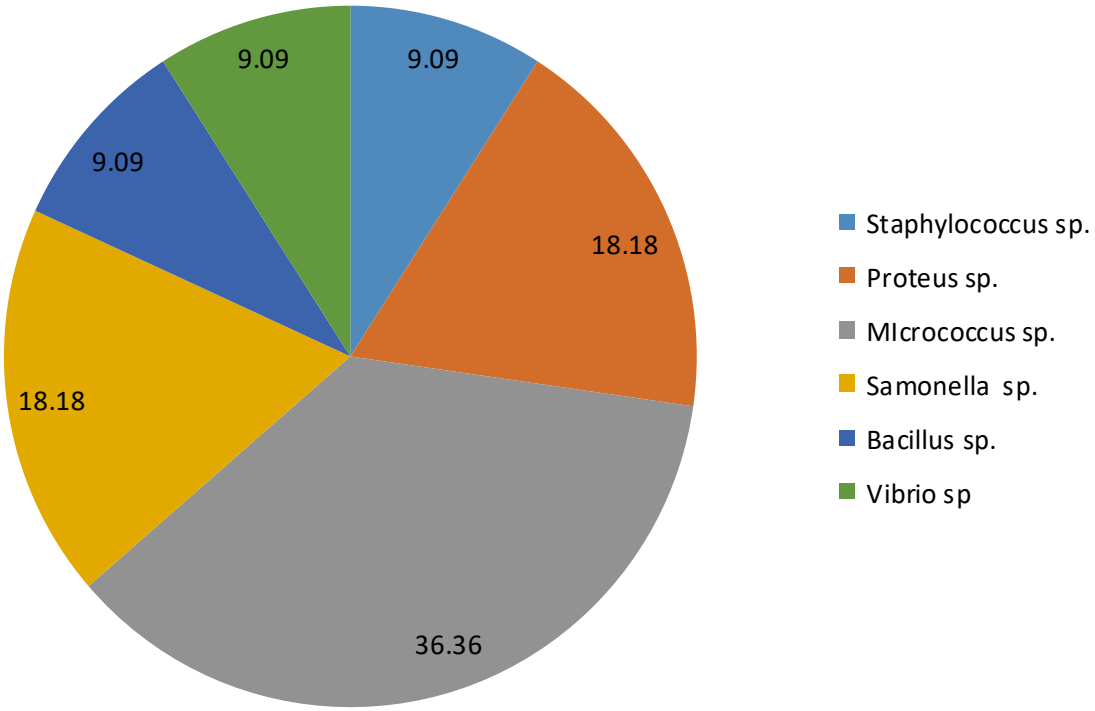

Fig 2: Percentage Occurrence of Bacterial Isolates Obtained from Pipe-borne Water and Swabs

In Table 4, the antimicrobial susceptibility pattern of Gram Positive isolates from samples were shown. The antibiotics susceptibility pattern of the isolates revealed that most Gram's positive isolate

were sensitive to all the antibiotics tested and *Bacillus sp.* was the only resistant specie as it was resistant to erythromycin.

Table 4: Antimicrobial Susceptibility Pattern of Gram Positive Isolates from Pipe-borne Water and Swabs

Sample code	Organism	CN	AML	RD	S	NB	CH	CPX	E	LEV	APX
SU1	*Staphylococcus sp.*	S	S	I	S	S	S	S	S	S	S
SU3	*Micrococcus sp.*	S	S	I	S	S	S	S	S	S	S
ST2	*Micrococcus sp.*	S	S	I	S	S	S	S	S	S	S
OW1	*Micrococcus sp.*	S	S	I	S	S	S	S	S	S	S
OW2	*Bacillus sp.*	S	S	I	S	S	S	S	R	S	S
OW5	*Micrococcus sp.*	S	S	I	S	S	S	S	S	S	S

Key: SU= Swab of Untreated tap, ST= Swab of Treated tap, OW = Omoku Water, S= Sensitive, I= Intermediate, R= Resistant. CN= Gentamycin, AML = Amoxil, RD= Rifampicin, S= Streptomycin, NB = Norfloxacin, CH= Chloramphenicol, CPX= Ciproflox, E = Erythromycin, LEV = Levofloxacin, APX = Ampiclox.

In Table 5 is reported the antimicrobial susceptibility pattern of Gram negative Isolates. The Gram's negative isolates were resistant to PN and NA except for salmonella that was sensitive for PN.

Table 5: Antimicrobial Susceptibility Pattern of Gram Negative Isolates from Pipe-borne Water and Swabs

Sample code	Organism	CN	AU	CPX	SXT	S	PN	CEP	OFX	NA	PEF
SU2	*Proteus sp.*	S	S	S	S	S	R	S	S	R	S
ST1	*Proteus sp.*	S	I	S	S	S	R	I	S	R	I
ST3	*Salmonella sp.*	S	I	S	S	S	S	S	S	R	S
OW3	*Salmonella sp.*	S	I	S	S	S	S	S	S	R	S
OW4	*Vibrio sp.*	S	S	S	S	S	I	I	S	S	S

Key: SU= Swab of Untreated tap, ST= Swab of Treated tap, OW = Omoku Water, S= Sensitive, I= Intermediate, R= Resistant. CN= Gentamycin, AU= Augmentin, CPX= Ciproflox, SXT = Septrin, S = Streptomycin, PN = Amplicin, CEP = Ceporex, OFX = Tarivid, NA= Nalidixic acid, PEF = Reflacine.

The Physicochemical analysis of the treated and Untreated Bonny pipe-borne water shows that the untreated water had some parameters that exceed the limits specified by regulatory bodies (WHO). Specifically, the pH, conductivity, total dissolved solids, alkalinity, salinity, turbidity, chloride, iron, sulphate, phosphate, lead, zinc and nitrate levels were within standard while odour, taste, chlorine and total Hardness of the treated water were above the regulatory limits and was as a result of chlorine used during treatment. According to WHO these parameters that were above the standard are needed in monitoring the quality of community drinking water as such water could be rejected.

The microbiological quality of the Bonny pipe-borne water were within regulatory standards. However, this could be due to the high chlorine level in the treated water which could be causing some toxicity to the body as revealed by the chemical analysis result. No faecal coliform was

found in the water samples analyzed. However pathogenic organisms were found in Omoku water samples. Therefore, reliance of Omouku people on tap water sources, may cause serious health problems if proper treatment facilities do not begin to function properly and proper personal hygiene are not practiced. Like the general saying "water is life", but microbiologically speaking, "quality water is life".

Characterisation of Microorganisms isolated from swab and water samples revealed the following genera; *Staphylococcus* sp., *Micrococcus* sp., *Proteus* sp., *Salmonella* sp., *Bacillus* sp., and *Vibrio* sp.

CONCLUSION

Further research such as Animal studies should be done to understand the harmful effects and toxicity of consumption of Bonny pipe-borne water on human body.

Measures should be put in place to ensure proper quality control of the Bonny pipe-borne water to ensure that the water meets regulated standard. There should be regular monitoring and also inspection by regulatory bodies, regulatory bodies should also ensure they sanction defaulters of the existing water quality standard. Under-ground pipes should be properly checked for breakages that may lead to microbial contaminations.

To determine the actual biocide inhibiting microbial growth in the untreated water, the volatile and non-volatile components of the water should be analyzed using High Performance Liquid Chromatography Methods and Gas Chromatography-Mass Spectrometry (GC-MS). Also, to ascertain the absence of Microorganisms in the water, the total Viable but non-cultural Microorganisms should be determined using appropriate techniques.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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