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Research Article

BIO- PHYSICOCHEMICAL ASSESSMENT OF SACHET WATER SOLD IN OGOJA METROPOLIS, NIGERIA

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ABSTRACT
In the Bio-physicochemical assessment of sachet water sold in Ogoja Metropolis, twenty samples of four (4) different brand of packaged sachet water labelled A- D, commonly found in Ogoja metropolis of Cross River State, Nigeria were examined for microbiological and physicochemical properties to determine their portability. Standard /conventional methods were employed for the detection of coliforms and other bacteria. Physical examinations were rigorously carried out for assessment of organoleptic attributes such as taste, colour and odour. Microscopic examinations for sediments and other debris were also carried out. Microbiological examination revealed the presence of the following pathogens, *Proteus mirabilis*, *Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia spp* and *Chromo bacterium spp* at varying concentrations. Chemical analysis revealed the presence of metals ranging from Sodium (Na), Potassium (K) and Zinc (Zn) in all the samples, Lead (Pb), Copper (Cu), and Chromium (Cr) in some of the samples and absence Cadmium (Cd) was apparent from the study. Other chemical compounds such as sulphates, chlorides, nitrates were detected at tolerable limits. Physical examination of samples showed a variable level of turbidity, colour, pH, hardness, acidity and alkalinity. The result also revealed variable level of taste but none had objectionable odour. The microbiological and physicochemical indices of contamination detected from some samples are indications that some of these sachets of water do not meet the NAFDAC/WHO standard and so may not be portable. They may also serve as possible sources of waterborne related diseases within Ogoja metropolis.

Keywords*: Microbiological, Organoleptic, Physicochemical, Portability, Water-borne*

INTRODUCTION
The availability and accessibility of fresh clean water is a key to sustainable development and an essential element in health, food production and poverty reduction (Adekunle *et al.,* 2004).Water constitutes four fifths of the body's weight and performs various biological roles and supports the internal functions of animals and plants. It is necessary for proper digestion of food and flushing toxins out of the body (Atlas, 2009). Recent statistics show that 1.2 – 2.4 billion people suffer from lack of portable water and secure sanitation respectively. Nigeria in particular is one of the developing countries in which more than half of the population lacks access to potable water. Access to potable water, more than any environmental factor, is a right to all human beings and not a privilege. Since life is impossible without water people are compelled to accept and use water from whatever source despite the devastating consequences of polluted water on human health (Steiner *et al*., 1997). Water has found its widest use in the industry as a medium of heat transfer and heat exchangers. It also functions as raw material in the beverage and chemical industry.
Report by Food and Agricultural Organization (FAO), 2005 revealed that in African countries particularly Nigeria, water related diseases have been interfering with basic human development. Degradation of water quality erodes the availability of water to the ecosystem, increasing financial cost for human users, and decreasing species diversity and abundance of resident communities.
The provision of potable water to the rural and urban population is necessary to prevent health hazards. Before water can be described as potable, it has to comply with certain biological, physical, chemical, and microbiological standards which are designed to ensure that the water is potable and safe for drinking.

Portable water is defined as water that is free from disease-producing micro-organisms and chemical substances deleterious to health (Edet *et al*., 2011)

There are several sources that water can be obtained from among which includes streams, lakes, rivers, ponds, rain, springs and well, but unfortunately clean, pure and safe water only exist briefly in nature and is immediately polluted by prevailing environmental factors and human activities (Kolade *et al*.,1998). Water from most sources is therefore unfit for immediate consumption without some sort of treatment otherwise it does not meet the W.H.O standard. These water bodies are closely interconnected and may affect each other directly though they have different hydrodynamics (Raymond, 1992). At a certain level, minerals may be considered contaminants that can make water unpalatable or even unsafe. These substances can be the result of human activities or can be found in nature. Deep ground water is generally of very high bacteriological quality (i.e. pathogenic bacteria or the pathogenic protozoa are typically absent), but the water may be rich in dissolved solids, especially carbonates and sulphate of calcium and magnesium. There may be a requirement to reduce the iron or manganese content of this water to make it acceptable for drinking, cooking, and laundry.

Consequently, a number of small scale industries are packaging and marketing factory-filled sachet drinking water, popularly called "pure water" that many consider a safer source of portable water (Dodoo *et al.,* 2006). Those who cannot afford the factory bagged sachet go for cheaper unbranded "ice water" which is hand-tied pipe borne water (Obiri-Danso *et al*., 2003)

The National Agency for Food and Drug Administration and Control (NAFDAC) is mandated to enforce compliance with internationally defined drinking water guidelines, but regulation of the packaged water industry aimed at good quality assurance has remained a challenge to the agency. To control this menace of contaminated water in sachets, NAFDAC declared a possible 'gradual' nationwide ban on sachet water to allow manufacturers of sachet water to start winding down or change to bottle packaging. Successful implementation of this ban has remained far from reality as the sachet water market is witnessing tremendous growth, especially among the poor and middle social classes. Because of the high use of sachet water utilized on daily bases in Cross River State, the State Government in 2005 commissioned the Cross River State Water Board Commission (CRSWBC) to take care of portable water within the state which Ogoja metropolis is not an exception.

The risk of infection and diseases as well as the public health burden caused by pathogens (Rahman *et al*., 2001) has therefore made access to safe water supply a serious issue across the globe. It becomes imperative to determine the biophysiochemical state of some sachet water sold within Ogoja metropolis in Cross River State, Nigeria.

MATERIALS AND METHODS

Study Site and Sample Collection

A total of 20 samples of sachet water from four (4) companies were purchased and analysed within 8 hours of collection. Sachet water samples were aseptically collected from the sources using sterile bottles. The water samples were kept between the temperature of 4 -10 ^{0}C and transported to the laboratory less than two hours of collection and analyzed within 24 hours. A total of twenty water samples were collected from Ogoja metropolis of Cross River State between the hours of 8.00 a.m. and 10.00 a. m, when the sampling points were free from contaminations

The biophysical examination of sachet water samples

The water samples were examined for pH, turbidity, odour, taste, presence of sulphate, phosphate, chloride, conductivity, appearance, magnesium, calcium, fluoride, total hardness, total dissolved solid and bacterial load of sachet water e.t.c. odour, sliminess and taste were evaluated by perception with the sense organs upon opening each sample while pH was determined by a combined glass electrode and pH meter (Mettle-Toledo, Essex M3509 Type 340).Sachet water contains heavy metals and consumers may be exposed to this hazard (Orisakwe *et al*.,2012).

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 microorganisms 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 mL of the prepared molten media were added. The cultured plates were allowed to cool and solidify then, they were incubated at 37 ^{0}C 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 (2000)

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 ^{0}C for 24 hours. 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 24 hours at 37 ^{0}C and observations recorded.

pH/ conductivity determination

The pH was determined using Electronic pH meter (Digital) Model Exner GMBH, D4040 NEUSSI with a combined electrode. Known buffer solutions of pH 4, and pH 9 were prepared and used to standardize the equipment. The pH readings were taken and recorded, while samples conductivity was measured using conductivity meter (Radio-meter Copen-Hagen CDM 83).

Alkalinity determination

Fifty (50) mL of the water sample were pipette into clean 150 mL capacity conical flask and three drops of phenolphthalein indicator added. There was a little change observed, indicating the presence of hydroxide and carbonate. The samples were titrated with 0.05M H_2SO_4, until the colour disappeared and the titre values recorded as F value. To the colourless solution, 3 drops of methyl orange indicator was added and further titrated until the colour changed from yellow to permanent reddish or orange red colour and recorded as M. The readings were then computed.

Chloride determination

Using the 50 mL sample for alkalinity HCO_3 and CO_2- determination, 1mL of potassium chromate indicator was added into the samples, and 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. 50 mL of the sample was measured into a 250 mL beaker, and diluted to 150 mL with distilled water. 1 mL concentrated (HCl), and 4 drops of methyl orange indicator was added. The samples were placed on hot plate and 10 mL of 10% barium chloride solution was measured into them and boiled for 5 minutes. The samples were then left overnight for filtration.

Sodium and potassium determination

Serial dilutions were made from the stock solutions (1000 mg/L) of sodium and potassium and analyzed by a Flame photometer model PFP7 Jenway. The operational procedure of the manufacturer was followed. After the dilution of the samples, the fuel and flame adjustment control were set, compressor and equipment on. The appropriate filter was placed in position and the nebulizer tube was inserted into a beaker with distilled water, and aspirated for 15 minutes. The control knob was used 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.

Heavy metal determination

Atomic Absorption Spectrophotometer (AAS) was used. The procedure for 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.

STATISTICAL ANALYSIS

All determinations were done in triplicates and data obtained were presented as mean ± SEM using Microsoft Excel 2007.

RESULTS

The results of the physicochemical properties were within the tolerable limit of WHO/NAFDAC standards for portable water except for temperature and alkalinity which were above the tolerable limits (Table 1). Though Cadmium and Chromium were not detected in most of the samples, it appears some of the samples were contaminated with Zinc and Lead causing the rise in their concentration above the WHO/NAFDAC standards. All other heavy metals were within the limits (Table 2). The determination of microbial load of the samples showed the presence of *Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia spp, Chromobacterium spp at varying concentrations* (Table 3)**.**

DISCUSSION

The aim of introducing sachet water to consumers was to provide safe, hygienic, affordable and instant drinking water to the public. Although this is a laudable idea, current trend seem to suggest that sachet drinking water could be a route for transmission of most water borne diseases and metal toxicity. The assessment of mineral levels showed high levels of lead (Pb) in samples A and D, while the others were within the WHO/NAFDAC standard. High levels of lead have been associated with lead toxicity whose primary site is the central nervous system and peripheral nervous system (Wartburton *et al*., 1998). The presence of Pb in the central nervous system reduces neuro-psychological functioning leading to drastic reduction in intelligent quotient (Wartburton *et al*., 1998). Lead in amounts over the primary drinking water standard of 0.05mg/L may cause nervous system disorders and brain or kidney damage. Since lead accumulates in the body tissue, it is especially hazardous to the foetus or to children under three years of age. Appropriate interventions are required to minimize heavy metal contamination of underground water (Wartburton *et al*., 1998). The most likely cause for the high levels of Pb in the samples described above may be traceable to the source in which they were obtained, the materials used in the production process and the storage materials of the water before being transferred into "sachet bags". However, the physicochemical nature of the water samples which is very important in determining the potability of water was within standard acceptable limits.

Table 1.The physicochemical properties of some sachet water in Ogoja metropolis of Cross River State, Nigeria

Physicochemical parameters	Sample A	Sample B	Sample C	Sample D	WHO/NAFDAC Standard
Taste	Satisfactory	Satisfactory	Satisfactory	Satisfactory	Unobjectionable
Temperature ^{0}C	28.6± 0.01	29.7± 0.02	28.3± 0.01	29.3± 0.01	Ambient
Conductivity µScm^{-1}	120± 0.03	98± 0.01	136± 0.02	138± 0.03	1000
pH	7.3± 0.03	9.0± 0.01	7.1± 0.01	7.5± 0.03	6.5-8.5
Odour	Satisfactory	Satisfactory	Satisfactory	Satisfactory	Unobjectionable
Colour	Colourless	Colourless	Colourless	Colourless	Unobjectionable
Turbidity (NTU)	0.31± 0.01	0.24± 0.04	0.45± 0.02	0.33± 0.01	5.0
Appearance	Satisfactory	Satisfactory	Satisfactory	Satisfactory	Unobjectionable
TDS (ppm)	42.4± 0.03	38.7±0.02	33.8±0.06	45± 0.03	500
Alkalinity (mg/L)	65± 0.01	245±0.04	54.3±0.03	58.7±0.01	98-200
Hardness (mg/L)	73± 0.03	82.3±0.02	77.5±0.03	78 ±0.06	61-150
Nitrates (mg/L)	23.2± 0.01	19.4±0.03	21.3±0.01	18.2±0.03	50
Chloride (mg/L	66.4± 0.01	54.6± 0.01	47.8± 0.03	55.6± 0.02	250
Sulphates (mg/L)	45.5± 0.03	47.3± 0.01	32.5± 0.02	26.8± 0.01	10-100

Values are presented as mean ± SEM compared with WHO/NAFDAC standards

Table 2: Distribution of Light and Heavy Metals in Some Sachet Water Sold in Ogoja Metropolis of Cross River State, Nigeria

Parameters	Sample A	Sample B	Sample C	Sample D	WHO /NAFDAC Standard
Na	10.3± 0.10	12.5± 0.05	19.6± 0.01	7.8± 0.21	200
K	0.93± 0.01	1.33± 0.04	3.2± 0.03	2.5± 0.01	12.0
Cu	ND	ND	0.21± 0 .01	ND	1.3
Pb	1.2± 0.03	ND	ND	0.1± 0.04	0.010
Zn	0.2± 0.01	0.42± 0.02	0.33± 0.02	0.31± 0.01	3.00
Cr	ND	ND	0.23± 0.03	0.5± 0.01	0.05
Cd	ND	ND	ND	ND	0.003

Values are presented as mean ± SEM compared with WHO/NAFDAC standards; ND = not detected

Table 3: Distribution and Frequency of Occurrence of Microorganisms Isolated From Some Sachet Water Sold in Ogoja Metropolis of Cross River State, Nigeria.

Microorganisms	Sample A	Sample B	Sample C	Sample D

Serratia spp	-	-	+	+
Proteus mirabilis	-	-	+	+
Klebsiella pneumonia	+	+	+	+
Chromobacterium spp	+	-	-	+
Pseudomonas aeruginosa	-	-	+	-

Key: *- = not detected, + = present*

Turbidity for example, refers to water clarity. The greater the amount of suspended solids in the water, the more turbid it appears, and the higher the measured turbidity. Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and bacteria (Dinrifo *et al*., 2010). The turbidity values fell within the WHO standard. This suggests that the samples are portable. Specific conductance or conductivity, measures how well the water conducts an electrical current, a property that is proportional to the concentration of ions in solution. Conductivity is often used as a surrogate of salinity measurements and is considerably higher in saline systems than in non-saline systems (Dodds, 2002). The conductivity values of the water samples also fell within the WHO acceptable limit considered suitable for human consumption.

Chloride in the form of sodium chloride (NaCl), potassium chloride (KCl) or calcium chloride ($CaCl_2$), is one of the major inorganic anions in fresh and waste-water but in potable water, the salty taste produced by it varies and depends upon the chemical composition of the water. While some waters containing 250 mg chloride in a litre may have a detectable salty taste if the cation is sodium, others may not have if their dominant cations are calcium and magnesium. High chloride content may harm metallic pipes and structures as well as growing plants, cause hypertension in humans and increase concentration of other metals in water (WHO,2003). The chloride content limit recommended by WHO is 250 mg/L and all the samples analysed in this study had values far lower than this limit. Sulphate occurs naturally in drinking water and low concentration is associated with restriction of the growth of phytoplankton. Health concerns regarding high levels of sulphate concentration in drinking water have been linked with diarrhoea due to its laxative effects. In drinking water, this ion has a secondary maximum contaminant level (SMCL) of 250 mg/dm^3 which is a value provided as a guideline for States and public water works (WHO/FAO, 2004). Sulphate doses of 100 to 2000 mg/L have a cathartic effect on humans, resulting in purgation of the alimentary canal. The sulphate values however in this study were within the permissible limit given by WHO.

The microbiological assay carried out in this study revealed organisms previously reported by some other researchers (Okonko *et al.,* 2008; Prasanna and Reddy, 2009). The organisms were similar to those commonly encountered in water and aquatic environments (Prasanna and Reddy 2009). In a certain study the presence of *P. aeruginosa* in the water of mobile vendors and ground water was reported. The presence of bacteria in some brands of sachet water examined in this study was really baffling. It is assumed that bacterial contamination might have been from the water source (Okonko *et al.,* 2008). The other likely sources of contamination may be as a result of improper handling, processing and purification procedures as well as unhygienic handling after production. It may also be attributed to more of the following such as contamination of treated water by organisms harboured in connecting tubes to the packaging machines, lack of or poor quality control system, otherwise the level of treatment of the water source would have been identified before packaging. Poor treatment mechanism and the possibility that the equipment or machines used in the purification process were not functioning well.

The microbial contaminations of packaged drinking water could also be influenced by factors such as their raw water source, treatment process employed and hygienic practices observed in production (Oyedeji *et al.*,2010). In a study, *K. pneumoniae* was reported as the most predominant organism (Taulo *et al*., 2008). Bacteria from genus *Klebsiella*causes numerous infections in human. A variety of nosocomial and community acquired (food-borne) infections are caused by *K. pneumoniae*, one of the deadliest pathogens of *Enterobactericeae*. Also the presence of *P. aeruginosa*, in this study, a pathogenic

organism renowned for its high resistance to antibiotics, is a cause for concern. Abed and Alwakeel (2007), reported that there is contamination with Pseudomonas species in 6.7 % of the water sampled. Presence of *P. aeruginosa* and *P. mirabilis* in some vended sachet water was also reported by Oladipo *et al* (2009), in Ogbomoso, Nigeria. The *P. aeruginosa* isolated from the sachet water could probably have come from the raw food materials, apron, dust and palms of the handlers. *P. aeruginosa* isolated from these samples could be an evidence of cross contamination. *P. mirabilis* has also been reported by Oladipo *et al.*, (2009), in a study on the microbiological quality and safety of sachet water, and attributed it to burst pipes along distribution lines of drinking water and unhygienic handling of water right from treatment plant used in the production of such water (Okonko *et al*., 2008; Oladipo *et al.*, 2009) The presence of these microbes in water may be unnoticed even in transparent packaged water and may pose a potential risk to consumers. The consumption of such contaminated water may eventually lead to the widespread of infections and can ultimately cause an outbreak of epidemic. The possible health hazards of drinking contaminated or poorly treated water is tremendous, as water related diseases continue to be one of the major health problems globally. However, to ensure that the microbial characteristics of sachet water is safe for human consumption, the Nigerian National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health Organization (WHO), recommends that potable water for human consumption should not contain any microorganism that is known to be pathogenic and the coliform number per 100 mL of water must be zero (WHO, 2003). It is important to note that the findings of this study suggests that some of the brands of sachet water on sales in Ogoja metropolis of Cross River State, South-Southern Nigeria, revealed the presence of pathogenic organisms in concentrations that make the products unfit for human consumption and do not conform to the international standards. There is therefore a need for NAFDAC to intensify efforts in the routine monitoring of activities in the packaged drinking water industry. The safety of sachet drinking water should be ensured through comprehensive regulatory programs at the Federal, State and Local levels. NAFDAC regulations for packaged waters should be protective to public health and there should be continuous adoption of packaged water quality standards. Assessment of sachet water quality at some stage of production; pre-production, production and postproduction stages at the factories is therefore suggested in order to ensure their quality and safety. In the light of the above, all water that fails NAFDAC and WHO regulations should be retracted or retreated before they are released to the public for use. The efforts made on batch number, production date and expiry date of all samples vended in public should be intensified by concerned regulatory bodies. High premium should also be placed on ascertaining compliance with Good Manufacturing Practice (GMP) with emphasis on management of raw water source to the consumer product point as recommended by the International Bottled Water Association. Application o GMP, strict process control and personal hygiene should be maintained at processing facilities.

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