PHYSIOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SACHET WATER VENDED IN PARTS OF EKITI STATE, NIGERIA

BY

OGUNDAISI, Isiaka Olalekan

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CERTIFICATION

This is to certify that this is an original and independent research project carried out by OGUNDAISI, I.O. (WMA/12/0493) in the Department of Water Resources Management and Agro-meteorology, in partial fulfilment for the award of Bachelor of Agriculture (B.Sc.) in Water Resources Management and Agro-meteorology, Federal University Oye-Ekiti.

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Mr. J. Adeyeye

Supervisor

Date :.....

.....

Prof. J. O. Ogunwole

Head of Department

Date :.....

DEDICATION

I dedicate this project to Almighty God who has been my guidance and my provider throughout my study years in this great institution and to my wonderful family for all the love, care and support they have given me. I am forever grateful.

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ABSTRACT

Quality water should be colourless, tasteless, odourless and free from faecal contamination. This study investigates the physiochemical and microbiological characteristics of sachet packaged water vended in four local government areas of Ekiti State, Nigeria of contamination of commercially vended packaged water and their public health implications. The four Local Government Areas were Ado-Ekiti L.G.A, Ikere L.G.A, Ijero L.G.A and Ikole Local Government Areas of Ekiti State. Five sachet waters vended in the four Local Government Areas each were purchased make a total of twenty. The sachet packaged waters were transported to the laboratory for the analysis. The physiochemical parameters analysed were odour, colour, pH, turbidity, Conductivity, total dissolved solid, Hardness, zinc, copper, iron, calcium, chloride, nitrate, sulphate, potassium, sodium and magnesium while the microbial parameters were total coliform count, E. coli, S. aeurus and the total plate count. The results were compared with the WHO and NAFDAC standards for drinking water. All the physiochemical and the microbial characteristics were in conformity with the standards except S.aeurus for which 15 percent of the samples were tested positive showing that not all the vended sachet waters are safe for drinking purpose. The research work therefore recommends that microbiological assessment of drinking water quality should be done periodically with the regulatory bodies, there should be regular monitoring, inspection and sanctions by regulatory bodies to enforce existing water regulations and that necessary environmental education should be intensified to inculcate the right attitude and consciousness towards environmental preservation. Finally, it recommends that regulatory agencies involved in food and beverages should be empowered in their regulatory and monitoring functions to check adulteration of these products.

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LIST AND DEFINITION OF SYMBOLS

NAFDAC: Nigerian National Agency for Food and Drug Administration and Control

WHO: World Health Organization

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CHAPTER ONE
INTRODUCTION

1.1 Background

Water is an essential resource for life and good health. Lack of water to meet daily needs is a reality today for one in three people around the world. Globally, the problem is getting worse as cities and populations grow, and the needs for water increase in agriculture, industry and households (WHO, 2009).

In recent times, packaging of potable water in form of bottled water or sachet water has gradually gained wider acceptability in our major cities. Demographic and socioeconomic variables are largely responsible for the choice and preference for this product. Sachet water is a mineral water of about 0.5 L meant for human consumption. It is usually packaged and sold to members of the public in sealed nylons. This occurs mostly in motor parks, markets, public functions and street corner shops. The growing popularity of this potable water unit in our cities singles it out as one of the fastest growing small scale businesses in Nigeria today. Sachet water notably, offers the most accessible and quickest means of assuaging the feeling of thirst. The relatively cheap and inexpensive nature of the sachet water compared to bottled water makes it a cherished and preferred commodity in the hands of many.

Water and sanitation remain the major primary drivers of public health. Access to potable water supply and sanitation remain basic human needs that should be satisfied in adequate quantities that meet at least minimum health standards. Agriculture and food security are also critically dependent on water availability as the planting time and crop yield are both determined the onset, duration and the amount of rain that is recorded in a rainy season. Modern environmental sanitation requires large quantity of water particularly for sewage as well as industrial waste. Water supply and sanitation lead to welfare improvement because it is not only an important sector in its own rights, it is also cross cutting affecting infant mortality, maternal mortality, nutritional values, environmental hygiene, etc. Therefore, the type of access and quantum of water supply as well as the quality of sanitation facilities available to a household or community determines the quality of life of the people and the potential for poverty alleviation (Nigeria Vision 20:2020).

The Nigerian 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. Water in sachets is readily available and affordable but there are concerns about their purity. The integrity of the hygienic environment and the conditions where

the majority of the water in sachets are produced has also been questioned (CAMON, 2004). Although nationally documented evidence is rare, there are claims of past outbreaks of water borne illnesses that ensued from consumption of polluted water in sachets. An understanding of their microbiological quality and safety are therefore imperative and should be a cause of concern to consumers, water suppliers, regulators and public health authorities (Dufor A *et. al*, 2003).

As the human population grows tripling in the past century while, simultaneously, quadrupling its demand for water Earth's finite freshwater supplies are increasingly strained, and also increasingly contaminated by domestic, agricultural, and industrial wastes (UNESCO, 2006).

1.2 Statement of problem

The quality of drinking water is an important environmental determinant of health. Widespread production and consumption of inadequately processed or contaminated packaged drinking water can lead to waterborne disease outbreaks. To safeguard public health, it is important that available packaged water is duly registered and regularly analysed.

1.3 Scope of study

The scope of this research study is to examine the microbial and the physiochemical characteristics of sachet packaged water vended in selected L.G.As of Ekiti State. The LGAs include Ado-Ekiti, Ikole, Ikere and Ijero L.G.As of the State.

1.4 Aim

The aim of the study is to determine the quality of the sachet packaged water vended in four local governments in Ekiti State of Nigeria.

1.5 Specific Objectives

The specific objectives of the study include:

1. To determine the physiochemical characteristics of the sachet packaged water.
2. To assess the microbiological quality of the sachet packaged water.
3. To ascertain the suitability of sachet packaged water for human consumptions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Earth's Water

Water covers 71% of the Earth's surface (CIA, 2008). It is vital for all known forms of life. On Earth, 96.5% of the planet's crust water is found in seas and oceans, 1.7% in groundwater, 1.7% in glaciers and the ice caps of Antarctica and Greenland, a small fraction in other large water bodies, and 0.001% in the air as vapour, clouds (formed of ice and liquid water suspended in air), and precipitation. According to Gleick, P.H., ed. (1993) and Water Vapour in the Climate System Special Report (1995), only 2.5% of this water is fresh water, and 98.8% of that water is in ice (excepting ice in clouds) and groundwater. Less than 0.3% of all freshwater is in rivers, lakes, and the atmosphere, and an even smaller amount of the Earth's freshwater (0.003%) is contained within biological bodies and manufactured products. A greater quantity of water is found in the earth's interior (Crocket, Christopher; 2015).

The water on Earth moves continually through the water cycle of evaporation and transpiration (evapotranspiration), condensation, precipitation, and runoff, usually reaching the sea. Evaporation and transpiration contribute to the precipitation over land. Large amounts of water are also chemically combined or adsorbed in hydrated minerals.

Safe drinking water is essential to humans and other life forms even though it provides no calories or organic nutrients. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion lack accesses to adequate sanitation (MDG Report, 2008). There is a clear correlation between access to safe water and gross domestic product per capita; however, some observers have estimated that by 2025 more than half of the world population will be facing water-based vulnerability (Kulshreshtha, 1998). A report, issued in November 2009, suggests that by 2030, in some developing regions of the world, water demand will exceed supply by 50% ("Charting Our Water Future: Economic frameworks to inform decision-making" PDF).

Water plays an important role in the world economy. Approximately 70% of the freshwater used by humans goes to agriculture (Baroni et al., 2007). Fishing in salt and fresh water bodies is a major source of food for many parts of the world. Much of long-distance trade of commodities (such as oil and natural gas) and manufactured products is transported by boats through seas, rivers, lakes, and canals. Large quantities of water, ice, and steam are used for cooling and heating, in industry and homes. Water is an excellent solvent for a wide variety of chemical substances; as such it is widely used in industrial processes, and in cooking and

washing. Water is also central to many sports and other forms of entertainment, such as swimming, pleasure boating, boat racing, surfing, sport fishing, and diving.

2.2 Water sources, sanitation and hygiene

2.2.1 Water sources

A safe water supply has been defined as a source which is likely to supply water which is not detrimental to health (Hamner et. al., 2006). Safe water sources include: a household piped water connection; a public stand pipe; a borehole; a protected dug well; a protected spring and a rainwater collection system (WHO/UNICEF 2000; Cairncross and Valdmanis 2006). If these sources are capable of providing 20 litres per capita per day at a distance which is no greater than one kilometre from the user's dwelling then they are considered to be improved sources (Hamner et. al, 2006; O'Hara, Hannan and Genina 2008). Cairncross and Valdmanis (2006) refer to these as reasonable sources, which are usually house connections and public facilities. However, according to the Global Water Supply and Sanitation Assessment 2000 Report, some countries have used more stringent definitions of an improved water source than others (WHO/UNICEF 2000). Unimproved water sources are identified as: unprotected wells and springs, vendors and tanker-trucks, and bottled water (WHO/UNICEF 2000). Cairncross and Valdmanis (2006) note that what one group of consumers may consider to be a perfectly satisfactory water supply system may be unsatisfactory for another group, as this may depend on social and cultural differences between groups. They further state that "water supply is not a single, well-defined intervention... but can be provided at varying levels of service with varying benefits and differing costs" (Cairncross and Valdmanis 2006, 771).

Most of the benefits conferred by water supply revolve around access to water in quantity, and this can be based on the technology used to supply it (Cairncross and Valdmanis, 2006). However from the literature reviewed it seems that more attention is placed on the technology (for example household connections) with the assumption that the appropriate quantity will follow.

Reservations about what is meant by the term "improved water source" have been acknowledged internationally (WHO/UNICEF 2000), due to arguments that it is too focused on the distance to the source and the quantity supplied. This is important because an ample water source which is nearby does not necessarily guarantee access. It is also worth noting that increased water access does not guarantee increased water use, therefore other factors must come into play (Fewtrell and Colford 2004). These other factors include cost (affordability by users) and the reliability of supply.

Cairncross and Valdmanis (2006) have indicated that if a water supply is considered to be improved it would provide water of a better quality, convenience and reliability than traditional sources classified as not improved. Convenience would include regularity of supply, cost and the number of users per source.

Reports have indicated that although statistics may show high percentages of improved water sources, sometimes this does not reflect the situation on the ground (O'Hara, Hannan and Genina, 2008). According to WHO/UNICEF (2000), in terms of water supply and sanitation, national consolidated data may not be representative of the poorest and most vulnerable individuals because they are usually hidden in the national totals and averages.

Clasen and Cairncross (2004) have pointed out that in terms of potable water quality a greater focus needs to be placed on point of use treatment. Fewtrell and Colford (2004) also point out that there has been a tendency to focus on the provision of household connections which does not include household water storage. This therefore brings to the fore the issue of household water management as a means of addressing potential water and sanitation related health issues. Nath (2003) agrees that although public health concerns are usually raised in the institutional setting, there is a tendency to not acknowledge the home as a setting of equal importance. It was further reported that improving health status requires an improvement in attitude concerning hygiene in the home and health education, both in tandem with community water supply and environmental sanitation programs.

A study conducted by Eshcol, Mahapatra and Keshapagu (2009) in India showed that faecal contamination of treated pipe borne water after collection was associated with water handling and hygiene practices in urban slums. They noted that the water was supplied on alternate days (intermittently), hence necessitating up to 48 hours of storage in households before subsequent supply. This resulted in dramatic increases in contamination after collection; hence it was acknowledged that until the problem of intermittent supply is resolved, the biggest impact to health must be made at the household level. As a coping strategy various household practices could be done to limit the contamination of stored water before drinking. These include: collecting water in ways which limit its contamination; storing water properly (for example completely covering containers); and treating stored water before drinking.

2.2.2 Sanitation

Cairncross and Valdmanis (2006) acknowledged that sanitation refers to excreta disposal but also includes other environmental health interventions. The term sanitation therefore also loosely falls under the broader definition of environmental sanitation, which refers to arrangements which cover issues related to drainage of storm-water and effluents, flood management, collection and disposal of garbage and removal of human excreta (Pandve 2008; Rautanen 2010). Pandve (2008) further highlights that environmental sanitation involves not only the facilities which are provided by governmental authorities but also includes the attitude of the community. This is due to the fact that a better environment can result, if community members work towards the same goal.

Public latrines fail to provide an adequate solution to the community excreta disposal needs because of problems with inadequacies in their maintenance and inaccessibility at night by the elderly, disabled and young children. It should be noted that these inadequacies sometimes lead to open defecation or inappropriate excreta disposal which sometimes reach sensitive aqueous environments or pose risks of human contact (Cairncross and Valdmanis 2006; WHO/UNICEF 2000). This therefore means that just having these public latrines constructed is not a clear cut solution to resolving sanitation problems.

The ability to engage in good sanitation depends on the availability of water which is used for cleaning and elimination of wastes. Water availability therefore influences the type and functionality of the sanitation facilities which exist (Pandve 2008) and as such it is not unusual for the two to be studied in relation to each other.

There have been differences in opinion as to the combined effect of water and sanitation services on users. According to Esrey et. al. (1991), from the public health standpoint (as it relates to diarrhoeal disease), the combined effect of water and sanitation is no greater than either component separately. However Cairncross and Valdmanis (2006) have considered the effects to be both independent and additive.

In the Caribbean it has been reported that rural sanitation gets much less attention and financial support than urban sanitation. It has also been reported that generally, only a small fraction of industrial and municipal wastewater is treated before being disposed into terrestrial and aquatic environments (Vassell 2009; Smith 2008).

Smith (2008) noted that the critical aspects of sanitation which have been identified in the Caribbean are as follows: interagency and inter-ministerial cooperation; behavioural change (cultural norms and practices); development of community sanitation programs; development of school sanitation programs; development of micro-financing enterprises and the introduction

of regional and local technologies. Smith (2008) further mentioned that the absence of central collection/treatment systems in high water table areas and the improper disposal of garbage/plastic bottles were problems.

2.2.3 Hygiene

In terms of hygiene, it may refer to a practice which is either personal or domestic. Personal hygiene refers to the use of water for cleaning parts of the body and domestic hygiene refers to water used to clean items in the home such as food, utensils and floors (Esrey et. al. 1991). In many articles reviewed, hygiene practice was usually considered as part of water and sanitation research. This is because all three components (water, sanitation and hygiene) commonly impact human health. These components also influence each other, for example, poor hygiene has been shown to be a result of low water availability and inconvenient water supply (such as low pressure, intermittence and crowding) (Karn and Harada 2002; Prüss-Üstün et. al. 2004). Poor hygiene would also be expected to have sanitary consequences. As such, WHO and UNICEF have considered hygiene information as an important component of their work on water and sanitation issues (WHO/UNICEF 2000). In terms of the combined importance of all three components: water supply, sanitation and hygiene promotion on the reduction of diarrhoea disease have been regarded as both independent and additive to one another (Cairncross and Valdmanis 2006).

Interventions which promote hand washing with soap as a single personal hygiene practice have been shown to be most effective when compared to other behaviours (Cairncross and Valdmanis 2006). Kawata (1978) also notes that the belief that water availability for personal hygiene is of prime importance for diarrhoea control is not uniform among researchers. Other important factors such as wastewater disposal, solid waste management and human settlement issues have been shown to have implications on health (Nath 2003; Kawata 1978).

A literature review on water, sanitation and hygiene by Fewtrell and Colford (2004) found that, generally hygiene interventions which comprise hand-washing and hygiene education in child care centres significantly contribute to a reduction in diarrhoeal disease. Metwally et al. (2007) stressed that public access to appropriate information to increase awareness and changes in hygiene patterns are important to public health. They further claimed that the result would lead to a greater tendency of the public to protect themselves from infectious diseases.

Tumwine et al. (2002) highlighted hygiene practices as a key compliment to improved water and sanitation programs. They warned that if the hygiene component was not included, some of the environmental health benefits would be lost.

It can also be said that the adequacy of water is a necessary condition to good hygiene. This is because when there is poor sanitation, supplying enough water per capita enables residents to practice good hygiene. This in turn safeguards public health by enabling residents to protect themselves from sanitation related diseases.

2.2.4 Disease types and transmission routes

Waterborne diseases are primarily caused by human and animal faecal contamination (San Martin, 2002; Eshcol, Mahapatra and Keshapagu, 2009). However, these infections are usually of human origin and to a lesser extent caused by animals (Prüss-Üstün et al. 2004). Since safe water can become contaminated with faecal matter during collection, transport and storage (Clasen and Cairncross 2004), it is important to devise ways of limiting contamination on these levels.

Water is an ideal medium for the transmission of diseases from faecal origin. Humans interact with water in different ways and as such sanitation based diseases may be transmitted through various routes. According to Clasen and Cairncross (2004) these routes have been classified by White, Bradley and White (1972) as shown below (Table 1).

Cairncross and Valdmanis (2006), reported that most diarrhoeal diseases are transmitted through water-washed and not waterborne routes (although the most notable epidemics such as cholera and typhoid are waterborne), hence the significance of the hygiene component of WASH interventions.

In terms of the faecal-oral disease group, it should be noted that water is the ideal but not the only medium which facilitates the faecal-oral pathway (Orlando, 2001). Prüss-Üstün et al. (2004), note that the predominant pathway of infection will depend on the survival characteristics of the pathogen, the local infrastructure in place and human behaviour.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study Area

Ekiti is a State in western Nigeria, declared a state on 1 October 1996 alongside five others by the military under the dictatorship of General Sani Abacha. Geographically, it is located between longitudes $4^0$45' to $5^0$45' East of Greenwich Meridian and on latitude $7^0$15' to $8^0$5' North of the equator. It shares boundaries with Kwara State in the north, Kogi State in the north-east Osun State in the south and south-east. The state enjoys 2 distinct wet and dry seasons with a population of 2,398,957 (NPC, 2006). The state, carved out the territory of old Ondo state, covers the former 12 local government areas that made up the Ekiti Zone of old Ondo state. On creation, it had 16 Local Government Areas (LGAs), having had an additional four carved out of the old ones.

The research work was carried out in four local government areas (LGAs) of Ekiti state Nigeria. The LGAs are; Ado, Ikole, Ijero and Ikere Local Governments Areas. The four Local Governments Areas were selected based on geo-political zones of the state.

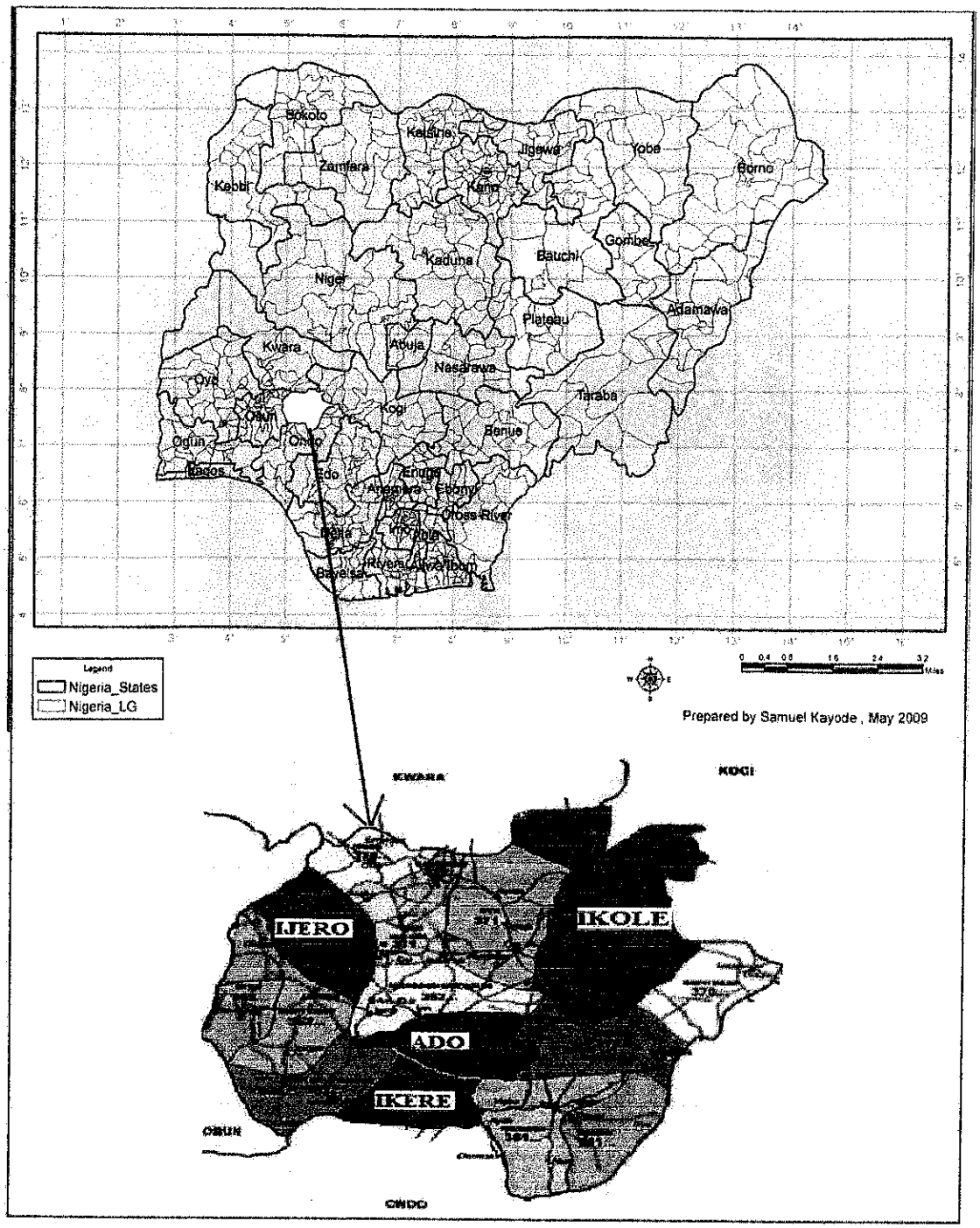

FIGURE 1: MAP OF NIGERIA SHOWING THE SAMPLED LGAs IN EKITI STATE

Source: Google, (2012).

3.2.1 Data Collection

Five different sachet packaged water each were procured from four local government areas of Ekiti State making twenty samples. The samples were Owosola, Esinkun, Femog, Marg darem, Diploma, kollege, Aboluwaji, Mike&Vic, Luason, Olamide, Adeb, Segun, Real noble, Remark, King, P.k, Poli, and Olaayo. Sample identifications label were recorded on site and transported in ice packed to Federal Polytechnic Ado-Ekiti laboratory for analysis. Tables 1, 2, 3 and 4 below showed the various brands of sachet packaged water obtained for the study.

Table 1: Sachet packaged water taken from Ado-Ekiti Local Government Area

SN	BRAND	SAMPLE CODE	NAFDAC NUMBER
1	ADEB WATER	A1	C1-4731L
2	OLAAYO WATER	A2	B1-7110L
3	OLAMIDE WATER	A3	01-4404L
4	P.K WATER	A4	01-4404L
5	POLI WATER	A5	01-8104L

Table 2: Sachet packaged water taken from Ikere Ekiti Local Government Area

SN	BRAND	SAMPLE CODE	NAFDAC NUMBER
1	SEGUN WATER	B1	01-7775L
2	LUASON WATER	B2	C1-1775L
3	OLAMIDE WATER	B3	01-4404L
4	KOLLEGE WATER	B4	D1-0467L
5	ADEB WATER	B5	C1-4731L

Table 3: Sachet packaged water taken from Ijero Ekiti Local Government areas

SN	BRAND	CODE	NAFDAC NUMBER
1	KING WATER	C1	C1-7895L
2	REMAK WATER	C2	01-4524L
3	MIKE & VIC WATER	C3	A1-3804L
4	REAL NOBLE WATER	C4	C1-3458L
5	ABOLUWAJI WATER	C5	A1-7956

Table 4: Sachet packaged water taken from Ikole Local Government areas

SN	BRAND	CODE	NAFDAC NUMBER
1	OWOSOLA WATER	D1	C1-7419L
2	ESINKUN WATER	D2	A1-1667L
3	MARG DAREM WATER	D3	C1-6557L
4	FEMOG WATER	D4	01-2405L
5	DIPLOMA WATER	D5	C1-7953L

3.2.2 Water analysis

3.2.2.1 pH

The pH of the sample was electrometrically measured using a well calibrated HANNA Hi208 pH meter.

3.2.2.2 Turbidity

Turbidity of the sample was determined after vigorously shaken using Labtech AVI-654 Turbidity meter.

3.2.2.3 Electrical Conductivity

The Electrical Conductivity was measured using DDS-307 Conductivity meter. This was used to estimate the total amount of dissolved salt or solid in water measured in ohms/cm at 25 ^{0}C.

3.2.2.4 Total Hardness

Determination of total hardness was achieved thorough shaking of sample and measuring 25 mL into a cylinder. This was made up to 50 mL with distilled water and then poured into a conical flask; 2 mL of a buffer solution (ammonium chloride) with a drop of Eriochrome black indicator were added to it. The sample was gently shaken and was titrated with a solution of 0.02EDTA as a titrant to a blue coloration as end point.

3.2.2.5 Determination of Nitrate

KNO_3 was weighed (0.7218g) and dissolved in 100ml of distilled water (stock nitrate solution), from the stock nitrate solution, 0,5,10,15,20,25 were diluted in 50 mL of distilled water, 2 mL was taken from each standard and 2 mL of sodium salicylate was added into each and also evaporated into dryness on hot plate, 2 mL of conc. H_2SO_4 was added into the samples and allowed to stand for 10 minutes. 15 mL of distilled water was added into both sample and 2 mL of sodium hydroxide-potassium tartrate solution was added into both samples. Yellow colour was observed. The absorbance was read on U.V spectrophotometer.

3.2.2.6 Chloride Determination

The sample was well agitated, followed by measuring 100ml into a conical flask. 1 mL of standard potassium chromate, k_2CrO_4 (an indicator) was added and the solution was titrated with standard silver nitrate solution, $AgNO_3$ to a reddish-brown colouration as end point.

3.2.2.7 Alkalinity Test

For samples with pH above 8.3, 50 ml of sample was transferred into a 250 mL Erlenmyer flask. Several drops of phenolphthalein indicator were added until the colour of the solution turned pink. The solution was titrated against 0.02 N HCl until colour changes from pink to clear (pH 8.3). The volume of acid used for the titration was recorded, then proceeded to step 2. If the sample pH is below 8.3, Several drops of bromocresol green indicator were until the colour of the solution turned blue, titrated against 0.02N HCl until the colour changes to yellow (pH 4.5), then proceeded to step 1.

3.2.2.8 Determination of sodium and potassium using flame photometer

The beaker was half filled with the water sample. The photometer and the air compressor were switched on. Then the filter selector was set to the required position i.e. either Na or K. The nebulizer inlet tube was inserted in a beaker containing 100ml of distilled water (blank). It was allowed to for 30 minutes for the temperature to stabilize. A set of calibration standard was prepared for sodium and potassium solutions. While aspirating blank, the blank control was adjusted so that the photometer display 0.0 It was allowed for 20 seconds for a stable reading and then adjusted for coarse and fine controls for a convenient reading. The procedure was repeated for different concentration of the standards. The readings of the water samples were then taken. The procedure was repeated for other water sample.

3.2.2.9 Determination of sulphate in water

In determination of sulphate in water, 100ml of the water sample was measured into a conical flask and 20ml of standard buffer solution A was added to the solution. A magnetic stirrer was added into solution and it was placed on the mixing machine. Then 1g of Barium chloride was added into the solution ($BaCl_2$). The solution was stirred for exactly 1 minute at a constant speed. Immediately after stirring, the solution was poured into 10mm cell vial tube and read with U.V Spectrophotometer at a wavelength of 420 nanometre (nm). The procedure was repeated for other water sample.

3.2.2.10 Determination of heavy metals in water sample

In determination of heavy metals in water, twenty millilitres (20 ml) of the water sample was measured and transferred into 250 ml conical flask. The water sample was digested by the addition of 20 ml of aqua regia (mixture of HCl and HNO_3, ratio 3:1). The beaker was covered with an inverted funnel to prevent excessive loss of sample and heated over a heating mantle at $90^{O}C$ until the volume reduced to about 5ml. The conical flask and funnel were washed with distilled water. The solution was cooled, filtered through Whatman No. 1 filter paper and transferred quantitatively to a 50 mL volumetric flask and made up to the mark with distilled water. The filtrate was transferred into plastic sample bottle and kept. The digested sample was analysed for metals using Buck Scientific 210VGP atomic absorption spectrophotometer (AAS). The wavelengths settings for the metals are 213.9 nm, 324.8 nm and 248.3 nm for Zinc, Copper and Iron respectively.

3.2.2.11 Microbiological Analysis

The Coliscan membrane filter method developed for Escherichia coli and total coliforms was used. The sample was filtered through a 47mm, 0.45 m pore size membrane filter. The filter funnel was then rinsed twice with at least 20 ml of sterile diluent to complete the filtration. The filter was then transferred to a petri dish containing a pad saturated with 2 mL of the Coliscan MF broth, the dish was inverted and the content incubated at about 350^{0}C for 24 hours.

The sum of blue/purple and pink/magenta colonies was the total Coliform Positive count. Blue/purple colonies were counted as E. coli. Pink/magenta colonies were counted as other than E. coli coliforms. Clear or white colonies were counted as non-coliforms.

Enumeration and isolation of total and faecal coliform count

Total coliforms were estimated using the most probable number (MPN) method. MacConkey's lactose bile salt broth with bromocresol purple as indicator was used for the presumptive tests. With a sterile pipette, 50 ml of each of the water sample was aseptically dispensed into 50ml double strength broth, another 10 ml of the sample into each of the five tubes containing 10 ml double strength broth and another one ml of the sample was then inoculated into each of the second five culture tubes containing 5ml single strength MacConkey broth with Durham's tubes. Inoculated tubes of MacConkey broth were incubated at 37° C for 24 to 48 hours. After 24-48 hours of incubation, the cultures were observed for the presence of acid production and gas formation

A sterile pipette was used to transfer 1ml of the culture from the positive presumptive fermentation tubes into tubes containing 5ml brilliant green lactose bile broth aseptically and incubated for 24-48 hours at 37°C. Following incubation, culture positive tubes were inoculated into MacConkey agar for total Coliform and Eosin Methylene Blue agar for faecal coliform and incubated at 37 ° C and 44 ° C respectively.

Determination of heterotrophic plate count/ total viable count

Heterotrophic plate count of all water samples were determined using dilution plate method technique and standard plate count agar medium. Serial dilutions were prepared (using peptone water) and 1 ml of the sample or dilution was transferred to a sterile, empty petridish. Plate count agar was melted by heating in boiling water and then allowed to cool in a water bath to 44 - 46° C. Approximately 15 ml of the agar medium was poured into the petridish containing the sample. The sample and agar was mixed thoroughly by rotating the plate several times.

When the media has solidified, the plates were inverted and incubated at 35 °C for 48 to 72 hours. Following the appropriate length of incubation, suitable plates from different dilutions were selected and the visible colonies were counted using a colony counter. Then the average colonies were counted and expressed as colony forming unit per ml of water.

Identification of isolates

Representative isolates from total coliforms and total viable counts were identified. Standard isolation techniques were employed. .Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase and Urease tests.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

The result of the physiochemical, microbial, the mean and standard deviation parameters of sachet packaged water vended in the selected L.G.As are given in Tables 5a, 5b, 6 and 7 below.

Table 5a: Physiochemical characteristics of the sampled sachet packaged water

Sample Location	Sample Code	Colour	Odour	pH	Turbidity (NTU)	Conductivity (µS/cm)	TDS (mg/L)	Hardness (mg/L)
ADO L.G.A	A1	Colourless	Odourless	7.8	0.43	60	36	0.96
	A2	Colourless	Odourless	8.1	0.39	30	17	0.57
	A3	Colourless	Odourless	7.9	0.45	56	33	0.89
	A4	Colourless	Odourless	7.8	0.51	75	46	1.26
	A5	Colourless	Odourless	7.8	0.37	29	15	0.47
IKERE L.G.A	B1	Colourless	Odourless	8.3	0.33	22	12	0.51
	B2	Colourless	Odourless	8.1	0.51	65	39	0.96
	B3	Colourless	Odourless	8.1	0.49	62	37	0.96
	B4	Colourless	Odourless	7.9	0.45	55	30	0.87
	B5	Colourless	Odourless	7.8	0.43	60	36	0.96
IJERO L.G.A	C1	Colourless	Odourless	8.1	0.33	21	11	0.47
	C2	Colourless	Odourless	8.0	0.41	35	21	0.65
	C3	Colourless	Odourless	7.8	0.53	76	45	1.19
	C4	Colourless	Odourless	7.9	0.44	52	31	0.89
	C5	Colourless	Odourless	7.7	0.66	92	57	1.33
IKOLE L.G.A	D1	Colourless	Odourless	7.8	0.56	64	39	0.89
	D2	Colourless	Odourless	7.7	0.69	90	56	1.33
	D3	Colourless	Odourless	7.8	0.71	103	64	1.45
	D4	Colourless	Odourless	7.8	0.71	102	63	1.45
	D5	Colourless	Odourless	7.8	0.54	64	39	0.96
WHO MAXIMUM PERMISSIBLE LIMIT		Unobjectionable	15	7-8.5	25	1000	500-1500	500
NAFDAC				6.5-8.5		1000	1000	100

Table 5b: Physiochemical characteristics of the sampled sachet packaged water

Sample Location	Sample code	Zinc (mg/L)	Copper (mg/L)	Iron (mg/L)	Calcium (mg/L)	Chloride (mg/L)	Nitrate (mg/L)	Sulphate (mg/L)	Potassium (mg/L)	Sodium (mg/L)	Magnesium (mg/L)
ADO L.G.A	A1	ND	0.002	0.010	9.23	0.98	1.45	6.01	0.812	2.204	0.730
	A2	ND	0.002	0.001	7.56	0.67	1.02	5.80	0.769	1.903	0.801
	A3	ND	0.002	0.010	9.78	0.78	1.80	6.34	0.800	2.001	0.607
	A4	ND	0.002	ND	10.33	0.56	1.78	6.89	0.581	2.667	0.791
	A5	ND	0.002	ND	7.11	0.40	1.45	5.86	0.591	2.303	0.827
IKERE L.G.A	B1	ND	0.010	ND	6.67	0.78	1.56	5.08	0.770	1.997	0.709
	B2	ND	0.020	0.01	9.01	0.33	1.22	6.54	0.812	1.780	0.821
	B3	ND	0.030	0.01	9.11	0.66	1.67	7.01	0.903	2.005	0.961
	B4	ND	0.010	0.02	9.56	0.66	1.33	6.09	0.667	1.803	0.837
	B5	ND	0.002	0.010	9.23	0.98	1.45	6.01			
IJERO L.G.A	C1	ND	0.100	ND	6.60	0.56	1.80	4.54	0.941	2.411	0.802
	C2	ND	0.070	0.01	7.23	0.78	1.60	6.09	0.766	2.007	0.701
	C3	ND	0.200	ND	10.44	0.67	1.45	6.34	0.712	2.301	0.527
	C4	ND	0.100	ND	9.67	0.33	1.09	7.01	0.800	1.901	0.801
	C5	ND	0.200	0.10	11.43	1.33	1.54	7.68	0.650	2.201	0.904
IKOLE L.G.A	D1	ND	0.070	0.10	9.01	0.98	1.93	6.54	0.830	2.220	0.741
	D2	ND	0.090	0.20	11.01	1.67	1.02	6.67	0.809	2.003	0.800
	D3	ND	0.070	0.10	13.23	0.56	1.45	7.80	0.672	2.403	0.709
	D4	ND	0.100	0.20	13.23	0.67	1.87	7.87	0.907	1.908	0.805
	D5	ND	0.100	0.01	9.01	0.67	1.67	6.08	0.702	2.003	0.753
WHO MAXIMUM PERMISSIBLE LIMIT		3.0	2.00	0.3	-	100	50	100			
NAFDAC		5.0	1.00	0.3	-	100					

ND = Below Detectable Limit

Table 6: Microbial characteristics of the sachet packaged water vended in the selected LGAs

Sample Location	Sample Code	Total Coliform (cfu/ml)	E. coli (cfu/ml)	Total Plate Count (cfu/ml)	S. aeurus (cfu/ml)
Ado L.G.A	A1	Negative	Negative	0.21×10^2	Positive
	A2	Negative	Negative	0.23×10^2	Negative
	A3	Negative	Negative	0.10×10^2	Negative
	A4	Negative	Negative	0.17×10^2	Negative
	A5	Negative	Negative	0.09×10^2	Negative
IKERE L.G.A	B1	Negative	Negative	0.21×10^2	Negative
	B2	Negative	Negative	0.18×10^2	Negative
	B3	Negative	Negative	0.06×10^2	Negative
	B4	Negative	Negative	0.18×10^2	Negative
	B5	Negative	Negative	0.21×10^2	Negative
IJERO L.G.A	C1	Negative	Negative	0.12×10^2	Negative
	C2	Negative	Negative	0.20×10^2	Negative
	C3	Negative	Negative	0.17×10^2	Positive
	C4	Negative	Negative	0.18×10^2	Negative
	C5	Negative	Negative	0.18×10^2	Negative
IKOLE L.G.A	D1	Negative	Negative	0.26×10^2	Negative
	D2	Negative	Negative	0.0×10^2	Negative
	D3	Negative	Negative	0.20×10^2	Positive
	D4	Negative	Negative	0.18×10^2	Negative
	D5	Negative	Negative	0.12×10^2	Negative

Table 7: The mean and standard deviation of the physiochemical parameters of sachet packaged water vended in the selected LGAs

PARAMETERS		ADO L.G.A	IKERE L.G.A	IJERO L.G.A	IKOLE L.G.A	WHO 2015	NAFDAC 2015
Copper (mg/L)	Mean	0.002	0.014	0.134	0.086	2	1
	STD	0	0.01	0.06	0.02		
Iron (mg/L)	Mean	0.007	0.013	0.055	0.122	0.3	0.3
	STD	0.005	0.005	0.063	0.08		
Calcium (mg/L)	Mean	8.8	8.72	9.07	11.10	75	75
	STD	1.4	1.16	2.08	2.11		
Chloride (mg/L)	Mean	0.68	0.68	0.73	0.91	250	250
	STD	0.22	0.24	0.37	0.45		
Nitrate (mg/L)	Mean	1.5	1.45	1.5	1.59	0.5	0.1
	STD	0.31	0.18	0.26	0.37		
Sulphate (mg/L)	Mean	6.18	6.15	6.33	6.99	100	100
	STD	0.45	0.72	1.18	0.80		
pH	Mean	7.88	8.04	7.90	7.78	7.0-8.9	6.5-8.5
	STD	0.13	0.19	0.16	0.04		
Turbidity (NTU)	Mean	0.43	0.44	0.47	0.66	25	5.0
	STD	0.05	0.07	0.13	0.08		
Conductivity (µS/cm)	Mean	50	52.8	55.2	84.6	1000	1000
	STD	20.01	17.60	29.05	19.49		
TDS (mg/L)	Mean	29.4	30.8	33.0	52.2	500-1500	1000
	STD	13.16	11.03	18.38	0.27		
Hardness (mg/L)	Mean	0.83	0.85	0.91	1.22	100-500	100
	STD	0.32	0.20	0.36	0.27		
Potassium (mg/L)	Mean	0.71	0.79	0.77	0.78		
	STD	0.11	0.10	0.11	0.10		
Sodium (mg/L)	Mean	2.22	1.9	2.16	2.1	250	200
	STD	0.30	0.12	0.21	0.76		
Magnesium (mg/L)	Mean	0.75	0.832	0.75	0.76	1.5	1.5
	STD	0.09	0.10	0.14	0.04		

4.2 DISCUSSION

The colour and the odour are unobjectionable. The pH of the sachet water sample in Ado-Ekiti L.G.A ranged from 7.8 to 8.1, samples from Ikere L.G.A ranged from 7.8 to 8.3, and that of Ijero L.G.A ranged from 7.7 to 8.1, while that of Ikole L.G.A 7.7 to 7.8.

The turbidity of the sampled sachet packaged water ranged from 0.33 NTU found in samples from Ijero and Ikere LGAs to 0.71 NTU in samples from Ikole L.G.A. The turbidity values of the sampled waters were very low when compared with the NAFDAC and WHO standards. The total hardness of the water samples were generally below 1.5mg/L.

The concentration of Zinc in the water samples were below detectable limit while only samples A4, A5, B1, C1, C3, and C4 were examined to be below for Iron concentration and all other samples were found to be below the permissible limit. Copper concentration in the water samples from the four L.G.As ranged from 0.002 mg/L found in all the water samples taken from Ado LGA and sample B5 to 0.2 mg/L gotten from samples C3 and C5 from Ikere L.G.A. The Mean of each parameter of the water samples from the various local government areas were in conformity with the WHO and NAFDAC set standards.

Total coliforms are seen to be absent from all the sachet water sampled from the four local government areas, also in the same manner is the amount of E. coli also found to be absent in the water samples. Of all the sampled sachet waters, A1, C3 and D2 were found to possess S. aureus. The total plate count ranges from $0.0x10^2$cfu/ml in D2 to $0.26x10^2$ cfu/mL from D1.

The concentration of copper in the analysed sachet packaged water ranged from 0.002 mg/L to 0.100 mg/L with a mean value of 0.059 mg/L. The obtained value were below the permissible limit of 2.00 mg/L and 1.00 mg/L of WHO and NAFDAC respectively. This is similar or contrary to Alhassan A.J (Yakasai et al 2008) when reported higher concentration of 0.019 mg/L to 0.15 mg/L of WHO and NAFDAC in his studies of sachet packed water in Kano state.

The concentration of iron in the analysed sachet packaged water samples ranged from the 0.001 mg/L to 0.20 mg/L. The obtained value were below the permissible limit of 0.30 mg/L and 0.30 mg/L of WHO and NAFDAC respectively. This is similar or contrary to Alhassan A.J (Yakasai et al 2008) when reported higher concentration of 0.001 mg/L to 0.95 mg/L of WHO and NAFDAC in his studies of sachet packed water in Kano state.

Generally for the four LGAs studied, the calcium concentration was lower compared to the permissible limits set by NAFDAC and the WHO (75 mg/L). The means ranged from 8.8±1.4 mg/L in Ado-Ekiti to 11.1±2.11 mg/L in Ikole. The mean magnesium concentrations in the water samples were less when compared to the WHO permissible limit (1.5 mg/L). The average

concentration of magnesium is lowest in water samples from Ado and Ijero LGAs (0.75 mg/L) and highest in samples from Ikere L.G.A (0.832 mg/L).

Calcium and magnesium salts in water contribute to the water hardness. The low amount of these substances in the water is evident in the low level of the water hardness as seen in table 7. The average hardness values of the sachet water samples ranged from 0.83 mg/L in Ado to 1.22 mg/L in Ikole. The sachet water vended in the study area can be generally classified as soft. Although there was no health-based guideline value is proposed for hardness in drinking water, water with a hardness of less than 100 mg/L may, have a low buffering capacity and so be more corrosive for some metals such as copper and lead (WHO, 2011).

The mean chloride concentration the water samples ranged from 0.68 mg/L to 0.91 mg/L. These values were below the permissible limits. WHO (1999) stated that high chlorine concentration gives an undesirable salty taste to water. Conductivity of the examined sampled ranged from 21 µS/cm to 103 µS/cm with mean value of 50 µS/cm, 52.8 µS/cm, 55.2 µS/cm, and 84.6 µS/cm for Ado, Ikere, Ijero and Ikole LGAs respectively. The mean values were below the permissible limit set by NAFDAC and WHO.

The average nitrate concentrations for the various LGAs were 1.5 mg/L, 1.45 mg/L, 1.5 mg/L and 1.59 mg/L for Ado, Ikere, Ijero and Ikole LGAs respectively. The concentration levels were below the maximum permissible limit given by NAFDAC and WHO. Nitrate is the most highly oxidized of Nitrogen compounds commonly present in natural water and it is the product of aerobic decomposition of organic nitrogenous matter (S. E. Shinde et al, 2011). Nitrate level in drinking water should not exceed 50 mg/L to protect against methaemoglobinaemia (blue-baby syndrome) in bottle-fed infants.

The total dissolved solids in the water samples ranged from 29.4 mg/L to 52.2 mg/L which was below the given standards. Low loaded TDS imparts flat, insipid taste to drinking water (Marier *et. al* 1979).

Total plate count is an indication of bacterial populations on a sample. S. aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious, S. aureus can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections.

Since the total coliform counts and the E. coli are absent, other organisms may be more appropriate indicators of persistent microbial hazards such as enterococci and Clostridium perfringes.

These pathogens may be naturally present in the environment but are generally not harmful to individuals with intact immune systems but may be able to cause disease in people with impaired local or general immune defence mechanisms, such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy or those with acquired immunodeficiency syndrome, AIDS (Ugochukwu S. *et. al* 2015). If water used by such persons for drinking or bathing contains sufficient numbers of these organisms, they can produce various infections of the skin and the mucous membranes of the eye, ear, nose and throat.