BACTERIA AND FUNGI ISOLATED FROM SELECTED SACHET WATER MARKETED IN AHMADU BELLO UNIVERSITY, ZARIA MAIN CAMPUS AND THEIR EFFECTS ON DRINKING QUALITY

BY

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A THESIS SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OFMASTER OF SCIENCE (M.Sc.) DEGREE IN BOTANY

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DECLARATION

I declare that the work in this thesis entitled "BACTERIA AND FUNGI ISOLATED FROM SELECTED SACHET WATER MARKETED IN AHMADU BELLO UNIVERSITY, ZARIA MAIN CAMPUS AND THEIR EFFECTS ON DRINKING QUALITY"was carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria under the supervision of Prof. A.U. Khan and Dr. D.B. Dangora. The information derived from the literatures has beendulyacknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Ijai AyubaTHLIZA Signature_______________ Date_______________

CERTIFICATION

This thesis entitled "BACTERIA AND FUNGI ISOLATED FROM SELECTED SACHET WATER MARKETED IN AHMADU BELLO UNIVERSITY, ZARIA MAIN CAMPUS AND THEIR EFFECTS ON DRINKING QUALITY" by Ijai Ayuba THLIZA meets the regulations governing the award of master degree in Botany of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary.

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DEDICATION

This work is dedicated to my Late Mum, I really miss you, and may God grant you eternal rest.

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ABSTRACT

This study focuses on isolating and identifying bacteria and fungi contaminants from sachet waters sold within the University's main campus, and determines the effects of these microbes on the quality of the sachet water. A sample size of 720 sachet water of 6 different brands were collected at random, and then subjected to storage, while their microbial and physicochemical parameters were analyzed. The colour, taste, odour, turbidity, temperature, pH, electrical conductivity, total dissolved solids, total hardness, chloride, sulphate, nitrate, phosphate.and microbial parameters of the stored samples were analysed weekly, for a period of 6 months. The study utilizedthe modified membrane filter method using commercially prepared Potato Dextrose Agar (PDA) for the isolation of fungi, and the multiple tube fermentation technique with MacConkey broth and Nutrient agar used as reagents for bacteria isolation. Two-way analysis of variance test was employed to analyse the data for fungi and bacteria count at different storage periods. This study revealed that, out of the 720 sachet water samples, fungal species were isolated in 24 and bacterial species were isolated in 54 while 642 were devoid of fungi and bacteria contaminants. Out of the 6 selected brands, fungi were isolated in five brands, while bacteria were isolated in all the six brands. Among the microbes isolatedare*Aspergillus* species,*Penicillium* species,*Fusarium* species, *Staphylococcus aureus, Escherichia coli and Bacillus cereus* were the predominant isolates. All the physicochemical parameters fall within the recommended limits set by Standard Organisation of Nigeria for Drinking Water. The findings revealed the presence of coliform and other pathogens in the samples which indicate risk involved in consumption of such products and therefore could be hazardous to human health.To safeguard the health of the consumers, there is need for regular monitoring of the

production and quality by National Agency for Food and Drug Administration and Control.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Water is one of the most essential commodities for the survival of all lives. It is abundant in nature and occupies about 70% of the earth's crust (Kalpana *et al*., 2011). Water is the biological medium on earth and the only common substance that exists in nature in all three physical states of matter: solid, liquid and gas. It is the most universally used solvent and common route of transmission of diseases. Unavailability of good quality drinking water is wide spread and this has serious health implications. In developing nations of the world, 80% of all diseases and over 30% of deaths are related to drinking water (Dada and Ntukekpo, 1997). According to World Health Organization statistics, only 36% of Nigerians have access to potable water and 6% have access to improved sanitation (WHO, 2014). An estimated 748 million people all over the world lack access to potable water and close to 2.5 billion persons are not provided with adequate sanitation (WHO, 2014). Water is said to be potable when the physical, chemical and microbiological qualities conform to specified standard. To achieve this, such raw water is subjected to purification processes that range from simple long-term storage to enable sedimentation of some suspended solid to aeration, coagulation, flocculation and disinfection among other treatments (Ajewole, 2005).

The demand for safe drinking water in Nigeria cannot be overemphasized, considering the inability of government to provide adequate pipe borne water to its citizens. Moreover, treated pipe borne water may be contaminated due to poor maintenance of broken and leaking pipes, especially those close to gutter and drainages. Consequently, a number of small scale water producing industries are packaging and marketing factory-filled sachet drinking water popularly called “pure water” that are considered as a safe source of potable water (Dodoo *et al.*, 2006). Sachet water, a brand of packaged water has therefore, gradually become the most widely consumed liquid for both the rich and the poor in Nigeria (Sunday *et al*., 2011). Hygiene, purity, tastes, and, most importantly, safety is probably amongst various reasons for sachet water consumption (Sunday *et al*., 2011). Unfortunately, the problems of its purity and health concerns have begun to manifest (Oladipo*et al.*, 2009). Sachet water like any other food product must be processed and packaged under aseptic conditions free from every possible source of contamination. The potability of this sachet water is found uncertain considering the fact it is collected from almost every available water source ranging from rainwater to tanker-borne water, most of which are rusty and unwashed (Dibua *et al.*, 2007). The potential danger associated with sachet water is contamination, which is a factor of the source of the water itself, treatment, packaging materials, dispensing into packaging materials and closure (Omalu *et al*., 2010).

Several studies conducted on the microbial quality of bottled and sachet water have reported violations of international quality standards. The most widely used indicator for microbial water contaminant is coliform group of microorganisms. Rutz (1996) reported that, sachet water vending machine may not be free of microorganisms, because bacteria like *Streptococcus faecalis,* have been isolated from sachet water producing machine. Sachet water have been reported to contain bacteria such as *Bacillus* specie, *Pseudomonas* species, *Klebsiella* species, *Streptococcus* speciesand oocysts of *Cryptosporidia* species(Omalu *et al.,* 2010). Under prolonged storage of packaged water at favorable environmental conditions, total aerobic heterotrophic bacteria can grow to levels that may be harmful to humans (Warburton *et al*., 1992). Although the microbial quantity levels in processed water are often initially low, they can evolve rapidly to high levels during storage (Stickler, 1992). Fungi have been reported from all types of water, from untreated water to treated water (Ribeiro *et al*., 2006). Water treatment appears to reduce the fungi and other microbes in water, without removing all of them (DEFRA, 2011). Many of the fungi that have been isolated from treated drinking water are known to be pathogenic, particularly *Aspergillus* and *Candida* (Nikaeen and Mirhendi, 2008). Fungi growing in drinking water source cause modification in taste, odour and composition of water (Kelley *et al*., 2003; Nazim *et al*., 2008; Ahmed *et al*., 2010).

1.2 Statement ofResearchProblem

The quality of drinking water is of growing concern, because of the microbial contamination found in drinking water in many places throughout the world. In Nigeria, most sachet water are below the World Health Organization Standard, and therefore, are of doubtful quality, thereby posing health risk to consumers (WHO, 2006; Kalpana*et al.*, 2011).

1.3 Justification

Water of good drinking quality is of basic importance to human physiology, and man's continued existence depends very much on its availability. An average man of 35-63kg body weight requires about 3 liters of water in liquid and food daily to keep healthy (Wordlaw et *al.*, 2004). Unfortunately, the quality of water sold to public in many places in Nigeria may not be wholesome (Mendie, 2004). According to the Institute of Public Health Analysts of Nigeria (IPAN), 50% of the "pure water" sold in the street of Lagos may not be fit for human consumption (Osibanjo *et al.*, 2000). The high prevalence of diseases such as diarrhea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (Mead *et al.*, 1999). Poor supply of tap water has made people to constantly use well, boreholes and sachet water. Poor quality of water combined with unsafe storage is prevalent in Zaria and it has contributed to increasing incidences of water borne diseases such as cholera and typhoid (Musa *et al.*, 2008). Research on the microbial and physiological contaminants and their effects on drinking quality has received little attention in Kaduna State (Ante *et al.*, 2007; Musa *et al*., 2009; Yahaya *et al.*, 2011). Considering the myriad brands of sachet water marketed in Ahmadu Bello main campus coincide with the increasing population of students using the water, there is need to determine the quality of the water. This will provide information on the safety and microbiological quality of the sachet water marketed in Ahmadu Bello University, Zaria main campus. This prompted the need to carry out this study in the above mentioned institution.

1.4 Aim and Objectives

The aim of this study is to isolate fungi and bacteria contaminants and assess the drinking quality of different types of sachet water marketed within Ahmadu Bello University, Zaria main campus.

The specific objectives were to;

1. Isolate fungi and bacteria from different types of sachet water marketed within Ahmadu Bello University, Zaria main campus.

2. Identify the fungi and bacteria associated with the sachet water marketed within Ahmadu Bello University, Zaria main campus.

3. Determine the effect of fungi and bacteria on the quality of sachet water marketed within Ahmadu Bello University, Zaria main campus.

1.5 Hypotheses

1. Fungi and bacteria do not occur in the different types of sachet water marketed within Ahmadu Bello University, Zaria main campus.

2. There was no significant difference in the fungi and bacteria associated with different sachet water marketed within Ahmadu Bello University, Zaria main campus.

3. There was no significant effect of fungi and bacteria on the quality of sachet water marketed within Ahmadu Bello University, Zaria main campus.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Drinking Water and its Sources

Water is the principal need of all life on earth, and is an essential component for all forms of lives, from microorganisms to man (Priyanka *et al.,* 2010). It is an essential substance for the maintenance of all life processes. It is abundant in nature, yet most people lack adequate and safe drinking water due to increase in human population, coupled with human activities which lead to pollution of drinking water sources and failure from government to provide potable water to its citizens, more especially in developing countries. Water is indispensible for life, since it is essential part of human nutrition and it is also required for maintenance of personal hygiene, food production and prevention of diseases (Adenkunle *et al.,* 2004, Oparaocha *et al.,* 2010, Edema *et al*., 2011, Adegoke *et al*., 2012).

The three major sources of drinking water are ground water, surface water and rain water. Surface fresh water is widely used as source of drinking water worldwide (CAWST, 2009). Of particular concern among people without access to improved drinking water sources are those who rely on surface water sources, such sources include rivers, dams, lakes, ponds and canals, and are often the most susceptible to pollution and most likely to have poor water quality (CAWST, 2009). Since 1990, use of surface water sources has decreased significantly and accounts for only a small proportion of drinking water sources in most regions where only 2% of the rural population in Southern Asia and 5%

of the rural population in South-Eastern Asia use surface water sources while in sub-Saharan Africa 20% of rural dwellers still rely on surface water sources (WHO, 2011). Surface water is generally unsafe to drink without treatment (CAWST, 2009).

Nigeria has adequate surface and ground water resources to meet current demands for potable water but variation in geographical climatic condition has lead to scarcity in some locations especially in the north (USAID, 1990). The Nigerian government held the role of provision of water and sanitation services to its citizens but after fifty-four years of water supply development in Nigeria, it is regrettable that only few number of the population has no access to safe drinking water (FMWR, 2000). However, the public sector has not been successful in meeting more than a small portion of the demand for water and sanitation of residential and commercial users. Services are in critically short supply in many communities in Nigeria (FMWR, 2000). Many households often end up purchasing water from private vendors much more expensively than from the public supply. Water supply services where they exist, are unreliable and of low quality and are not sustainable because of difficulties in management. Many water supply systems show extensive deterioration and poor utilization of existing capacities, due to under maintenance and lack of funds for operation. Rural people in the country still depend very much on rivers, streams, ponds, and shallow wells for their water needs.

All human activities such as food production, energy, health and environmental sanitation depend very much on water but the challenges associated with water has cause hunger and thirst, high rates of disease outbreak and death, lost of productivity and economic crises, and degraded ecosystems. These impacts elevate water to a crisis of global concern. There is the tremendous economic waste involved in people spending so much time and effort in search of water. Lack of water often means relatively low levels of personal hygiene and environmental sanitation and because water is needed for most productive activities, inadequate access to it limits the livelihood options of the people, particularly in rural areas (Gbadegesin and Olorunfemi, 2009).

2.2 Availability and Accessibility of Safe Drinking Water

According to WHO(2008), safe drinking water is water that does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. The safety of water in public health is determined by its microbiological, physical, chemical and radiological quality of these, microbiological quality is usually the most important (ADWG, 1996). The provision of an adequate safe drinking water was one of the eight components of primary health care identified in 1978 by the International Conference on Primary Health Care (Dufour *et al*., 2002). Having better water and sanitation is essential in breaking the cycle of poverty since it improves people's health, strength to work, and ability to go to school (CAWST, 2009). Lack of safe drinking water is a serious threat to national development and may have a negative impact on the health status of its citizen and the overall performance of the work force. In many developing countries, availability of water is a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system (Okonko *et al*., 2008).

The United Nations General Assembly adopted Resolution 64/292 recognizing that safe and clean drinking water and sanitation is a human right essential to the full enjoyment of life and all other human rights (WHO, 2011). Nigeria in the last four decades has witnessed a steady decline in the quality of service rendered by most public water agencies (Amori and Makinde, 2012), which may be due to increase in population, deteriorations of the water supply facilities, poor management of resources, water pollution and lack of commitment from the government towards proving adequate and safe drinking water to its citizens which resulted in high cost of production of potable water. In the urban and rural areas of Nigeria, pipe borne water is supplied is through treatment plants owned by government while communities without water infrastructure depend directly on rivers, wells and boreholes as sources of drinking water, such sources of water are not suitable for human consumption as they are usually not treated and are contaminated by human and animal excretaand agricultural and industrial wastes (FMWR, 2000).

Accessibility and availability of safe drinking water is very important for human survival. People's access to safe drinking water and adequate sanitation encourage personal, domestic and community hygiene which will improve the quality of life of millions of individuals (WHO, 2008). Access to safe drinking water is important as health and development issues at regional, national and local level. Interventions in improving access to safe water favour the poor whether in rural or urban areas, and can be effective part of poverty alleviation strategies (WHO, 2008). Despite global efforts toward the Millennium Development Goal's target, millions of people lack access to safe drinking water while billions people are not provided with improved, adequate sanitation and majority of these are in southern Asia (25%) and sub Saharan Africa (37%)(WHO, 2011). In many developing countries, availability of water is a matter of great concern to families and communities more especially those who depend on non public water supply system (Okonko *et al*, 2008). The demand for treated water has exceeded the supply capacity of the few available water treatment plants and consequently, water supply is irregular, epileptic and grossly inadequate. The inadequate investment in water infrastructure over the past few decades has restricted or even eliminated pipe water access on an increasingly large fraction of the urban population (Justin *et al.,* 2012).

According to USAID (1990), access to improved water and sanitation is a daily challenge for most Nigerians and the problem is particularly acute in Northern Nigeria where only few of the population have access to safe drinking water and adequate sanitation in which this situation leads to high prevalence of water borne diseases, threatening the livelihood of small holder farmers and contribute to low level of school enrolment especially among girls. The global water crises claims more lives through disease than any war claims through guns (USAID, 1990). This water scarcity results in further marginalization of living conditions and generates high rate of morbidity, particularly in most densely populated and generally poorest urban areas (Gaisie and Gyau-Boakye, 2007).

The demand for good quality water for drinking and other purposes is no doubt exceeding supply especially in some regions of developing countries where drought has claimed thousands of lives and lifted economic and social damage (Frederiksen, 1996). Water safety is affected by pollution from industry and wastewater, poor sanitation, weak infrastructure, unreliable services, and the need for collection, transportation and storage in the home (WHO, 2011). Contaminated drinking water, along with inadequate supplies of water for personal hygiene and poor sanitation, are the main contributors of diarrhea. The World Health Organization estimates that 88% of diarrheal disease is caused by unsafe water, inadequate sanitation and poor hygiene as a result, more than 4,500 children die every day from diarrhea and other diseases (CAWST, 2009).

In Nigeria the inadequacy of safe water and improved sanitation service is manifested in the prevalence of water and sanitation related diseases. Diarrhea which results from poor hygiene habits and consumption of poor quality water is the second main cause of infant mortality after malaria and the third main cause of under five mortality (FMWR, 2000). The prevalence of diarrhoea is higher in the rural than urban areas and in the northern zone than in the south of Nigeria (FMWR, 2000). The high morbidity and mortality rates and the impact of these diseases are due to a combination of inadequate health care, water supply and sanitation services and unhygienic practices. The drive for poverty reduction in Nigeria recognizes water supply and sanitation as an important component (FMWR, 2000).

2.3 Packaged Drinking Water

The challenges associated with drinking water have encouraged the production of packaged drinking water by private enterprises that have little knowledge about manufacturing practices (Edema *et al*., 2011). The increasing demand for safe drinking water has led to production of bottled water and sachet water popularly called "pure water" as an alternative, and considered it being safe for drinking. Bottled water consumption has been steadily growing up in the last three decades at a global level and only individuals who have good financial status can afford bottled water while the low income earners are left with no option but to consume sachet water that is cheaper and affordable (Adegoke *et al.,* 2012).

Studies have shown that most bottled water manufacturers in Nigeria also engage in sachet water packaging and obtain their raw water mostly from local municipal pipe water or well water (Oyedeji *et al.,* 2010). Worldwide, sales of bottled water increase by an average of 12% every year, and consumers may have reasons for purchasing bottled water, such as taste, convenience, but for many consumers, safety and potential health benefits are important considerations (Magda *et al*., 2008). Results of studies conducted on the quality of bottled water in many countries to assess its suitability for human consumption indicated variation in the quality. The quality of water may vary from one source to another based on several parameters such as water sources, type of water purification, and storage tanks (Mufeed, 2006). There are concerns about chlorine byproducts, contaminants such as lead, nitrates and microorganisms contamination in municipal water supplies however, some microorganisms, which are normally of little or no public health significance, may grow to higher levels in bottled water (WHO, 2008).

Study conducted by International Bottled Water Association revealed that 25% of all bottled water is simply tap water placed in a bottle (Magda *et al*., 2008). In addition, the quality of bottled water can also substantially vary among brands as well as with time and with different production runs depending on its source, treatment technology, manufacturing operations, packaging material and shelf life before use, although bottled water should have a shelf life of 30 days unopened but most bottled water companies' labels show that their water is valid to 1 or 2 years (Magda *et al.*, 2008). On the other hand, bottled water are most commonly disinfected with ozone, which provides a residual disinfection for a limited time and subsequently does not leave a residual taste like tap water, which uses chlorine as a final disinfectant(Magda *et al.*, 2008). The length of time chlorine and ozone remain active in water depends on many factors including temperature. However, bottle water may be in distribution and storage conditions for several weeks, which may adversely affect its quality. According to (Magda *et al.*, 2008) in mid 1970s there was an outbreak of Cholera in Portugal due to consumption of bottled water and the Center for Disease Control and Prevention reported in 1994 an outbreak of cholera in the United States associated with bottled water.

The introduction of sachet water in Nigeria was a laudable idea but studies suggested that this innovative idea is not risk free (Alli *et al.,* 2011). The sachet drinking water was introduced into Nigeria as less expensive means of accessing drinking water than bottled water (Oyedeji *et al.,* 2010). It also acts as improvement over the former types of drinking water packaged for sale to consumers in hand filled and tied in polythene bags (Oyedeji *et al.,* 2010). Today, the easy accessibility to drinking water in packaged forms has resulted in big thriving water industries with several millions of litres of these products consumed every year by Nigerians (Ogundipe, 2008). Most people in the cities and rural communities depend on sachet water as their ultimate source of drinking water. The integrity of sachet water is doubtful in fact, it has been reported that most vendors do not treat their sachet water before selling to the public and many who are engaged in the production do not follow strictly standard set by Federal Environmental Protection Agency and World Health Organisation (Okpako *et al*., 2009; Oladipo *et al*., 2009). The consumption of sachet water in Nigeria is on alarming rate and people are not mind full of the source, quality and possible consequence associated with sachet water consumption. Occasionally, contamination of sachet water may occur either during processing, transportation and improper handling by hawkers and source of the water used for the production (Adegoke *et al.,* 2012). Apart from health implication, contaminated drinking water also has serious socio-economic and political implication (Mukhtar and Oyeyi, 2005). This become of concern to public health worker and right thinking individual when considering the fact that public health including nursing mothers patronize the vendor to procure water for their small children (Oladipo *et al.*, 2009).

Despite its popularity, studies conducted on the bacteriological quality of sachet water in some African cities have catalogued various levels of contamination (Adenkunle *et al*., 2004; Ifenyi *et al.*, 2006; WHO, 2011). Most of the brands fall below World Health Organisation standard for drinking water and are therefore of doubtful quality. Adherence to production and analytical standard are doubtful as most of the factories are observed to lack the appropriate technology for achieving these (Alli *et al*., 2011). The standard of hygiene in the various stages of production of bottle and sachet water varies among various manufacturers while some employ sophisticated techniques such as ozonization and reserve osmosis. Most use ordinary boiling of well water and exclusion of particles by use of unsterilized filtration materials (Oyedeji *et al*., 2010).

Researches conducted in different parts in Nigeria and Ghana have shown the existence of various forms of harmful bacteria and fungi in the sample of sachet water analyzed. Edema *et al.*(2011) examined the bacteriological quality of sachet water sold in Nigeria in which, the result indicated that 85% contain *Escherichia coli* and 65% of the polythene sachet used is not food grade quality and impacted polyester taste in the water.

Study conducted by Adegeko *et al*. (2012) on sachet water in Abia State of Nigeria revealed that 5 brands out the 10 met up international standard for drinking water. Ezeugwunne *et al*. (2006) isolated *Streptococcus faecalis, Pneumonia* and *Staphylococcus aureus* from sachet water examined in Nnewi, Nigeria. Nwachukwu and Emeruem (2007) examined the presence of antibiotic resistant bacteria in sachet water in which various heterotrophic bacteria are resistant to eight common antibiotics. Nwosu and Ogueke (2004) isolated *E. coli* from sachet water examined in Owerri metropolis, Nigeria. Study from Abakaliki, Nigeria by Afiukwa *et al*. (2010) revealed that all twenty brands of sachet water sampled contained eleven strains of bacteria which are multi-drugs resistant while Ngozi *et al*. (2010) isolated *Klebsiella* species and *Enterobacter* species in sachet water sampled in Abakaliki.

Study in Port Harcourt by Alli *et al.*(2011) on 100 different brands of sachet water indicated that 20% of the water samples harboured parasites which were attributed to the environmental condition of the area where they are produced.Okpako *et al.,* (2009) conducted a study on the quality of drinking water in Calabar, Nigeria in which 75% of the samples contained at least two fungi species. Similarly, Dada (2009)reported22% non compliance with the regulatory standard from 100 samples of 10 brands of sachet water in Lagos. Moreover, another study conducted in Lagos, Nigeria revealed that bacteriological characteristics of sachet water deteriorate considerably as products moved further down the distribution chain. Less than 7% of sachet water contamination took place after the production(Omalu *et al.,* 2010). Egwari and Aboaba (2002) in Lagos, southwest Nigeria conducted bacteriological study of sachet water found no enteric pathogen in the water. Study conducted in Ibadan by Ajayi *et al.,* (2008), showed that bottled water sampled was in compliance but 30% of sachet water sampled did not comply. Indeed 5% of 78 samples showed positive coliform count. A second study from Ibadan conducted by Oyedeji*et al*. (2010) who examined 16 brands that include samples from Ile-ife, one brand of bottled water result indicated positive coliform count while all the sachet water brands showed bacterial growth. Study conducted by Adenkunle *et al*. (2004) on quality of sachet water in Ibadan, Nigeria indicated 6.4% bacterial growth. Taiwo *et al*. (2012) analysed the bacteriological properties of 20 brands of sachet water in Abeokuta the capital of Ogun State, Nigeria, and found that all the sachet water were within the permissible limits, and they concludes that there is improvement in the quality of the sachet water produced in Abeokuta metropolis. However, Popoola *et al*. (2007), conducted a study on potable water in Abeokuta and isolated several bacteria species were isolated from stored water samples which have significant health implications. Oyagade and Fasuan, (2004) reported the occurrence of antibiotic resistant strains of *Escherichia coli* in water used for drinking purposes by Nigerians communities which signals additional danger with the use of water stored for long period of time*E. coli* were the most consistent bacteria species encountered in the water samples at the different stages of storage. Onifade and Iiori (2008) examined quality of sachet water in Ondo State, Nigeria where the result indicated *E. coli*whichexceeded the international drinking water limit. Oladipo *et al*., (2009) isolated fourteen bacteria, including *Bacillus, Pseudomonas* species, *Enterobacter aerogenes* and *Proteus mirabilis* from the sachet water tested in Ogbomos, Nigeria.

Ante *et al.* (2007) conducted a research on the potability of sachet water in Kaduna State, where the microbial parameters exceeded World Health Organisation Standard for drinking water. Similarly, a study conducted by Yahaya *et al.*(2011) on public drinking water in parts of Zaria, Nigeria observed the occurrence and elevated number of coliform bacteria within the period of the research.. Kalpana *et al*. (2011) also examined quality of sachet water sold in Kebbi State University and the nearby market in the city and reported to be contaminated. Microbial quality of sachet water marketed in Maiduguri metropolis eastern Nigeria was examined by Muaza *et al*. (2012) revealed the presence of coliform, 95% of the sachet examined are not fit for human consumption and hazardous to human health.

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Obiri-Danso *et al*. (2003) examined the microbial quality of sachet drinking water and bottle water sold on the street of Kumasi, Ghana, concluded that bottled water in the Ghanaian market is of good microbial quality. Study by Dodoo *et al.*(2006) in the cape coast of Ghana indicated high level of contamination by microorganisms. Addo *et al*. (2009) reported that, the quality of sachet water in Teshi-Nungua, Ghana contain coliform bacteria. Quality of bottle and sachet water in Tarka-Nsuaem municipality of Ghana assessed by Nana and Amorin (2011) revealed that, both microbial and the physicochemical parameterssatisfy the international and Ghana drinking water standard. Emmanuel and Solomon, (2012) examined the quality of sachet water and bottle in the Bolgatanga Muncipality of Ghana, the study revealed that except for low level of fluoride ion in some brands, sachet and bottled water brands sold in Bolgatanga municipality possessed acceptable physicochemical characteristics. However, the bottled water brands generally possessed good bacteriological characteristics, while the bulk of sachet water brands were contaminated by coliform bacteria.

In Nigeria, the National Agency for Food and Drug and Administration Control is the parastatal under the Federal Ministry of Health charged with the responsibility for the regulation and control of imported and locally processed food, drugs and water products (Omotayo and Denloye, 2002). To ensure strict adherence to international standards, National Agency for Food and Drug Administration Control’s regulation for bottled and sachet water in Nigeria has been put at the standards established by the World Health Organisation, and according to these standards, potable water for human consumption must be free of microbial indicators of feacal contamination, and *coliform* count per 100mL of drinking water be zero (WHO, 1997; Pierre, 1999).

Several studies on microbial quality of bottled and sachet water have indicated violation of international quality standard which is adopted governmental organization such as National Agency for Food and Drug Administration and Control, Federal Environmental Protection Agency and Standard Organization of Nigeria. Surveillance carried out by National Agency for Food and Drug Administration and Control between 2004 and 2005 revealed that some producers of packaged water indulge in quick practices such as packaging of untreated water, production under unhygienic condition, illegal production of unregistered water in unapproved premises, use of non-food grade sachet and release of packaged water for distribution and sale without date marking, these malpractices compelled the agency to formulate guideline for the production of wholesome packaged water (Edema *et al*., 2011).

2.4 Microbial Quality of Drinking Water

The microbiological quality of drinking water is of serious concern to consumers and public health authorities. Certain microorganisms, including various bacteria, viruses, fungi and parasites, are well-known water contaminants, of which several may lead to waterborne disease and epidemics (Hageskal *et al.,* 2009). Bacteria are probably the most frequently studied group of microorganisms with respect to the quality of drinking water. Pathogenic viruses in water are also of great importance as viruses are the most common cause of gastrointestinal infection worldwide (Mara and Horan, 2006). The level of microorganisms in drinking water generally dependent on good manufacturing practices. The presence of microorganisms in processed water can deteriorate and the potability of the water before it reaches the consumer(Mara and Horan, 2006). To ensure that the microbiological characteristic of drinking water is safe for human consumption the

Nigeria base National Agency for Food and Drugs Administration and Control in association with World Health Organisation recommended that potable water for human consumption should not contain any microorganism that is known to be pathogenic (WHO, 1984).

2.4.1 Bacteria associated with drinking water

The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority, this is so because studies have reveal several disease outbreak associated with untreated or poorly treated water containing bacteria pathogen that have been isolated from sachet water. The water contamination with fecal bacteria is a common and persistent problem that has a direct impact on the public health, and in economic and social aspects (Stewart *et al.* 2007). *Coliform* in water, though harmless to human health had been shown to portend the most probable presence of other pathogenic microorganisms like *Salmonella typhi*, *E. coli*, *Pseudomonas*, *Vibrio* species,*Shigella* species, *Aeromonas hydrophilia* among others (Egwari and Aboaba, 2002).

Several studies on the microbial quality of bottled and sachet water have reported violations of international quality standards (Edema *et al.*,2011). In a Canadian study, screening of bottled water for indicator bacteria revealed that 3.7% of the samples had total *coliforms* and 23.3% of the 3,460 samples had more than 100 colonies of heterotrophic bacteria per ml of sample (Warburton *et al.*, 1992). In a study by Banu and Menakuru (2010) in India on the microbial properties of sachet water, *Klebsiella, Pneumonia* and *Proteus mirabilis* were isolated and are antibiotic resistant.

The quality monitoring sachet water in Nigeria has been documented (Adenkunle *et al.*, 2004; Onifade and Ilori, 2008; Dada, 2009).

Acinetobacter species are Gram-negative, oxidase-negative, non-motile coccobacilli (short plump rods), common species are *Acinetobacter baumannii, Acinetobacter iwoffii* and *Acinetobacter junii.* They are usually commensal organisms, but they occasionally cause infections (WHO, 2011). They are opportunistic pathogens that may cause urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections (Cheesbrough, 2000; WHO, 2011). *Acinetobacter* species are ubiquitous inhabitants of soil, water and sewage environments. *Acinetobacter* has been isolated from 97% of natural surface water samples (WHO, 2011). The organisms have been found to represent 1.0–5.5% of the heterotrophic plate count (HPC) flora in drinking-water samples and have been isolated from 5–92% of distribution water samples (Bifulco *et al.,* 1989). In a survey of untreated groundwater supplies in the United States of America, *Acinetobacter* species were detected in 38% of the groundwater supplies at an arithmetic mean density of 8/100 ml (Bifulco *et al.,* 1989). *Acinetobacter* species are part of the natural microbial flora of the skin and occasionally the respiratory tract of healthy individuals. *Acinetobacter* species are detected which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms. However, *E. coli* (or, alternatively, thermotolerant coli- forms) cannot be used as an indicator for the presence/absence of *Acinetobacter* species (WHO, 2011).

Bacillus species are Gram positive aerobic spore-formers and most members of the genus are saprophytic prevalent in soil, water and air and on vegetation (WHO, 2011). *Bacillus* species are classified into the subgroups *Bacillus polymyxa, Bacillus subtilis* (which includes *Bacillus cereus* and *Bacillus licheniformis), Bacillus brevis* and *Bacillus anthracis*. Although most *Bacillus* species are harmless, few are pathogenic to humans and animals. *Bacillus cereus* causes food poisoning similar to *Staphylococcal* food poisoning (WHO, 2011). Some strains produce heat-stable toxin in food that is associated with spore germination and gives rise to a syndrome of vomiting within 1 to 5 hours of ingestion (WHO, 2011). Other strains produce a heat labile enterotoxin after ingestion that causes diarrhoea within 10 to 15 hours. *Bacillus cereus* is known to cause bacteraemia in immune compromised patients as well as symptoms such as vomiting and diarrhoea. *Bacillus anthracis* causes anthrax in humans and animals. They form part of the heterotrophic plate count bacteria, which are readily detected in most drinking-water supplies. Drinking-water has been identified as a source of infection of pathogen. *Bacillus* species are often detected in drinking-water supplies, even supplies treated and disinfected by acceptable procedures. This is largely due to the resistance of spores to disinfection processes (WHO, 2011).

*E. coli*is present in large numbers in the normal intestinal flora of humans and animals, where it generally causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia, acute diarrhoea, abdominal cramps, nausea, vomiting and fever in infants, headache and meningitis (Nataro and Kaper, 1998). *E. coli* are enteric organisms, and humans and animals are the major reservoir.. Waterborne transmission of pathogenic *E. coli* has been well documented for recreational waters and contaminated drinking-water (WHO, 2011).There is no indication that the response of enteropathogenic strains of *E. coli* to water treatment and disinfection procedures differs from that of other *E. coli*.

Staphylococcus aureus is an aerobic, non-motile, non-spore-forming, catalase and coagulase positive, Gram-positive coccus, usually arranged in grapelike irregular clusters. The genus *Staphylococcus* contains at least 15 different species. Although *S. aureus* is a common member of the human microflora, it can produce disease through two different mechanisms. One is based on the ability of the organisms to multiply and spread widely in tissues, and the other is based on the ability of the organisms to produce extracellular enzymes and toxins (WHO, 2011). Gastrointestinal disease (enterocolitis or food poisoning) is caused by a heat stable staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids (WHO, 2011). *S. aureus* is relatively widespread in the environment but is found mainly on the skin and mucous membranes of animals. The organism is a member of the normal microbial flora of the human skin and is found in the nasopharynx of 20–30% of adults at any one time. *Staphylococci* are occasionally detected in the gastrointestinal tract and can be detected in sewage. *S. aureus* can be released by human contact into water environments such as swimming pools other recreational waters. It has also been detected in drinking-water supplies (WHO, 2011). Hand contact is by far the most common route of transmission. Inadequate hygiene can lead to contamination of food. The consumption of foods containing *S. aureus* toxins can lead to food poisoning within a few hours (LeChevallier and Seidler, 1980). Although *S. aureus* can occur in drinking-water supplies, there is no evidence of transmission through the consumption of such water (WHO, 2011). Although *Staphylococci* are slightly more resistant to chlorine residuals than *E. coli*, their presence in water is readily controlled by conventional treatment and disinfection processes (Lechevallier and Seidler, 1980). As faecal material is not their usual source, *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable indicator for *S. aureus* in drinking-water supplies (WHO, 2011).

The genus *Yersinia* is classified in the family enterobacteriaceae and comprises seven species. The species *Yersinia pestis*, *Yersinia pseudotuberculosis* and certain serotypes of *Yersiniaenterocolitica* are pathogens for humans. *Yersinia*species are Gram-negative rods that are motile at 25 °C but not at 37 °C (WHO, 2011). *Y. enterocolitica* penetrates cells of the intestinal mucosa, causing ulcerations of the terminal ileum (WHO, 2011). Yersiniosis generally presents as an acute gastroenteritis with diarrhoea, fever and abdominal pain (Waage, 1999). Domestic and wild animals are the principal reservoir for *Yersinia* species. Pigs are the major reservoir of pathogenic *Y. enterocolitica*, whereas rodents and small animals are the major reservoir of *Yersinia pseudotuberculosis* (WHO, 2011). Pathogenic *Y. enterocolitica* has been detected in sewage and polluted surface waters, however, *Y. enterocolitica* strains detected in drinking-water are more commonly non-pathogenic strains of probable environmental origin (WHO, 2011). At least some species and strains of *Yersinia*seem to be able to replicate in water environments if at least trace amounts of organic nitrogen are present, even at temperatures as low as 4 °C (Ostoff, 1994). Owing to the long survival and/or growth of some strains of *Yersinia* species in water, *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable indicator for the presence/absence of *Yersinia* species in drinking-water (WHO, 2011).

2.4.2 Fungi associated with drinking water

Fungi are eukaryotic, heterotrophic and many fungal species can survive in oligotrophic environments (DEFRA, 2011). Fungi are a diverse group of organisms belonging to the kingdom Eumycota which comprises of five phyla, namely Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota (Kirk *et al.,* 2001). Some fungi are primarily adapted to aquatic environments and are naturally found in water. These fungi are zoosporic, and many belong in phyla Chytridiomycota while the rest of the phyla are primarily adapted to terrestrial environments (Hageskal *et al.*, 2009).

Fungi are widely distributed in nature and can occur as unicellular yeast or filamentous and, multicellular molds. Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments (Kirk *et al.,* 2001). Fungi are cosmopolitan in origin and various environmental factors; wind, moisture, temperature and air pollution affects and alter the density and frequency of fungi species in any medium while temperature, water potential, humidity and pH have critical influence on the growth and survival of fungi (Tanveer *et al*., 2011). In 1980s and 1990s, more cases of health problems caused by fungal contaminated drinking water were reported from Finland and Sweden (Muittari *et al.*, 1980; Aslund 1984). In the last decade, several studies conducted in several countries on drinking water had results which indicated that the recovery of fungi varied between 7.5–89 % positive samples, and that the levels of fungi in the samples varied considerably in the various investigations (Kelley *et al.*, 2003; Hapcioglu *et al*., 2005; Kanzler *et al*., 2007; Kennedy and Williams, 2007). In addition, results from studies of taste and odour problems in drinking water indicated the occurrence of fungi as an important causative factor for these sensoric changes (Nystrom *et al.,* 1992; Montiel *et al*., 1999). Statistically, it has been established that the odds for fungal recovery are three times higher in surfaces sourced water compared with ground-sourced water and fungi are more commonly recovered from cold water than from hot tap water (Hageskal *et al.,* 2007). Fungi have been reported from all types of water which include bottled and sachet drinking water (Mirian *et al*., 2007; Okpako *et al.,* 2009). The most commonly isolated genera from drinking water are *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialophora* and *Acremonium* (DEFRA, 2011).

Fungi can enter drinking water distribution systems through deficiencies in treated water storage facilities, cross-connections, mains breaks and intrusions, and during installation of water storage and distribution facilities (DEFRA, 2011). Oni and Olayeni (2003) reported that purification procedures such as chlorination do not eliminate fungal spores, which implies that perhaps the treatment given to our sachet water is usually not effective enough to eliminate these microorganisms (DEFRA, 2011).

Studies established that fungi can survive treatment, and that most of the water treatment methods are not sufficient against fungi. Many of the taxa most frequently isolated from drinking water has the potential to secrete pigment called melanin which provide protection against range of stresses making them resistance to water treatment (Langfelder *et al.*, 2003). In a study by Kelley (2003) sand filtration was reported to give better removal of fungi than coagulation with iron. Chlorination used for disinfection of water was found to be insufficient (Frankova´ and Horecka, 1995), whereas the use of chlorine dioxide and ozone were reported as the most effective water treatment methods against fungal spores (Kelley *et al.*, 2003). A frequently used water treatment method in developed countries is ultra violet radiation. With respect to fungi in drinking water, ultra violet may have a positive effect (Kanzler *et al.*, 2007). However, as the ultra violet sensitivity of fungi often is related to pigmentation, fungi with pigmented spores, such as *Aspergillus* and *Penicillium*, have better protection against radiation and are less sensitive to ultra violet (Waipara, 1998). The abiotic factors that influence fungal taxa isolated from drinking water are accumulation of organic and inorganic materials, water temperature, water flow rate and nutrient concentration while the biotic factors are interaction between fungi with bacteria, protozoa and viruses (DEFRA, 2011).

They are involved in different forms of diseases, including allergies to fungal antigens, production of toxins, or direct invasion of hosts. Several species of fungi are capable of infecting healthy hosts and causing diseases ranging from mucosal to life threatening disseminated infections (Mirian *et al.*, 2007). Fungal infections are difficult to treat since the agents are eukaryotes, as human cells (Mirian *et al*., 2007). Some fungi species isolated from water samples are known to be strong allergenic skin irritants or may cause infections in immune suppressed individuals such as Acquired Immune Deficiency Syndrome, cancer, and organ transplant patients and persons with asthma or various respiratory problems (Hageskal *et al.*, 2006).

Penicillium species have been frequently recovered from water in the various studies performed. Several of the species in both genus *Penicillium* and *Aspergillus* are known to produce mycotoxins in food and beverages (Moreau, 1979; Pitt and Hocking, 1999). In a study by Kelley *et al.* (2003) concluded that mycotoxins and other metabolites can be produced by fungi in water. Mycotoxins produced in water will of course be extremely diluted, and are perhaps of minor concern. Nevertheless, water is occasionally stored in cisterns or reservoirs, or even in bottles, for prolonged periods. In such cases, the concentrations of mycotoxins may increase. Large amounts of water are consumed daily, and daily intake over many years of even small amounts of mycotoxins may be hazardous to human health (Paterson, 2006). According to Nikaeen and Mirhendi (2008), *Aspergillus* species in drinking water are involved in the production of health risks while De-hoog *et al*. (2000) described that many species of *Aspergillus* are found in water and are causative agents of kidney and liver disorders, allergic sinusitis burns, otitis media and increase the risk of invasive infections.

2.5 Health Implication of Microorganisms in Drinking Water

Previous studies have revealed that pathogens gain entry into drinking water supplies even in industrialized countries. Break-through during treatment and ingress through cracked pipes are well documented causes of waterborne outbreaks of cryptosporidiosis and *E. coli*. (Craun and McCbe, 1993; Swerdlow *et al.,* 1997). The microbiological safety of drinking water remains a challenging problem in developing countries. The prevalence of water related diseases in developing countries is determined by the quality of the drinking water. The intake of unwholesome water could have devastating effects on our health as unsafe drinking water is a key determinant of many microbial diseases with serious complications in immune competent and immune compromised individuals. The quality and quantity of available water have implication on the health status of a community. Transmission of water borne disease is still a major public health concern despite considerable effort and modern technology being utilized for the production of safe drinking water. Water related diseases continue to be one of the major health problems globally (Oladipo *et al.,* 2009). It is very difficult to control diarrhoea, without a reliable supply of safe water to maintain hygiene. This reliable supply of safe water is most often beyond the reach of people and they rely on these cheap vended sachet water. Mortality in children under five years from water related diseases annually is estimated to be about 4 million in developing countries while 2.3 billion people worldwide have mortality and morbidity associated with water related ailment (WHO, 1997). Water-related diseases are estimated to cause 1.8 million deaths each year, mostly in developing countries and have been the major cause of mortality and morbidity (WHO 2010). Diseases caused by contaminated water are among the ten most prevalent waterborne diseases (WHO, 2010). Water the most vital resource for all kinds of life on this planet, can be extremely dangerous when it becomes the vehicle of transmission of diseases (WHO, 2010).

It is important to recognize that what appears to be harmless to the healthy individual may be potentially fatal to children and immune compromised persons. The consumption of drinking water contaminated with pathogenic microbes of faecal origin is a significant risk to human health in the developing world, especially in remote rural areas and industrial areas. According to Edema *et al.*(2011) the incidence of bacterial water-borne infections particularly typhoid fever caused by *S. typhi*, is increasing in Nigeria as large proportion of the popular sachet-packed water is microbiologically unsafe for human consumption, constituting potential health risk; and this can be linked to the increasing incidence of typhoid fever among other food and water-borne. The fungi recovered from water in previous studies performed include some yeast, but are mostly filamentous fungi. A wide diversity of mould species has been isolated from drinking water. *Aspergillus fumigatus* has been one of the most significant fungal pathogen causing infections in immune compromised patients in hospitals are facing a greater challenge with respect to opportunistic fungal infections (Denning *et al*., 2006).

2.6 Physicochemical Quality of Drinking Water

Most chemicals arising in drinking water are of health concern. A few chemical contaminants have been shown to cause adverse health effects in humans as a consequence of prolonged exposure through drinking water. Some substances of health concern have effects on the acceptability of drinking-water that would normally lead to rejection of the water at concentrations significantly lower than those of health concern. Several research findings such as those of Egwari and Aboaba (2002) showed that water from several boreholes and wells in some urban centers in Nigeria were heavily contaminated with lead and toxic organic wastes. Although, Shelton *et al.* (2005) suggested that the permissible limit for lead in drinking water is 0.05µg/l but the current limit by WHO standards is 10µg/l or 0.01mg/l. It has been established that long term accumulation of lead in body tissues has neurotoxic, nephrotoxic, fetotoxic, and teratogenic effects on man just like most heavy metal contaminants. Research conducted by Musa *et al.,* (2008), indicated high concentration of arsenic in sources of drinking water in Zaria. Garba *et al.,* (2012), indicated that southern Kaduna sources for drinking water have high concentration of arsenic than northern Kaduna, the concentration is higher than maximum allowed in drinking by both Standard Organization of Nigeria (2007) and World Health Organization (2008). Similar condition was observed in Karaye Local Government Area, Kano State revealed high levels greater than the maximum permissible limit sets by the World Health Organization (0.01mg/L) was detected and hence, the water samples are not fit for human consumptions. People that are prominently consuming the water from these areas are vulnerable to arsenic related diseases such as; cardiovascular problems, convulsions, inflammation of the liver, cancer, birth defects, organ damage disorder of the nervous system and damage to the immune system (Garba *et al.,* 2008). The International Agency for Research on Cancer (IARC) recognized arsenic compounds as group one carcinogen (Garba *et al.,* 2008).

Research has indicated high concentration of magnesium in drinking water gives unpleasant taste to the water (WHO, 2011). Hardness is usually known to be caused calcium and magnesium. Public acceptability of the degree of hardness of water may vary considerably from one community to another due to differences in sources of water

(WHO, 2011). The taste threshold for the calcium ion is in the range of 100–300 mg/l, depending on the associated anion, and the taste threshold for magnesium is probably lower than that for calcium (WHO, 2011). In some instances, consumers tolerate water hardness in excess of 500 mg/l (WHO, 2011). Calcium, which is essential for nervous system and for the formation of bones, is commonly present in all water bodies where it usually comes from leaching of rocks. High concentrations of chloride give a salty taste to water. Taste threshes hold for the chloride anion depend on the associated cation and are in the range of 200–300 mg/l for sodium, potassium and calcium chloride. Concentrations in excess of 250 mg/l are increasingly likely to be detected by taste, but some may become accustomed to low levels of chloride-induced taste (Agunwamba, 2000).

Drinking-water should ideally have no visible colour. Colour in drinking-water is usually due to the presence of coloured organic matter (primarily humic and fulvic acids) associated with the humus fraction of soil. Colour is also strongly influenced by the presence of iron and other metals, either as natural impurities or as corrosion products (WHO, 2011). It may also result from the contamination of the water source with industrial effluents and may be the first indication of a hazardous situation (WHO, 2011). Most people can detect colour above 15 True Colour Units (TCU) in a glass of water. Levels of colour below 15 TCU are often acceptable to consumers (CAWST, 2009). High colour from natural organic carbon (e.g. humics) could also indicate a high propensity to produce by products from disinfection processes.

Odour in water is usually caused by volatile substances associated with organic and inorganic chemical materials such as algae and hydrogen, respectively. In many cases, aesthetic problems will be prevented by optimizing conventional treatment processes such as coagulation, sedimentation and chlorination (WHO, 2008). However, if specific treatment is deemed necessary, aeration, granular or powdered activated carbon and ozonation are generally effective techniques in removing organic chemicals and some inorganic chemicals, such as hydrogen sulphide, that cause tastes and odours (WHO, 2010). Tastes and odours caused by disinfectants are best controlled through careful operation of the disinfection process and pretreatment to remove precursors. Manganese can be removed by chlorination followed by filtration. Techniques for removing hydrogen sulphide include aeration, granular activated carbon, filtration and oxidation (WH0, 2008). Ammonia can be removed by biological nitrification. Precipitation softening or cation exchange can reduce hardness. Other taste- and odour causing inorganic chemicals (e.g. chloride and sulphide) are generally not amenable to treatment. Taste can be influenced by temperature, total dissolved solids, and pH (ADWG, 1996).Cool water is generally more palatable than warm water, and temperature will have an impact on the acceptability of a number of other inorganic constituents and chemical contaminants that may affect taste (Sunday *et al*., 2011). High water temperature enhances the growth of microorganisms and may increase problems related to taste, odour and colour (WHO, 2011).

Usually pH has no direct impact on consumers, it is one of the most important operational water quality parameters. Careful attention to pH control is ne-cessary at all stages of water treatment to ensure satisfactory water clarification and disinfection (WHO, 1997). For effective disinfection with chlorine, the pH should preferably be less than 8; however, lower pH water (approximately pH 7 or less) is more likely to be corrosive

(WHO, 2011). The pH of the water entering the distribution system must be controlled to minimize the corrosion of water mains and pipes in household water systems (WHO, 2008). Alkalinity and calcium management also contribute to the stability of water and control its taste. Failure to minimize corrosion can result in the contamination of drinking-water and in adverse effects on its taste and appearance. The optimum pH required will vary in different supplies according to the composition of the water and the nature of the construction materials used in the distribution system, but it is usually in the range 6.5–8.5 (WHO, 2011). The presence of sulfate in drinking-water can cause noticeable taste, and very high levels might cause a laxative effect in unaccustomed consumers (WHO, 2011). Taste impairment varies with the nature of the associated cation; taste thresholds have been found to range from 250 mg/l for sodium sulfate to 1000 mg/l for calcium sulfate. It is generally considered that taste impairment is minimal at levels below 250 mg/l. No health-based guideline value has been derived for sulfate (WHO, 2008).

The palatability of water with a total dissolved solids (TDS) level of less than about 600 mg/l is generally considered to be good; drinking water becomes significantly and increasingly unpalatable at TDS levels greater than about 1000 mg/l (WHO, 2010). The presence of high levels of TDS may also be objectionable to consumers, owing to excessive scaling in water pipes, heaters, boilers and household appliances. No health-based guideline value for TDS has been proposed ((WHO, 2011). Turbidity in water is caused by suspended particles or colloidal matter that obstructs light transmission through the water. It may be caused by inorganic or organic matter or a combination of the two. Microorganisms (bacteria, viruses and protozoa) are typically attached to particulates, and removal of turbidity by filtration will significantly reduces microbial contamination in treated water (WHO, 2011). Turbidity in some groundwater sources is a consequence of inert clay or chalk particles or the precipitation of non- soluble reduced iron and other oxides when water is pumped from anaerobic waters, whereas turbidity in surface waters may be the result of particulate matter of many types and is more likely to include attached microorganisms that are a threat to health (WHO, 2010). Turbidity in distribution systems can occur as a result of the disturbance of sediments and biofilms but is also from the ingress of dirty water from outside the system. In addition, turbidity can seriously interfere with the efficiency of disinfection by providing protection for organisms, and much of water treatment is directed at removal of particulate matter before disinfection (WHO, 2008). This not only will increase the efficiency of disinfection by chemical disinfectants such as chlorine and ozone, but is an essential step in ensuring the effectiveness of physical disinfection processes such as ultraviolet irradiation, because light transmission through water is impaired by particulates (WHO, 2011). Removal of particulate matter by coagulation and sedimentation and by filtration is an important barrier in achieving safe drinking-water. Achieving low turbidity by filtration (before disinfection) of water from surface sources and groundwater where raised turbidity occurs for instance, where these are under the influence of surface water is strongly recommended to ensure microbially safe water (WHO, 2010),

Turbidity can also have a negative impact on consumer acceptability of water as a result of visible cloudiness. Although turbidityfrom groundwater minerals or from post-precipitation of calcium carbonate from lime treatment) is not necessarily a threat to health, it is an important indicator of the possible presence of contaminants that would be of concern for health, especially from inadequately treated or unfiltered surface water (WHO, 2011). Data are emerging that show an increasing risk of gastrointestinal infections that correlates with high turbidity and turbidity events in distribution (WHO, 2011). This may be because turbidity is acting as an indicator of possible sources of microbial contamination. Turbidity is measured by nephelometric turbidity units (NTU) and can be initially noticed by the naked eye above approximately 4.0 NTU. However, to ensure effectiveness of disinfection, turbidity should be no more than 1 NTU and preferably much lower (WHO, 2011). Large, well-run municipal supplies should be able to achieve less than 0.5 NTU before disinfection at all times and should be able to average 0.2 NTU or less (WHO, 1997). Surface water treatment systems that achieve less than 0.3 NTU prior to disinfection will have demonstrated that they have significant barriers against pathogens that adsorb to particulate matter. Of particular importance is the fact that this will be a good indicator that they are removing chlorine-resistant pathogens such as Cryptosporidium (WHO, 2008). Occasionally, turbidity can be caused by minute air bubbles released when water has high dissolved air content.

Ajayi and Adesida (2009) conducted a research on quality of sachet water in Akure, Nigeria found that the concentration of uranium and thorium exceeded the World Health Organisation (2011) limits for uranium and thorium. Orisakwe *et al.* (2006) studied heavy metals in sachet water in the Eastern Nigeria and found that there were excess concentrations of lead, cadmium and copper. Okeri *et al.* (2009) examined trace metals in drinking water in Benin City, Nigeria in which the concentration of copper, iron, manganese and zinc were within the recommended level by international drinking water guidelines. Okafor and Ogbonna (2003) discovered that water used for production of fruit juice has high concentration of nitrite in Southeastern Nigeria. Ashaye *et al.* (2001) examined the physicochemical properties of sachet water in Ibadan, Nigeria, the result indicated low level of total dissolved solids and variable mineral content.

Concentrations of trace elements in water vary because of physiological, environmental and other factors. Some trace elements have several roles in living organisms. Some are essential components of enzymes where they attract substrate molecules and facilitate their conversion to specific and products (Okafor and Ogbonna, 2003). In excessive concentrations, however trace elements can negatively affect growth, reproduction and other biological functions. Some people prefer bottled water to sachet water as they believe that it is hygienically prepared. Some people drink only bore hole water or well water as it is the only available source especially in rural areas where bottled and sachet water are not produced or too expensive to buy. High concentration of lead was observed in bottle while borehole had least concentration of lead. The United States Environmental Protection Agency has classified lead as being potentially hazardous and toxic to most forms of life (USEPA, 2006). It has been found to be responsible for quite a number of ailments in humans such as chronic neurological disorders especially in fetuses and children. High concentration of zinc was observed in tap water while arsenic concentration was highly significant in borehole, sachet and well water (Nana and Amorin, 2011).

2.7 Bio-indicators of Water Quality

The microbiological quality of drinking water relies on the presence or absence of indicator organisms. The indicator organisms that are well known are the total coliforms, and thermotolerant coliforms or alternatively *E. coli*. Heterotrophic plate counts (colony counts) have been used to assess the general bacterial content of drinking water. An effective indicator organism for inferring the presence of pathogenic microorganisms in drinking water should: always be present when pathogens of like origin are present; be present in relatively large numbers so that they can be detected after considerable dilution; not be present in the absence of contamination, be easy and quick to detect, survive in water as long as water-borne pathogens, and be of similar sensitivity to disinfection as pathogens (ADWG, 1996). Coliforms may sometimes be present when there is no faecal contamination in the water or, the absence of thermotolerant coliforms does not necessarily guarantee the absence of faecal contamination (ADWG, 1996; WHO, 2011).The coliform group of organisms is generally accepted as the most suitable set of organisms to indicate faecal contamination. Although as a group they may not be exclusively of faecal origin, they are present in very high numbers in the faeces of warm-blooded animals. These are the coliform bacteria which can survive better, longer and are easier to detect than other pathogens (Agunwamba, 2000). Coliform bacteria are commonly found in soil, on vegetation, and in surface water. They also live in the intestines of warm-blooded animals and humans. Some coliform bacteria strains can survive in soil and water for long periods of time. Coliform bacteria are not likely to cause illness. However, because coliform bacteria are most commonly associated with sewage or surface waters, the presence of coliform bacteria in drinking water indicates that the other disease-causing organisms (pathogens) may be present in the water system (ADWG, 1996).

Coliforms comprise *E. coli*, other thermotolerant coliforms such as*Citrobacter* and regrowth bacteria such as *Klebsiella*, and other related enterobacteria. They are the most sensitive but least specific indicator group for faecal contamination (WHO, 2011). The total coliforms serve as an indicator because: as a group, they include thermotolerant coliforms which are a direct indicator of faecal contamination; their high numbers and their ability, along with some other more resilient faecal pathogens, to survive longer in natural water than *E. coli*enable them to indicate less recent or more remote incidents of faecal pollution; their detection can indicate that conditions may favour the presence of free living opportunistic pathogens such as *Aeromonas*, *Pseudomonas* and *Legionella*; their relative abundance and hardiness makes them a useful indicator of the efficiency of water treatment and disinfection processes, and their absence is a good indication of the absence of contamination and where coliforms are found in water supply systems they usually outnumber thermotolerant coliforms, and thus may be easier to detect (ADWG, 1996).

There are three different groups of coliform bacteria; each has a different level of risk. Total coliform, faecal coliform and *E. coli*are all indicators of drinking water quality (USEPA, 2006). The total coliform group is a large collection of different kinds of bacteria. *E coli*is a subgroup of faecal coliform. Total coliform bacteria are commonly found in the environment (e.g. soil or vegetation) and are generally harmless. If only coliform bacteria are detected in drinking water, the source is probably environmental and faecal contamination is not likely. However, if environmental contamination can enter the system, there may be a way for other pathogens to enter the system. Therefore, it is important to determine the source and resolve the problem. Faecal coliform bacteria are a subgroup of the total coliform group. They appear in great quantities in the intestines and feaces of people and animals. The presence of fecal coliform in a drinking water sample often indicates recent faecal contamination and there is a greater risk that pathogens are present than if only the total coliform bacteria are detected. *E. coli* is a subgroup of fecal coliform. Most *E. coli* are harmless and are found in greater quantities in the intestines of people and warm-blooded animals. Some strains, however, may cause illness. The presence of *E. coli* in a drinking water sample indicates recent faecal contamination.

According to WHO (2011), monitoring water quality is used to determine whether a treatment or other process is effective in removing target organisms. Very occasionally, pathogen testing may be performed to verify that a specific treatment or process has been effective, however, microbial testing included in verification, operational and surveillance monitoring is usually limited to testing for indicator organisms. The concept of using *Escherichia coli* as indicators of feacal pollution is well established practice in the assessment of drinking water quality.

According to WHO (2008), some organisms could be used as indicators for both faecal pollution and treatment process efficacy. However, it has become clear that one indicator cannot fulfill these two roles and that a range of organisms should be considered for different purposes. According to WHO (2011), heterotrophic bacteria can be used as operational indicators of disinfection effectiveness and distribution system cleaness, *Clostridium perfringens* and coliphage can be used to validate the effectiveness of treatment system. *E. coli* has traditionally been used to monitor drinking water quality and it remains an important parameter in monitoring and undertaken as part of verification or surveillance.

Thermotolerant coliforms can be used as an alternative to the test for *E. coli* in many circumstances. Water intended for human consumption should contain no feacal indicator organism. In the majority of cases monitoring for*E. coli* or thermotolerant coliforms provides a degree of assurance because of their large numbers in polluted waters. However, increased attention has focused on the shortcomings of traditional indicators, such as *E. coli*as indicator organisms for enteric viruses and protozoa (WHO, 2011). Viruses and protozoa more resistance to environmental conditions or treatment technologies, including filteration and disinfection, may be present in treated drinking water in the absence of *E. coli*. Retrospective studies of waterborne diseases outbreaks have shown that complete reliance on assumptions surrounding the absence or presence of *E. coli* may not ensure safety (ADWG, 1996). Under certain circumstances, it may be desirable to include more resistant microorganisms, such as bacteriophages and/or bacterial spores, as indicators of presence of microbial harzards (WHO, 2011). Their inclusion in monitoring programmes including control and survelliance programmes should be evaluated in relation to local circumstances and scientific understanding. Such circumstances may include the use of source of water known to be contaminated with enteric viruses and parasites or where such contamination is suspected as a result of the impacts of human and livestock waste.

2.8 Effect of Storage on Quality of Drinking Water

Water supply from the mains that is presumed safe is often stored in plastic tanks and other plastic containers for several months without considerations for the possible implications of storage on its quality (Popoola *et al.*, 2007). Studies have shown that water may become contaminated at any point between collection, storage and usage

(Tambekar *et al.,* 2006). Even when the quality of drinking water at the point of treatment or in the distribution system is microbiologically safe, it can deteriorate during storage in the absence of a residual disinfectant, consequently, there is a high risk of contamination between the time of collection and the time of consumption (Momba *et al.,* 2010).

The effect of long term storage in household containers on the microbiological quality of drinking water is well documented. During storage, the deterioration of drinking water quality is associated with the phenomenon of regrowth and survival of pathogenic microorganisms on the surface of the household container (Momba and Kaleni, 2002). Safe household water management to protect against microbiological contamination should therefore begin with safe storage and handling. Also, storing water in containers and handling procedures of water at home, hotels or restaurants cause water quality deterioration to such extent that the water becomes potential risk of infections to consumers (Jagals *et al*., 1999). A study has shown that natural bacteria community appears to be highly preserved throughout the packaging process (Vachee*et. al*., 1997). The rapid growth of bacteria after the water is packaged may be due to oxygenation of the water during the process, the increased surface area from the packaging materials, the increase in temperature during storage and the trace amounts of nutrients arising from the packaging materials (Leclere and Moreau, 2002). Bacteria usually occur in greater numbers in plastic containers than in glass bottles as plastic tends to be more permeable to external oxygen and extraneous vapours. Similarly, Olayemi(2005) and Eniola *et al.*(2006) highlighted the importance of a few days of indoor storage in improving the physical and microbiological quality of water.

Rosenberg(2003) observed increases in pH during storage which could be due to activities of the resident flora and or their death, which results in the release of inorganic substances such as ammonia. It is likely that if the time of storage is increased, all suspended materials present in the water could settle out under gravitational pull and this could probably be the basis for the influence of storage on bacteriological quality of the water. Sedimentation of suspended materials in the water due to gravitational force could also contribute to the decline in bacterial populations (Salle,1973; Eniola *et al*., 2006).

The reduction in population of total bacteria as the day of storage increased was observed by Payment *et al* (1997). Decline in the bacterial population can be attributed to the death of the resident bacteria during the storage period due to depletion of nutrients (Olayemi, 2005). Study conducted by Sunday *et al.*(2011) revealed that prolonged storage cause an increase in pH and the presence of dissolved oxygen coupled with availability of organic materials and nutrients aid continuous and rapid proliferation of bacteria in sachet water. It also revealed that sachet water when stored at room temperature for long period can increase total aerobic heterotrophic bacteria to a level that may be harmful to human health (Sunday *et al.*, 2011). Georgia (1999) postulated that water can only be stored for a short time after which it begins to deteriorate. However, for water to be stored , it is expected to meet the quality requirement of potable water, also, such stored water should be examined periodically to determine the degree of deterioration with a view of deciding on the type of usage such water can be put to. Improving domestic water storage and handling practices have been shown to be of health benefit to consumer (Mintz *et al.*, 1995).

Field investigations have identified certain practises and vessel characteristics that are associated with the contamination of water or the disease resulting there from, such as using large-mouth vessels to collect and store water (Mintz *et al*., 1995), transferring water from collection vessels and accessing water by dipping hand held utensils rather than via a tap or by pouring (Swerdlow *et al.*, 1997).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Ahmadu Bello University, Zaria main campus at the Department of Biological Sciences. The main campus is located in Samaru, a suburb of Zaria in Kaduna State, Nigeria. Samaru falls between longitude $7^{o}37'22.25''$ E to $7^{o}40'36.89''$ E and latitude $11^{o}9'14.07''$ N to $11^{o}10'9.08''$ N(Mortimore, 1970).

3.2 Sample Collection

Seven hundred and twenty sachet water from six brands marketed within Ahmadu Bello University, Zaria, Nigeria were randomly collected from the production companies for a period of six months.

3.3 Experimental Design

From each brand of sachet water, a bag of 20 sachets was purchased each month, which was divided into two, half (ten sachets) was stored at ambient temperature, while the other half (ten sachets) were refrigerated. At collection, samples collected were analysed for microbial and physicochemical to assess the quality of the water. Subsequently, each week a single sachet was randomly selected from the stored samples for determination of microbial load and physicochemical properties such as colour, taste, odour, turbidity, temperature, pH, electrical conductivity, total dissolved solids, total hardness, chloride, sulphate, nitrate and phosphate.

3.4 Culture Media for Isolation of Fungi and Bacteria

Commercially prepared Potato Dextrose Agar (Titan Biotech) was used for isolation of fungi. Similarly, MacConkey broth (Titan Biotech), Nutrient agar (Titan biotech) and Mannitol Salt Agar (Oxoid) were used for isolation of bacteria.

3.5 Culture Media Preparation for Isolation of Fungi and Bacteria

The recommended quantity of all culture media was weighed into conical flask and the appropriate quantity of distilled water was added to it. The suspension was boiled to completely dissolve the agar. Subsequently all the media were sterilized by autoclaving at 121°C at a pressure of 15 pound for 15 minutes. After sterilization, 15 ml of each medium were aseptically dispensed into sterile petri dishes and allowed to solidify. The petri dishes were labeled accordingly.

3.6 Methods for Isolation of Fungi

Many methods for isolation of fungi have been mentioned in literatures; however, there is no standard method for isolation of fungi in water (DEFRA, 2011). Based on the result obtained from pilot study, the modified membrane filter reported by Cheesbrough (2000) was adopted. From each sample 100 ml was dispensed into two sterilized test tubes each and centrifuged. The centrifuged samples were filtered through a small disc of sterilized filter paper of 0.45μm pores and the membrane filter was placed on Potato Dextrose Agar plates in duplicates and labeled accordingly. All isolates from the media were subcultured into Potato Dextrose Agar (PDA) slant labeled appropriately and refrigerated for further assay.

3.7Method for Isolation of Bacteria

The method used for enumeration of bacteria was multiple tube fermentation technique as described by Cheesbrough, (2000). Presumptive test was done using MacConkey broth. Using 10 ml sterile disposable syringe, 10 ml of the sample was withdrawn and dispensed aseptically into five tubes containing 10 ml of MacConkey broth double strength then 50 ml of the sample was dispensed into one tube containing 50 ml of MacConkey broth double strength. Each bottle containing an inverted Durham tubeaccordingly. The test tubes were closed tightly and shaken to distribute the sample uniformly throughout themedium and to make sure the inverted Durham tube was fullof broth and there wasno air bubble trapped inside it.The test tubes were incubated at 37ºC for 24 hours. After 24 hours the tubesfrom the presumptive fermentation test showing gas andacid formation were recorded and the corresponding Most ProbablyNumber (MPN) index was determined from theProbability table.Those positive tubes were streaked unto Nutrient agar plates from which distinct colonies were picked and subcultured into Nutrient agar slant labeled appropriately and refrigerated for further assay.

3.8 Identification of Fungi

Fungi isolates were identified based on macroscopic and microscopic characteristics. The macroscopic characteristics such as colours, shapes and textures of the colonies on PotatoDextrose Agar plates were examined. The microscopic characteristics such as the vegetative and reproductive structures were studied, for this purpose, a drop of Lacto phenol cotton blue was placed on clean slide, little portion of the colony was picked using sterilized inoculating loop, placed on the Lacto phenol cotton blue, gently squashed, covered with cover slip and viewed under the microscope objectives as described by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson*et et al*. (2004).

3.9 Identification of Bacteria

Pure cultures of bacteria isolates were Gram stained to identify the bacteria type on the basis of cell shapes and Gram reaction. During Gram staining, a drop of distilled water was placed on clean microscopic slide, distinct colonies on the agar plates were picked using sterilized wire loop which was then smeared thinly on the slide. The smears were allowed to air dry and heat-fixed by passing the slide over Bunsen burner flame. The heat-fixed smear was then allowed to cool and was Gram stained. The smears were examined microscopically under microscope using oil immersion objective as described by Cheesbrough (2000). The isolates were further subjected to various biochemical tests for identification using Microgen test kits as described by Barrow and Feltham (1993).

3.10 Determination of Physicochemical Parameters of Sachet Water

3.10.1 Colour of the samples

The colour of the sample was measured using a model AF7103 Lovibond comparator in which 10 ml of the sample was poured into cuvette and was inserted into the machine andthe reading was noted and recordeddescribed by APHA (2005).

3.10.2 Taste and odour of the samples

Small volumes of each sample was tasted with the tongue and then immediately rinsed with taste free distilled water after each sample while 20 mL volume of each water sample was poured into a clean beaker, then shaken vigorously and then brought close to the nose to test for any odour present, the result was recorded accordingly as proposed by Yakassai (2009) and Muazu *et al.*(2012).

3.10.3 Turbidity of the samples

A 10 ml of deionised water was poured into a cuvette which was used to standardized the machine and 10 mL of each sample was poured into cuvettes which was inserted into the machine andthe reading was noted and recorded at 430nm on D70 Jackson turbidity meter as described by APHA (2005).

3.10.4 Temperature, pH, Total Dissolved SolidS (TDS) and Electrical Conductivity

A 40 mL volume of each sample was poured into a beaker; the rod of the Hanna machine (HI 98129, HANNA) was inserted into the beakercontaining the sample and the machine was turned on andthe reading for each of the parameter was noted and recorded.

3.10.5 Total hardness of the samples

Twenty five miles of the water sample and 25ml of distilled water were transferred into 250 ml conical flask, then 2 ml of buffer solution and 0.1g of Errochrome black T dye were added, which weretitrated with ethylenediamine tetra acetic acid (EDTA) as described by APHA (2005).

3.10.6 Chloride of the samples

One hundredmiles of the water sample was transferred into 250 ml conical flask, two to three drops of potassium chromate was added and the content was swirled for a few minutes which was then titrated against silver nitrate solution until dirty reddish precipitate was obtained (APHA,2005).

3.10.7 Sulphate of the sample

Twenty five miles of the water sample and 25ml of distilled water were transferred into 250 ml conical flask. One gram of barium chloride (BaCl) was added, stirred and allowed to stand for 30 minutes. The colour intensity was then measured at 430nm on Sherwood 175colorimeter (APHA, 2005).

3.10.8 Nitrate of the samples

One hundred miles of water sample was poured into a clean dry crucible and kept in an oven at 100^oC till dryness. It was then removed and allowed to cool after which 2 ml of phenol disulphonic acid was added and swirled round uniformly, after 10 minutes, 10 ml of distilled water was added in which 5ml of ammonia solution was added. Colour change was read at 430nm on Sherwood 175 colorimeter (APHA, 2005).

3.10.9 Phosphate of the samples

One hundred miles of the sample was transferred into a 250 ml conical flask, 1ml of ammonium molybdate reagent and 1 drop of stannous chloride were added which was allowed for 12 minutes. Colour change was read at 600nm (APHA, 2005).

3.11Statistical Analysis

Two-way analysis of variance (ANOVA) test was employed to analyze the data for fungi and bacteria count and physicochemical parameters at different storage period. Where significance occurred between the treatments, the means were separated using LSD (Least Significant Difference) test (Analyse it, 2008).

CHAPTER FOUR

4.0 RESULTS

From the 720 sachet water samples obtained from six brands and analysed for the presence of fungi and bacteria, 9 fungal species and 17 bacterial species were isolated.

4.1 Distribution of Fungi Species in Different Brands of Sachet Water

Nine fungi species were isolated from six brands of sachet water but their distribution was not even. *Aspergillus fumigatus and Aspergillus niger*were isolated from FTT.*Aspergillus niger*,*Pencillium glabrum*,*Fusarium oxysporum, Odiodendron griseuum, Rhizoctonia solani and Trichoderma viride*were isolated from OJT. *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Odiodendron griseuum and Trichoderma viride* were isolated from BST. *Trichoderma viride*was isolated from LST. *Cephalosporium curtipes, Odiodendron griseum,Rhizoctonia solani*and *Trichoderma viride*were isolated from DFT while BRT was devoid of any fungus (Table 4.1).The nine fungal species encountered were identified by comparing their macroscopic and microscopic characteristics with already described species using identification keys by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson *et al*., (2004) (Appendices 1-10).

Table 4.1: Fungi Species Isolated and their Distribution from Six Different Brands of Sachet Water Marketed in Ahmadu Bello University, Zaria

	Brands of Sachet Water					
Fungal species	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]
Aspergillus flavus	-	-	✓	-	-	-
Aspergillus fumigatus	✓	-	-	-	-	-
Aspergillus niger	✓	✓	✓	-	-	-
Penicillium glabrum	-	✓	-	-	-	-
Fusarium oxysporum	-	✓	✓	-	-	-
Cephalosporium curtipes	-	-	-	-	-	✓
Oidiodendron griseum	-	✓	✓	-	-	✓
Rhizoctonia solani	-	✓	-	-	-	✓
Trichoderma viride	-	✓	✓	-	✓	✓

✓ = Present, - =Absent, [x] - Codes representing the trade names of brands of the sachet water samples

4.2Prevalence Occurrence of Fungal Species Isolated from Six Brands of Sachet Water

It is evident from Table 4.2 that *Aspergillus niger*was more prevalence out of the three *Aspergillus*species as it was isolated in three different samples with higher percentage of 12.50% followed by*Pencillium glabrum* and *Fusarium oxysporumn*with 8.33%each while *Aspergillus flavus*, *Aspergillus fumigatus*, *Cephalosporium curtipes*, *Odiodendron griseum*, *Rhizoctonia solani*and *Trichodern viride*had the same percentageof 4.17% each.

4.3Colony Counts of Fungi Isolated from Six Brands of Sachet Water

There was no significant difference in the mean of the fungal colony count (P□ 0.05) between initial count at collection and when stored at room temperature and when refrigerated in the brands expect OJT which was significant (P□ 0.05). It is evident that ambient temperature fovours the multiplication of fungi as the higher colony count was recorded at ambient temperature (Table 4.3).

4.4Distribution of Isolated Bacteria Species from Six Brands of Sachet Water

Seventeen bacteria species were isolated from six brands of sachet water but their distribution was not even(Table 4.4). The seventeen bacterial species isolated were identified base their macroscopic, microscopic and biochemical test. They showed variation in the colony colour, shapes and Gram reaction. (Appendices 11-16). Similarly, the *Staphylococcus* species and the *Bacilli* species varied in their reaction to biochemical substrates (Appendices 17-19)

Table 4.2 Percentage of Occurrence of Different Fungal SpeciesIsolated From Sachet Water Marketed in Ahmadu Bello University, Zaria

	Brands of Sachet Water					
Fungal Species	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]
Aspergillus flavus	0(0.00)	0(0.00)	1(4.17)	0(0.00)	0(0.00)	0(0.00)
Aspergillus fumigatus	1(4.17)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Aspergillus niger	2(8.33)	2(8.33)	3(12.50)	0(0.00)	0(0.00)	0(0.00)
Penicillium glabrum	0(0.00)	2(8.33)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Fusarium oxysporum	0(0.00)	2(8.33)	1(4.17)	0(0.00)	0(0.00)	0(0.00)
Cephalosporium curtipes	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(4.17)
Oidiodendron griseum	0(0.00)	1(4.17)	1(4.17)	0(0.00)	0(0.00)	1(4.17)
Rhizoctonia solani	0(0.00)	1(4.17)	0(0.00)	0(0.00)	0(0.00)	1(4.17)
Trichoderma viride	0(0.00)	1(4.17)	1(4.17)	0(0.00)	1(4.17)	1(4.17)

[x] = Codes representing trade names of brands of sachet water, Values outside parenthesis represent occurrence, Values in parenthesis represent Percentage of occurrence.

Table 4.3: The Mean (±S.E.) Value of Fungal Colony Count of Six Brands of Sachet Water Marketed in Ahmadu Bello University, Zaria

		Mode of Storage	
Brands of Sachet Water	After Collection	Ambient Temperature	Refrigerated
FTT[x]	0.33 ± 0.33^{a} (0.00-2.00)	0.17 ± 0.10^{a} (0.00-2.00)	0.00 ± 0.00^{a} (0.00-0.00)
OJT[x]	1.17 ± 0.48^{a} (0.00-3.00)	0.29 ± 0.14^{b} (0.00-2.00)	0.00 ± 0.00^{b} (0.00-0.00
BST[x]	0.33 ± 0.33^{a} (0.00-2.00)	0.33 ± 0.19^{a} (0.00-3.00)	0.00 ± 0.00^{a} (0.00-0.00)
BRT[x]	0.00 ± 0.00^{a} (0.00-0.00)	0.00 ± 0.00^{a} (0.00-0.00)	0.00 ± 0.00^{a} (0.00-0.00)
LST[x]	0.00 ± 0.00^{a} (0.00-0.00)	0.08 ± 0.08^{a} (0.00-2.00)	0.04 ± 0.04^{a} (0.00-0.00)
DFT[x]	0.17 ± 0.00^{a} (0.00-1.00)	0.08 ± 0.08^{a} (0.00-2.00)	0.00 ± 0.00^{a} (0.00-0.00)

Means with different superscript across the rows are significantly different (P□ 0.05),[x] = Codes representing trade names of brands of sachet water, Range in parenthesis

Table 4.4 Bacteria Species Isolated and their Distribution from Six Different Brands of Sachet Water Marketed in Ahmadu Bello University, Zaria

	Brands of Sachet Water					
Bacteria Species	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]
Staphylococcus aureus	✓	✓	✓	✓	✓	✓
Staphylococcus lugdeninsis	-	✓	-	-	-	✓
Staphylococcus saprophyticus	✓	-	-	-	-	-
Staphylococcus warneri	-	✓	-	-	-	-
Staphylococcus xylosus	-	-	-	-	-	✓
Escherichia coli	✓	✓	✓	✓	✓	-
Yersinia enterocolitica	✓	-	-	-	-	-
Acinetobacter iwoffii	-	-	-	✓	-	✓
Acinetobacter haemolyticus	-	✓	✓	-	-	-
Providencia stuartii	-	-	-	-	✓	-
Edwardsiella tarda	✓	✓	-	-	-	-
Morganella morganii	-	-	-	-	✓	-
Providencia acalifaciens	-	-	✓	-	-	-
Enterobacter agglomerans	-	-	✓	-	-	-
Bacillus cereus	✓	✓	✓	✓	-	-
Bacillus lentus	-	-	✓	-	-	-
Bacillus mergaterium	-	-	-	-	✓	-

✓ = Present, - =Absent, [x] =Codes representing trade names of brands of sachet water samples

4.5 Prevalence Occurrence of Bacteria Species Isolated from Six Brands of Sachet Water

Seventeen different bacteria species were isolated from six different brands of sachet water. Their distribution and percentage of occurrence varied. *Staphylococcus aureus*was found in all the six water samples and recorded the highest percentage of occurrences 5 (9.26%) followed by *Escherichia coli* and *Bacillus cereus* which were found in five and four samples respectively and each had 3 (5.56%) as percentage of occurrence. Rest of the fourteen bacteria species were recorded from one sampleeach even though their percentage occurrences varied (Table 4.5).

4.6 Mean Value of the Most Probable Number of Bacteria Isolated

Table 4.6 showed the mean value of the Most Probable Number per 100 ml of six brands of sachet water. At collection and when the samples were subjected to two conditions there was significant differences in their mean (P□ 0.05) even though it was not high. The high colony count at collection was (6.00) in all the samples and when stored at ambient temperature, DFT had the highest colony count of (6.00) while FTT, OJT, DFT recorded (9.00)each.

4.7 Physical Information for Labeling Compliance of Sachet Water Samples

Table 4.7 showed the results of physical examination for labeling compliance of the sachet water samples analysed. All the brands displayed manufacturer's name, address and National Agency for Food and Drug Administration and Control number. Vital information such as Manufacturing date, expiry date and nutritional information was not displayed, only LST displayed information on the nutritional value among the water sample analysed.

Table 4.5: Percentage ofOccurrence of Different Bacteria Species Isolated From Sachet Water Marketed in Ahmadu Bello University, Zaria

	Brands of Sachet Water					
Bacteria Species	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]
Staphylococcus aureus	3(5.56)	2(3.70)	3(5.56)	2(3.70)	1(1.85)	5(9.26)
Staphylococcus lugdeninsis	0(0.00)	1(1.85)	0(0.00)	0(0.00)	0(0.00)	1(1.85)
Staphylococcus saprophyticus	1(1.85)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Staphylococcus warneri	0(0.00)	1(1.85)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Staphylocccus xylosus	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.85)
Escherichia coli	2(3.70)	1(1.85)	2(3.70)	1(1.85)	3(5.56)	0(0.00)
Yersinia enterocolitica	1(1.85)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Acinetobacter iwoffii	0(0.00)	0(0.00)	0(0.00)	2(3.70)	0(0.00)	1(1.85)
Acinetobacter haemolyticus	0(0.00)	1(1.85)	1(1.85)	0(0.00)	0(0.00)	0(0.00)
Providencia stuartii	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.85)	0(0.00)
Edwardsiella tarda	1(1.85)	2(3.70)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Morganella morganii	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.85)	0(0.00)
Providencia acalifaciens	0(0.00)	0(0.00)	1(1.85)	0(0.00)	0(0.00)	0(0.00)
Enterobacter agglomerans	0(0.00)	0(0.00)	1(1.85)	0(0.00)	0(0.00)	0(0.00)
Bacillus cereus	3(5.56)	2(3.70)	3(5.56)	1(1.85)	0(0.00)	0(0.00)
Bacillus lentus	0(0.00)	0(0.00)	1(1.85)	0(0.00)	0(0.00)	0(0.00)
Bacillus mergaterium	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.85)	0(0.00)

[x] =Code represent trade names of brands of sachet water, Values in parenthesis represent percentage of occurrence, Values outside parenthesis represent occurrence.

Table 4.6: The Mean (±S.E.)Value of the Most Probable Number (MPN/100 ml) of Six Brands of Sachet Water Marketed in Ahmadu Bello University, Zaria

		Mode of Storage	
Brands of Sachet Water	After Collection	Ambient Temperature	Refrigerated
FTT[x]	3.00±1.03[a] (0.00-6.00)	0.46±0.18[c] (0.00-3.00)	2.46±0.48[b] (0.00-9.00)
OJT[x]	2.83±1.05[a] (0.00-6.00)	1.17±0.26[c] (0.00-4.00)	2.04±051[b] (0.00-9.00)
BST[x]	1.67±0.95[a] (0.00-6.00)	0.50±0.19[c] (0.00-4.00)	0.54±0.30[b] (0.00-6.00)
BRT[x]	2.50±1.15[a] (0.00-6.00)	1.13±0.22[b] (0.00-3.00)	0.67±0.35[c] (0.00-6.00)
LST[x]	1.00±1.00[a] (0.00-6.00)	0.25±0.12[c] (0.00-2.00)	0.42±0.26[b] (0.00-6.00)
DFT[x]	3.17±0.91[a] (0.00-6.00)	1.17±0.38[c] (0.00-6.00)	2.71±0.60[b] (0.00-9.00)

[x] =Code represent trade names of brands of sachet water, Mean with the same superscript across the rows are not significantly different (P□ 0.05), Range in parenthesis

Table 4.7: Physical Information for Labeling Compliance of Sachet Water Marketed in Ahmadu Bello University, Zaria

Sample code	Registration Number	Manufacturing date	Expiry date	Nutritional information	Batch number	Producer's name and address
FTT[x]	+	-	-	-	-	+
OJT[x]	+	-	-	-	-	+
BST[x]	+	-	-	-	-	+
BRT[x]	+	-	-	-	-	+
LST[x]	+	-	-	+	-	+
DFT[x]	+	-	-	-	-	+

(+) Present(–) Absent, [x] = Codes representing trade names

4.8 Physical Parameters of Six Brands of Sachet Water

The result showed that the samples colourless, odourless, tasteless and not turbid after collection and after storage at ambient temperature and when refrigerated (Table 4.8).

4.9 Physicochemical Parameters of Six Brands of Sachet Water After Collection

There was significant difference (P□ 0.05) in pH, conductivity, total dissolved solids, hardness and chloride while there was no significant difference (P□ 0.05) intemperature, nitrate, sulphate, and phosphate among the brands of sachet water samples at collection. BST recorded the highest pH mean value of 7.40 (7.10-7.87) while the temperature ranged from 24.8-29.1°*C*with highest mean of 27.05°C in BST. OJT had the highest for mean for conductivity, total dissolved solid, chloride, nitrate, sulphateandhardnesswhile LST had the highest mean value of 15.75 mg/L with range of (10.0-27.5 mg/L) for phosphate(Table 4.9).

4.10 Physicochemical Parameters of Six Brands of Sachet Water Determined After Refrigerated

There was no significant different (P□ 0.05) among the brands analysed for pH, temperature, nitrate, sulphate hardness and phosphate while conductivity, total dissolved solids and chloride were significantly different (P□ 0.05). BST had the highest mean value of 7.26 while FTT had the least 6.93 for pH. Temperature ranged from 11.30-15.0°*C* with the highest mean of 13.20°*C* in BRT while OJT had the least 12.25°*C*.OJT had the highest for mean for conductivity, total dissolved solid, chloride, nitrate and hardness. BST recorded the highest mean 25.92 mg/L for sulphate. Highest mean value recorded for phosphate was 16.50 mg/L in DFT (Table 4.10).

Table 4.8: Physical Parameters of Six Brands of Sachet Water From Sachet Water Marketed in Ahmadu Bello University, Zaria

	Brands of Sachet Water						
Parameters	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]	Standar by SON. 2007
Colour	0.00	0.00	0.00	0.00	0.00	0.00	15 TCU
Odour	Odourless	Odourles	Odourless	Odourless	Odourles	Odourless	Unobjectionable
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Unobjectionable
Turbidity	0.00	0.00	0.00	0.000	0.000	0.00	5 NTU

SON =Standard Organization of Nigeria, TCU= True Colour Units, NTU= Nephelometric Turbidity Units, [x] = Codes representing trade names

Table 4.9: Mean Values (±S.E.) of Physicochemical Parameters of Six Brands of Sachet Water Determined after Collection

	Brands of Sachet Water						
Parameters	FTT^{x}	OJT^{x}	BST^{x}	BRT^{x}	LST^{x}	DFT^{x}	Standard by SON, 2007
pH	6.98±0.08^{bc} (6.8-7.2)	7.04±0.13^{bc} (6.8-7.67)	7.40±0.12^{a} (7.1-7.87)	7.02±0.08^{bc} (6.77-7.33)	6.83±0.07^{c} (6.5-6.95)	7.25±0.08^{bb} (6.96-7.5)	6.5-8.5
Temperature (°C)	26.88±0.53^{a} (24.9-28.4)	27.00±0.63^{a} (24.8-29)	27.05±0.62^{a} (25-29.1)	26.90±0.54^{a} (25-28.5)	26.82±0.25^{a} (25-28.5)	26.70±0.46^{a} (25.1-28.2)	Ambient
Conductivity (µs/cm)	209.33±48.80^{b} (63-406)	358.17±28.84^{b} (269-476)	74.50±4.24^{c} (61-91)	143.00±3.20^{bc} (135-158)	143.83±6.03^{bc} (125-169)	73.50±2.77^{c} (63-79)	1000
TDS (ppm)	102.00±23.78^{b} (32-203)	182.33±13.52^{c} (136-232)	38.17±2.89^{c} (30-51)	71.50±1.65^{bc} (67-79)	74.0±2.86^{b} (69-87)	37.67±0.95^{d} (33-39)	500
Chloride (mg/L)	31.83±3.75^{a} (20-42)	95.83±15.06^{b} (22-120)	30.00±4.24^{b} (16-43)	43.83±7.51^{b} (24-76)	40.33±5.34^{b} (26-63)	36.67±8.53^{b} (15-71)	250
Nitrate (mg/L)	2.09±0.14^{a} (1.7-2.55)	2.23±0.15^{a} (1.9-2.9)	2.00±0.18^{a} (1.25-2.4)	1.93±0.24^{a} (0.8-2.4)	2.18±0.06^{a} (2-2.4)	2.08±0.27^{a} (1.35-32)	50
Sulphate (mg/L)	23.25±3.83^{a} (11-34)	18.58±5.23^{a} (3-38)	25.50±2.93^{a} (17-34)	14.25±5.26^{a} (3-37)	19.80±5.56^{a} (1.8-35.5)	23.66±4.62^{a} (3-33.5)	100
Hardness (mg/L)	110.00±18.81^{a} (40-164)	135.00±38.28^{b} (48-316)	100.00±11.45^{a} (48-124)	140.67±50.56^{a} (36-384)	125.33±29.04^{a} (40-248)	133.33±28.49^{a} (84-268)	150
Phosphate (mg/L)	16.50±2.52^{a} (11-26.5)	15.21±2.06^{a} (11-21.5)	15.42±2.23^{a} (10-22.5)	14.58±1.43^{a} (11.5-21)	15.75±2.66^{a} (10-27.5)	14.50±3.96^{a} (1.5-30.5)	NA

Mean with the same superscript across the rows are not significantly different (P□ 0.05) TDS= Total Dissolved Solids, SON= Standard Organisation of Nigeria, NA=Not Available, ppm= part per million, ^{x} = Codes representing the trade names of sachet water, Range in parenthesis

Table 4.10: Mean Values (±SE) of Physicochemical Parameters of Six Brands of Sachet Water when Refrigerated

	Brands of Sachet Water						
Parameters	FTT	OJT	BST	BRT	LST	DFT	Standard by SON, 2007
pH	6.93±0.13[a] (6.7-7.64)	7.03±0.15[a] (6.7-7.69)	7.26±0.18[a] (6.2-7.52)	7.11±0.06[a] (6.9-7.34	7.11±2.66[a] (10-27.5)	7.02±0.11[a] (7.01-7.8)	6.5-8.5
Temperature (°C)	12.40±0.76[a] (11.30-15.0)	12.25±0.62[a] (11.80-14.0)	12.63±0.55[a] (11.90-14.5)	13.20±0.58[a] (11.70-14.4)	12.47±0.57[bc] (11.50-13.4)	12.42±0.57[a] (10.9-14.1)	Ambient
Conductivity (µs/cm)	207.00±41.89[b] (54-347)	345.00±35.94[a] (238-480)	76.50±2.75[c] (66-87)	141.17±4.02[bc] (128-157)	146.67±6.15[d] (126-171)	73.00±2.08[c] (66-80)	1000
TDS (ppm)	99.33±20.73[b] (32-174)	176.17±20.09[a] (120-240)	37.67±1.61[c] (33-45)	70.83±1.79[bc] (66-79)	74.00±3.77[bc] (63-90)	37.17±0.99[c] (34-40)	500
Chloride (mg/L)	30.00±3.34[b] (18-40)	74.50±13.82[a] (17-111)	23.50±4.70[b] (9-38)	31.17±7.50[b] (20-68)	36.50±3.96[b] (27-53)	33.50±7.63[b] (10-60)	250
Nitrate (mg/L)	2.02±0.13[a] (1.6-2.4)	2.19±0.09[a] (2-2.5)	1.89±0.17[a] (1.2-2.3)	1.83±0.02[a] (0.9-2.1)	2.05±0.06[a] (1.9-2.3)	1.87±0.27[a] (1.35-3)	50
Sulphate (mg/L)	23.02±3.88[a] (10-33.5)	23.60±5.64[a] (3-41)	25.92±3.03[a] (17-34)	16.50±6.21[a] (1.5-43)	22.25±4.96[a] (3-3.5)	25.03±2.98[a] (14.5-34)	100
Hardness (mg/L)	111.17±17.00[a] (36-156)	135.00±30.61[a] (48-272)	88.00±8.39[a] (72-128)	130.67±42.78[a] (40-344)	127.67±24.47[a] (80-248)	121.33±23.44[a] (72-232)	150
Phosphate (mg/L)	15.92±2.62[a] (11-26.5)	14.71±1.98[a] (11-21.6)	14.67±2.16[a] (9.5-21.5)	14.25±1.30[a] (11.5-20)	16.25±2.67[a] (10-28)	16.50±3.01[a] (11-30.5±)	NA

Mean with the same superscript across the rows are not significantly different (P□ 0.05) TDS= Total Dissolved Solids, SON= Standard Organisation of Nigeria, NA=Not Available, ppm= part per million, [x] = Codes representing the trade names of sachet water, Range in parenthesis

4.11 Physicochemical Parameters of Six Brands of Sachet Stored at Ambient Temperature

The results shows that there was no significant different (P□ 0.05) among brands sachet water for pH, temperature, nitrate, sulphate hardness and phosphate while conductivity, total dissolved solids and chloride were significantly different (P□ 0.05) among the brands when stored at ambient temperature. The highest pH meanof 7.30 was recorded in DFT while OJT had the least 7.09. Temperature ranged from 23.4-28.4°C with highest mean of 25.97°C in BRT while LST had the least24.53°C. OJT had the highest for mean for conductivity, total dissolved solid, chloride and nitrate. BST had 25.42 mg/L as the highest mean while BRT had the least mean of 15.33 mg/L for sulphate. BRThad the highest mean of 142.0 mg/L for hardness while BST had the least mean of 100 mg/L. DFT had the highest mean of 16.73 mg/L while BRT has the least mean of 14.58 mg/L for phosphate. All the physicochemical parametetrs analysed at ambient temperatureare within the permissible limit set by Standards Organisation of Nigeria, 2007 (Table 4.11).

Table 4.11: Mean Values (±S.E.) of Physicochemical Parameters of Six Brands of Sachet Water at Ambient Temperature

	Brands of Sachet Water						
Parameters	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]	Standard by SON, 2007
pH	7.10±0.13^{a} (6.7-7.64)	7.09±0.15^{a} (6.7-7.69)	7.01±0.18^{a} (6.2-7.52)	7.18±0.06^{a} (6.9-7.34)	7.13±2.66^{a} (10-27.5)	7.30±0.11^{a} (7.01-7.8)	6.5-8.5
Temperature (°C)	25.00±0.76^{a} (23.7-28.4)	25.01±0.62^{a} (23.4-27.4)	25.00±0.55^{a} (23.6-27.1)	25.97±0.58^{a} (23.4-27.4)	24.53±0.57^{a} (23.6-27.2)	25.32±0.57^{a} (23.5-27.4)	Ambient
Conductivity (µs/cm)	201.33±41.89^{b} (54-347)	345.17±35.94^{c} (238-480)	75.67±2.75^{c} (66-87)	153.00±4.02^{bc} (128-157)	144.50±6.15^{bc} (126-171)	73.67±2.08^{c} (66-80)	1000
TDS (ppm)	102.00±20.73^{b} (32-174)	163.00±20.09^{b} (120-240)	37.17±1.61^{c} (33-45)	72.50±1.79^{c} (66-79)	73.00±3.77^{bc} (63-90)	36.50±0.99^{c} (34-40)	500
Chloride (mg/L)	25.33±3.34^{b} (18-40)	81.67±13.82^{a} (17-111)	26.17±4.70^{b} (9-38)	33.17±7.50^{b} (20-68)	35.83±3.96^{b} (27-53)	30.67±7.63^{b} (10-60)	250
Nitrate (mg/L)	2.02±0.13^{a} (1.6-2.4)	2.20±0.09^{a} (2-2.5)	1,93±0.17^{a} (1.2-2.3)	1.88±0.02^{a} (0.9-2.1)	2.08±0.06^{a} (1.9-2.3)	2.14±0.27^{a} (1.35-3)	50
Sulphate (mg/L)	23.17±3.88^{a} (10-33.5)	19.33±5.64^{a} (3-41)	25.42±3.03^{a} (17-34)	15.33±6.21^{a} (1.5-43)	19.50±4.96^{a} (3-3.5)	25.17±2.98^{a} (14.5-34)	100
Hardness (mg/L)	105.33±17.00^{a} (36-156)	132.67±30.61^{a} (48-272)	100.00±8.39^{a} (72-128)	142.00±42.78^{a} (40-344)	139.33±24.47^{a} (80-248)	119±23.44^{a} (72-232)	150
Phosphate (mg/L)	16.63±2.62^{a} (11-26.5)	15.33±1.98^{a} (11-21.6)	15.25±2.16^{a} (9.5-21.5)	14.58±1.30^{a} (11.5-20)	16.27±2.67^{a} (10-28)	16.73±3.01^{a} (11-30.50)	NA

Mean with the same superscript across the rows are not significantly different (P□ 0.05)TDS= Total Dissolved Solids, SON= Standard Organisation of Nigeria, NA=Not Available, ppm= part per million, [x] = Codes representing the trade names of sachet water, Range in parenthesis

CHAPTER FIVE

5.0 DISCUSSION

Water is an essential part of human nutrition, but one of the greatest concerns of water consumers with respect to the quality of drinking water is microbial contamination (Payment 1997, WHO, 2010).. Previous studies conducted in Nigeria and other parts of the world have revealed microbial load in packaged drinking water. Fungi in water have mostly been overlooked, but may be regarded as a chronic problem in drinking water. Bacteria are probably the most studied group of microorganisms with respect to quality of drinking water. However, fungi have received increased focus as drinking water contaminants in the last decade due to its ability to cause acute disease (Hageskal *et al*., 2009).

It is evident that measurement of different parameters in all the identified fungi species was slightly different from the already described species which could be due to environmental condition and the nutrition medium used. This has already been reported by Klich (2002) and Samson et al. (2004).Five of the sachet water brands analysed in this study were found to be contaminated with varied number of fungi species. The occurrence of varied number of fungal species in different sachet water indicates the status of the treatment rendered to the water during production. The presence of fungal species in any sample may be an indication indicates treatment.(Gunhild *et al.,* 2006; Omalu *et al.,* 2010).

Mean of the colony count also varied even though it was not significantly different (P□ 0.05). OJT recorded six species of fungi and with higher mean of colony counts than other samples which indicated high level of contamination. No fungus was isolated in BRT which indicated that the treatment was probably effective.

The highest percentage of occurrence by *Aspergillus niger,* could be attributed to its ability to secrete pigments called melanin, which provides protection against a range of stresses making them resistant to water treatment coupled with inadequate treatment (Langfelder *et al*., 2003). *Aspergillus flavus, Aspergillus fumigatus, Cephalosporium, Odiodendron griseuum, Rhizoctonia solani* and *Trichoderma viride* had the lowest percentage of occurrence of 1(4.17%) each. This varied number of fungal species with varied percentage occurrence and colony count could be attributed to variation in level of treatment, hygiene practices, sources of water used for production, unhygienic working environment, poor storage, inadequate treatment and lack of sterilized materials.The presence of fungal species in the five samples that are contaminated is probably due inadequate treatment while the absence of any kind of fungi in one of the brand analysed indicates adequate or moderate treatment of the water respectively. Similar observation was made by Hageskal. (2006); Okpako *et al.* (2009); Tanveer*et al*., (2011).

However, the standard for drinking water by World Health Organization and Standards Organisation of Nigeria did not provide information about the occurrence and implication of fungi on human health in drinking water (SON, 2007 and WHO, 2011). The occurrence of these fungi may however lead to diverse effects on human health as they have the potential of producing mycotoxins. The concentration of these substance may increase during storage of water due increase in the population of the fungi species, hence, daily intake of such water containing mycotoxin could result in bioaccumulation in the body which could be hazardous to human health.

Aspergillus flavus, *Aspergillus fumigatus* and *Aspergillus niger*are known to produce aflatoxins, ochratoxins and fumitremoigin which are carcinogenic and capable of causing kidney and liver disorders, invasive and non invasive aspergillosis, allergic and sinusitis(Bryce, 1992; Bennett and Klich, 2003 and Samson *et al*., 2004). *Penicillium glabrum*produce citromycetin which is known to cause allergy, asthma and some respiratory problems (Cooley *et al.,* 1998; Frisvad *et al.,* 1998; Samson *et al*., 2004 and Gunhild *et al.*, 2006).

Fusarium oxysporum are capable of producing fusaric acid, monilifornun, gibepyrones and naphthoquinone pigment which *has* been recognized as an agent of superficial infections (Guarro and Gene, 1995 and Samson*et al*., 2004). *Trichoderma virides* produces trichothecenes, alamethicins, emodin, trichotoxin and suzukacillin and has been reported to cause mycosis and allergy in humans (Jaakkola *et al.,* 2002; Tang *et al.*, 2003; Samson *et al*., 2004 and Okpako *et al.,* 2009).

Seventeen different bacteria species were isolated from all the sachet water samples. Most of the bacteria species isolated have been reported in previous studies (Abiodun, 2010; Waziri, 2012; Ayoade *et al*., 2013). All the sachet water was however contaminated with varied number of bacteria species even though their distribution varied among the brands. The frequent occurrence of the bacteria species could be attributed to lack of proper knowledge on the application of disinfectants, poor hygiene practices, use of unsterilized equipmentduring production. The Most Probably Number which defined the degree of contamination and the microbiological quality of drinking water, going by the zero tolerance levels stipulated by regulatory agency for coliforms in drinking water, none of the brands meet the existing standards.. Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality (Olayemi, 2005).

The variation in the number of bacteria present, percentage occurrence and mean of the Most Probably Number could be adduced to variation in the level of treatment. Similar observation was made byOyedeji *et al*. (2010); Muazu *et al.*(2012) and Musa and Iyoha (2014).The potential health effects that may be caused by these bacterial species include abscesses, ulcers, food poisoning, inflammation of breast and conjunctivitis in new born, nausea, vomiting, diarrhea, urinary tract infections, appendicitis, meningitis, abdominal pain , pneumonia and bacteraemia (Cheesbrough, 2000;WHO, 2011).

All the sachet water samples analysed registered with appropriate regulatory agency but none of the sachet water producers indicated manufacturing date, expiring date and batch number on the sachet, therefore not complying with the labeling compliance as stipulated by the WHO (1997) and SON, 2007.

All the physico-chemical parameters thereby falls within the range set by Standards Organization of Nigeria (2007) for drinking water both at collection and when subjected to storage at different condition. The samples were colourless, odourless, tasteless and non turbid. This finding differed from the result obtained by Popoola *et al*. 2007, who reported the development of taste, odour and suspended particles after 12 week of storage. The difference could be adduced to difference in the time of storage. The pH obtained in this study at collection and when stored at ambient temperature and refrigerated falls within the range set by SON, 2007 this could be attributed to the fact that the treatment was adequate. The pH value obtained in this study will not have any implication such as acidosis when consumed. This finding is similar to the result obtained by Sunday *et al*. (2011). The temperature obtained is within the permissible limit by SON for drinking water. Though temperature does not have direct health impact, the temperature obtained in this study may favour the growth of microorganisms. The value of conductivity and total dissolved solids obtained in this study at collection and when stored at different conditions are within the permissible by SON, 2007 which could be attributed to adequate treatment. Both are of none health importance but may serve as an indicator of the level of contamination and may influence the aesthetical quality of the drinking water. This finding confirmed the result obtained by Eric and Isaac, (2013).

The concentration of chloride obtained during this study was within the permissible limit set by SON, (2007) this could be attributed that the treatment was adequate. This result contradicts result obtained by Emmanuel and Solomon (2012). The difference could be adduced to difference in source of water used during production. Chloride ions are of non cumulative toxins, an excess amount of which, if taken over a period of time can constitute a health hazard and high concentration of chloride may result in taste problem (Emmanuel and Solomon, 2012). Nitrate value obtained in all the samples are within the recommended limit by SON, 2007 for drinking water. This could be attributed to the fact that the source of the water has low level of nitrogen coupled with adequate treatment during production. This finding is in accordance with the result of Onweluzo and Akugbazie (2012). The water samples may not cause health problem such as cyanosis because of the low level of nitrate in the samples. The concentration of sulphate, hardness and phosphate are within the permissible limit by SON (2007). This could be adduced to the fact that the source of the water used for the production is of low level of sulphate, calcium, magnesium. This finding is in agreement with the findings of *Ackah et al.* (2012) and Onweluzo and Akugbazie (2012)

The variation in the means could be attributed to the fact that, the source of the water used for production has different level of element composition which may also coupled with differences in the level of treatment and utilization of the different substrates by microorganisms Similar observation was made by Onweluzo and Akuagbazie (2010), Sunday *et al.*(2011), Muazu *et al*. (2012) and Eric and Isaac (2013)

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

1. Nine fungal species were isolated from five brands of sachet water while one was devoid of any fungus contaminant. However, seventeen bacterial species were isolated from all the six brands of sachet water samples.

2. The predominant fungal species identified were *Aspergillusniger*, *Penicllium glabrum*and *Fusarium oxysporum* whereas the predominant bacterial species associated with the sachet water were *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus.*

3. The presences of these fungal species and bacterial species could has significantly affected the quality of the water and may be hazardous to human health.

6.2 RECOMMENDATIONS

1. Considering the high patronage of sachet water in the area of study, it is recommended that all manufacturing industries must adhere to NAFDAC guidelines and all the existing laws should be enforced.
2. Furthermore, to safeguard the health of the people there is need for regular monitoring of the quality of the water and the environment they are produced by NAFDAC.
3. In addition, there is need for awareness programs in order to educate the general public on the potential health implication associated with consumption of such products.

4. There is also need for awareness programs in order to educate the general public on the effect of storing potable water for long periods. Further research should also be conducted on other microbes such as virus.
5. The need for the University to set up microbiology laboratory for testing the sachet water that is brought into the school.

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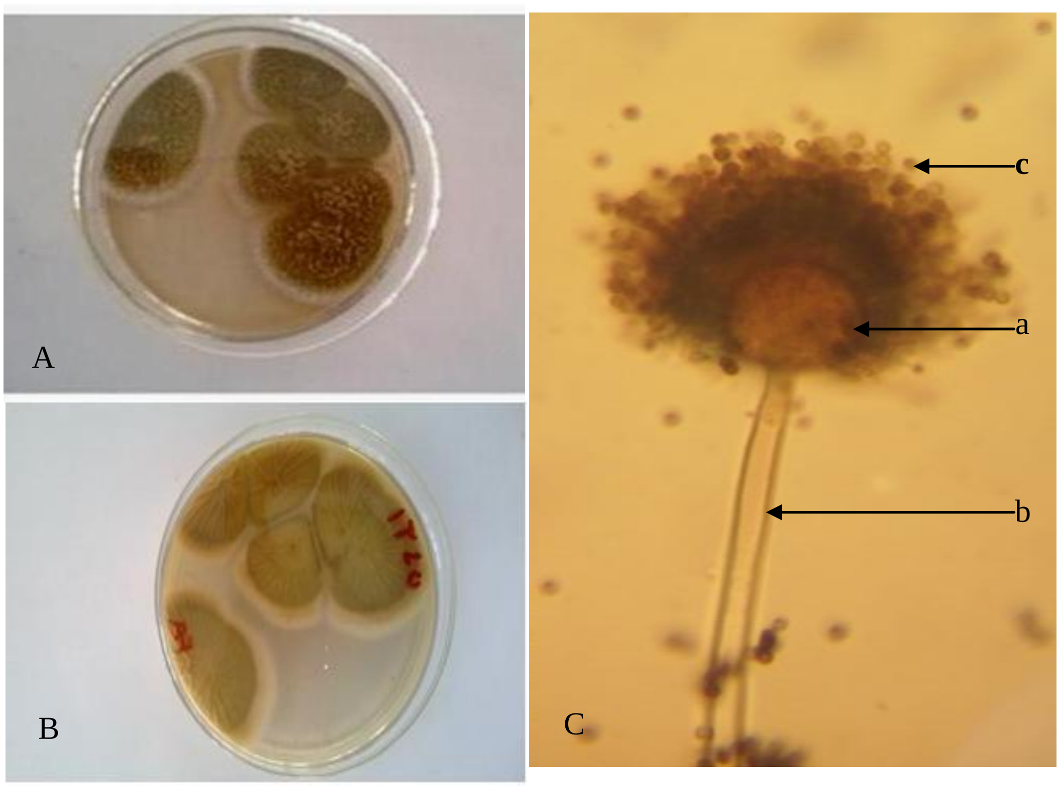
APPENDICES

Appendix 1: Description of the Macroscopic and Microscopic Characteristics of the Fungal species Isolated

Fungal species	Macroscopic Characteristics	Microscopic Characteristics
Aspergillus flavus	The upper surface of colonies was olive green with white edge, granular surface and green coloration on the reverse side on Potato Dextrose Agar. The Colonies were ovoid in shape and varied in size from 2.5-3.7 cm with an average of 3.1 cm in diameter within 5 days at room temperature	The conidiophore was thick walled, hyaline and slightly roughened, erect, long, aseptate with a vesicle at the top with phialides and with short conidial chains. The length of conidiophore measured ranged from 110-320µm with an average length of 290.20 µm. The width of the conidiophore near the vesicles measured 21-45 µm, 22-44 µm at the middle and 23-45 µm at the base. The vesicles measured 57-103 µm in diameter with an average of 80 µm. The conidia measured 2-4 µm in diameter with an average of 3.9 µm
Aspergillus fumigatus	The colony was widely spread, dark green with smooth white edges and spongy surface and brown on the reverse side on Potato Dextrose Agar. The colonies were round in shape and measured 7 cm in diameter within 5 days at room temperature	The conidiophore was long, narrow at the base and broad near the vesicle with smooth walled hyaline. The length of conidiophore ranged from 100-120µm, width near the vesicle and measured 9-12 µm, 7-9 µm at the middle and 7-8 µm at the base. The vesicle measured 30-35 µm with an average of 30 µm. The conidial head is grayish near the apices with an irregular in shape. Conidia measured 4-6 µm in diameter with an average of 4.2 µm in diameter.
Aspergillus niger	The colonies were widely spread, black, with smooth white edges and spongy surface densely packed and brown on the reverse side on Potato Dextrose Agar. The colonies were round in shape and measured 2-4.5 cm with an average of 3 cm within 5 days at room temperature .	The conidiophore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head. The length of the conidiophore ranged from 100-102 µm with an average 100 µm. The width of the conidiophore near the vesicle measured 10 µm, at the middle measure 10 µm and 9 µm at the base. The conidial head was blackish in colour and measured 4-6 µm in diameter with an average of 5 µm.
Penicillium glabrum	The colonies were green with cottony upper surface, white border and brown on the reverse side on Potato Dextrose Agar. The colonies were round in shape and varied in size, measured 1-1.5 cm in diameter with an average of 2 cm within 5 days at room temperature.	The conidiophore was long, thick walled and hyaline. The conidiophore ranged from 100-125 µm in diameter with an average of 100 µm, near the vesicle it measured 12 µm, 11 µm at the middle and 12 µm at the base. The conidial head was bluish near the apices with an irregular shape and the conidia measured 5-6 µm in diameter with an average of 4µm.
Fusarium oxysporum	The colony was pink with white patch on the surface and on the reverse side was brown in coloration on Potato Dextrose Agar. The colony was round in shape and measured 4 cm in diameter within 5 days at room temperature.	The macroconidia are canoe shaped, multiseptate which contain 3-6 septations and slightly pointed at the end. Measured 29-100 µm in length with an average of 75 µm. The width measured 20-30 µm with an average of 35 µm. The microconidia were ovoid or oblong borne single with no septation and single celled. The conidia measured ranged between 20-40 µm in length with an average length of 30 µm. The width measured between 9-18 µm with an average of 18 µm.
Cephalosporium curtipes	The colony is white with smooth edge and tuft surface and on the reverse side is brown in colour growing on Potato Dextrose Agar. The colony is round in shape and measured 4 cm in diameter within 5 days at room temperature	The hyphae are hyaline, branched, measured 20-30µm in diameter and bear the conidiophores on aerial branches. The conidiophore ranged from 25-50 µm, with 3-4 side branches. Conidia are developed at the tip of the conidiophore forming a globose head and measured 4-8 µm with an average of 4 µm.
Oidiodendron griseum	The colony was white with irregular edge and spongy surface and brown in colour on the reverse side on Potato Dextrose Agar. It measured 3 cm in length within 5 days at room temperature	The conidiophore was irregularly branched at the upper portion and the branches segmented into rod-shaped conidia which were in chains. The conidiophore measured 80-120 µm in length with an average of 80 µm. The conidia measured 30-40 µm with an average of 30 µm.
Rhizoctonia solani	The colony was white with cottony surface and on the reverse side is brown in coloration on Potato Dextrose Agar. It measured 4 cm within 5 days at room temperature	Dark mycelial hyaline, long mycelium cell and branched at the upper part, no septation of branches set off from the main hyphae. The mycelium measured 100-250 µm in length with an average of 160 µm
Trichoderma viride	White colony with folded cottony upper surface, creamy colored spores and a yellowish brown on the reverse side when growing on Potato Dextrose Agar. It measured 7 cm within 7 days at room temperature	The conidiophores hyaline was irregularly branched. Lateral branch below with short branches near the apex. Phialides are in group of 2-3. The conidia are round, clustered at the end of phialide and measured 3-4 µm with an average of 2.5 µm. The conidiophore measured 80-110 µm in length with an average of 90 µm.

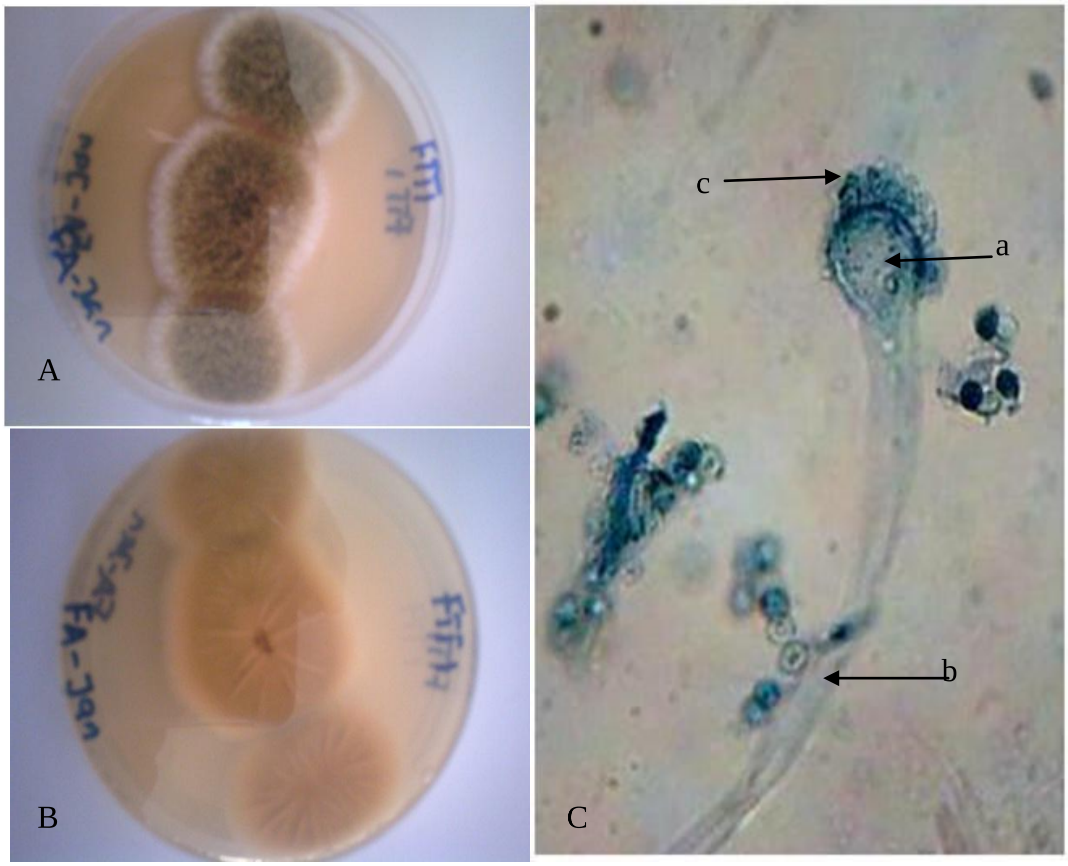


Appendix 2: Pure culture of *Aspergillus flavus*(×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= vesicle, b:=conidiophore, c=conidium

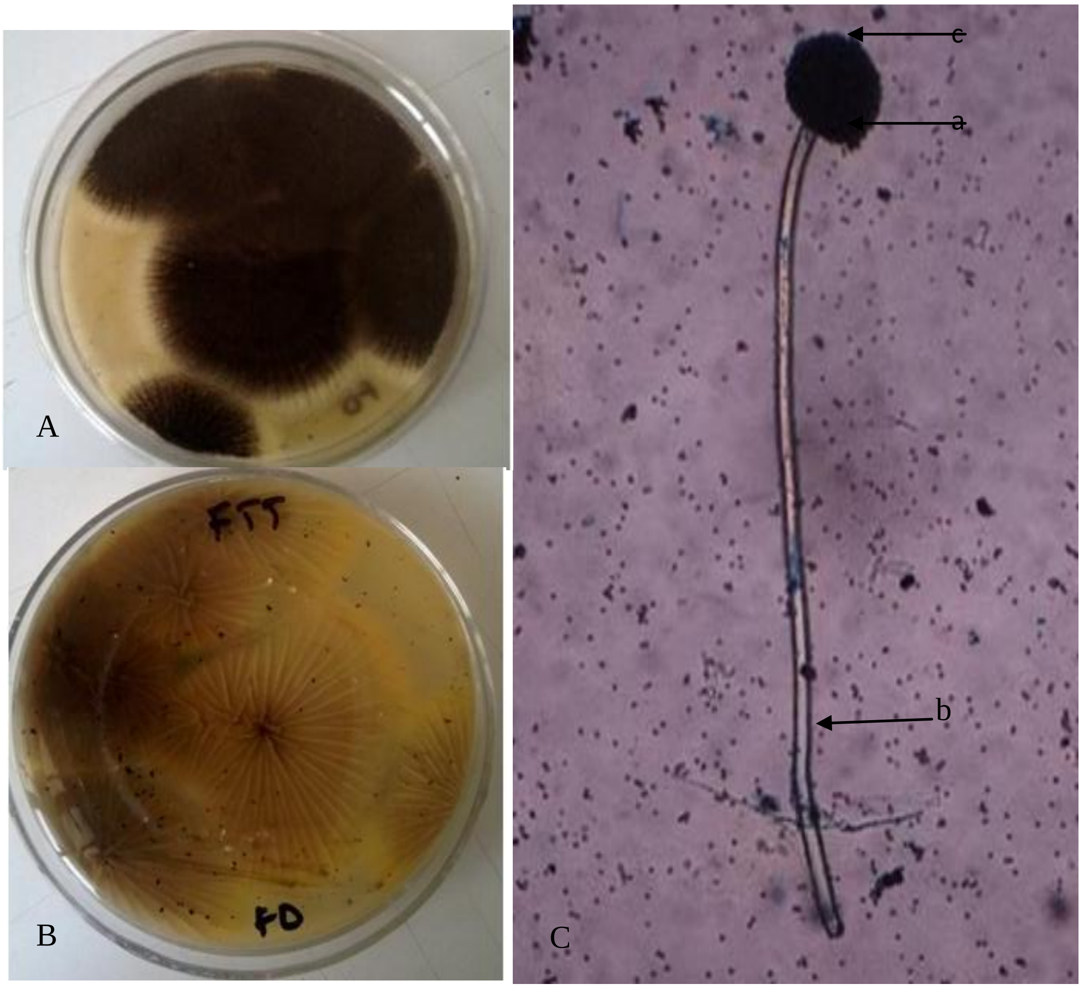


Appendix 3: Pure culture of *Aspergillus fumigates* (×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= vesicle, b =conidiophore, c=conidium

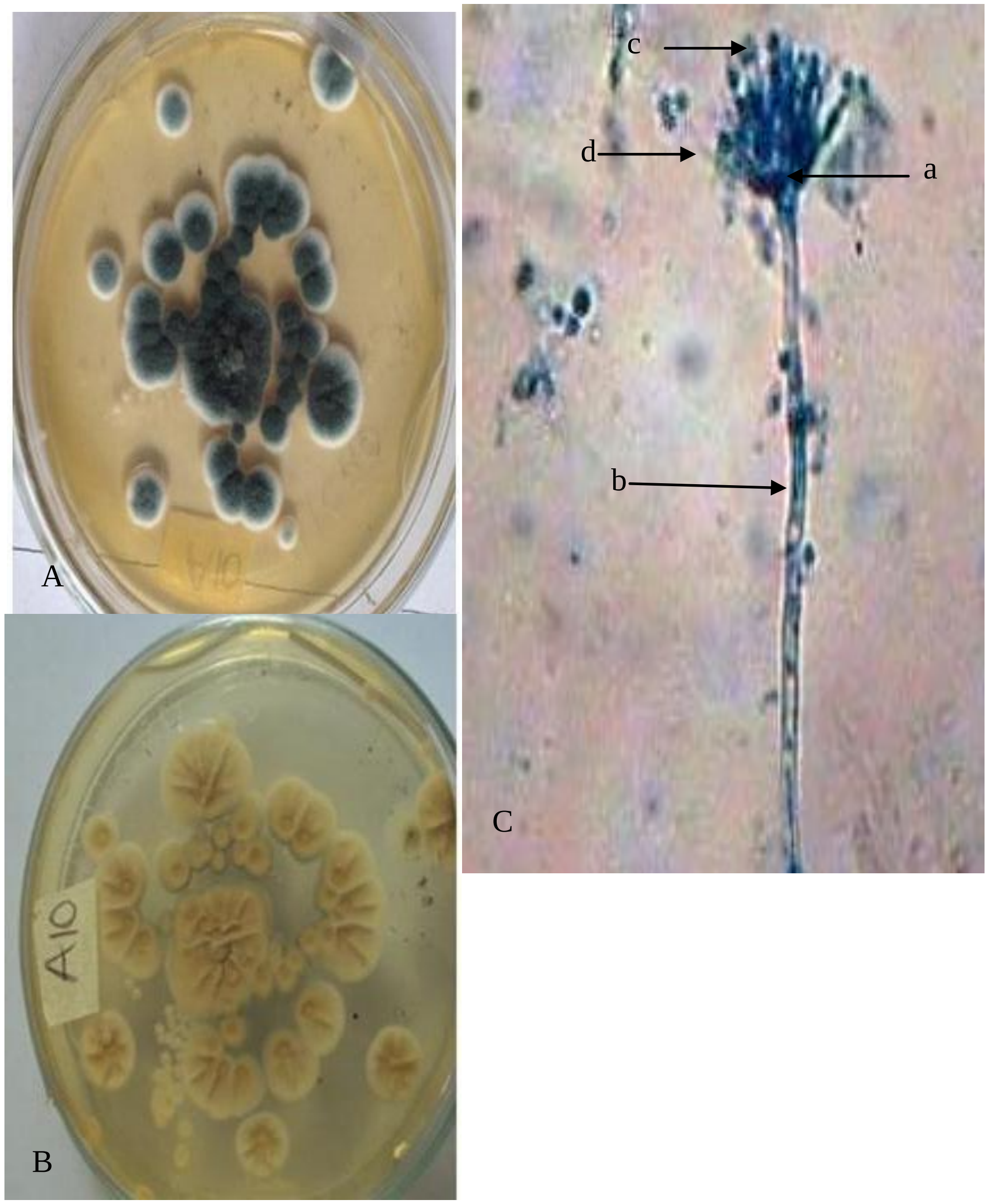


Appendix 4: Pure culture of *Aspergillus niger*(×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= vesicles, b:=conidiophore, c=conidium

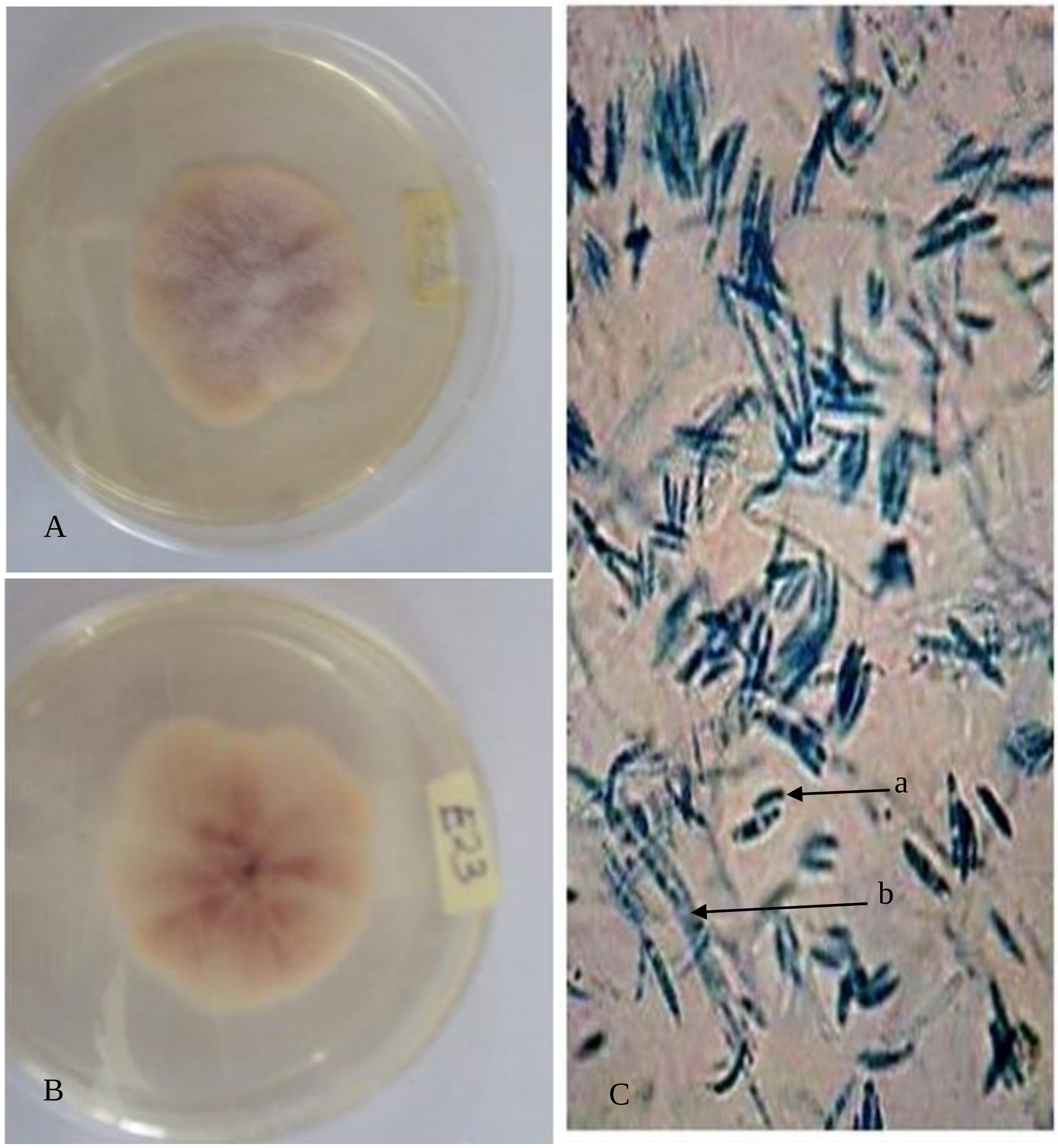


Appendix 5: Pure culture of *Pencillium glabrum*(×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= vesicles, b=conidiophore, c=conidium, d=phialides

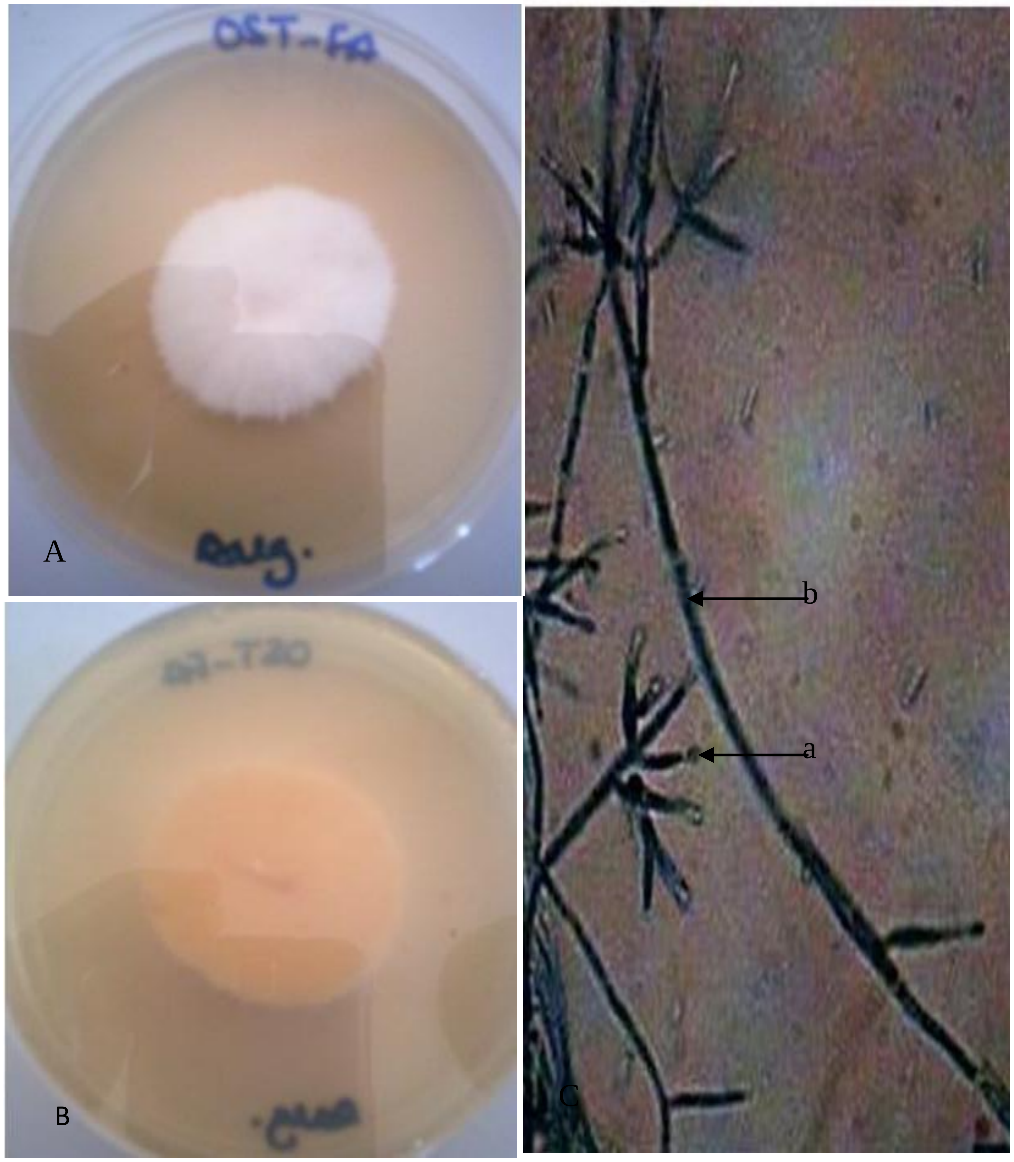


Appendix 6 : Pure culture of *Fusarium oxysporum*(×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= microconidium, b=macroconidium

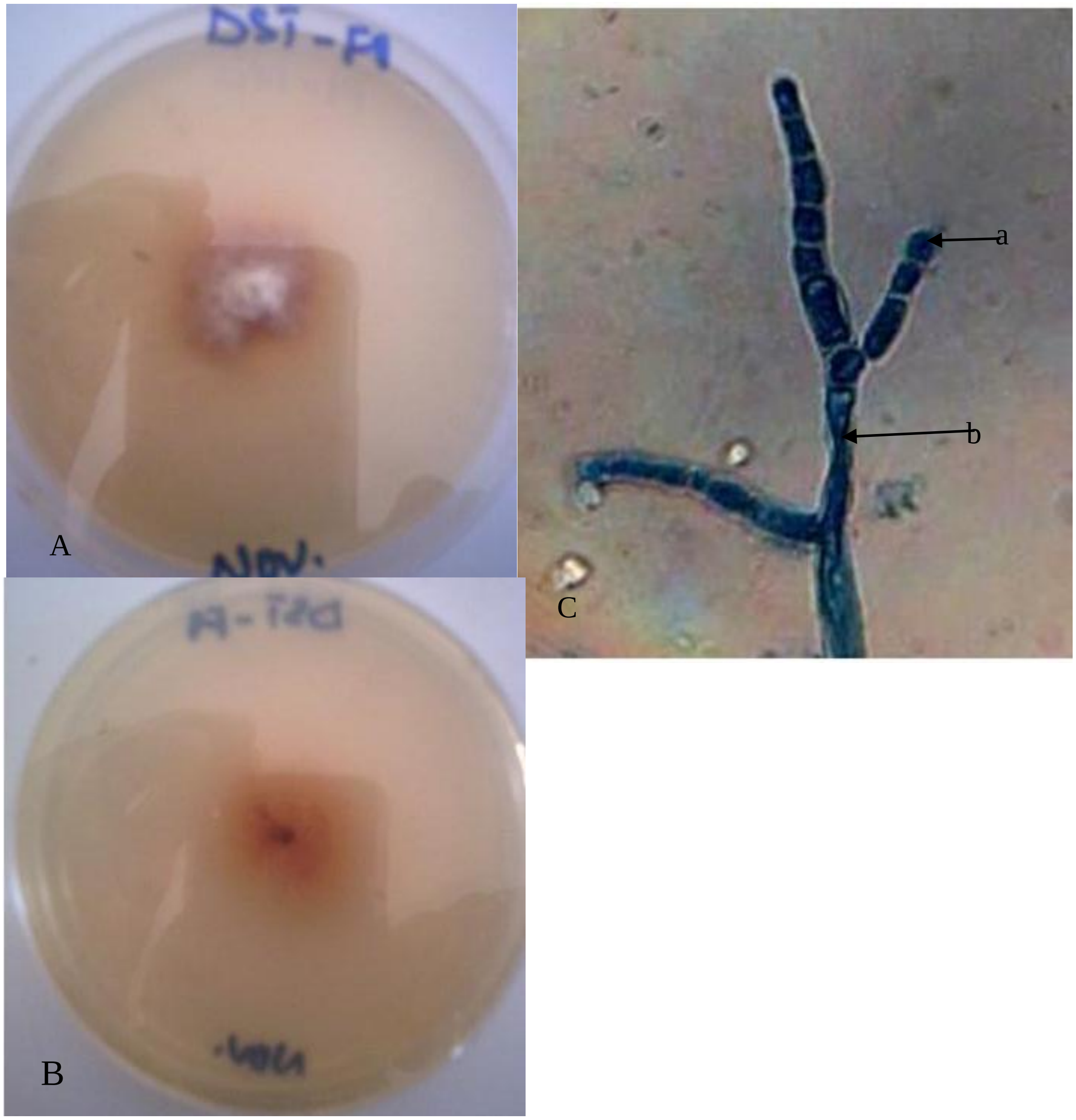


Appendix 7: Pure culture of *Cephalosporium curtipes*(×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= conidium, b:= conidiophore

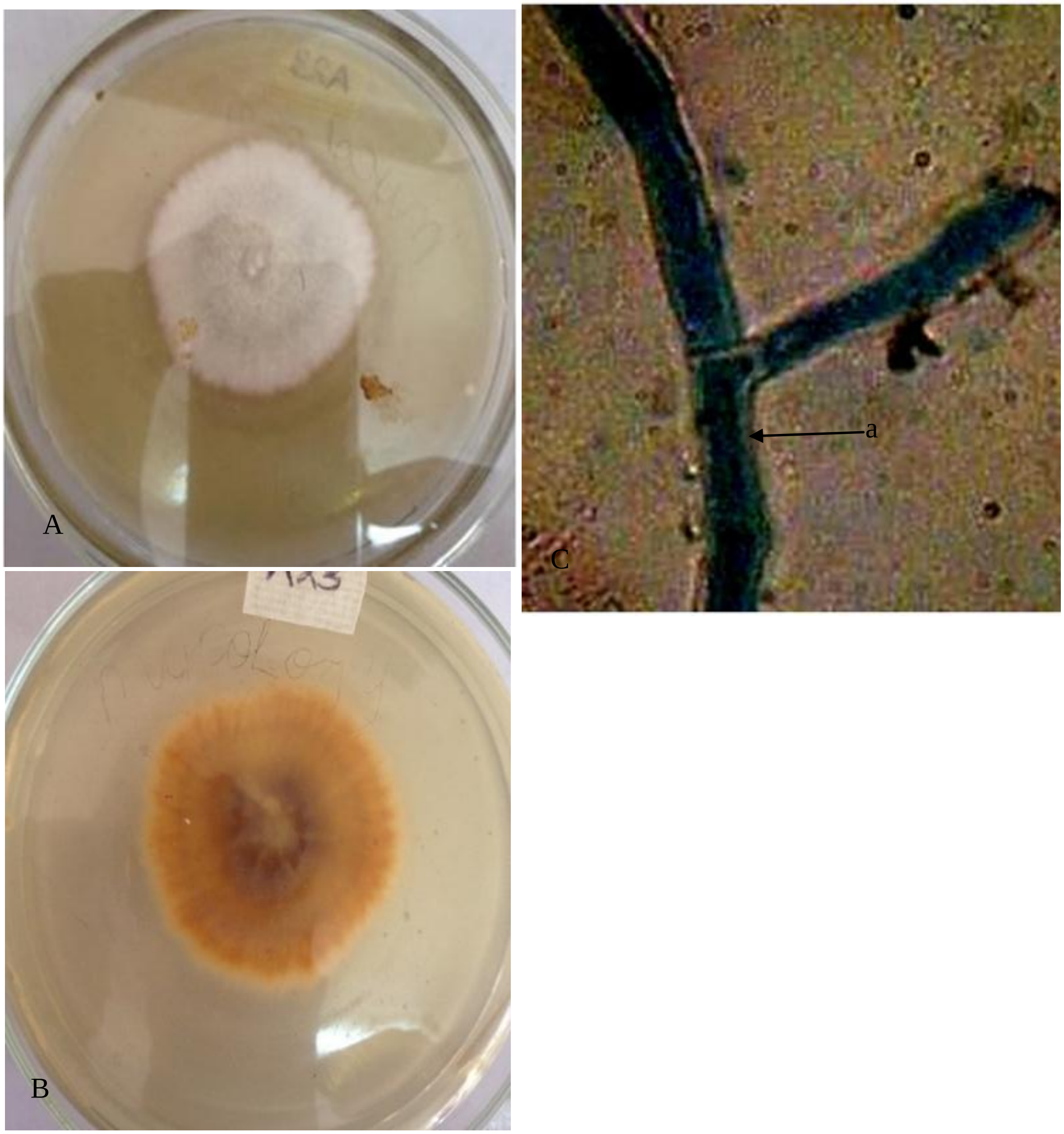


Appendix 8: Pure culture of *Oidiodendron griseum* (×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= conidium, b:= conidiophore

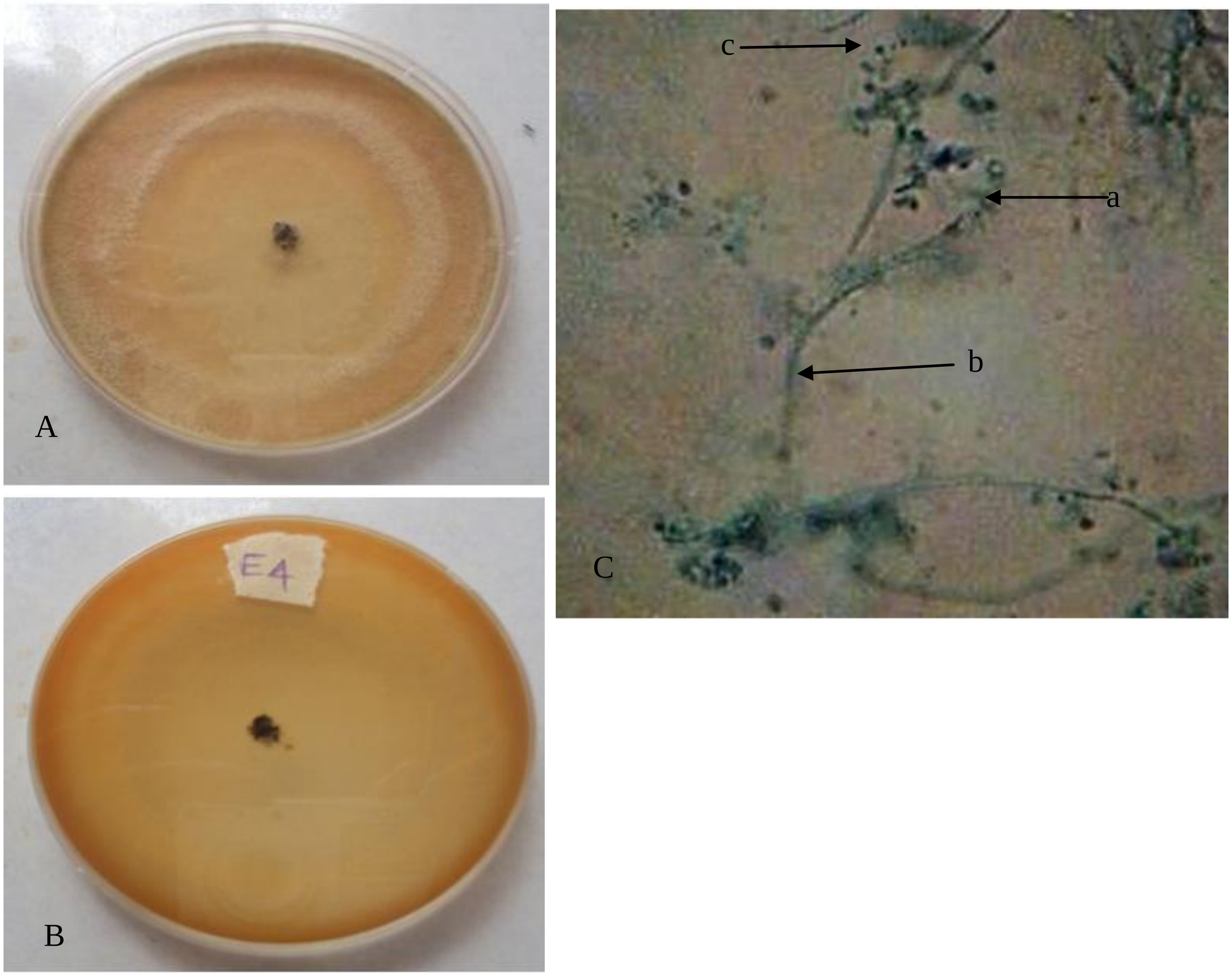


Appendix 9: Pure culture of *Rhizoctonia solani* (×40)

A=Upper surface

B=Reverse side

C=Micrograph: a= conidiophore

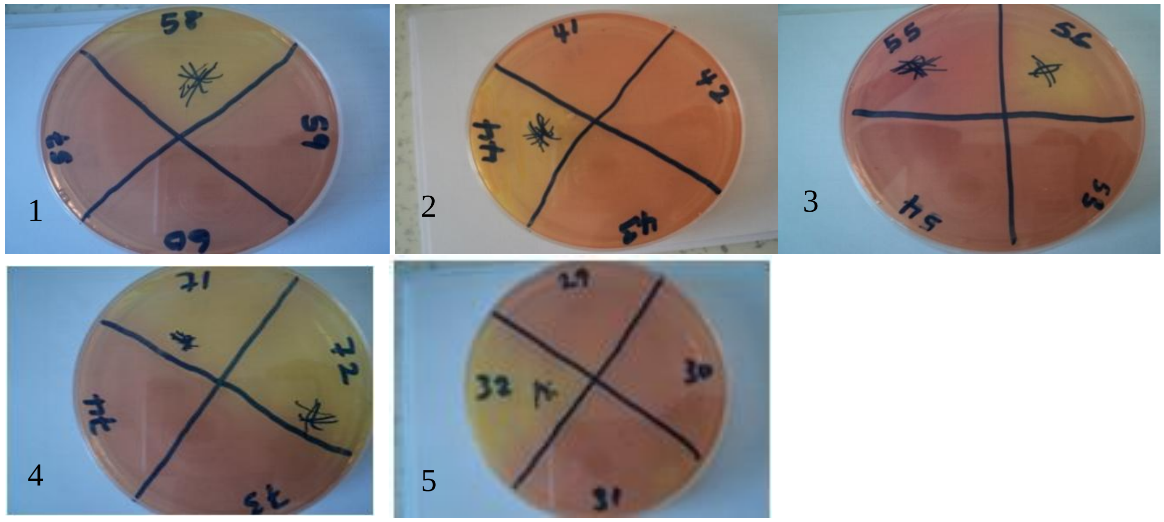


Appendix 10: Pure culture of *Trichoderma viride*(×40)

A=upper surface

B=reverse side

C=Micrograph: a=phialide, b=conidiophore, c=conidium



Appendix11: Pure culture of *Staphylococcus*species

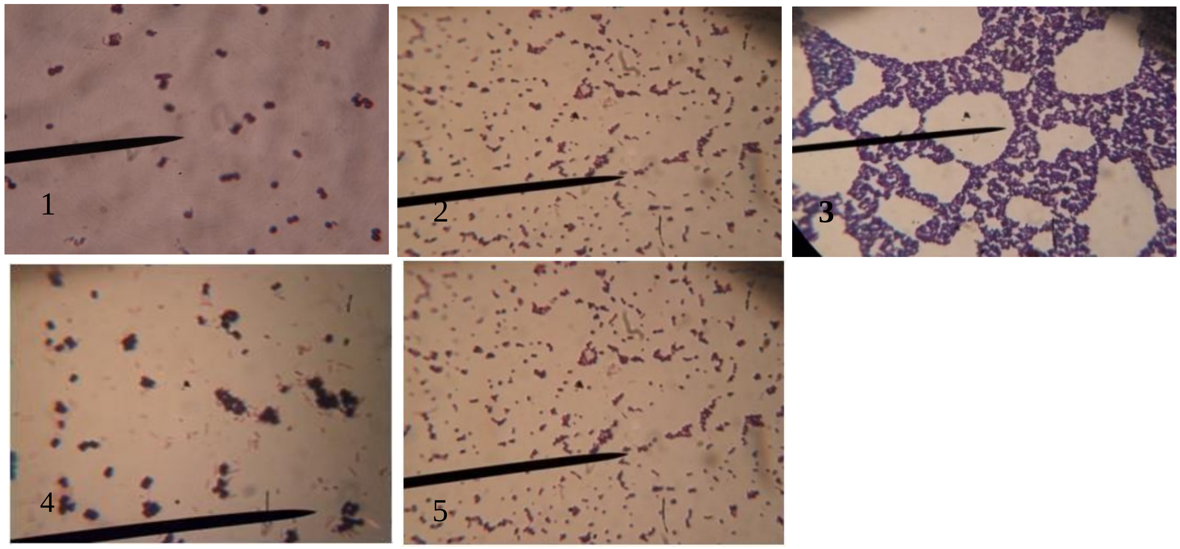
1=*Staphylococcus aureus*

2=*Staphlococcus lugdeninsis*

3=*Staphylococcus saprophyticus*

4=*Staphylococcus warneri*

5=*Staphylococcus xylosus*



Appendix 12: Micrographs of *Staphylococcus* species(×100)

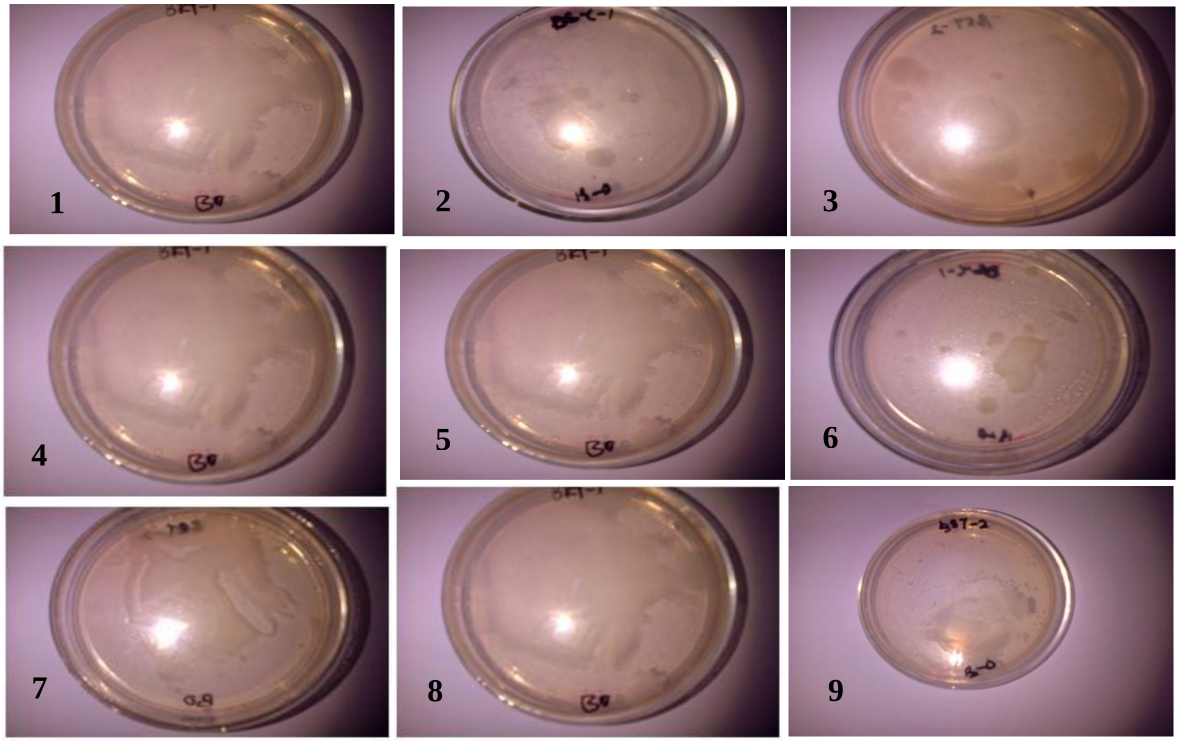
1=*Staphylococcus aureus*

2=*Stphlococcus lugdeninsis*

3=*Staphylococcus saprophyticus*

4=*Staphylococcus warneri*

5=*Staphylococcus xylosus*



Appendix 13: Pure culture ofGram negative *Bacillus* species

1=*Escherichia coli*

2=*Enterobacter agglomerans*

3=Yersinia enterocolitica

4=*Acinetobacter iwoffii*

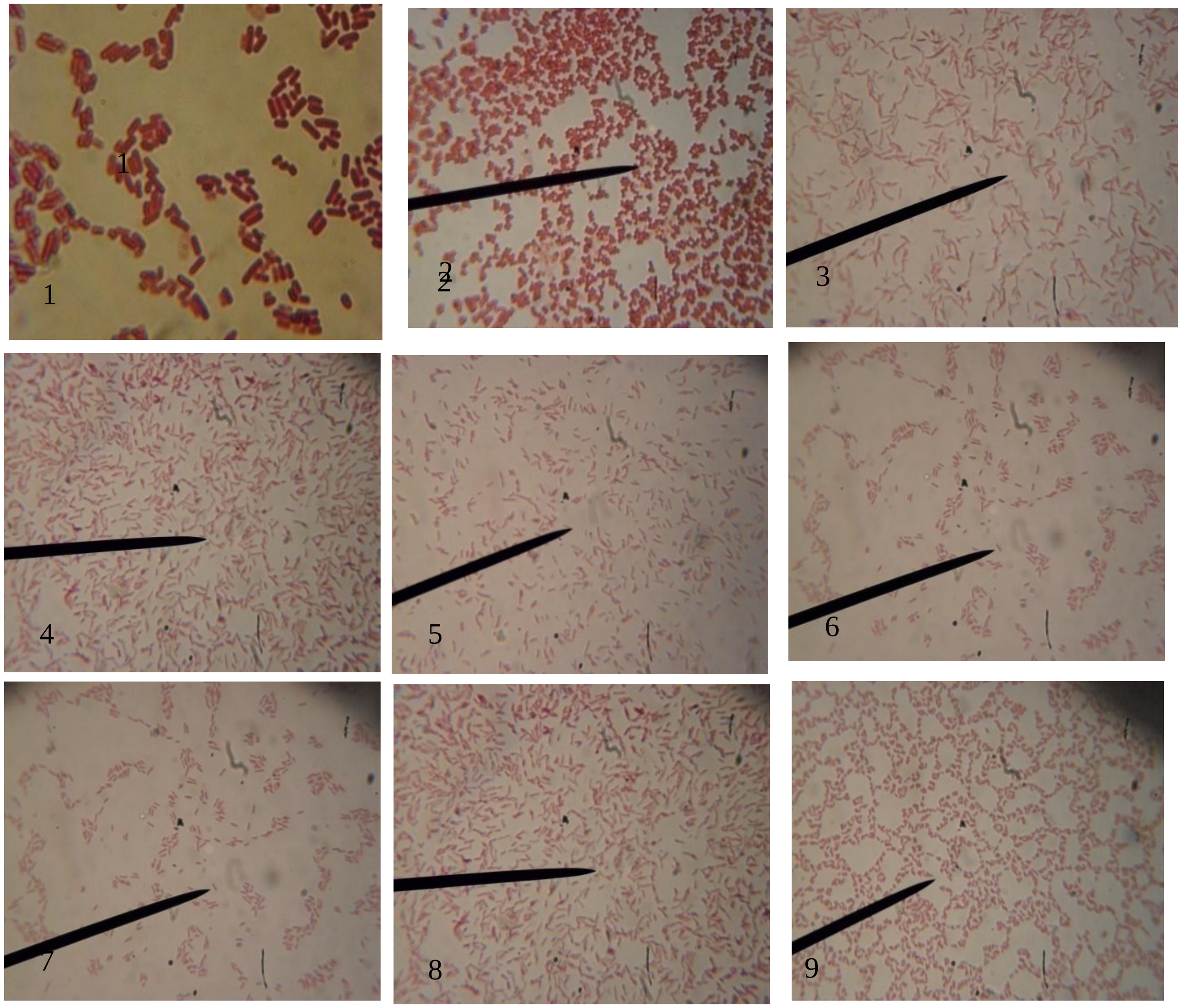
5=*Acinetobacter haemolyticus*

6=*Providencia stuartii*

7=*Edwardsiella tarda*

8=Morganella morganii

9=Providencia acalifaciens



Appendix14: Micrographs of Gram negative*Bacillus* species(×100)

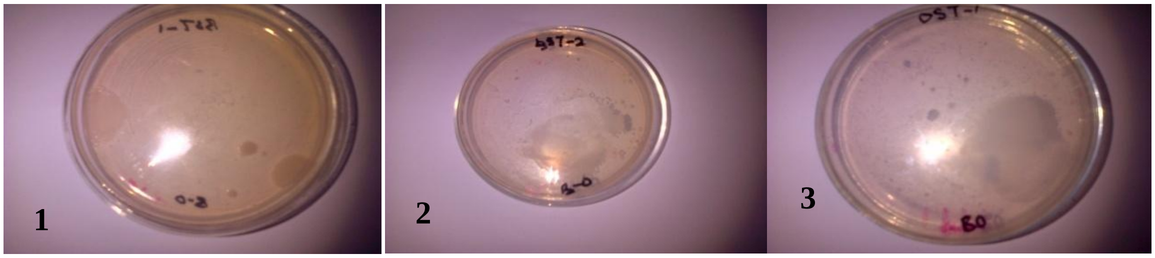
1=*Escherichia coli* 2=*Enterobacter agglomerans*

3=*Yersinia enterocolitica* 4=*Acinetobacter iwoffii*

5=*Acinetobacter haemolyticus* 6=*Providencia stuartii,*

7=*Edwardsiella tarda* 8=*Morganella morganii,*

9=*Providencia acalifaciens*

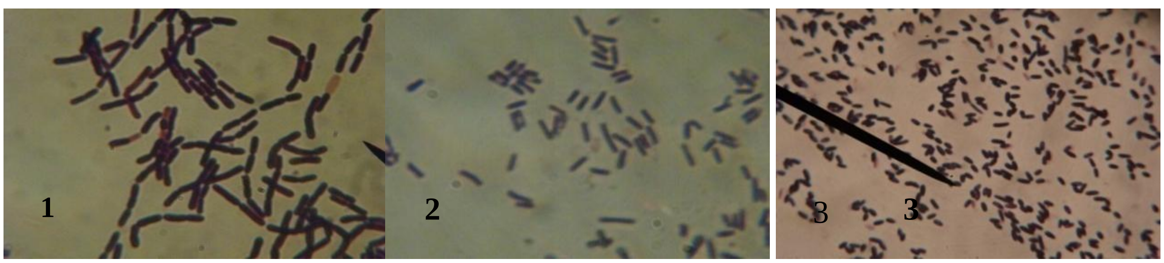


Appendix15: Pure culture of Gram positive *Bacillus* species

1=*Bacillus mergaerium*

2=*Bacillus cereus*

3=*Bacillus lentus*



Appendix16: Micrographs of Gram positive *Bacillus* species(×100)

1=*Bacillus mergaerium*

2=*Bacillus cereus*

3=*Bacillus lentus*

Appendix 17: Biochemical Characteristics of Gram Positive Cocci Isolated from Six Brands of Sachet Water

	Staphylococcs species				
Biochemical Substrates	*Staphylococcus aureus*	*Staphylococcus lugdeninsis*	*Staphylococcus saprohyticus*	*Staphylococcus Warneri*	*Staphylococcus xylosus*
Sucrose	+	-	+	+	+
Trehhalose	+	-	+	+	+
Mannitol	+	-	+	+	+
N-Acetyl Glucosamine	+	+	-	-	+
Mannose	+	+	+	+	+
Turanose	+	-	+	-	+
Glucosidase	+	+	+	+	+
Glucuronidase	+	-	+	+	+
Glucuronidase	+	+	-	+	+
Urease	+	-	+	+	+
Arginine	+	-	+	+	+
PYR Reagent	-	+	+	-	+
Catalase	+	+	+	+	+
Coagulase	+	+	-	+	+

✓ (+) Positive (-) Negative, [x] =Code represent trade names of brands of sachet water.

Appendix 18: Biochemical Characteristics of Gram Negative Bacilli Isolated from Six Brands of Sachet Water

Biochemical Substracts	Bacillus species								
	Escherichia coli	*Yersinia enterocolitia*	*Acinetobacter iwoffii*	*Acinetobacter .haemolyticus*	*Providenca stuartii*	*Edwardsiela tarda*	*Morganella morganii*	*Providenci, acalifacies*	*Enterobacte, agglomeras*
Oxidase	-	-	-	-	-	-	-	-	-
Mortility	+	+	-	-	+	+	+	+	+
Lysine	+	-	+	+	-	+	+	+	-
Ormithine	+	-	-	-	-	-	+	+	-
H. sulphide	-	-	-	-	-	+	-	-	-
Glucose	+	+	+	-	+	+	+	+	+
Mannitol	+	+	-	-	-	-	-	-	+
Xylose	+	-	-	-	-	-	-	-	-
B. gaactosidase	+	+	-	+	+	-	+	-	+
Indole	+	+	-	-	+	+	+	+	-
Urease	-	+	-	+	-	-	+	-	-
V. proskauer	-	-	+	-	-	+	-	+	+
Citrate	-	-	-	+	+	-	-	+	-
T. Deaminase	-	-	-	-	-	-	-	+	-

(+) Positive (-) Negative , H=Hydrogen, *B*=Beta, V=Voges, T=Tryptophan

Appendix 19: Biochemical Characteristics of Gram positive Bacillus Isolated from Six Brands of Sachet Water

	Bacillus species		
Biochemical Substracts	*Bacillus cereus*	*Bacillus lentus*	*Bacillus mergaterium*
Arabinose	-	+	+
Cellobiose	-	-	+
Inositol	-	-	-
Mannitol	-	-	-
Raffinose	-	+	-
Rhamnose	-	+	+
Salicin	+	-	-
Sorbitol	-	-	-
Sucrose	+	-	+
Trehalose	+	-	+
Xylose	-	+	+
Adonitol	-	-	+
Galactose	-	-	-
Methyl-D-mannosida	-	-	+
Methyl-D-glucosida	-	-	-
Inulin	-	+	-
Melezitose	-	-	-
Indole	-	+	+
ONPG	-	-	-
Nitrate	+	-	+
Agrginine dihydrolase	-	+	-
Citrate	+	+	+
Voges Proskauer	+	+	+

(-)=Negative, (+)-Positive