

# **COMPARATIVE STUDY OF QUALITIES OF SACHET AND BOTTLE WATER SOLD ON THE STREETS OF ABUJA, NIGERIA**

**A Case Study By Ibemesim Augustine Obinna, Nigeria**

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## **ABSTRACT**

A total of one hundred (100) brands of sachets and one hundred (100) brands of bottle water samples were collected from different locations in Abuja Metropolis and were analyzed physico-chemically and microbiologically.

The total count of most of the sachet water samples ranges between  $0.32 \times 10^2$  and  $1.95 \times 10^2$  CFU/ml, exceeding the limit of  $1.0 \times 10^2$  CFU/ml. The MPN of coliform counts ranged from 1 to 16 cfu /100 ml of sample. Total number of isolates 502, 201 (40.04%) *Escherichia coli*, 52 (10.36%) *Streptococcus faecalis*, 50(9.36%) *Pseudomonas aeruginosa* and 199 (39.64%) *Klebsiella* Species. The dominant bacteria isolates were *Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella* Species.

The entire bottle water samples analyzed showed no bacterial growth. Bacteriological analysis showed that 10/100 (10%) of the samples of sachet water tested showed positive coliform counts. These contaminants could be as a result of improper sterilization of the water before packaging or contamination due to poor handling during production, transportation or sales of such products. There were no traces of Heavy metals and all other chemical parameters were within permissible limits.

## **KEYWORDS**

Unsafe Water, TSIA Test, Packaged water in Abuja, Water-borne diseases, NAFDAC, Oxidase Test.

## **INTRODUCTION**

Safe and potable water supplies in urban centers in Nigeria are still inadequate in spite of over five decades of independence and several efforts from various governments.

In many developing countries, availability of water has become 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). Increase in human population has exerted an enormous pressure on the provision of safe drinking water in developing countries (Umeh et al., 2005). Towards the Millennium Development Goals – Action for Water and Environmental Sanitation is timely in the light of the problem of poor availability and access to good drinking water in many countries of the

world including Nigeria. “About one – fifth of the world’s population lack access to safe drinking water, and about half lack adequate sanitation. About 40 percent of the world’s population lives in countries with moderate to high water stress.

By 2025, this figure could rise to 50 percent. Yet, with the help of policy and legal reform, international cooperation, community and private sector participation, technical innovation – there are encouraging signs that the crisis could be averted. The connectivity between poverty, hunger, availability, affordability and access to drinking water to sustainable development is succinctly described by the goals of the millennium declaration. “The links between water, health and poverty are numerous and complex.

Access to safe water affects adequate sanitation which in turn drives the risk of water borne diseases especially in poor urban communities. The urban poor often spend up to 10 - 20 times more on water from vendors than piped water.

The inability of Government to consistently provide adequate water contributed to the proliferation of the so-called ‘pure water’ manufacture in Nigeria. The provision of drinking water that is not only safe, but tasteless, odourless and clean in appearance is top priority in any country that cares for good health, and poverty alleviation towards sustainable development. consumers cannot by themselves ascertain the quality of drinking water.

Naturally, water that appears dirty, discoloured, smelly or with unpleasant taste will be treated with grave suspicion by consumers, thus causing them to find an alternative. However, appearance and other organoleptic properties are not all there is to Water Quality Assurance (Akunyili, 2003). Unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical intoxication (Hughes and Koplan, 2005). Unsanitary water has particularly devastating effects on young children in the developing world.

Each year, more than 2 million persons, mostly children less than 5 years of age, die of diarrhea disease (Kosek et al., 2003; Parashar et al., 2003). For children in this age group, diarrheal disease accounted for 17% of all death from 2000 to 2003 (WHO, 2005), ranking third among causes of death, after neonatal causes and acute respiratory infections. Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitation (WHO, 2004) conditions affecting a large part of the world’s population (Hughes and Koplan, 2005).

An estimated 1.1 billion persons (one sixth of the world’s population) lack access to clean water and 2.6 billion to adequate sanitation (WHO, 2005; Hughes and Koplan, 2005). The principal objectives of municipal water are the production and the distribution of safe water that is fit for human consumption (Lamikanra, 1999; Okonko et al, 2008).

Abuja the Federal capital city of Nigeria is situated at the center of the map of Nigeria, bordered by Nasarawa, Niger, Kaduna and Kogi states. According to an official of Abuja water Board, the portable water scarcity has been a perennial problem of the local indigene. Worst hit areas are the core and inner areas like Abaji, Gwagwalada, Kuje, Bwari and others. Hence, the inhabitants mostly women and children have resorted to sourcing drinking water from dug wells, unprotected and protected springs, brooks and harvested rainwater throughout the seasons. (Sridhar et al, 1982; Sridhar, 1999).

Recently in Nigeria, drinking water is commercially available in easy-to-open 50-60ml polyethylene sacks known as sachet /pure water (Umeh et al., 2005).The water vending is a flourishing business in Abuja Nigeria and many people are lured into this business for getting easy returns. The major supply which has become popular among the medium and low income groups are the cheap nylon sachets either registered with the regulatory body(National Agency for Food and Drug Administration and Control NAFDAC) or without registration. Conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population.

Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading waterborne diseases in addition to being pleasant to drink, which implies that it must be wholesome and palatable in all respects (Edema et al., 2001; Okonko et al., 2008). A collaborative, interdisciplinary effort to ensure global access to safe water, basic sanitation, and improved hygiene is the foundation for ending cycle of poverty and diseases (Hughes and Koplan, 2005). At the end of 2000 United Nations (UN) Millennium Summit, member states adopted a set of 8 goals and related targets and indicators aimed at helping to end human. poverty and its ramifications (Sachs and McArthur, 2005). According to Hughes and Koplan, (2005), among these millennium.

Development Goals is a call to halve by the year 2015 the proportion of persons without sustainable access to safe drinking water and basic sanitation. Towards the end of March 2005, the UN launched the “International Decade for Action: Water for Life 2005-2015” (UN, 2005; Bartram et al., 2005). Success in reaching these targets will help achieve the other goals, increase work force productivity, and substantially reduce the amount of time that women and children spend collecting and storing water, which will free them to pursue other productive and educational activities (Hughes and Koplan, 2005).

According to Bartram et al., (2005), the WHO-sponsored International Network for the Promotion of Safe Household Water Treatment and Storage, a global collaboration of UN and bilateral agencies, non-governmental organizations, research institutions, and the private sector, could serve as a model for improving coordination of international efforts in this area of global safe water, sanitation, and hygiene. Innovative approaches towards improving water, sanitation, and hygiene must be implemented and evaluated. A number of studies conducted in a variety of geographic settings have shown that interventions such as point-of-use disinfection of water and educational efforts to improve personal hygiene help reduce disease prevalence (Clasen and Cairncross, 2004). These studies also highlighted the importance of tailoring such interventions to local situations (Hughes and Koplan, 2005).A recent study in an area in rural western Kenya that had turbid source of water found that household use of flocculants disinfectant Preparation helped to reduce the prevalence of diarrhea in children less than 2 years of age (Crump et al., 2005). Studies in refugee camps in Africa (Peterson et al., 1998) and urban slums in Asia (Luby et al., 2005) have reported that hand-washing with soap reduced the prevalence of diarrhea in all age groups and lowered the incidence of diarrhea and pneumonia in children less than 5 years of age.

## **AIMS AND OBJECTIVES**

1. To assess and compare the physicochemical and microbiological qualities of packaged water sold in Abuja Metropolis.
2. To find out if the packaged water sold, is in conformity or otherwise with the standards set by the regulatory body.

3. To identify the organism common to packaged water sold in Abuja.

## **LITERATURE REVIEW**

Water quenches our thirst and refreshes us. That's how it has always been but will it stay like that? We take our most valuable good for granted. To humans, water is indispensable. In the home, water is used for cooking, washing, bathing and other domestic uses. Industrially, water is the starting point of most processes. The chemist says that water is a universal solvent following his findings that most chemicals are soluble in water. For the biologist, it is even more important for the growth of organisms and for carrying out fermentation for the production of products useful to man. (Ibemesim, 1998)

However, one of the most widespread and directly accessible sources of water is rainfall itself. For municipal water supplies, rainwater is rarely used directly. It is most often used as a source of water supply in rural town and villages or communities which do not have any other source of water supply or for areas where water is very 'hard' and unpalatable (Johnson, 1981). Rain water is soft and it is most suitable for laundry purposes. Due to the absence of minerals, rain water is insipid and when in equilibrium with atmospheric gases (carbon dioxide) it is corrosive (Al-layla, 1978). Available water includes surface such as that provided by streams, rivers and lakes as well as ground water which is subsurface water that fill small openings (pores) of loose sediments (such as sand and gravels or rocks). (Jan et al., 2002).

An examination of water quality is basically a determination of the organisms, minerals and organic compounds contained in the water. The basic requirements for drinking water are that it should be free from pathogenic organisms, contain no compounds that have adverse effects on human health, be fairly clear, be non-saline, contain no compounds that cause an offensive taste or smell and cause no corrosion (Kott, 1974).

Jan et al. (2002), described water treatment to involve the conversion of water taken from the natural sources, the "raw water" into that suitable for domestic use. Ground water and surface water usually require more critical treatment than rain water. Harvested water also requires some form of treatment. Most important is the removal of pathogenic organisms and toxic substances such as heavy metals that can cause health problems. Storage of water may be regarded as a form of treatment. Schistomiasis ceracariae are normally unable to survive 48 hours of storage (Mann and Williamson, 1968). Also the number of faecal coliforms and faecal streptococci will be considered reduced when the raw water is subjected to storages (Smethurst, 1979).

Various water treatment processes called unit operations have been developed. Some serve a single purpose while others have multiple applications. Often, a treatment result can be obtained in different ways. Water treatment employs aeration, coagulation and flocculation, sedimentation, slow sand filtration and rapid filtration and disinfection. The principal methods of purifying water on a small scale are those used locally in areas where water harvesting is carried out include sedimentation, coagulation, boiling and filtration.

Boiling is the most satisfactory way of destroying disease-producing organisms in water. It is equally effective whether the water is clear or cloudy, whether it is relatively pure or heavily contaminated

with organic matter. Boiling destroys all forms of disease producing organisms usually encountered in water whether they are bacteria, viruses, spores, cysts or ova. To be safe the water must be brought to a good rolling boil (not just simmering) and kept there for 15-20 minutes. Boiling drives out the gases dissolved in the water and give it a flat taste but if the water is left for a few hours in a partly filled container, even though the mouth of the container is covered, it is wise to store the water in the vessel in which there will be no risk of re-contamination (Lorch, 1987)

## **MAIN SOURCES OF DOMESTIC WATER**

The major sources of drinking water in most of the rural areas include: Streams, Lakes, Rivers, Ponds, wells and Rainwater. These are mostly surface water and may have the following disadvantages. Water from deep wells and deep springs usually dissolves a lot of salts and other minerals and so the water becomes salty, sometime too salty or "hard" for any use unless the salts are removed which is expensive. This water generally needs pumping from great depth often to tanks or reservoirs before reaching the user (Cheesbrough, 2000).

Potable water in Abuja is normally obtained from Dams, lake and wells. The water bottled and sold in various stores and markets is normally obtained from springs or packaged from pipe supplies. All public supplies of drinking water are assumed disinfected before distribution. Potable water is often treated by chlorination. This makes the water free from any coliform organism no matter how polluted the original water may have' been (WHO, 2004).

Potable water is free of pathogens and toxic chemicals. Non- potable water is one contaminated with domestic and industrial waste. There are so many characteristics that make water not potable such as taste, smell, pH, colour/turbidity and mineral salts (WHO, 2005). Purification can be done by coagulation, which is by adding alum, Nitrogen aluminates or ferric chloride to the water. Using the sand bed method, filtration can be carried out or repair sand bed filters can also be used. For the correction of pH of potable water, limestone is added. Low pH will cause corrosion.

## **SOURCES OF WATER POLLUTION**

Waterborne pathogens make their entry into the water bodies through a number of sources. Recycling of treated/inadequately treated wastewater by mixing them with natural water bodies adds microorganisms. When septic tanks are built near the water bodies mixing or seeping of excreta may occur and this may act as a source of waterborne pathogens. Quite a large number of pathogens will be added if the population suffers from an enteric disease. Wastewater from abattoirs and animal processing plants also contribute to the waterborne pathogens. Droppings from nearby birds and faecal materials of domestic and wild animals including those of diseased ones are another potential source.

## **WATER RELATED HEALTH RISK**

Problems due to a lack of water: - In extreme cases of lack of water, life is simply not possible (dehydration and death) since water makes up more than 85% of the protoplasm. Less extreme shortages also have an impact on the health status of a population. They provoke an increase in the incidence of numerous diseases due to a lack of good hygiene; good personal hygiene requires a sufficient quantity of water (Ibemesim, 1998). The diseases linked to water can be classified into water-washed and water-borne diseases.

## **WATER-WASHED DISEASES**

These are diseases that resulted from lack of water for personal hygiene and they include Dermatological and Ophthalmic diseases, Diseases Transmitted by Lice, and Faeco-Orally Transmitted Diseases. Lack of personal hygiene particularly washing of clothes, hands and food, allows the transmission of these diseases from infected individuals (sick people or carriers) to uninfected individuals. These so called “dirty hands diseases” are: diarrhoea and dysenteries (bacterial, protozoan, or viral), cholera, typhoid and paratyphoid fevers, hepatitis A, poliomyelitis, and various helminthes diseases. Poor personal hygiene also encourages the proliferation of some disease vector like lice, resulting to itching, scratching and skin sores due to their bites. They could also transmit louse-borne typhus and recurrent fever.

## **WATER BORNE DISEASES**

Water should be harmless to health and have an appearance and taste (Medecins Sans Frontieres, 2008) acceptable to the population. Ideally the water supplied should meet the quality standard of the WHO. Quite a number of human pathogens find their way into a susceptible host through contaminated water. These pathogens often called waterborne pathogens, have the ability to survive at least for a short period in water and thus water may act as a route of transmission for them. Waterborne diseases are posing a serious threat to health since the potential of contaminated water to transmit disease is very high. Often they lead to epidemic.

According to a WHO survey about 30,000 people die from water-related diseases every day. About 80% of all illness in developing countries is water related (Jan et-al.,2002). Water plays an essential role in the spread of many communicable diseases and epidemics. Diarrhoeal diseases, mostly caused by poor hygiene and lack of safe water, are a major cause of morbidity and mortality among refugee and displaced populations. Large scale and severe outbreaks have occurred frequently, particularly in the initial phase of a refugee crisis situation. The most striking example is that reported among Rwandan refugees in Goma (Zaire) during the 1994 Genocide, where extremely high mortality rates were associated with explosive epidemics of Cholera and shigellosis (Medecins Sans Frontieres, 2008).

## **PROBLEMS DUE TO CHEMICALLY CONTAMINATED WATER**

Water may contain numerous dissolved chemical substances which come either from pollution (fertilizers, insecticides, pesticides, industrial waste etc), or from the composition of the rocks



themselves (fluorine, arsenic, iron etc). These substances may give the water a bad taste that it is undrinkable (for instance, if it contains too many salts or too much iron), but it may also in the long term, cause severe health problems for example: Methaemoglobineamia in babies due to high nitrate levels, arsenic poisoning etc. Unsafe Water contaminated with heavy metals, leads to renal failure, Hair loss, and Chronic Anemia. The possible presence of toxic substances in water is something which must be borne in mind, but in the situations considered here, the micro- biological quality of the water is a much more important and preoccupying problem.

## **PROBLEMS DUE TO MICROBIOLOGICALLY CONTAMINATED WATER**

Water may contain numerous pathogenic organisms and thereby become a means of transmission for many diseases. These includes: Typhoid and paratyphoid fever, Hepatitis A, Cholera, Poliomyelitis, Diarrhoea (caused by *Escherichia coli*, *Salmonellae*, *Yersinia Enterocolitica*), Viral gastroenteritis, Bacillary dysentery (caused by various species of shigellae), *Campylobacter* dysentery, Amoebic dysentery, *Giardia* (lambliasis), Balantidiasis, Helminthiasis (cause by *ascaris* and *trichuris*). It should be noted that these so-called “water-borne” diseases form part of the group of “water-washed” diseases as well. They may also be transmitted by any of the faeco-oral routes; dirty hands, dirty food, dirty water etc. Besides these diseases, water is also involved in the transmission of “water- based” diseases (in other words, those diseases of which the causative agent passes part of its life cycle in an aquatic plant or animal): The different schistosomiasis or bilharzias: diseases caused by helminths (worms) which are usually contracted by contact with infected water (washing clothes, bathing etc), but sometimes also via the oral route.

Dracunculiasis (Guinea worm), transmitted only by drinking infested water. (Medecins Sans Frontieres, 2006). Lastly, water may also transmit: Leptospirosis: a bacterial disease which is contracted primarily by contact with water contaminated with the infected urine of various animals (principally the rat), but also by drinking such water. All the infectious diseases transmitted by water with exception of guinea worm are linked to the pollution of the water by the excreta of humans or other animals (from the sick or from the healthy carriers). One last category of water related diseases is those with an insect vector which develops in or lives near to the water, for example malaria, dengue and yellow fevers, and onchocerciasis. (Medecins Sans Frontieres, 2006).

## **ASSESSMENT OF WATER QUALITY**

The only criteria really of importance to health are the presence or the absence of pathogenic organisms and of toxic concentrations of certain chemicals. There is no direct relationship between the appearance of a sample of water and its portability. (A cloudy sample may be safe, whereas a clear sample may be both chemically and biologically dangerous).

## **BIOLOGICAL ANALYSIS**

The pathogenic organisms which may be present in water are too numerous and too various to be identified individually in practice (bacteria, protozoa, helminths etc). As their presence is always linked to faecal pollution (except for guinea worm), it is preferable to look for organisms which are “indicators” of this pollution. The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern. Indicator organisms are bacteria such as non-specific coliforms, *Escherichia coli* and Faecal Streptococci that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage. Indicator organisms are used because even when a person is infected with more pathogenic bacteria, they will still be excreting many million times more indicator organisms than pathogens. It is therefore reasonable to surmise that if indicator organism levels are low, then pathogen levels will be very much lower or absent.

The count of those colonies which develop with a characteristic appearance gives the number of faecal coliforms in the sample of water. When Multiple Tube method is used and incubated at 37°C all the coliforms will develop (Total coliform count)/100ml and can easily be enumerated. (Cheesbrough, 2000).

## **NIGERIAN STANDARD FOR DRINKING WATER**

Nigerian Standard for Drinking Water Quality contains mandatory limits concerning constituents and contaminants of water that are known to be hazardous to health and/or give rise to complaints from consumers. The standard includes a set of procedures and good practices required to meet the mandatory limits.

## **DRINKING WATER QUALITY STANDARD USED IN NIGERIA**

In 2005, the National Council on Water Resources (NCWR) recognized the need to urgently establish acceptable Nigerian Standard for Drinking Water Quality because it was observed that the “Nigerian Industrial Standard for Potable Water” developed by Standards Organization of Nigeria and the “National Guidelines and Standards for Water Quality in Nigeria” developed by Federal Ministry of Environment did not receive a wide acceptance by all stakeholders in the country. Since water quality issues are health related issues, the Federal Ministry of Health, collaborating with the Standards Organization of Nigeria (the only body responsible for developing National Standards in Nigeria) and working through a technical committee of key stakeholders developed this Standard. The effective protection of public health against water related diseases requires a preventive integrated management approach, this includes:

- a) The protection of drinking water from catchments and source to its use by consumers.
- b) A collaborative multi-agency approach that involve all agencies with responsibilities in the management of water quality.
- c) Water quality standard that is comprehensive, realistic and implementable within the resources of the implementing agencies.



- d) The development of procedures and requirements that ensure good water quality management in order to meet the maximum allowable limits. These procedures also protect the environment.
- e) An independent surveillance agency with strong enforcement authority and functions decentralized to local government level.
- f) An effective drinking water quality data management system to enable generation of data for the development of coherent public health-centred policies and practices. (Standards Organization of Nigeria, 2007).

## **PACKAGED WATERS IN THE MARKET**

The water vending is a flourishing business in Abuja and many people are lured into this business for getting easy returns on investment. The major supply which has become popular among the medium and low income groups are the cheap nylon sachets either registered with the regulatory body or with no registration. There are about 200 brands or even more of packaged water available in the city at the time of this study although quite a large chunk are produced in neighbouring states like Nasarawa, Niger, Kaduna and Kogi and the number is increasing every day. They are broadly classified according to the packages and price tag as well.

Bottle water is usually manufactured and marketed by standard companies, both local and Multi-nationals. These waters are either drawn from natural springs, or deep boreholes some are even from public mains and treated according to the specifications like Aeration whereby water entering the plant is sprayed into the air through nozzles, producing a fountain-like effect. Breaking the water into small drops creates a proper oxygen balance, releasing trapped gases that can cause objectionable tastes and odors. Filtration by passing through various filters like sand and Activated Carbon filters, Reverse osmosis and disinfected appropriately using either ultra- violet radiation or ozonation or both. Ozone is used for disinfection and control of taste and odor causing compounds. This bottle water is available in 50cl, 75cl, 150cl and 20L jar bottles.

These are relatively expensive (at the rate of Naira 60, 100-400 <1-2USD) and is a class thing being very popular among hotels and restaurants and people from higher socioeconomic strata. The source is well protected. An in-house quality control laboratory usually checks the water quality.

Plastic packaged water, popularly called sachet “pure water” which is manufactured by small to medium scale industries (either in a shed or garage or in most cases in standard houses and factories) with a registered name and supposed to have been prepared under Government stipulated hygienic quality regulations. According to the specifications, the water is passed through a series of sand and activated carbon or suitable filtering media and Millipore or equivalent filters of a specific pore size, and disinfected using ultraviolet radiation for a specific period. They are packed in 50cl or 60cl nylon / plastic film sachets and sealed by a sachet packaging machine. They are put in larger sacks in dozens and transported to various distribution points in open pick-up vehicles. Sometimes the sealing is poor and quality control is rather questionable. The source in many cases is not well protected and

human errors in the manufacturing process are possible. The price is affordable (Naira 10 < a cent) mostly for the middle income groups. They are popular at social gatherings and public places and are hawked along the streets with reckless abandon thereby littering the environment with the nylon material after being consumed.

## **THE ROLE OF PACKAGED / “PURE WATER” PRODUCERS TOWARDS NATIONAL DEVELOPMENT**

According to Akunyili, regulation and control of packaged water is not only the responsibility of NAFDAC but also that of the manufacturers, consumers and other relevant government organs. This team approach to regulation is necessary because of the dire consequences of consuming poor quality water to public health. Consumption of contaminated or poorly produced water could result in water-borne diseases like cholera, typhoid fever, diarrhoea etc. Chemical contaminants such as lead, iron, nitrates, etc. in water also give rise to illness (e.g. liver and kidney problems) and even death. Water borne diseases resulting from contamination are treated with scarce funds thereby further impoverishing the masses and causing underdevelopment. Pure water manufacturers form a major part of the small medium scale industries (SMIs) in Nigeria. Studies have shown that about 10-15 percent of total manufacturing output is from the SMIs and this accounts for over 40 percent of gross domestic product (GDP). (Akunyili, 2003).

Packaged water especially the sachets (Pure water) production is a good poverty alleviation programme and should be encouraged. It is an industry that has immense potentials for job and income generation. With the number of pure water and bottled water outfits in the country (and judging by about 10,634 participants at NAFDAC water workshop) their retinue of staff should stand in the region of over fifty thousand strong workforce. This number excludes the chain of wholesalers and retailers that generate income from selling packaged water products. The disposal of waste generated from the production and use of packaged water constitutes one aspect of environmental/health hazard that must be tackled by all stakeholders. Waste-to-wealth programmes have created great wealth for many nations. The Agency therefore suggests that the Ministry of Environment and the private sector participate in the reactivation of recycling programmes that will generate employment, boost our economy, protect the environment and promote health. Some countries that are not endowed with oil wealth recycle cellophane bags, (which presently litter our streets) into other very useful materials (Akunyili, 2003). NAFDAC encourages the promotion and use of biodegradable packaging materials. They are often cost-effective and enhance proper disposal, thus reducing public health risks.

NAFDAC is concerned about this because, improperly disposed waste effects the health and well being of the people and negates the positive impact of our regulatory activities. It is in recognition of these facts that NAFDAC insists that there must be an environmentally friendly disposal instruction on the packaged water before it can be registered. This they enforce to the letter though environmental protection is not their mandate but a social responsibility. The packaged water industry has enormous export potentials. Nigeria's problem is not poor availability of water resources rather that of poor management of these resources. Well processed and properly packaged water can be exported to earn much needed foreign exchange. It is an embarrassment for Nigerians to import packaged water in any form. Manufacturers therefore must improve their standard as well as output to recapture the present market share taken by smuggled water/water based products (Akunyili, 2003). Self regulatory measures should be put in place by manufacturers of packaged water. This way the

Agency will focus better in assisting them to meet their optimal potentials in terms of product safety, quality, acceptability and marketability both locally and internationally. The result of our post marketing surveillance show that self regulation by pure water manufacturers still leave a lot to be desired (Akunyili, 2003).

## **STUDY AREA**

The study area is the municipal area of Abuja, which is made up of six local government councils. The city is the Federal Capital Territory of Nigeria with a population of over twelve million and also houses the Diplomatic community in Nigeria and all Federal Ministries and Parastatals and a lot of Universities owned by both Nigerians and expatriate community alike. However, the city is characterized by high and low level of environmental sanitation, no slums but scattered poor housing in some localities with lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations mostly the Indigenes.

## **SAMPLING OF WATERS**

From the well over 200 brands of water sold in the city at the time of this study, 200 samples were selected by simple random sampling method (here, the selection of units from a population is based on the principle of randomization. Every unit of the population has a calculable (non-zero) probability of being selected.

**RANDOM SAMPLING:** A subset of the population in which every member of the population has an equal likelihood of being selected.) from various vendors. The distributions of samples were as follows: bottle water 100 brands, Sachet water 100 brands were picked from the market for ease of calculation and also the higher the sample size the better the representation of each brand in the actual population. It was assumed that the bottle water quality will be satisfactory relying on the fact that the quality control of those factories producing bottle water is very high in the large scale industries. Therefore all the water samples from the 100 brands were collected from different sellers in different outlets. These were purchased directly from water vendors in the markets, Hotels, food serving areas (Bukhas) and motor parks in the six Local Government Areas of the metropolis (Abuja Municipal, Karshi, Kuje, Buwari, Gwagwalada and Abaji). The samples were collected and stored in cool boxes and transported to the laboratory. The number collected on a day are immediately processed for physico-chemical and bacteriological analysis as described in Standard Methods (APHA 1998). For bacteriological analysis, the bottles and sachets were opened under aseptic conditions.

## **METHODOLOGY**

The physical parameters included pH, temperature, total dissolved solids (TDS), oxidation reduction potential (ORP) and electrical conductivity (EC). Chemical parameters included cation and anion constituents: aluminum, calcium, chromium VI, iron, magnesium, zinc, chloride, cyanide, fluoride, nitrite, and nitrate, total alkalinity, total hardness and total and free chlorine. Hanna C-100 spectrophotometer (HI 83099 COD and Multiparameter Photometer made in UK) and chemical

reagents supplied along with the kit were used for analyses. Total hardness was determined using EDTA titration method using Eriochrome Blak T indicator. Chloride was determined using the silver nitrate titration method using potassium chromate indicator. Total alkalinity was measured titrimetrically using mixed indicator. For bacteriological analysis (Total and faecal coliforms), multiple tube method was used. The culture media used were MacConkey Broth (MB) and Brilliant Green Bile Broth (BGBB).

After inoculation of the media with the samples, the BGBB culture tubes were incubated at 37°C for 2 hours before transferring them to 44°C incubator for 18 hours. The MB cultures were incubated at 37°C for 18 hours. After the incubation period, the cultures were inspected for changes in colour and gas production. Those showing growth with or without gas production were noted. Those showing no changes in colour were re-incubated for additional 24 hours. The tubes showing changes in colour are counted and the MPN count expressed per 100 ml of sample as per the Mac-Grady's Probability Table. The cultures that showed growths were also sub-cultured on MacConkey agar plates to obtain discreet colonies to facilitate easy isolation and identification of the predominant organisms. Quality control and Quality Assurance were ascertained appropriately. Standard Methods for water analysis as described by the American Public Health Association (Mara and Oragui 1985, APHA 1998) were employed. The coliform counts were expressed as cfu/ 100 ml.

## **SAMPLING OF WATER**

A total of 200 packaged water samples comprising of 100 brands of bottle water and 100 brands of Sachet water were purchased directly from water vendors in the markets, food serving areas (Eateries, Bukhas), motor parks and retail outlets of some of the producers in the metropolis. However, the choice of 100 to 100 samples of sachet to bottle was for ease of calculation and also the higher the sample size the better the representation of each brand in the actual population. The samples were stored in cool boxes and transported to the laboratory without delay. The samples collected were processed within six hours of collection.

## **CULTURE MEDIA AND REAGENTS**

The culture media and reagents used for this project and their preparations are in appendix 2.

## **METHODS**

### **PRESUMPTIVE COLIFORM TEST BY:**

#### *MULTIPLE TUBE METHOD*

In this method, both single strength and double strength sterile MacConkey Broth (MB) were used. Here, 50mls of the Double strength MB was placed in a tube, and 10mls each, was placed into 5 tubes containing inverted Durham tubes for the collection of gas produced. 1ml of the water sample were added into each tube, mixed thoroughly and incubated aerobically at 37°C for 18-24 hours after which Statistical tables was used to derive the concentration of organisms in the original sample.

## **DETERMINATION OF VIABLE BACTERIAL COUNTS**

The numbers of tubes with positive presumptive test were sub-cultured on fresh Plate Count Agar (PCA) and the colonies were counted for each dilution, using the formula stated as follows. Plates showing total counts of about 20 colonies were selected and the number of viable bacterial per ml of sample was determined by multiplying the number of colonies counted by the dilution factor and capacity of pipette as expressed mathematically below;

Calculation: - Number of viable bacterial /ml

Number of colonies counted x dilution factor x volume of pipette = x orgs/ml.

## **IDENTIFICATION OF ISOLATES**

The isolates from Plate Count Agar were sub-cultured on MacConkey Agar and Nutrient Agar. Pure isolates of resulting growth were identified using morphological and biochemical methods as described by APHA, (1998). The sterility of each batch of test medium was confirmed by incubating one or two un-inoculated tubes or plates along with the inoculated tests. The un-inoculated tubes or plates were always examined to show no evidence of bacterial growth. After inoculation of the media with the samples, the MB cultures were incubated at 37°C for 18 hours. After the incubation period, the cultures were inspected for changes in colour and gas production. Those showing growth with or without gas production were noted.

Those showing no changes in colour were re-incubated for additional 24 hours. The tubes showing changes in colour were counted and the MPN count was expressed per 100 ml of sample as per the Mac-Grady's Probability Table. The cultures that showed growth were also sub-cultured on MacConkey agar plates to obtain discrete colonies to facilitate easy isolation and identification of the predominant organisms. Quality control and Quality Assurance were ascertained appropriately. Standard Methods for water analysis as described by the American Public Health Association (Mara and Oragui 1985, APHA 1998) were employed. The coliform count is expressed as cfu/ 100 ml.

## **GRAM STAINING**

Gram stain was done on each bacterial isolate and examined microscopically using oil immersion objectives. The reaction test was carried out on all the different isolates.

## **MOTILITY TEST BY HANGING DROP METHOD**

This was done to determine the presence of motile organisms. A ring of plasticine of about 2cm in diameter was made on a grease free slide. A loopful of a 24 hour broth culture of gram negative bacilli was placed at the center of a clean coverslip measuring about 22 x 22mm in dimension. The slide was gently pressed on the cover slip such that the drop of the culture was positioned at the center of the plasticine ring. The slide was inverted and the coverslip seen uppermost. The preparation was

examined under x10 and x40 objectives. Motility was indicated by movement of the bacterial cells within the hanging drop.

## **BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES**

Biochemical tests were carried out and all results obtained with reference to (Cheesbrough, 2000) were noted.

### *COAGULASE TEST*

This test is used to determine the ability of an organism to coagulate plasma by the action of the enzyme coagulase thereby converting fibrinogen to fibrin. It is used to differentiate between *Staphylococcus aureus* and non-coagulase *Staphylococci*. 0.5ml of 1:10 diluted human plasma was placed in a clean khan tube. 0.1ml of overnight broth culture of the organisms was added. This was mixed, incubated at 37°C for 30mins to 6hours and observed for clot formation. (observation was made at 1hr interval)

### *INDOLE TEST*

This determines the ability of an organism to breakdown tryptophan by the activity of an enzyme tryptophanase to release indole as a by-product. In a test tube containing a 24-hour broth culture of the test organism, 0.5ml of Kovac's reagent was added and shaken. This was observed for colour change at the top layer (ring). A rose pink-red colour ring was taken for positive result and no colour change for negative result.

### **OXIDASE TEST**

This is carried out to determine the oxidase producing ability of some organisms. The tetramethylparaphenylene diamine dihydrochloride solution is oxidized to a deep purple colour by oxidase enzyme produced by some organisms. A piece of filter paper was soaked in oxidase reagent, a smear of the suspected colony was made on the soaked filter paper. Purple colour indicated positive result within 10 seconds while negative showed no colour change.

### **CITRATE UTILIZATION TEST**

This is based on the ability of an organism to utilize citrate as its only source of carbon and ammonium as its only source of nitrogen. The citrate is metabolized to acetoin and carbon dioxide. A broth culture of the organism to be tested was inoculated into Simmon's Citrate Agar slope with a straight wire loop and incubated at 37°C for 24 hours. A change in colour from green to deep blue is considered positive.



## *UREASE TEST*

This test is used to determine the ability of an organism to produce the enzyme urease. The urease is able to decompose urea by hydrolysis to give ammonia and carbon dioxide. The ammonia produced makes the medium alkaline so that the colour of the indicator becomes pink. The test organism was stabbed into the medium using a sterile straight wire, and then a streak was made on the slope. This was incubated at 37°C overnight with the covers loosely capped. Colour change to red-pink for positive results while negative showed no colour change.

## **DISCUSSION AND CONCLUSION**

This study showed that 10 out of 100 brands of sachet water were contaminated by different organisms such as *Klebsiella* species *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* (Table 1). This finding agreed with that of Ibemesim A.O (2009), Umeh et al (2005) in which these organisms were isolated in addition to other organisms. Although physical examination of the water samples analyzed did not show any particulate object or discolouration of any type yet the presence of pathogenic bacteria in them calls for a serious concern. According to Umeh et al (2005), bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these organisms may pose a potential health risk to consumers.

The coliform count range of  $0.32 \times 10^2$  to  $1.95 \times 10^2$  recorded in this work (Table 2) is above the value recommended by the WHO. This might be unconnected to the improper sterilization, poor handling of the products in the course of production, transportation and sales of the products. This supports the earlier views of Osibanjo (1999) and Umeh et al (2005) that the sachet water being produced is of questionable quality. The implication therefore was that all the 200 water samples investigated carried NAFDAC (Registration) approval numbers and the products are popularly and freely served at open parties and social functions. Also, the possible contamination of sachet water at the point of production has been confirmed by Chaidez et al (1999) and Dan Rutz (1996) in which they reported that pure water vending machine may not be so pure, after all, because investigations found bacteria like *Escherichia coli* in the machine. However, it was gratifying to note that all the bottle water analyzed in this study were free from bacterial contamination which possibly showed that the manufacturers adhered strictly to the guidelines set up by NAFDAC and SON.

## **CONCLUSION**

The results obtained so far highlights the fact that communities in urban areas suffer from acute portable water shortages. To augment this situation, many entrepreneurs took to packaged water business – production and vending. There is a rush to get into business and as a result quality control has been compromised. Therefore, packaged water other than those in company sealed bottles could pose as a source of waterborne infection as this study has shown that the bottle water is obviously of better quality than the popular sachet water. Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet standard for portable water. There is, therefore, a need to monitor all those involved in water business to comply

with the guidelines to avert possible outbreak of water-borne diseases as a result of consumption of contaminated water.

## **RECOMMENDATION**

Tap water, bore-hole water, and publicly sold sachet and bottle water should be adequately treated before use and NAFDAC should ensure and enforce strict compliance to the standards as regards the production and sales of packaged sachet water. Packaged water consumers should be aware of a possible danger of consumption of poorly packaged water especially the sachet water and the potential health risk associated with such. Also NAFDAC should apart from educating the consuming public on the dangers of patronizing sachet pure water that does have NAFDAC approved numbers; producers should also be educated on how to maintain Good Manufacturing Practice (GMP) and companies that fail to maintain the standard should be properly sanctioned either by stipulating adequate fines to be paid or out rite withdrawal of their production Licenses.

Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet portable water standard. There is, therefore, a need to monitor all those involved in water manufacturing business to comply with the guidelines. The national regulatory bodies and Ministries of Health, Water Resources as well as those of Trades and Industries should exercise more stringent surveillance programmes and educate the producers and the consumers alike on the need to look for water quality, proper labeling and certification. To achieve this goal the manufacturers, the consumers and government should work together to achieve this common goal for the betterment of all.

## **APPARATUS USED**

1. Autoclave
2. Bunsen burner
3. Bijou bottles
4. Colony counter
5. Coverslip
6. Conical flasks
7. Glass slides
8. Hanna C100 spec
9. Incubator
10. Inoculating loop
11. Microscope
12. Measuring cylinders
13. Masking tape
14. Pipettes( various volumes)
15. Non absorbent cotton wool
16. Refrigerator
17. Racks

18. Weighing Balance
19. Test tubes

## **MEDIA PREPARATION**

All the culture media used in the work were prepared according to the manufacturer's instructions and specifications.

## **MEDIA AND REAGENTS USED**

### *MEDIA*

1. Nutrient Agar
2. MacConkey Agar
3. MacConkey Broth
4. Plate Count Agar, Triple Sugar Iron Agar(TSIA)
5. Distilled water
6. Peptone water

### *REAGENTS*

1. Crystal Violet
2. Oxidase Violet
3. Oxidase Kovac's reagent
4. Potassium Iodide
5. Acetone Neutral red iodine
6. Hanna Reagent for Physico-chemical analysis of water
7. Immersion Oil

### *TRIPLE SUGAR IRONAGAR (TSIA) TEST*

TSIA is used in the identification of gram negative bacilli, particularly, members of the enterobacteriaceae. TSIA detects three primary characteristics of bacteria; the ability to produce gas from the fermentation of sugars, the production of hydrogen sulphide and the fermentation of lactose and sucrose with acid production or non fermentation of lactose and sucrose with acid production or non fermentation with alkaline production. TSIA slope was inoculated with the suspension of the test organisms by stabbing through the butt with a straight wire and streaking the surface of the slope. It was incubated at 37°C overnight with cotton wool loosely plugged. Fermenting organisms produce an acid reaction (yellow colour) throughout the tube gas production and hydrogen sulphide production was also considered. Oxidizing organisms produce an alkaline reaction (red colour) at both slope and butt.(Cheesbrough, 2000).

## **RESULTS**

The total count of most of the water samples ranges between  $0.32 \times 10^2$  and  $1.95 \times 10^2$  CFU/ml, exceeding the limit of  $1.0 \times 10^2$  CFU/ml. The MPN of coliform counts ranged from 1 to 16 cfu /100 ml of sample. The total aerobic Mesophilic Bacteria Count cfu/100ml of those samples of sachet water that gave moderate growth is  $1.2 \times 10^2$ . Coliform, *Escherichia coli* counts of those samples that gave heavy growths are  $2.0 \times 10^2$  and  $1.0 \times 10^2$  respectively. The entire bottled water sample analyzed showed no bacterial growth. The dominant bacteria were *Escherichia coli*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*. The total counts for some of the water samples were generally high, exceeding the recommended standard limit for water (FAO, 1997; Okonko et al., 2008).

There were no traces of Heavy Metals in all the water samples analyzed and the chemical parameters were all within the permissible limits. The isolated bacteria species were identified to be same with those commonly encountered in water and aquatic environments as was also reported in a study on streams surface water in Wyoming in U.S.A. reported by Clark and Norris (1999) and reviewed by Banwo (2006). These identified isolates include *Bacillus cereus*, *Enterobacter aerogenes*, *Flavobacterium* sp., *Micrococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The presence of Coliforms and *Pseudomonas aeruginosa* reported in this study has also been reported by Umeh et al., (2005) in a study on the bacteriological quality and safety of pure water sold in Akwa, Nigeria using membrane filtration method. Umeh et al., (2005) reported the presence of enteric bacteria associated with fecal contamination and this include *Streptococcus faecalis*, *Citrobacter* species, *Proteus mirabilis*, *Providencia* species, *Micrococcus* species, *Escherichia coli*, *Shigella* species, *Enterobacter aerogenes*, *Serratia* species and *Klebsiella* species. Bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential health risk to consumers (Umeh et al., 2005).

## REFERENCES

1. Akunyili, D., (2003). The role of “pure water” and bottled water manufacturers in Nigeria. 29th WEDC International Conference Abuja, Nigeria, 2003, 85-88.
2. Al-Layla, M., Anis, K., Shedmin, K., & Ahamad, J., (1978). Water Supply Engineering Design, 3rd Edition.
3. American Public Health Association (APHA), (1998)., Standard Methods for Examination of Water and Wastewater, 18th Edition, Washington D.C.
4. Aramas, A. B., & Sutherland, J. P., (1999). A survey of the microbiological quality of bottled water sold in the UK and changes occurring during storage, *International Journal of Food Microbiology* 1997, 48: 1, 59.

5. Banwo, K., (2006). Nutrient Load and Pollution Study of Some Selected Stations along Ogunpa River in Ibadan, Nigeria. M.Sc. Dissertation. University of Ibadan, Ibadan, Nigeria, p. 107.
6. Bartram, J., Lewis, K., Lenton, R., & Wright, A., (2005). Focusing on improved water and sanitation for health. 2005 *Lancet* 365: 810-812
7. Chaidez, C., Rusin, P., Naranjo, J., & Gerba, C. P., (1999). Microbiological quality of water vending machines, *International Journal of Environmental Health Research*, 1999, 9: 3, 197–206
8. Cheesbrough, M.,(2000). *District Laboratory Practice in Tropical Countries Part 2*, 2nd Edition p.62-70,143.-145.
9. Clasen, T. F., & Cairncross, S., (2004). Household water management: refining the dominant paradigm. *Tropical Medicine International Health* 9:187-191
10. Crump, J. A., Otieno, P.O., Slutsker, L., Keswick, B. H., Rosen, D. H., & Hoekstra, R. M., et. al., (2005). Household based treatment of drinking water with flocculant disinfectant for preventing diarrhea in areas with turbid source of water in rural western Kenya: cluster randomized controlled trial. *BMJ* online published 26/07/2005. Available from <http://bmj.bmjournals.com/cgi/rapidpdf/bmj.38512.618681.E0v1>
11. Dan, Rutz., (1996). Pure water vending machines may not be so pure, <http://k.N@wN.ciin.com/HEALTH/960>
12. Edema, M. O., Omemu, A. M., & Fapetu, O. M., (2001). Microbiology and Physicochemical Analysis of different sources of drinking water in Abeokuta. Nigeria. *Nigerian Journal of Microbiology*2000, 15(1): 57-61.
13. Food and Agriculture Organization (FAO) (1997a). *Chemical analysis manual for food and water*, 5th Ed, FAO ROME 1: 20-26.
14. Food and Agriculture Organization (FAO) (1997b). *Annual Report on Food Quality Control*; 1: 11-13.
15. Hughes, J. M., Koplan, J. P., (2005). Saving Lives through Global Safe Water. *J. Emerging Infectious Diseases* 11 (10): 1636-1637.
16. Ibemesim, A. O., (1998). Physicochemical and Microbiological Qualities of Harvested Water. AB tech citation in FUTO P.2-16.
17. Ibemesim, A. O., (2009). Physicochemical and Microbiological Qualities of packaged water sold in Jos Nigeria.
18. Jan, D., & Robert, L., (2002). *Engineering in Emergencies. A handbook for relief workers* 3rd edition. ITDG Publishing. 103-105 Southampton Row, London WC1B4HL, UK.p 288-292.
19. Kott, Y., Rose, N., Sperber, S., & Betzer, N., (1974). Rapid Determination of presence of Enteric Bacteria in water; 12:484.

20. Lamikanra, A., (1999). *Essential Microbiology for students and practitioner of Pharmacy, Medicine and microbiology*, 2nd edn. Amkra books, Lagos, p. 406.
21. Lorch, W., (1987). *Handbook of water purification*. 2nd Edition. Ellis Horwood Ltd Chichester. P 66-72
22. Luby, S. P., Agboatwalla, M., Feikin, D. R., Painter. J., Billhimer, W., & Altaf A, et al.
23. Mara., D. D., & Oragui, J., (1985). Bacteriological methods for distinguishing between human and animal faecal pollution of water: Result of field work in Nigeria and Zimbabwe, *Bulletin of WHO*, 63: 4, 773-783.
24. Medecins Sans Frontieres., (2006). *Public Health Engineering in Emergency Situation*. 2nd Edition. MSF Books. Pgs 5-7. Medecins Sans Frontieres (2008).
25. NAFDAC., (2002). *Guidelines for Registration and Production of Package water in Nigeria*. website:www.NAFDAC.org.
26. Okonko, I. O., Adejoye, O.D., Ogunnusi, T. A, Fajobi, E. A., & Shittu, O.B., (2008). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. *African Journal of Biotechnology* 2007,7(3):617-621. Available online at <http://www.academicjournals.org/AJB>
27. Parashar, U., Bresee, J. S., & Glass, R. I., (2003). The global burden of diarrhoeal sease in children. *Bulletin of World Health Organization*,2003; 81:236
28. Peterson., E, A., Robert, S. L., Toole, M. J., & Peterson, DE. (1998). The effect of soap distribution on diarrhea: Nyamithuthu Refugee Camp. *International J. Epidemiology* 27:520-524
29. Sachs, J.D., McArthur JW (2005). The Millennium Project: a plan for meeting the millennium development goals. *Lancet* 365: 347-353
30. Smethurst, G., (1978). *Basic water treatment*, 2nd Edition, Thomas Telford Ltd. London.
31. Sridhar, M. K. C., Akinfenwa A., & Osifo, D. B., (1982). Water quality and management in Oyo State: Proceedings of a Workshop on Engineering Management of Water Supply System in Ibadan, 1-23
32. Sridhar, M. K. C. (1999). You, Your Health and the Environment, A Chapter in Nigeria's Endangered Environment: Agenda for a Millennium, Edited by Abiodun, J. O., Filani, M. A., Sridhar, M. K. C., & Olomola, A.O., Published by Obafemi Awolowo Foundation, Lagos, Africa Press Limited, pp. 185-203.
33. Standards Organization of Nigeria. (2007). *NIGERIAN INDUSTRIAL STANDARD NIS 554:2007*.



34. Umeh CN, Okorie OI, Emesiani GA. (2005). Towards the provision of safe drinking water: The bacteriological quality and safety of sachet water in Awka, Anambra State. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbe As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th November, 2005. p22
35. UN Millennium Project (2005). Task force on water and sanitation. Health, dignity, and development: what will it take? New York: Stockholm International Water Institute and United Nations Millennium Project.
36. WHO (1984). Health criteria and other supporting information– Guidelines for Drinking Water Quality, Geneva 2, 281-285
37. WHO (1995). Recommendations: Guideline for drinking water quality – 2 nd Edition, Geneva, Reprinted by Laxman Chand Arya for All India Travelers, Booksellers, Delhi 1: 47.
38. World Health Organization WHO. ( 2004). Water, sanitation and hygiene links to health: Facts and figures updated November 2004 [cited 17 Aug.2005]. Available from [http://www.who.int/water\\_sanitation\\_health/publications/facts2004/en/print.html](http://www.who.int/water_sanitation_health/publications/facts2004/en/print.html)
39. World Health Organization WHO. ( 2005). The WHO Report 2005-make every mother and child count. Geneva: The Organization; 2005.