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Microbial Contamination and Physico-chemical Characteristics of Some Sachet Water sold in Kashere Metropolis, Gombe State, Nigeria

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Abstract
The study focuses on isolating and identifying fungi and bacteria (coliform) contaminants from sachet waters as well as analyzing some physico chemical characteristics of sachet waters sold within Kashere metropolis, and determines the effects of these organisms on the quality of human life. Sample of 36 sachet water of 6 different brands were collected and analysed. Physicochemical parameters were analyzed using standard method. The Colour, Taste, Odour, Turbidity, Temperature, pH, Electrical conductivity, Total dissolved solids Nitrite, Phosphate were analysed monthly, for a period of 3 months. Standard plate count method was adopted using commercially prepared Potato Dextrose Agar (PDA) for the isolation of fungi, and the multiple tube fermentation technique with lactose broth and Nutrient agar used as reagents for bacteria isolation. Two-way analysis of variance was used to analyze the data for fungi and bacterial count at different months. The study revealed that, fungal species were isolated from all the 36 sachet water samples. Fungi and bacteria (coliform) were isolated in all the six brands. Among the microbes isolated are Aspergillus species, Penicillium species, Escherichia coli were the predominant isolates. All the physicochemical parameters fall within the recommended limits set by Standard Organisation of Nigeria for Drinking Water. The findings further 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.

Key words: Coliform, Contaminant, *Escherichia coli*, Physico – chemical, Potato Dextrose Agar

INTRODUCTION
Water is one of the most abundant and essential commodities of man occupying about 70% of the earth's surface, yet a greater percentage of the world's population, most especially in developing countries live without access to safe water (Hazen and Toranzos, 1990; Adriano and Joana, 2007).Water is a colourless, transparent, odourless, tasteless liquid that forms the seas, lakes, rivers, and rain fall as well as the basis of the fluids to living organisms (Molden, 2007). Water is a combination of hydrogen and oxygen atoms, with a chemical formula H_2O and known to be the most abundant compound (70%) on earth's surface (Orewole *et al.,* 2007). Water is an essential requirement of life for drinking, domestic, industrial and agricultural uses. Its quality and quantity which vary over space and time are important components in the integral development of any area. Any change in the natural quality of water may disturb the equilibrium system and it would become unfit for designated uses (Ato and Ayua, 2013).

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Drinking water that is safe and acceptable is a matter of high priority to National Agency for Foods and Drugs Administration and Control (NAFDAC) and other regulatory agencies in Nigeria and is expected to meet the Nigerian Industrial Standard. Furthermore, drinking water that is fit for human consumption is expected to meet the World Health Organization standard and be free from physical and chemical substances and microorganisms in an amount that can be hazardous to health (Denloye, 2004). It is a known fact that no single method of purification can eliminate 100% contaminants from drinking water. However, water can be and should be made safe for consumption within acceptable limits (Denloye, 2004). Sachet water is any commercially treated water, manufactured, packaged and distributed for sale in sealed food grade containers and is intended for human consumption. 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).

Water quality and quantity are much linked, but quality deserves special attention because of its implication on health and life (Isikwue and Chikezie, 2014). Sachet packaged drinking water is very common in Nigeria. It is mostly found as a major source of water at food canteens and sold by many food vendors in the country. The majority of the population consume it (Adiotomre and Agbale, 2015). Water to be used for human consumption must meet certain requirements. It must be free of all disease causing microorganisms, low in concentration of compounds that are acutely toxic or that have serious long term effect on health. Considering the various brands of sachet water marketed in Kashere metropolis which coincide with the increasing population using the water, there is a need to determine the quality of the water. This will provide baseline information on the safety and microbiological quality of the sachet water marketed in Kashere community.

The aim of this study is to assess some physico-chemical characteristics, fungal and bacterial contamination of different types of sachet water marketed within Kashere metropolis and these was achieved through isolating and identifying fungi and bacteria from different types of sachet water marketed within Kashere metropolis also to analyse some physico-chemical characteristics of the sachet water and finally examine the level of contamination of fungi and bacteria on the sachet water.

MATERIALS AND METHODS

Study Area

The study was carried out in Kashere metropolis $9^{0}52'$N, $11^{0}0'$E, Akko local Government area, Gombe state. It has an estimated area of 427km^2 The population and activities in the local government area have increased in the last six years which may be due to the establishment of the new Federal University. The people are predominantly farmers and traders.The experiment was carried out in Biology laboratory, Federal University of Kashere Gombe State.

Sample collection

Thirty six (36) sachet water from six brands made up of two from each brand marketed within Kashere Metropolis, were collected for a period of three months

Determination of Physicochemical Parameters of Sachet Water

Physicochemical parameters that were analyzed include: Temperature, pH, turbidity, Total dissolved solids, Phosphate, Nitrite, Conductivity, Taste and Odour.

Taste and odour
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).

Turbidity
The electrode was wetted thoroughly and then plugged into the turbidity meter before it was inserted into a 250ml beaker containing distilled water. The turbidity meter was then switched on and zero error was corrected. The distilled water was replaced with raw water samples and the reading was recorded.

Temperature and pH
The glass electrode and temperature probe was thoroughly wetted with distilled water. The pH meter (PHS-3D) was then switched on and was standardized. This was done by connecting glass electrode to the pH meter and inserting the electrode into the buffer 4 solution. This was allowed to stabilize and pH meter reading indicated 4.0. The same thing was repeated using buffer solution with pH of 9.0. The beaker containing buffer solution was then replaced with the sample and the electrode was inserted into it. This was allowed to stabilize and readings for both temperatures and pH were recorded.

Electrical conductivity and Total Dissolved Solids
The electrode was wetted thoroughly and then plugged into the conductivity meter before it was inserted into a 250ml beaker containing distilled water. The conductivity meter (DDSJ-308A) was then switched on and zero error was corrected. The distilled water was replaced with raw water samples and the reading was recorded. The mode from the conductivity meter is changed and the reading of TDS was recorded.

Nitrite
100ml of water sample was poured into a clean dry crucible and kept in an oven at 100^{o}C 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).

Phosphate
100ml 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).

Identification of Fungi
Fungi isolated were identified based on macroscopic and microscopic characteristics The macroscopic characteristics such as colours, shapes and textures of the colonies on Potato Dextrose 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 using atlas and manuals by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson *et al*., (2004).

Procedure for isolation of fungi

The dilution tubes were labelled as follows: 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} which were filled with 9ml sterile distilled water. A sterile pipette was used to transfer 1ml of the original sample into the tube labelled 10^{-1}. It was mixed thoroughly. A new sterile pipette was used to transfer 1ml of the 10^{-1} tube into the tube labelled 10^{-2}. It was mixed thoroughly. A new sterile pipette was used to transfer 1ml of the 10^{-2} tube into the tube labelled 10^{-3}. It was mixed thoroughly. 7.8g of PDA powder was dissolved in 200ml of distilled water for preparation of culture media. The edge of the bottom of agar plates was labelled with the samples name and the dilution factor of the sample to be plated. The media prepared was poured into 8 different 20ml bottle, sterilized and allowed to cool to 50-55^{0}C. 1ml of the diluted sample (10^{-1}) was transferred into a tube of agar and thoroughly mixed. The overlay was poured into the agar plate labelled 10^{-4} and the plate was gently swirled to spread the overlay and allowed to solidify. The same process was repeated for the other dilutions (10^{-2}, 10^{-3}, 10^{-4}) and incubated at 30^{0}C for 24-48hrs. After incubation, the plate was placed upside down on the colony counter and colonies were counted while viewing through the plate. A mark was placed over each colony counted and results were recorded.

Original cell concentration was calculated by multiplying the colony counts by the total dilution factor.

$$\text{CFU/ml} = \text{No of colonies} \times \frac{\text{No of ml inoculated}}{\text{Dilution factor}}$$

Presumptive Test

The sample dilutions were first inoculated into a series of fermentation tubes containing lactose broth and durham tubes. The fermentation tubes were then incubated at 35-37^{0}C. The tubes were observed at the end of 24 and 48 hours for gas production. Preparation of media was done through the following procedure.

Double strength lactose broth: 2.6g nutrient broth and 0.2g lactose was dissolved in 100ml of distilled water. 1ml bromothymol was added as indicator and sterilized, Single strength lactose broth: 1.95g nutrient broth and 0.15g lactose in 150ml of distilled water. 1.5ml bromothymol was added as indicator and sterilized.0.2% bromothymol: 0.02g of bromothymol was dissolved in 10ml of distilled water. Nutrient agar medium: 2.8g nutrient agar powder was dissolved in 100ml of distilled water. Eosin Methylene Blue Agar: 3.6g of EMB powder was dissolved in 100ml of distilled water and sterilized in bottles. Three tubes were set up for five dilution MPN representing 10, 1, 0.1, 0.01, and 0.001ml dilutions of the water sample. Three containers hold 10ml of double strength lactose broth and 10ml of the original sample. Three containers hold 9ml of single strength lactose broth and 1ml from the original water sample. Three containers hold 9.9ml of single strength lactose broth and 0.1ml from the original water sample. Three containers hold 9ml of single strength lactose broth plus 1ml of 10^{-1} dilution of the water sample to give 0.01ml of the original water sample. Three containers hold 9ml of single strength plus 1ml of 10^{-2} dilution of the water sample to give 0.001ml of the original water sample. Durham tube was inserted in all tubes and were incubated at 35-37^{o}C for 24-48 hrs.

The Most Probable Number (MPN) was calculated using the formula

$$\text{MPN/100ml} = \frac{\text{No of positive tubes}}{\sqrt{(\text{No. of ml of sample in negative tubes}) \times (\text{No. of ml of sample in all tubes}}}$$

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

Completed test

An isolated colony from a positive tube of Eosin Methylene Blue (EMB) was inoculated in Nutrient Agar slant and incubated. Grams staining and biochemical tests were carried out

RESULT AND DISCUSSION

Table 1: Mean Values (±S.E.) of Physico - chemical Parameters of Six Brands of Sachet Water

	Brand of sachet water						
Physicochemical parameters	**A**	**B**	**C**	**D**	**E**	**F**	**STD SON (2007)**
Temperature	28.63^{b} ±1.20	28.73^{b} ±1.10	28.87^{b}±1.23	29.90^{ab}±0.30	28.90^{a}±1.7	28.87^{b}±1.23	Ambient
pH	7.63^{a}±0.13	7.83^{a}±0.89	7.82^{a}±0.14	7.98^{a}±0.11	7.85^{a}±0.76	7.70^{a}±0.58	6.5-8.5
Turbidity	0.45^{a}±0.10	0.37^{a}±0.05	0.37^{a}±0.10	0.45^{a}±0.12	0.22^{a}±0.04	0.44^{a}±0.11	5 NTU
TDS	60.00^{c}±2.90	51.67^{c} ±6.00	61.33^{c}±5.93	72.33^{b}±15.77	49.67^{ab}±8.41	62.00^{c}±5.69	500
Phosphate	8.73^{a}±1.07	1.01^{a}±0.12	0.82^{a}±0.13	0.56^{a}±0.25	2.74^{a}±2.08	1.51^{a}±0.55	5
Nitrite	0.24^{a}±0.11	0.05^{a}±0.03	0.10^{a}±0.22	0.33^{a}±0.01	0.06^{a}±0.03	0.06^{a}±0.01	0.2
Conductivity	126.0^{d}±8.80	123.33^{d}±8.8 2	120.67^{d}±10.59	143.33^{c}±32.8 2	115.33^{b}±12.9 8	114.00^{d}±13. 01	100
Colour	U	U	U	U	U	U	U
Taste	U	U	U	U	U	U	U
Odour	U	U	U	U	U	U	U

U= Unobjectionable, O=Objectionable. Mean with the same superscript across the rows are not significantly different (P<0.05)

Table 3: Biochemical characteristics of *Escherichia coli*

Biochemical extracts	Escherichia coli
Hydrogen sulphide	-
Indole	+
Motility	+
Simmon's citrate	-
Catalase	+

(+) positive, (-) negative

Table 4: Percentage of Occurrence of Different Fungal Species Isolated from Sachet Water marketed within Kashere Metropolis

Name of fungi	A	B	C	D	E	F	TOTAL %
A.flavus	2(4.34)	1(2.17)	2(4.34)	0(0.00)	0(0.00)	3(6.52)	17.47
A. fumigatus	2(4.34)	2(4.34)	1(2.17)	0(0.00	0(0.00	1(2.17)	12.98
A.niger	3(6.52)	2(4.34	3(6.52)	2(4.34)	3(6.52)	2(4.34)	19.55
Penicillium glabrum	0(0.00)	2(4.34)	1(2.17)	0(0.00)	0(0.00)	3(6.52)	13.09
Cephalosporium curtipes	3(6.52)	2(4.34)	0(0.00)	0(0.00)	0(0.00)	2(4.34)	15.20
Rhizoctonia solani	2(4.34)	2(4.34)	2(4.34)	3(6.52)	1(2.17)	0(0.00)	21.71

All the 36 sachet water samples obtained from six brands of sachet water analysed for the presence of fungi were positive for MPN test , a total of six (6) fungal species were isolated in all samples.Fungi species isolated from six brands of sachet water include: *Aspergillus fumigatus*, *Aspergillus niger, Cephalosporium curtipes* and *Rhizoctonia solani* were isolated from sample A. *A. niger*, *Aspergillus flavus, A. fumigatus* and *C. curtipes* were isolated form sample B. *Pencillium glabrum*, *R. solani, A.* niger and *A. fumigatus* were isolated from sample C. *A. niger* and *R. solani* were isolated from sample D and E. *A. flavus, A. niger, A. fumigatus, P. glabrum* and *C. curtipes* were isolated from sample F. The six fungal species recorded 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). It is evident from Table 2 that *Rhizoctonia solani*, was more prevalent out of the six fungal species isolated,and A.fumigatus is the least in percentage as it was isolated from all samples followed by *Rhizoctonia solani*, *Aspergillus flavus* and *Cephalosporium curtipes*. with 15.20% has the lowest percentage occurrence.

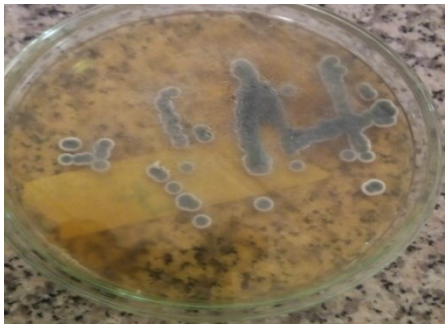

Plate i: Pure culture of *Penicillium glabrum*

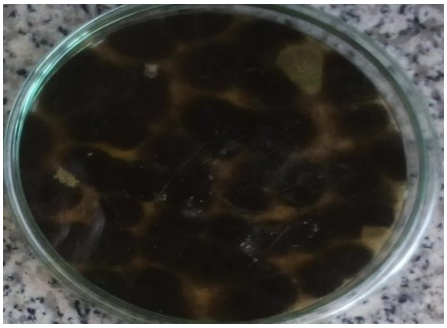

Plate ii : Pure culture of *Aspergillus niger*

Plate iii: Pure culture of *Rhizoctonia solani*

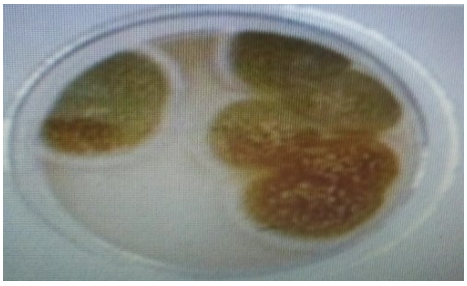

Plate iv Pure culture of *Aspergillus flavus*

Plate v. Pure culture of *Cephalosporium curtipes*

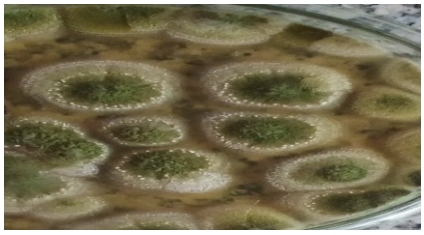

Plate vi: Pure culture of *Aspergillus fumigatus*

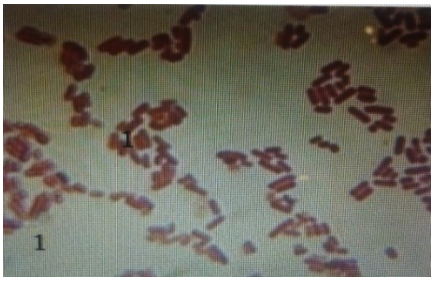

Plate vii : Micrograph of *Escherichia coli*

Table 4: The Mean (±S.E.)Value of the fungal colony count (CFU/ML) of Sachet water within Kashere, Gombe state.

	Month of analysis		
Brand of sachet water	April	May	June
A	2325.33^{f} ±0.88	21000.67^{f} ±0.67	1100.33^{f}±0.33
B	1700.00^{e}±1.15	300.00^{c}±0.58	201.00^{d} ±0.58
C	141.00^{a} ±0.58	881.00^{d} ±0.58	129.66^{c} ±0.88
D	290.00^{c}±1.15	119.67^{a} ±0.33	250.33^{e} ±0.88
E	280.67^{b}±0.33	141.00^{b}±0.58	49.33^{a} ±0.33
F	948.33^{d}±3.28	902.66^{e}±1.20	80.00^{b} ±0.58

Mean with the same superscript across the rows are not significantly different (P<0.05).

Key :A=Boddam shenidam, B= Raudatun,C= Uchetex,D= Na'eema,E= Aquasum,F= Charlin

Table 5: The Mean (±S.E.)Value of the Most Probable Number (MPN/100 ml) of *E.coli* in Sachet Water within Kashere , Gombe State.

	Month of analysis		
Brand of sachet water	April	May	June
A	453.00^{f} ±0.2887	30.9^{b} ±0.51	76.33^{b} ±0.4440
B	62.40^{b} ±1.2702	109.70^{e} ±0.8544	93.00^{c} ±0.5774
C	197.30^{e} ±0.1732	25.83^{a} ±0.5248	45.00^{a} ±1.1547
D	41.58^{a} ±0.5312	39.62^{f} ±0.3470	163.00^{d} ±1.7321
E	147.10^{c} ±0.6351	57.30^{c} ±0.3512	180.00^{e} ±0.5774
F	41.39^{c} ±0.3055	4.39^{d} ±0.3466	77.00^{b} ±0.5774

Mean with the same superscript across the rows are not significantly different (P<0.05),

Out of the six brand of sachet water analysed for the presence of fungi, 6 fungal species were isolated with *Aspergillus niger* having the highest percentage and all samples were positive for coliform test (Table 2).All the physicochemical parameters falls within the ranges set by Standards Organization of Nigeria (2007) for drinking water except sample A which had exceeded in phosphate and nitrite values (Table 1).

Sample A records the highest coliform and fungal colony count which may be as a result of the excess nutrient (Phosphate and Nitrite). While sample E had the lowest fungal colony count and sample D had the lowest coliform count (Table 4 and 5) the fungal species isolated from the samples collected was in line with the findings of Thilza *et al.,* (2015) who identified

the six fungal species in addition to *Oidiodendron griseum, Fusarium oxysporium and Trichoderma viride* in sachet water. Also research conducted by Okpako *et al.,* (2009) in Calabar indicates the presence of 8 fungal species in sachet water and bore hole.

All the six brands were positive for coliform test (Table 5). There was significant difference in the mean of the most probable number ($P<0.05$) among the three month of collection. Also there is significant difference among all six brands of sachet water. There is variation in mean of the most probable number which could be due to variation in the level of treatment.Similar observation was made by Oyedeji *et al*., (2010); Muazu *et al*., (2012).

There was significant difference ($P<0.05$) in total dissolved solids, temperature, conductivity, while there is no significant difference ($P<0.05$) in pH, Total Dissolved Solids, nitrite and phosphate among the brands. All samples analysed were colourless, odourless, tasteless and non turbid. This result is similar to that of Uduma, (2014) where samples were found to be colourless, odourless and tasteless but 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 associated to differences in the time of storage.

CONCLUSION

From the results obtained in this present research it can be concluded that satchet water sold within Kashere metropolis is unsafe due to the biological indices recorded which are likely to be an agent of contamination and hence posses potential health hazard of the water to humans despite some of the physicochemical characteristics conforming to the standards.

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