Fungal Assessment Of Some Selected Sachet Water Sold In Bwari Area Council Federal Capital Territory, Nigeria.

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Abstract- This study focused on isolating and identifying fungi from sachet waters sold in Bwari area council of Federal Capital Territory Nigeria, and also to determine the quality of the sachet water after treatment processes. Samples were collected from 4 different brands and then subjected to physicochemical analysis using standard methods of American Public Health Association (APHA) and fungal identification on potato dextrose agar. This study revealed 18 fungi isolates belonging to 5 fungal species were isolated across all 4 brands analyzed. There was no fungal isolate found in the source of water, but varying number of fungi were recorded as the product moved down the production line and across the distribution chain. Samples from the manufacturers (sachet water after treatment) and hand swab had the highest number of fungal counts. Brand B had the highest number of fungi (7) isolates; this was followed by brand C which had 5 isolates. *Aspergillus flavus* and *Trichoderma viride* were among the predominant isolates. The findings in this study revealed the presence of pathogenic fungi species in the sachet water after treatment processes.

Index Terms- Water, Sachet water, Fungi, *Aspergillus flavus*, WHO

I. INTRODUCTION

Potable water should be clean and treated according to guidelines set by the WHO for drinking water quality. Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, and must meet the standard guidelines for taste, odour, appearance and chemical concentrations, and also be available in adequate quantities for domestic purposes [1].

In the last 30 years, the presence of a high variety of fungi was reported from European water, including surface, ground and tap water intended for human consumption [2]. Some fungi are generally adapted to aquatic environments, and therefore naturally be found in water [3].

The Department for Environment, Food and Rural Affairs (DEFRA) stated that the most commonly isolated genera of fungi from drinking water are *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialophora* and *Acremonium* [4,5]. Purification procedures such as chlorination has been recorded not to eliminate fungal spores [6] which implies that perhaps the treatment given to our sachet water is usually not effective enough to eliminate these microorganisms. Most fungi frequently isolated from drinking water have the potential to secrete melanin pigment which provide protection against range of stresses making them resistance to water treatment [7]. However the use of chlorine dioxide and ozone were reported as the most effective water treatment methods against fungal spores [8].

The packaging and regulation of the water industry started in the early 80s in Europe [9] but in Nigeria packaged water industry started in the early 1990s. Water is packaged in sachet known as 'pure water' and bottle forms and are regulated by the National Agency for Food and Drug Administration and Control (NAFDAC). The potential danger associated with sachet water is contamination, which is a factor of the source of the water, treatment, packaging materials, dispensing into packaging materials and closure [10]. [11] observed that the most probable point of post-treatment microbial contamination of packaged waters is largely during distribution. Coupled with the possibility of back-seepage into bags that are not well-sealed, the regrowth potential of microorganisms is heightened as these packets spend ample time in the open air due to improper storage. Regardless of the level of treatment given to the water in sachets within the factory location, food handlers in the distribution line remain potential flaws by impeding safe transfer of the desired quality to the consumer, thus constituting a potential threat to public health [11]. it is very important to understand whether the production process, distribution and storage practices may affect the quality of this water, these has accentuated the need for this study in other to critically examine the prevailing problems.

II. MATERIAL AND METHODS

Sample Collection

Water samples were collected from four (4) different sachet water production factories in Bwari Area Council, FCT Nigeria. Samples were collected from the sources of water, final product (sachet water), at the distributor shops and from the hawkers as well as the hand swab of the factory workers were aseptically collected using sterile swab stick. Manufacturing dates, dates of expiry dates and batch numbers of the sachet water were noted. The samples were transported to the laboratory in ice-cold container for analysis.

Physicochemical Parameters

Physicochemical properties of the samples were determined using standard methods of American Public Health Association (APHA). Parameters include temperature, colour, turbidity, pH, electrical conductivity, total alkanlity, total Hardness, total dissolved Solids, chloride, iron, nitrate.

Preparation of Culture Medium for the Isolation of Fungi

Sabouraud dextrose agar used for the isolation, was prepared according to manufacturer's instruction. The molten medium was poured into conical flasks, plugged with aluminum foil. The medium was sterilized by autoclaving at 121°C at a pressure of 15 pound for 15 minutes. After sterilization, 15 ml of medium was aseptically dispensed into sterile petri dishes and allowed to solidify. The petri dishes were labeled accordingly. (5)

Isolation and identification of fungi

Each colony from the primary plates was sub-cultured onto fresh potato dextrose agar, each supplemented with 300 mgl^{-1} cefotaxime and 100 mgl^{-1} Kanamycin to inhibit bacterial growth. These were replicated three times. The sub-culture was carried out to purify the fungi isolates. During the sub-culturing inoculating loop flamed in a bursen-burner was used to pick the colony and smeared on the agar plate. This was further incubated at room temperature for 7 days. Fungal colonies were isolated upon formation, stained with lactophenol and observed under the microscope. Fungi so observed were identified using appropriate taxonomic guides [12].

III. RESULT

Physical Information for Labeling Compliance of Sachet Water Sold in Bwari Area Council

Table 1 shows the label information on all the sachet water analyzed. The result showed that all the samples collected had registration number, product name and address but did not have manufacturing date, expiry date or batch number. These are all information that are required on a label of a packaged water. Table 2 shows the mean values of the physicochemical parameters of four brands of sachet water final product obtained from the manufacturer in Bwari Area Council FCT Nigeria. All the physicochemical parameters analyzed at ambient temperature were within the permissible limit set by World Health Organization.

Table 1: Physical Information for Labeling Compliance of Sachet Water Sold in Bwari Area Council

Samples	Registration Number	Manufacturing Date	Expiry Date	Batch Number	Product Name and Address
Brand A	+	-	-	-	+
Brand B	+	-	-	-	+
Brand C	+	-	-	-	+
Brand D	+	-	-	-	+

Brand = Codes representing the trade names of sachet water from location A, B, C and D

Table 2: Physicochemical Parameters of the source of water for Brands of Sachet Water Sold in Bwari Area Council

Parameters	Brands of Sachet Water Final Product				
	Brand A	Brand B	Brand C	Brand D	WHO Standard 2018
Colour	10.00	13.00	11.00	12.00	15 TCU
Odour	Odourless	Odourles	Odourless	Odourless	UO
Taste	Tasteless	Tasteless	Tasteless	Tasteless	UO
Turbidity	3.70	3.20	2.80	3.50	<4NTU
pH	7.11 ± 0.12^{a}	7.08 ± 0.14^{a}	7.02 ± 0.16^{a}	7.17 ± 0.05^{a}	6.5-8.5
Temperature (°C)	25.02 ± 0.74^{a}	25.03 ± 0.60^{a}	25.01 ± 0.53^{a}	25.95 ± 0.56^{a}	Ambient
Conductivity (µs/cm)	202.31 ± 39.98^{a}	344.17 ± 34.93^{b}	75.66 ± 2.73^{c}	152.01 ± 4.03^{d}	1000
TDS (ppm)	101.00 ± 21.71^{a}	162.01 ± 20.08^{a}	38.16 ± 1.62^{b}	72.51 ± 1.79^{b}	<600
Chloride ion (mg/L)	25.35 ± 3.33^{a}	81.68 ± 13.81^{b}	26.18 ± 3.75^{a}	33.18 ± 7.52^{a}	<250
Nitrate (mg/L)	5.04 ± 0.12^{a}	1.40 ± 0.08^{a}	1.20 ± 0.18^{a}	5.40 ± 0.02^{a}	50
Hardness (mg/L)	104.33 ± 17.00^{a}	132.67 ± 29.69^{a}	100.02 ± 8.40^{a}	142.00 ± 43.18^{a}	<200

Iron (mg/L)	0.02±2.63[a]	0.05±1.99[a]	0.03±2.18[a]	0.03±1.32[a]	<0.3

Keys: Brand = Codes representing the trade names of sachet water from location A, B, C and D. TDS= Total Dissolved Solids, TCU= True Colour Units, NTU= Nephelometric Turbidity Units. Mg/l= milligram per liters. ppm= part per million. a, b, c, d= parameters with the different superscript across the rows are significantly different.

Table 3 showed that a total of eighteen (18) fungi belonging to five (5) genera and five species were isolated. The isolates were identified by studying their macroscopic and microscopic characters and were compared with already described species using identification keys [12]. Figure 1 showed that brand B had the highest count of fungi isolated. It is also evident from Table 4 that *Aspergillus flavus* was more prevalence while *Fusarium* spp was the least. Table 5 showed that no fungi was isolated from the source of water, but the final product and the sachet water collected from the hawkers had the highest number of fungi isolated. There was no significant difference in the mean of the fungal colony count at ($P \geq 0.05$) as the sachet water moved down the production and across the distribution chain.

Table 3: Description of the Macroscopic and Microscopic Characteristics of the Fungal species Isolated from Sachet Water

Fungal Isolates	Macroscopic	Microscopic	Probable Identity
F1	The colonies were widely spread, black, with smooth white edges and spongy surface White with typical black spores	The conidiophores was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head. the conidial head was blackish in colour	*Aspergillus niger*
F2	The colonies were green with white border and brown on the reverse side on Potato Dextrose Agar. The coloines varied in size with an average of 2cm within a few days	The conidiophore was long, thick walled and hyaline. The conidial head was bluish near the apics with an irregular shape	*Penicillium glabrum*
F3	The colony was pink with white patch on the surface and brown on the reverse The colony was round and measured 4cm	The macroconidia are canoe shaped, slantly pointed at the end. Measured 29-100 in length	*Fusarium oxysporum*
F4	The upper surface of the coloines was olive green, with white edge, green coloration on the reverse side	The conidiophore has thick walled hyaline and roughened.	*Aspergillus flavus*
F	White colony with folded cottony upper surface and a yellowish brown on the reverse side	The conidiophores hyaline was irregularly branched. The conidia are round, clustered at the end of phialide	*Trichoderma viride*

F=fungi isolated from all the sachet water analyzed

Table 4: Fungi Associated with Sachet Water Sold in Bwari Area Council, FCT-Abuja

Isolates	Frequency	Percentage (%)
Aspergillus flavus	9	50
Trichoderma viride	5	27.8
Aspergillus niger	2	11.1
Pencillium spp	1	5.6

Fusarium spp	1	5.6
Total	**18**	**100**

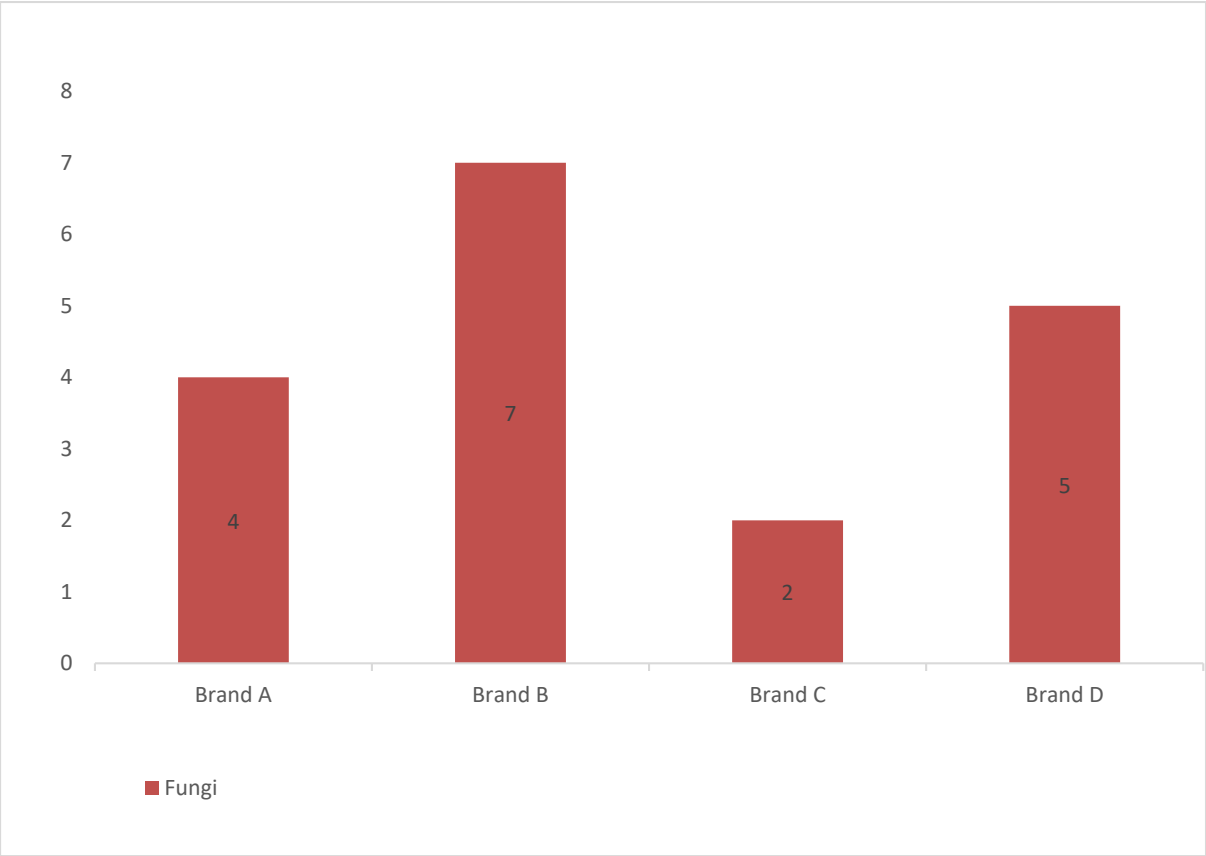

Figure 1: Distribution of fungi across the four (4) brands of sachet water analyzed.

Table 5: Frequency of occurrence of Fungi in Sachet Water

Samples	Isolates	Frequacy
Source	*Aspergillus flavus*	0
	Trichoderma viride	0
	Aspergillus niger	0
	Pencillium spp	0
	Fusarium spp	0
	Total	**0**
Final product		3
	Aspergillus flavus	2
	Trichoderma viride	0
	Aspergillus niger	0
	Pencillium spp	0
	Fusarium spp	**5**
	Total	3
Handswab		0
	Aspergillus flavus	0
	Trichoderma viride	0

	Aspergillus niger	1
	Pencillium spp	**4**
	Fusarium spp	
Distributor	**Total**	1
		1
	Aspergillus flavus	1
	Trichoderma viride	1
	Aspergillus niger	0
	Pencillium spp	**4**
	Fusarium spp	2
Hawker	**Total**	2
		1
	Aspergillus flavus	0
	Trichoderma viride	0
	Aspergillus niger	**5**
Total	*Pencillium* spp	**18**
	Fusarium spp	
	Total	

IV. DISCUSSION

It was observed that all sachet water analyzed exhibited 100% compliance as regard to the product names, manufacturers' addresses, and NAFDAC number. However, non of the sachet water had manufacturing or expiry date, this information is very essential, as they tell the consumer if the water sample is still within its shelf life or not. Furthermore, all the sachet water studied were observed to be without batch number. Batch number is essential for any food product especially when there is need to recall the product from the market in the case where an abnormality has been discovered with the product [13]. The act of noncompliance by the production factories as observed in this present study is of great concern, as the sachet water sold are likely to pose health risk when consumed.

All the physicochemical parameters analyzed falls within the range set by [14] for drinking water. High temperatures have been reported to have the tendency of developing undesirable taste and odour in water with time [19]. Hardness in water can be tolerated for up to 500 mg/l for some consumers [14] but depending on other factors such as pH and alkalinity, water with a hardness of above 200 mg/l may cause scale deposition in the treatment works and distribution system. The minimum and maximum values for hardness obtained in this study were 100-142 mg/l, which were within the non-hard water value set by a researcher [14]. Nitrate value recorded in all the samples were also within the recommended limit by WHO for drinking water. This could be as a result of the fact that the source of the water having low level of nitrogen coupled with adequate treatment during production. This finding is in accordance with the result of [15]. The water samples may not cause health problem such as cyanosis because of the low level of nitrate in the samples. Iron is an essential component of the red blood cell. Iron has little or no health concern, but it is still considered as a nuisance in excessive quantities.

Fungi isolated from this study include *Aspergillus flavus* which was nine (9) representing 50% and the most frequently isolated. This was followed by *Trichoderma viride* 5 representing 27.8%, *Aspergillus niger* 2 (11.1%) while both *Pencillium spp* and *Fusarium spp* each were 5.6% being the least fungi isolated. Most of the fungi species isolated have been reported in previous studies [16], [5]. Brand B had the highest count of fungi isolated among all the brands analyzed as represented in figure 1.

From this study it can also be seen that as the sachet water moved across the production process it was observed that no fungi was isolated from the source of water. However, Fungi was isolated from the sachet water taken from the factory, from hand swabs of the factory workers and was also isolated from sachet water gotten from the distributors and the hawkers. From this we could assume that treatment options were not the issue here because no fungi was isolated from the source of water but the handling and the packaging processes involved could have introduced this fungi into the water along the way. The guide for good hygienic practices for packaged water in Europe states that every person working in a food handling area is to wear suitable, clean and where necessary, protective clothing [17].

The polyethylene water bags or pure water nylon as it is called in Nigeria used in packing this water should be stored under a ultra-violent light [18], as this could easily introduce microorganism into the water during packaging. Most of the water factories in this study do not store their packaging materials under sterile condition. Fungi contamination after treatment could also be as a result of fungi have spores that travel through the air within the environment and these fungi require warmth and humidity to grow [19] and easily settle on the sachet of the water and when these sachets are not aseptically opened, they contaminate the water in the sachet. Fungi have received increased focus as drinking water contaminants in the last decade due to its ability to cause acute disease [3].

V. CONCLUSION

Production processes, storage and distribution should be taken into consideration as all this has been shown to affect the water quality. Isolation and distribution of organisms across the

various stages of production shows that while the raw source of was free from fungi, microbial isolates were still found in the sachet water after production. This shows that more effective handling and packaging options needs to be developed and adopted by this water companies. It is also important that water production factories adhere to good hygienic practices for packaged water especially personal hygiene of their workers. (Personal protective covering are worn in restricted area). It is also recommended that all manufacturing industries must adhere to NAFDAC guidelines and all the existing laws should be enforced. Manufacturing and expiry dates should be included on the package water.

VI REFERENCES

[1] Kirkwood, A. Safe water for Africa DFID Conference features. Africa Health. 1998;2: 9-11.

[2] Novak Babič, M., Gunde-Cimerman, N., Vargha, M., Tischner, Z., Magyar, D., Veríssimo, C., Sabino, R., Viegas, C., Meyer, W., & Brandão, J. Fungal Contaminants in Drinking Water Regulation? A Tale of Ecology, Exposure, Purification and Clinical Relevance. *International Journal of Environmental Research and Public Health*. 2017; *14*(6), 636.

[3] Hageskal, G., Nelson, L. and Ida, S. The Study of Fungi in Drinking Water (Review). *Elsevier Journal of Mycological Research*, 2009;113:165-172

[4] Department of Environmental, Food and Rural Affair. A Review of Fungi in Drinking Water and the Implication for Human Health DEFRA, 1st ed. BIO Intelligence Service; Paris, France. 2011; pp 5-105.

[5] Thliza, L. A., Khan, A. U. and Dangora, D. B. Fungi Contamination of Some Selected Brands of Sachet Water Marketed in Ahmadu Bello University, Zaria, Nigeria. *Journal of Microbiology Research* 2015;5(1): 23-30

[6] Oni, O.O.O. and Olayeni, F. (2003). Nigeria Quality of Sealed Polythene Water in Kaduna and Lagos 29th WEDC International Conference Abuja, Nigeria. pp34-56

[7] Langfelder, K., Streibel, M., Jahn, B., Haase, G. and Brakhage, A. A., (2003). Biosynthesis of Fungal Melanins and their Importance for Human Pathogenic Fungi. *Fungal Genetics and Biology*. 2003;38 (2): 143-158.

[8] Kelley, J., Kinsey, G., Paterson, R. and Brayford, D. (2003). *Identification and Control of Fungi in Distribution Systems. Denver, USA*. Awwa Research Foundation and American Water Works Association. 2003. [Online]. Accessed: January 6, 2022. Pp1-33

[9] Yael, P. and Tamar, O. (2011). Water and health – Bottled Drinking Water. ©Encyclopedia of Life Support Systems (EOLSS)-UNESCO. www.eolss.net. [Accessed: January 24. 2022].

[10] Omalu, C. I., Eze, G. C., Olayemi, K., Gheri, S., Adeniran, L. A., Ayanwale, A. V., Mohamed, A. Z. and Chukwuemeka, V. Contamination of sachet water in Nigeria: Assessment and health impact. *The Online Journal of Health and Allied Sciences.* 2010;9: 15-20

[11] Dada, A. C. Towards a successful packaged water regulation in Nigeria. *Scientific Research and Essay*. 2009;4(9):921-928

[12] Samson, A. R., Hoekstra, S. E. and Prisvad, C. J. (2004). *Introduction to Food and Airborne Fungi*, 7th ed. Utrecht: Centraal Bureau Voor Schimmelcultures. 2004. Pp. 12-124

[13] Ibrahim, M. D., Umaru, M. and Akindele, A. A. Qualitative Assessment of Sachet and Bottled Water Marketed in Bauchi Metropolis, Nigeria. Chemical and Process Engineering Research 2015;37: 11-23.

[14] World Health Organisation (2017). Guidelines for drinking-water quality. 4th ed incorporating the 1st Addendum. World Health Organization. Geneva, Switzerland.

[15] Onweluzo, J. C. and Akuagbazie, C. A. Assessment of The Quality of Bottled And Sachet Water Sold In Nsukka Town. *Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension*, 2010;9(2): 104-110

[16] Okpako, E. C., Osuagwu, A. N., Duke, A. E. and Ntui, V. O. Significance of Fungi in Sachet and Borehole Drinking Water in Calabar, Nigeria. *African Journal of Microbiology Research.* 2009; 3:56-61.

[17] European Federation of Bottled Waters (2012). Guide to Good Hygienic Practices for Packaged Water in Europe. Belgium Brussels. 112pp

[18] National Agency for Food & Drug Administration & Control (NAFDAC) Food Safety & Applied Nutrition (FSAN) Directorate (2018). Review Date: 31/05/2023 Doc. Ref. No: FSAN-GDL-002-01 Effective Date: 01/06/2018. p1-11

[19] University of Worcester. What are fungal spores Henwick Grove, WR2 6A

https://www.worcester.ac.uk/about/academic-schools/school-of-science-and-the-environment/science-and-the-environment-research/national-pollen-and-aerobiology-research-unit/What-are-fungal-spores.aspx. Accessed on September 7, 2021.

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