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Evaluation of Bacterial and Fungal Isolates of Biofilm of Water Distribution Systems and Receptacles in Abuja, Nigeria

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ABSTRACT

The present study was carried out to determine the prevalence of bacterial and fungal organisms isolated from biofilms of water distribution systems and Receptacles. Our results clearly demonstrated that the overall prevalence rate of the microorganisms was 36.36%. The frequency of occurrence of the bacterial isolates urns: Ligionella pneumophila (3.33%), Aeromonas hydrophila (6.67%), Pseudomonas aeroginosa (25.00%), Mycobacterium avium (1.67%), coliforms (25.00%), E. coli 16.67%>, Salmonella typhi 18.33, and Streptococcus species 3.33%>. Fungal organism isolated included Aspergillus niger (50.00%), Penicillium rubrum 20.00%, Fusarium species (30.00%). Heterotrophic bacterial counts yielded bacterial load ranging from 3.0 x 10^1 to 1.2 x 10^7 CFU/ml. These were analyzed statistically and the results of X^2 showed that P = <0.01, with the Standard Deviation of 3.5 x 20^4 + 2.0 x 10^5 and1.15 x 10^2 x 10^2 ± 1.1 x 10^3 for both copper and stainless steel pipes respectively. Biofilms in Nigerian drinking water distribution systems and receptacles could provide nutrients for microbial growth, biosynthesis and proliferation. It is necessary to treat water distribution plants and provide adequate public health education in order to safeguard human health and animal health especially in developing counties.

Keywords: Biofilms, Receptacles, Distribution system, Microorganisms, Water.

INTRODUCTION

Biofilms accumulating on the inner surface of the tubing of water distribution systems are responsible for high levels of contamination of pipe-borne water. [25]. Uncertain ramifications of fungi and bacteria in portable water have led to a limited number of investigations which showed that both bacteria and fungi could be present in a significant proportion of water distribution systems. However, species abundance and diversity are extremely variable [7]. The concentration of microorganisms in water distribution systems and receptacles is often-attributed to factors such as unhygienic water source, poor water ambient temperature patterns, inadequate treatment conditions, stagnation and lack of adequate maintenance of the distribution systems [3]. A number of surveys have globally demonstrated that the majority of water distribution systems are supplied by Tap water [28]. The European Union (EU) and the Centre for Disease Control (CDC) guidelines recommended that tap water should be delivered at <100 CFU. ml^{-1} at 22°C and <20 $CFU.ml^{-1}$ at 27°C [28]. However, once the water enters the distribution systems (pipes, receptacles and fittings) number of microorganisms may begin to proliferate. The numbers may increase as high as 1.6 x 10^5 $CFU.ml-^1$ having been recovered in the outflow, there by posing high public health risk [8, 15].

The presence of water-borne microorganisms and biofilms are associated with the taste and odor problems of water, contamination of water in food and beverage industries [12]. The presence of biofilm in water distribution system could enhance provision of carbon and other nutrients required for microbial biosynthesis. This could permit the survival and proliferation of variety of bacterial pathogens including: *Legionella pneumophila*, *Aeromonas hydrophila*, *Mycobacterium* species, *Pseudomonas aeroginosa* and *Candida* species, and many other fungi, viruses and protozoa [8, 9]. These organisms are associated with a variety of illness and symptoms including diarrhea, gastroenteritis, stomatitis, cholera, food poisoning, typhoid fever, candidiasis, leptospirosis, arteriosclerosis, chronic sinusitis, chronic wound infection, cystic fibrosis, endocarditis, kidney stones, osteonecrosis and severe periodontal diseases [28].

In Nigeria, the presence of bacterial organisms and fungus in drinking water and within biofilms of water distribution systems has received limited attention. The causal relationships between bacterial and fungal occurrence and water quality have not been fully documented. The evaluation of microbial isolates within biofilm of water distribution systems still remains obscure. To the best of our knowledge, there is scanty literature to demonstrate conclusively that certain bacteria and fungi are integral parts of biofilm in water distribution systems. Our research therefore is the first to demonstrate that bacteria and fungi organisms are associated with biofilm of University of Abuja water distribution systems and receptacles. This finding is intended to be an eye opener to public health authorities in Nigeria to intensify effort in the effective maintenance and high quality control of Nigerian water distribution systems and other developing countries of the world.

MATERIALS AND METHODS

Sample Collection and processing of samples

The samples were randomly collected from willing participants after formal clearance with the Maintenance Department of the University of Abuja on the regularity of washing and disinfection of the water distribution systems. Twenty-seven biofilm samples each were collected from water tanks, water receptacles, floor pipes, tap water, PVC pipes, iron pipes, copper pipes and stainless steel, and 31 samples from iron pipes totaling 220. The collection methods used was based on that of [19] adopted by [24] with slight modification. Sterile swab sticks were used to gently scrub an area of $1cm^2$ containing the biofilm in the inner surfaces. The swabs "were immediately transferred into tubes containing 9ml of 0.1% ($^v/v$) sterile peptone water and arranged in cold ice packs sealed cooler for onward transfer to National Agency for Food and Drug Administration and Control (NAFDAC) reference field laboratory Kaduna, Nigeria for laboratory analysis. The duration of collection of the samples lasted for four months from February, 2013 to June, 2014.

Bacterial isolation

This was done based on standard methods [13]. The isolates were selectively cultivated on M-Fc nutri Disks (pre-prepared pads impregnated with selective medium for the detection of conforms, *E.coli* and other bacteria organisms in water and food stuffs supplied by Wagtech International Ltd, UK. The remaining diluted samples of 9ml of 0.1% ($^v/_v$) sterile peptone water (Total Volume, 10ml) was filtered under vacuum by using a vacuum Alteration unit (Wagtech Int. Ltd, UK) through a micro pore paper and placed on the M-Fc Nutri disks and incubated at 44.5°C for 24hrs. Other bacterial-isolates were detected and characterized by the methods of [21].

Fungal Isolation

This was done based on the methods of [7|. The swab containing fragments of biofilm were used. About $0.5cm^2$ of surface matrix were removed and plated on potato dextrose agar (Difco) or sabarauds dextrose agar (Difco). The inoculated samples were placed at room temperature and monitored for growth at 24hrs interval. The fungal isolates were identified visually and microscopically using appropriate taxonomic guides.

Microscopic identification of fungi was done using the methods of [4,14]. A drop of 95% ethanol was placed on a microscope slide. Using a sterile inoculating needle, a small portion of fungal growth was gently removed and placed on 95% ethanol and then it was gently spread with two dissecting needles for easier identification. On evaporation of almost all the ethanol used, a drop of lacto-phenol cotton blue was gently added. The arrangement was covered with a cover slide and then examined using light microscope, at X10 then viewed at X40.

Determination of Microbial Load

This was done based on the by methods adopted by [10] as adopted by [18] with slight modification. The microbes

were picked aseptically using a sterile loop from the nutrient agar unto universal bottle which is capped and shaken vigorously by hand for about 2 minutes to dislodge the microorganisms into the fluid. The resulting fluid (Buffered peptone water) which served as the test sample.

Serial doubling dilutions were prepared from the test samples as shown thus: 1:10,1:10^2, 10^3…….1:10^{10}. This was done by transferring dispensed 1ml aliquot into the test tube using micropipette. Starting with the highest dilution, 0.1ml of the test dilution (after agitation) was dispensed onto plate count agar also called PCA (Oxoid, Ltd, Basing stoke, Hamsphire, England) plates in duplicate. The inoculums were spread evenly over the entire surface of the PCA using a sterile bent spreader. All plates were incubated at 37°C aerobically overnight. All the colonies were counted from the duplicates and the mean of the counts were determined.

Statistical Analysis

All the values were expressed as mean+ standard deviation while analysis of chi square (X^2) was used to analyze the extent of variation between groups and P values equals or less than 0.5 were considered as significant [20]. Graphed instant 3.0 for windows USA @ computer software was used to analyze the data.

RESULTS

Table 1 shows the various samples that were collected and the prevalence of microorganisms from the pipe walls. From the Table, the highest prevalence was from the Copper pipes (59.2%) while the lowest prevalence was from the CPVC pipes (11.1%). Table 11 shows the microorganisms (bacteria) isolated from our study. Other Coli form bacteria and *Pseudomonas aeroginosa* competed with the highest prevalence rate each of 25.00% respectively, while *Legionella pheumophila* had the lowest prevalence rate of 1.67%. On the other hand, Table III showed the fungi isolation rate which the highest was for *Aspergillus niger* at the prevalence rate of 50.00% while *Penicillium rubrum* had the lowest isolation rate of 20.00%. Figure 1 and figure 2 present chats that concurrently showed the differences in the prevalence of both the bacterial organisms and fungi isolated from the water distribution systems and receptacles. Table IV shows the heterotrophic bacterial counts. The average bacterial counts for copper pipes ranged between 3.5 x 10^4 + 2.0 x 10^5 and 3.0 x 10^1 to 1.2 x 10^7 CFU/ml. While the average bacterial counts for stainless steel also ranged between 1.15 x 10^2 + 1.1 x l'O^3. The Colony Forming Units (CFU) of the bacteria counts ranged between 1 x 10^1 CFU/ml to 1.4 x 10^3 CFU/ml. Based on the obtained average; and the heterotrophic bacterial counts, there was a statistically significant difference regarding the contamination level of the water collected from, copper pipes and stainless steel with the Qui-Square test values giving P <0.01.

Table 1: Prevalence of Microorganisms in pipe walls and Receptacles in University of Abuja

Pipes/Receptacles	Number collected	No. Positive	% Positive
Water Tanks	27	15	55.5
Other Receptacles	27	10	37.0
Floor pipes	27	5	18.5
Tap water	27	6	22.2
CPVC pipes	27	3	11.1
Copper pipes	27	16	59.2
Stainless steel	27	15	55.5
Iron pipes	31	10	37.0
Total	**220**	**80**	**36.36%**

Table 2: Species of Bacteria Identified from 220 samples in University of Abuja Water Distribution Systems

S/N	Bacteria	Frequency	% Prevalence
1	*Legionella pheumophila*	2	3.33
2	*Aeromonas hydrophila*	4	6.67
3	*Pseudomonas aeroginosa*	15	25.00
4	*Mycobacterium avium*	1	1.67
5	Other coliform bacteria	15	25.00
6	*Escherichia coli*	10	16.67
7	*Salmonella typhi*	11	18.33
8	*Streptococcus* species	2	3.33
	TOTAL	**60**	**100**

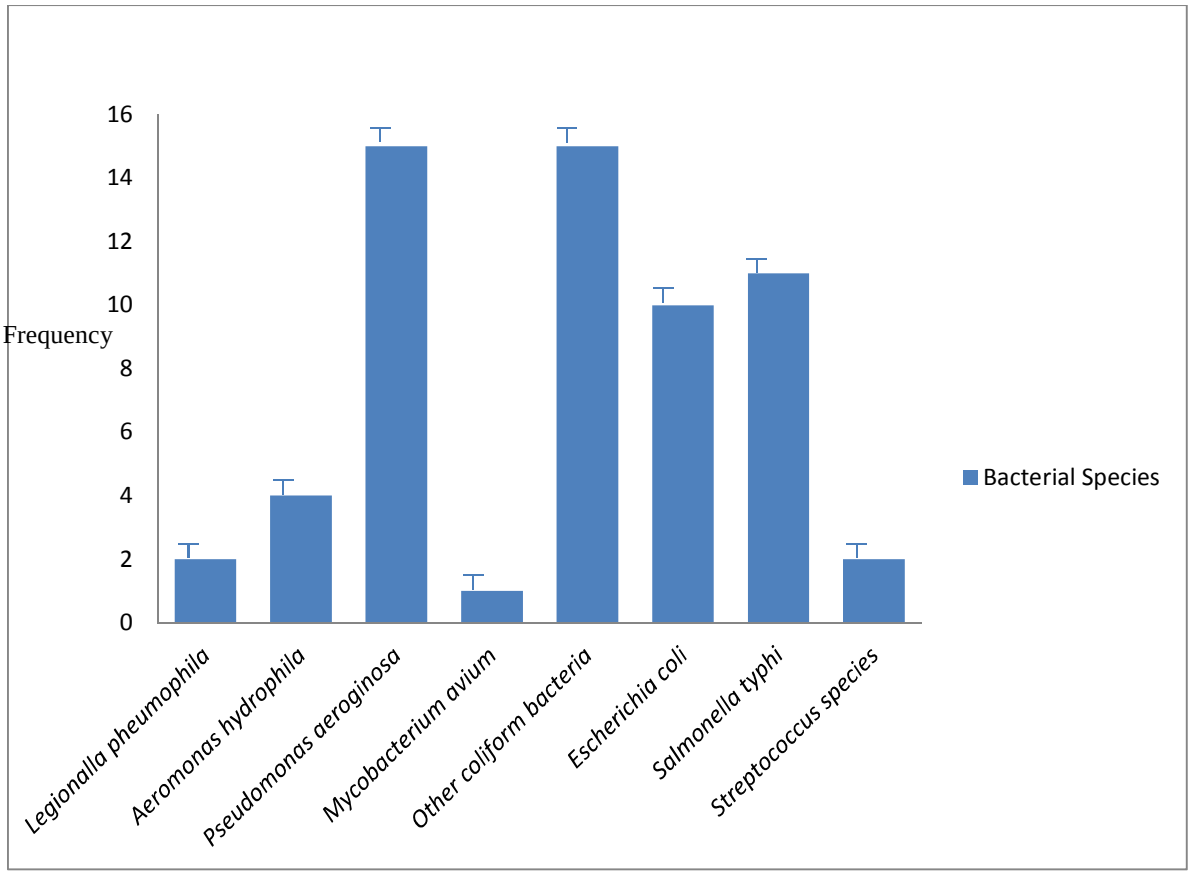

Fig. 1: Chart showing the variation of species of bacterial isolates in University of Abuja water distribution systems

Table 3: Species of fungal isolates from 220 samples university of Abuja water Distribution Systems

S/N	Fungi	Frequency	% Prevalence
1	*Aspergillus niger*	10	50.00
2	*Penicillium rubrum*	4	20.00
3	*Fusarium species*	6	30.00
	Total	**20**	**100**

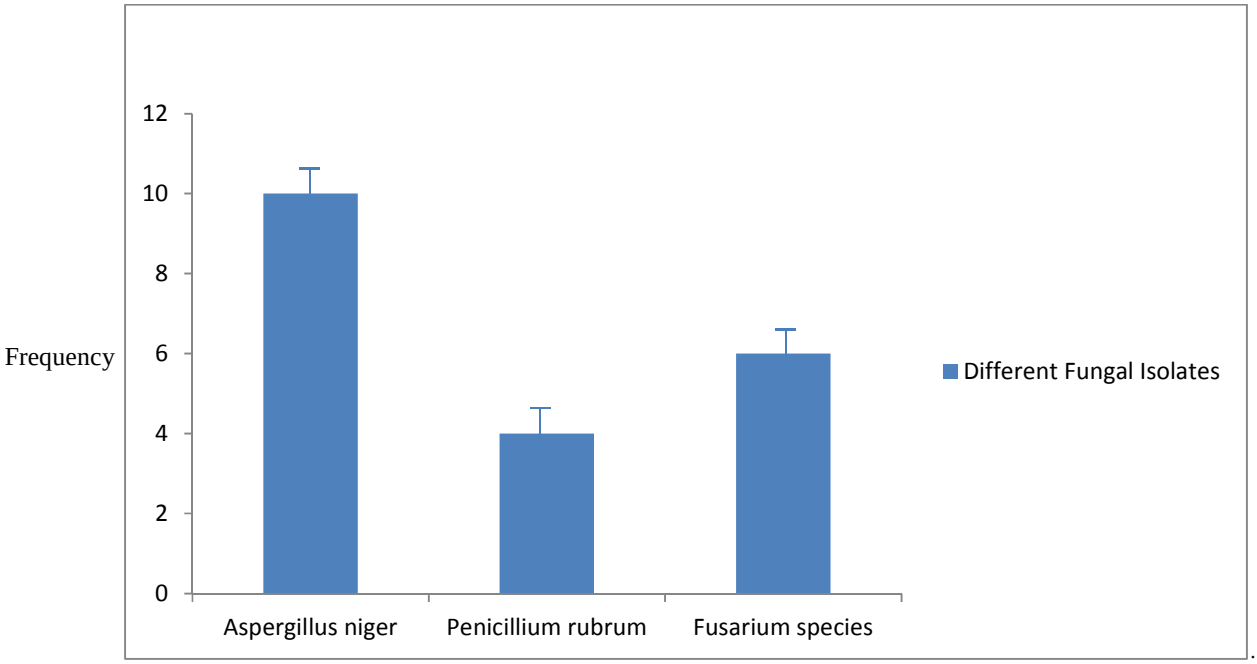

Fig. 2: Chart showing the various of species of fungi isolates in University of Abuja water distribution systems

Table 4: The Determination of the number of Microbial load (counts) in Water Distribution Systems

Unit	Copper	Stainless Steel
1	2.5×10^5	1×10^1
2	3.5×10^6	2×10^2
3	4.0×10^5	4×10^2
4	3×10^1	1.5×10^2
5	4×10^2	1.6×10^2
6	5 x 104	1.7×10^2
7	6.0×10^5	1.1×10^2
8	2.5×10^5	1.05×10^2
9	2.0×10^5	1×10^1
10	1×10^6	2.0×10^1
11	1.2×10^7	1×10^2
12	1.0×10^7	1×10^2
13	2×10^8	2×10^2
14	9.0×10^4	1.3×10^2
15	1×10^6	1.4×10^3
16	10.3 x 103	1.5×10^1
17	2.5×10^6	1.2×10^1
18	3.0×10^6	0.5×10^1
19	4×10^7	0.6×10^1
20	2.3×10^6	0.7x10*
Average + SD	**$3.5 \times 10^4 + 2.0 \times 10^5$**	**$1.15 \times 10^2 + 1.1 \times 10^3$**

DSSCUSSION

Bacterial Counts

Microorganisms are integral parts of biofilm in water distribution systems. This accounts for the high microbial count indicated in this study. The constant flow of water through the pipe is also a determinant factor in the high preponderance of bacteria count [13]. Our studies show lower concentration in CFU/ml in stainless steel than in copper pipes. This variation may be attributed to the heterogeneous distribution of the bacteria within the water sample. When new, the pipes may not show biofilm, this will affect the bacterial count either in the water or the reservoir. The same count behavior can occur if the unit is old but the lines have been recently replaced [3, 14].

The importance of the values of the total plate counts of the bacterial load is useful in the sense that our values exceeded the safety range of 100 CFU.m^{-1} and 20 CFU.m^{-1}values recommended by the EU and CDC and other regulatory bodies worldwide concerning the number of organisms expected to be found in potable water [15, 29]. The differences in the values of the total bacterial count may indicate the presence of other organisms which may still be found in the biofilms whose health implications have not been isolated. This research provides a window dressing for the conduct of more research using higher molecular techniques to develop policies for monitoring, provision of adequate sanitation and treatment for water distribution plants in Nigeria.

Prevalence of bacterial organisms

Most bacterial organisms isolated in our study were predominantly environmental organisms which could cause opportunistic infections in immunocompromised individuals [5]. Occurrence of coliforms and other associated bacteria such as E. *coli* and *Salmonella typhi* were enormous in water distribution systems as indicated by our study. The contamination of water by coliform bacteria could arise when the water is contaminated by fecal matter especially human feces. These organisms could be associated with many debilitating diseases including: diarrhea, cholera and typhoid fever [9]. It is therefore essential to break the fecal - oral cycle by preventing fecal matter from entering into water sources or by treating the drinking water with disinfectants to kill the pathogens. However, these approaches need to be operated alongside hygienic practices such-as hand washings, pipe replacement and maintenance, which may reduce the level of person-to-person infection.

The detection and enumeration of fecal *Streptococci* are also indication for fecal contamination [19]. The assumption is that if indicators are detected, pathogens such as viruses and protozoa could also be present. Therefore, appropriate action is required to provide adequate sanitation. However, the time taken to carry out analysis means that if contamination is detected, the contaminated water will be well on one way to the consumer and properly drunk, by the time the result has been obtained/more damages have already been accomplished. Therefore, monitoring itself is not an adequate means of assuring drinking water safety. Multiple barriers are needed to be put - in place at all times whatever is the size of the distributive system.

The occurrence of *Psendomonas aeroginosa* and *Aeromonas hydrophila* is an indication that opportunistic pathogens may multiply within the distribution system of water [1, 16]. Though most of these organisms are harmless, but may multiply and mutate in the water distribution system given suitable conditions to produce virulent strains [8]. These pathogens when consumed may be responsible for disease or ailments in both healthy, immune compromised and weakened patients especially the young and the very aged individuals. The resultant diseases may include: wound infections, diarrhea, cellulites, meningitis and gastroenteritis [25]. *Aeromonas hydrophila* is an emerging and re-emerging and potential water-borne pathogen and could give rise to a variety if infections in man, fishes, wild, zoo, laboratory animals and other livestock [18]. '

The isolation of the bacterium *Legionella pneumophila* in water distribution system is higher than that ascertained within the -distribution systems of both hot and cold water in Morocco [21]. The pathogen is responsible for 'Legionnaires' disease in animals and Pontiac fever in man. This disease is characterized by high fever, myalgia, headache, digestive disorder and pneumonia [29]. The presence of these organisms may be responsible for safety hazards associated with water quality often reported in developing countries. Its presence indicates the need to protect water quality deterioration and enforce the control the possible spread to the population at risk.

Prevalence of Fungi

In our study, the most frequently isolated mould was the genus *Aspergillus.* These findings are in consistence with the works conducted [11]. *Aspergillus* species were identified as the most common genera of fungi associated with water [2]. The fungus is capable of producing aflatoxins which are very important in the pathogenesis of major diseases such as aflatoxicosis in man is known to be associated with the production of aflatoxins Bi, B2, Gl and G2, the most potent hepato-carcinogenic toxin ever characterized [7, 27]. The toxin is capable of producing a wide range of disease in humans, ranging from hypersensitivity to invasive infections associated with angio-invasions. The confirmation of *A, niger* in our studies further concurs with previous studies [1]; [19]. *A. niger* could also be involved as a common allergen in many opportunistic invasive infections in hospitalized immunocompromised patients [6].

Fusarium species were isolated at frequency rate of 30% this is higher than the previous studies which isolation rate was. 19.5% [2.]. Several scientific works are being undertaken to further demonstrate the occurrence of *Fusarium* species in water distribution systems [23]. *Fusarium* species have been recognized as an agent involved in superficial

infections such as keratitis, and cutaneous infections, onchomycosis, and infections of wounds and brains [22].

The occurrence of *Penicilliurn* species which were specifically abundant in the water distribution system clearly lends supports to the ability of the organisms to survive water treatment plants. *Penicillium* hyphae have been incriminated diseases such as allergy, asthma and other chronic or acute respiratory diseases [24; 26]. The presence of *Penicillium* in water could seriously suggest its role as an active mycotoxin producer [25]. This question of possible mycotoxin production by *penicillium* merited further investigation on this fungus to evaluate its potentials in the production of *mycotoxins* in food, beverages and other regulated food products.

CONCLUSION

From the results of this study, it is believed that a wide variety of bacteria and fungi such as: Coliforms (25.00%), *E.coli* (16.67%), *Salmonella typhi* (18.33%), *Pseudomonas aeroginosa* 25%, *Legionella pheumophila* (3.33%), *Aeromonas hydrophila* (6.67%), *Streptococcus* species (3.33%), *Aspergillus niger* (50%) *Fusarium* species (30%), *Penicillium rubrum* (20%) still exists in drinking water supplies in Nigeria and Africa in general. The average bacterial counts at CFU/mls were subjected to statistical analysis and X^2 + SD for copper $3.5 \times 10^4 + 2.0 \times 10^s$ and stainless steel $1.15 \times 10^2 + 1.1 \times 10^3$. This shows level of association between type of distribution system and microorganisms. Epidemiological studies needs to be intensified with routine surveillance and reporting is needed, using higher molecular techniques such as Polymerase Chain Reaction (PCR), Restricted Fragment Length Pleomorphism (RFLP), to reveal other microbes of interest.

REFERENCES

[1] Acliti, Abaidya NS, and Madhulika S, *European Journal of experimental biology,* **2016**, 6(2) 21-25.
[2] Ana BG, Russell RMP, and Nelson L, *Intl.]. Hyg. Envtal. Hlth,* **2006,** 209:257-264.
[3] Auwal H, and Taura D. W, *Greener]. Biol. Sc,* **2013**, 3(5):179-186.
[4] Carnio J, *RSBO, J,* **2012,** 9 (3): 245-253.
[5] Chei MM, Cromar NJ,Mcclure, N. and Fallow field, HJ. *J. Appl Microbiol,* **2000**, 91: 646-651.
[6] Dange VU, and Peshive SA, *European Journal of experimental biology,* **2015**, 5(10) 57 – 61.
[7] Doggett MS, *Appl Env. Microbiol.* **2000,** 66(3): 1249-1251.
[8] Dougrouz N, Lilian - Sunqur E, Goskay, D, and Tiiretgen, I. *J.Environ. Mont Asses.* **2012,** 184 (133-139).
[9] Fawell J, and Mark JN, *British Med Bull.* **2003,**68(1): 199-208.
[10] Feglo P, and Nkansali M, *Afric. J. Microbiol. Res.* **2010**, 4(22): 2375-2380.
[11] Fernendez Cuenca, F, Lopez-Cartes, LE. and Rodriquez-Bang, *J Infec Clin Microbiol,* **2011**.29:40-46.
[12] Grant M.O, *Fungal Nutrition and Physiology.* New York, N.Y: John Willey and Sons.**1996**, 34-36.
[13] Idramsa ES, Haranto LN, Parastoeti P, and Eko P, *European Journal of Experimental Biology,* **2015**, 5(9): 20 – 26.
[14] Kadam SS, and Agrawal BA, *European Journal of Experimental Biology,* **2015**, 5(6): 13-22.8
[15] Kurup, R, Roland, P, John C, and Vincent, R, Microbiological and physiochemical analysis of drinking water in Georgeton, Guyana. /. *Nat. See,* **2010,** 8(8): 261-264.
[16] Mailafia S, *Nig. Vet. J,* **2013**, 21(4): 112-116.
[17] Mailafia S, Molecular epidemiology of *Aeromonas* species from fishes, poultry and human diarrheic stools in Zaria, Nigeria, Ph.D Research Thesis, Ahmadu Bello University, Zaria Nigeria. **(2008),** Pp. 23-75.
[18] Marques SC, Rezende JOS, Alves LF, Silver BC, Ronaldo E, Luiz A, and Coli HR, *Braz J Microbiol,* **2007**, 38(3): 538-54
[19] Mead C, and Currow J, Health risks from microbial Growth and Biofilms in Drinking water Distribution systems. Office water **(460).** US Environmental Protection Agency (EPA), 2002.
[20] Mekkour M, Elkhahill, BD, and Nozha C, *Wld.]. Em. Sc.* 2012, 2(2): 11-15.
[21] Musa MO, Aleisa A, Halim M, Sahovic E, Gyer M, Chaudhri N, Almoharab F, Seth P, Aslam M, and Aljurf M, *British J Haematol,* **2000,** 108*:* 544 -545.
[22] Okpoko EC, Osuagwu AN, Duke AE, and Ntui VO, *African Journal of Microbiology Research,* **(2009),** 3(2): 56-61.
[23] Olaleye ON, Detection of Fecal Coli forms in biofilms of water tankers in Odongiuyan, Ikorodu - Aperi - urban Lagos settlement. *Proceedings of the Environmental Management Conference,* Federal University of Agriculture, Abeokuta, Nigeria, **2011**, 459 - 464.
[24] Rechab SO, Kereru GP, Kutima LH, Nyagah CG, Njonge FK, Wambaru RW, and Cheruiyot K, *European*

Journal of experimental biology, **2015**, 5(11): 43 – 46.
[25] Scwab CJ, and Straus DC, *Adv. Microbiol*. **2004**,55:215-237.
[26] Suresh N, and Nelson R, *European Journal of Experimental biology*, **2016**, 6(1): 15-21.
[27] Walker BD, Bradshaw DJ, Fulford MR, Martin MV, and Marsh PD, Controlling mixed species of biofilm contamination in dental unit water systems. **2003**, 3(4): 56-58.
[28] Williams JF, Andrews N, and Santiago JI, *Compend, Con. Edu. Den.* **1996**, 17:691-694.