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MICROBIAL WATER QUALITY: AN ASSESSMENT FROM DELTA STATE UNIVERSITYCAMPUSES, ABRAKA

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ABSTRACT: *This study was aimed at assessing the microbiological quality of water collected from water storage tanks present in campus I and campus II of Delta State University, Abraka, Delta State, Nigeria. Ten (10) water samples were collected in triplicates from both campuses and assessed for the bacterial, fungal and coliform counts. There was significant bacterial growth of the water samples. The mean heterotrophic counts of the water samples ranged from 0.9x103 to 3.5x105 CFU/ml in campus I while the mean heterotrophic counts in campus II ranged from 0.3x103 to 2.7x105. The mean coliform counts ranged from 8.5-64.66 CFU/ml and 29.07-270.67 CFU/ml for campus 1 campus II respectively. Eleven (11) bacterial isolates were obtained which include: Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cerus, Salmonella typhi, Vibrio cholerae, Micrococcus luteus, Klebsiella pneumonia, Proteus mirabilis and Shigella dysenteriae. The results showed that Escherichia coli in Campus 1(15.09%) and Campus 2 (21.54%) had the highest percentage of occurrence from the thirty samples assessed for bacteriological contamination in both campuses, while Vibrio cholerae (1.89%) and Proteus mirabilis (1.54%) had the least occurrences in campus I and II respectively. Percentage of occurrence of fungal isolates shows that Fusarium subglutinans (32.26%) and Rhizopus microsporus (64.29%) had the highest occurrences in campus I and II respectively while Rhizopus microsporus (6.45%) and Aspergillus fumigatus (4.76%) had the least occurrence for both campuses respectively. Therefore, the high microbial loads observed in the study could be as a result of inadequate hygienic or sanitary practices of the storage tanks within the university community. Hence, proper hygiene and sanitary practices is required to reduce contamination and the likelihood of water borne diseaseoutbreak.*

KEYWORDS: Microbial, Water quality, Ecosystem, Gastrointestinal infection, Fungal infection.

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INTRODUCTION

Water is one of the most abundant compounds in the ecosystem with earth having approximately 70% of water and all living organisms on earth require water to survive (Basavaraja *et al*., 2011). Water makes the difference between living and non-living things. The importance of water for the maintenance of life cannot be over emphasized (Shekha *et al*., 2013). Water quality is essential for public health and obtaining clean water is a major concern of people living in developing countries. This is the situation found in Nigeria especially in the rural areas where the provision of clean and safe drinking water is a problem; and from a public health perspective, access to potable and good water supply is a serious issue (Anyanwu and Okoli, 2012).Water is important for its use in domestic and developmental purposes (Nwachukwu and Ume, 2013). A large percentage of the population in developing countries do not have access to clean and safe drinking water and therefore resort to use water from sources like shallow wells, streams, boreholes and springs with high probability of contamination. Therefore, a good knowledge of the physicochemical parameters of raw water quality is important in determining itssuitability for use (Nwachukwu and Ume, 2013).

Water also plays a crucial role in the socio-economic development of human population (Eniola *et al*., 2007). The deterioration in the quality of river and lake water is common in many aquatic systems and is due to point and non-point sources of pollution (Pisinaras *et al*., 2007). Factors such as economic and geographical locations are also responsible for diminished water quality (Reiff et al., 1996). Water supplied to rural communities is usually of poorer quality than that supplied to urban areas (Welch *et al*., 2000). According to Owa (2013), water pollution arises from several activities and the effect has led to an irreversible change in the ecosystem which is dangerous to plants and animals. It causes approximately 14,000 deaths per day as a result of contamination of water due to untreated sewage in developing countries and approximately 700 million Indians do not have access to good toilet facilities and an estimated 1000 Indian children die of diarrhea every year as well as other countries, Owa (2013). Also, an estimated 500 million Chinese lack access to safe drinking water. Contamination of water by pathogenic microorganisms is a great concern for water consumers with respect to drinking water quality (Hageskal *et al*., 2009). Water is frequently polluted with different harmful pollutants as a result of increased human population, industrialization use of fertilizers in agriculture and man-made activity. It has been established statistically that the rate of fungal recovery are three times higher in surface sourced water as well as ground water and their rates of recovery are higher in cold water and shower water than from hot tap water (Hageskal *et al*., 2007). Those most susceptible to water infection are children, the elderly, pregnant women and immune-compromised persons (Gerba, 1996) so there is need to ensure potable water supply to these groups. The study was aimed at assessing the microbial agents that may gain access to water storage tanks at different sites in campus I and II of Delta State University, Abraka and to proffer solutions or measures to prevent contamination and spread of waterborne disease and outbreaks.

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MATERIALS AND METHODS

Study Area: The research was carried out at Abraka in Ethiope East local government Area of Delta State, Nigeria. Abraka is located at 5^0 47′ 0″ North and $6^0$6′0″ East.

Collection of Samples: Ten (10) water samples were collected in triplicates from water tanks in campus I and II of Delta State University, Abraka campus, Delta State. The containers were sterilized and closed until they were required for the collection of the water samples. Before filling, the container was held by thebase in one hand and a spirit lamp was held close to the tap connected to the tank and flamed for thirty seconds and the water allowed to run for at least sixty seconds before the water samples were collected. The cap of the containers were securely tightened and labeled for proper identification. The sample containers were transported to the Microbiology laboratory and analyzed immediately (Cheesbrough, 2004)

Microbiological Analysis

- Total Bacterial Count

Tenfold serial dilution of the water samples were carried out using a sterile pipette. An aliquot (1ml) of each water sample was homogenized with 9ml of distilled water in sterilized test tubes. Using standard pour plate method, 0.1ml of the serially diluted water (10^{-3} and 10^{-5}) were aseptically withdrawn from the test tubes for each sample and were introduced in sterile petri-dishes required for the Nutrient agar and MacConkey agar. Both agar's were autoclaved, cooled and introduced into the petri dishes. The petri dishes were swirled gently to evenly distribute the innoculum and allowed to solidify. They were incubated in an inverted position in an incubator at37ºC for 24hours and the viable colonies were counted as previously described by Cheesbrough (2004). The isolates were characterized and identified based on their cultural, morphological and biochemical characteristics (cheesbrough, 2004)

- Total Fungal Count

Tenfold serial dilution of the water samples were carried out using a sterile pipette. An aliquot (1ml) of each water sample was homogenized with 9ml of distilled water using sterilized test tubes. Using standard pour plate method, 0.1ml of the serially diluted water (10^{-3} and 10^{-5}) were aseptically withdrawn from the test tubes for each sample and were introduced into the petri dishes required for the Potato dextrose agar. The Potato dextrose agar was autoclaved, cooled and 250mg of chloramphenicol added to inhibit bacterial growth. The agar were introduced into the petri dishes, swirled gently to evenly distribute the innoculum and allowed to solidify. They were incubated in an inverted position at room temperature (25ºC) for 72 hours as previously described by Cheesbrough (2004).

- Total Coliform Count (Most Probable Number/ Multiple Tube Method)Presumptive Test

Three sets of five test tubes containing MacConkey broth were prepared aseptically with durham tubes inverted into the medium. Each set received specified amount of the medium. Each set received an amount ten-fold less than the first i.e. 10ml, 1ml and 0.1ml. The mouth of the

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containers were flamed and the water withdrawn using a sterile pipette. 10ml, 1ml and 0.1ml of the water sample were introduced into five test tubes each. This was repeated for the other water samples. In all, five test tubes for each set of volume were used for each sample. The test tubes were then stoppered with sterile cotton wool wrapped with aluminium foil paper and incubated aerobically in a test tube rack at 37ºC for 24-48 hours. After incubation, schanges in the colour of the broth and gas production were observed. The probable number of coliform organisms in the water was determined from McCrady's probability table (Cheesbrough, 2000). The data were analyzed using Statistical Package for the Social Science (SPSS).

RESULTS

Table 1 shows the total and mean heterotrophic count of the bacterial isolates from both campuses. The mean heterotrophic counts ranged from 0.9 x 10^3 to 3.5x10^5 CFU/ml in campus I while the mean heterotrophic counts in campus II ranged from 0.3x10^3 to 2.7x10^5 CF/ml. Table 2 represents the mean coliform counts of the water samples at different locations in both campuses in Colony forming units per milliliter (CFU/ml). It was observed that the water samples obtained from water tanks in campus II had higher coliform counts as compared to the water samples obtained from water tanks in campus I. Eleven (11) bacterial isolates were obtained; *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cerus*, *Salmonella typhi*, *Vibrio cholerae*, *Micrococcus luteus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Shigella dysenteriae* as represented in Table 3. While table 4 is represented the fungal isolates which includes: *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium subglutinans*, *Mucor* sp. and *Rhizopus microsporus*. Table 5 shows the total and mean heterotrophic count of the bacterial isolates from both campuses. The mean heterotrophic counts ranged from 0.9 x 10^3 to 3.5x10^5 CFU/ml in campus I while the mean heterotrophic counts in campus II ranged from 0.3x10^3 to 2.7x10^5 CF/ml. Table 6 shows the percentage of occurrence of the bacterial isolates. The data collected shows that there is no significant difference between the occurrence of bacterial isolatesobtained from both campuses. The result shows that *Escherichia coli* (15.09%), (21.54%) had the highest percentage of occurrence from the thirty samples assessed for bacteriological contamination in both campuses, while *Vibrio cholerae* (1.89%) and *Proteus mirabilis* (1.54%) had the least occurrences in campus I and II respectively. Presented in Table 5 is the percentage of occurrence of fungal isolates obtained from both campuses. The data collected shows that there was no significant difference between the fungal isolates obtained from both campuses. The result shows that *Fusarium subglutinans* (32.26%) and *Rhizopus microsporus* (64.29%) had the highest occurrences in campus I and II respectively while *Rhizopus microsporus* (6.45%) and *Aspergillusfumigatus* (4.76%) had the least occurrence in campus I and II respectively.

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Table 1: Total Heterotrophic Bacterial Counts

Count		Heterotrophic count (CFU ml)						Mean Heterotrophic (CFU ml)	
Campuses	**Sites**	**1st Trip**		**2nd Trip**		**3rd Trip**			
		10^{-3}	**10^{-5}**	**10^{-3}**	**10^{-5}**	**10^{-3}**	**10^{-5}**	**10^{-3}**	**10^{-5}**
1	Staff Quarters	$1.3x10^3$	$5.2x10^5$	$1.0x10^3$	$4.8x10^5$	$0.4x10^3$	$0.6x10^5$	$0.9x10^3$	$3.5x10^5$
	Primary School	$3.2x10^3$	$1.9x10^5$	$2.2x10^3$	$2.0x10^5$	$1.2x10^3$	$0.8x10^5$	$2.2x10^3$	$1.6x10^5$
	Secondary School	$0.8x10^3$	$1.9x10^5$	$1.8x10^3$	$1.4x10^5$	$1.5x10^3$	$1.0x10^5$	$1.4x10^3$	$1.4x10^5$
	Guest House	$1.7x10^3$	$3.3x10^5$	$1.4x10^3$	$2.9x10^5$	$1.5x10^3$	$1.4x10^5$	$1.5x10^3$	$2.5x10^5$
	Main Supply	$3.0x10^3$	$1.4x10^5$	$1.7x10^3$	$1.9x10^5$	$4.3x10^3$	$3.7x10^5$	$3.0x10^3$	$2.3x10^5$
2	Abraka Hall	$3.2x10^3$	$1.1x10^5$	$3.0x10^3$	$1.4x10^5$	$0x10^3$	$0x10^5$	$2.1x10^3$	$0.8x10^5$
	Council Hall	$1.7x10^3$	$1.5x10^5$	$1.1x10^3$	$1.5x10^5$	$1.2x10^3$	$1.7x10^5$	$1.3x10^3$	$1.6x10^5$
	Ethiope Hall	$2.0x10^3$	$3.1x10^5$	$1.2x10^3$	$3.0x10^5$	$0.7x10^3$	$1.9x10^5$	$1.3x10^3$	$2.7x10^5$
	Boys Quarters	$0x10^3$	$1.0x10^5$	$0.2x10^3$	$1.1x10^5$	$0.6x10^3$	$0.3x10^5$	$0.3x10^3$	$0.8x10^5$
	Cooperative Building	$1.4x10^3$	$1.2x10^5$	$0.9x10^3$	$0.7x10^5$	$0.8x10^3$	$0x10^5$	$1.0x10^3$	$0.9x10^5$

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Table 2: Coliform counts of water samples

		Coliform count (CFU/ml)			Mean coliform count(CFU/ml)
Campuses	**Sites**	**Ist trip**	**2nd trip**	**3rd trip**	
1	Staff Quarters	5.5	8.2	33	15.57
	Primary School	46	26	8.3	26.77
	Secondary School	43	17	1.2	20.4
	Guest House	140	33	21	64.66
	Mainly Supply	10	5.5	10	8.5
2	Abraka Hall	24	31	8.2	21.07
	Council Hall	54	24	9.2	29.07
	Ethiope Hall	95	76	220	130.33
	Boys Quarters	210	25	17	84
	Cooperative Building	430	350	32	270.67

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Table 3: Identification of bacterial isolates

Bacterial Isolates	Cultural characteristics on nutrient Agar and Eosin Methylene blue agar	Biochemical characteristics																						
		Gram	Shape	Catalase	Oxidase	Glucose	Lactose	H_2S	Acid	Gas	Motility	Citrate	Indole	Arabinos	Cellobios	Fructose	Galactose	Maltose	Mannitol	Mannose	Raffinose	Ribose	Sucrose
Escherichia coli	Large thick greyish white on Nutrient agar, reflecting green metallic sheen on Eosin methylene blue agar	-	Rods	+	-		+	+	-		+	+	-	+		+	-		-+	-+		-	-		-
																						v		
Enterobacter aerogenes	Smooth thick white colonies on Nutrient agar, colonies with dark centres on Eosin methylene blue agar	-	Rods	+	-		+	+	-		+	+	+	-+		-+		+	+	+	-+		+	+
Klebsiella pneumoniae	White viscous mucoid colonies on Nutrient agar, pink colonies on eosin methylene blue agar	-	Rods	+	-	+	+	-	+		+	-	+	+	+	+	-	+	+	+	+	+	+	+

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Pseudomonas aeruginosa	Greenish colonies, smooth raised and round	-	Rods	+	+	-	-	-	- -	+	+	-	--	-	-	-	-	-	--	-			
Staphylococcus aureus	Smooth raised and round creamy colonies	+	Cocci in Clusters	+	-	+	+	-	+ -	-	+	-	- -	+	+	+	+	+	- +	+			
Bacillus cerus	Large greyish-white granular colonies	+	Rod Shaped with round edges	+	-	+	-	-	+ +	+	+	-	-V	+	-	+	-	-	-+	V			
Bacillus subtilis	Large, white, flat colonies	+	Rods	+	V	+	V	-	+	-	+	+	-	+	+	+	V	+	+	+	+	+	+
Salmonella typhi	White clear colonies	-	Rods	+	-	+	-	+	+	-+	+	-	-	--	+	+	+	+	-+	-			
Vibrio cholerae	Moist, translucent round colonies	-	Curved rods	+	+	+	V	-	+	-+	+	+	-	-+	+	+	+	+	-+	+			

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Micrococcus luteus	Circular, entire convex and creamyyellow pigmented colonies	+	Cocci in pairs, irregular clusters or irregular clusters or tetrad	+	+	-		-		-		--+	-		-+	+	+	-+		+	+	-+		-									
Proteus mirabilis	Creamy, mucoid and swarming colonies	-	Rods	+	-	+	-	+	+	-	+	+	-	+	+	+	-	+	+	+	+	-	-										
Shigella dysenteriae	Small, convex, smooth and transparent colonies	-	Rods	+	-	+	-	-	+	+	-	-	-	+	+	-	-	+	+		+	+	-										

Key: + = positive - = negative v = variable

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Table 4: Identification of fungal isolates

Fungal isolates	Hyphae	Spores	Cultural Appearance	Size
Aspergillus niger	Septate	Conidial heads are dark brown to black, radiate and biseriate with metulae twice as long as the phialides. Conidia brown and rough-walled	White to becoming black to deep brown with conidial production with the reverse yellow	3.8 – 5µm in diameter
Aspergillus fumigates	Septate	Uniseriate and columnar heads with the phialides limited to the upper two-thirds of the vesicle and curving to be parallel to each other	Blue-green	400 x 500 µm in diameter
Fusarium Subglutinans	Mostly non- septate or occasionally with one septum	Phialides with conidia arranged in fake heads in the aerial mycelium	Yellow-green to brown	20 x 50µm in diameter
Mucor Spp	*Scarcely or non-septate*	Large spherical, non-apophysate sporangia with pronounced columellae and conspicuous collarette at the base of the columella following sporangiospore dispersal	White-yellow becoming dark-gray with the development of sporangia	3.5 – 5.0 µm in diameter
Rhizopus microsporus	Non-septate/ Aseptate	Phialides are all flask-shaped and the conidia clustered at the tips of the phialides. Columellae are subglobose to somewhat conical	White becoming gray-brown on surface with reverse pale white	40 – 350 µm in diameter

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Table 5: Percentage occurrence of bacterial isolates

Bacterial Isolotes	Occurrence	
	Campus 1N (%)	Campus 2N (%)
Escherichia coli	8 (15.09)	14 (21.54)
Enterobacter aerogenes	5 (9.43)	9 (13.85)
Pseudomonas aeruginosa	8 (15.09)	5 (7.69)
Staphylococcus aureus	6 (11.32)	8 (12.31)
Bacillus cerus	0 (0)	3 (4.62)
Bacillus subtilis	2 (3.77)	2 (3.08)
Salmonella typhi	3 (5.66)	3 (4.62)
Vibrio cholera	1 (1.89)	3 (4.62)
Micrococcus luteus	3 (5.66)	0 (0)
Klebsiella pneumonia	*8 (15.09)*	*10 (15.38)*
Proteus mirabilis	*3 (5.66)*	*1 (1.54)*
Shigella dysenteriae	*6 (11.32)*	*7 (10.77)*

$P > 0.05$

Key

$P > 0.05$ = No significant difference$P < 0.05$ = Significant difference

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Table 6: Percentage occurrence of fungal isolates

Fungal isolates	**Occurrrence**	
	Campus 1 N (%)	**Campus 2 N (%)**
Aspergillus niger	3 (9.68)	10 (23.81)
Aspergillus fumigates	8 (25.81)	2 (4.76)
Fusarium subglutinans	10 (32.26)	0 (0)
Mucor spp.	8 (25.81)	3 (7.14)
Rhizopus microsoporus	2 (6.45)	27 (64.29)

$P > 0.05$

Key

$P > 0.05$ = No significant difference$P < 0.05$ = Significant difference

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DISCUSSION

The mean heterotrophic counts of the water samples ranged from $0.9x10^3$ to $3.5x10^5$ CFU/ml in campus I while the mean heterotrophic counts in campus II ranged from $0.3x10^3$ to $2.7x10^5$. The mean coliform counts ranged from 8.5-64.66 CFU/ml and 29.07-270.67 CFU/ml for campus 1 campus II respectively. These counts were higher than the allowable limit of 1.2 $x10^2$ CFU/ml by local and international bodies. The high counts observed in the study might be due to contaminations from faecal materials such birds that are always found perching on the unclosed lid of the water tanks. This result is in agreement with the report of Munawwar and Asma (2016). Eleven (11) bacterial isolates were identified which includes; *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cerus*, *Salmonella typhi*, *Vibrio cholerae*, *Micrococcus luteus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Shigella dysenteriae*. These bacterial isolates are common in the gastrointestinal tract (GIT) of birds, other animals and humans that are causal agents of various illnesses (Kuitcha *et al*., 2010; Uzoigwe and Agwa, 2012). Among the bacterial isolates it was revealed that Escherichia coli in Campus 1(15.09%) and Campus 2 (21.54%) had the highest percentage of occurrence from the studied samples obtained from both campuses while *Vibrio cholerae* (1.89%) and *Proteus mirabilis* (1.54%) had the least occurrences in campus I and II respectively. Hence, the presence of indicator bacteria such as *Escherichia coli* which are prevalent in the digestive tract of warm blooded animals indicates faecal contamination of these waters (Popoola *et al*., 2007). Also, the presence of *Bacillus cerus* in the water samples is also of major concern and might lead to the ubiquitous distribution of organisms and its ability to form endospores (McKillip, 2010). Furthermore, the presence of *Staphylococcus aureus* is also of great concern as it is known to produce enterotoxins a causative agent for diseases (Aydin, 2007). Five (5) fungi species were also recovered from the water samples which includes; *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium subglutinans*, *Mucor* sp. and *Rhizopus microsporus*. The isolates were similar to the findings of Thomas and Thangavel (2017). Who worked on the isolation of fungi from the surface of water vembanadu wetland agroecosystem. Percentage of occurrence of fungal isolates showed that *Fusarium subglutinans* (32.26%) and *Rhizopus microsporus* (64.29%) had the highest occurrences in campus I and II respectively while *Rhizopus microsporus* (6.45%) and *Aspergillus fumigatus* (4.76%) had the least occurrence for both campuses respectively. Thus, the presence of fungi in the water storage tanks is an indication of the presence of mycotoxins since species of *Aspergillus* are known to produce aflatoxins, a

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carcinogenic substance of health significance. In this modern era, the incidence of nosocomial fungal infections has dramatically increased. It has been reported that *Aspergillus* is the second most significant cause of hospital acquired fungal infection, and aspergillosis usually occur in immune-compromised patients (Richardson and Richardson, 2015; Perlroth *et al*., 2007). Anaissie *et al*. (2002) reported that *Aspergillus* species obtained from hospital water system was proven to be the highest airborne *Aspergillus* propagule density (2.95 CFU/m3) for bathroom waters. They further recorded that water from tanks had higher colony-forming units than the municipal watersduring their study.

The contamination of these water tanks could also be due to the proximity of boreholes to septic tank from which the water is pumped where some of these bacterial isolates must have sip into the ground. Moreover, information obtained from the users of these water storage tanks indicate that these tanks are not usually washed or disinfected for years. This is similar to the report of Lévesque *et al*. (2008) whose study documented a similar reason for gross contamination of water obtained from water storage tanks.

CONCLUSION

The high microbial load observed in the study might be responsible for gastrointestinal (GIT) infections and enteric fever which are very common among students and staff that use this water for drinking and domestic uses. Therefore, proper hygiene and sanitary practices is required to reduce contamination and the likelihood of water borne disease outbreak. `

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