JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

ENVIRONMENTAL ASSESSMENT OF BACTERIAL FAECAL POLLUTION MARKERS IN OVERLYING WATERS FROM AN EARTHEN FISH POND

Adewale Olalemi[1]*, *Busayo Oluyemi*[1]

Address(es): Dr. Adewale Olalemi,
[1]Federal University of Technology, Akure, School of Sciences, Department of Microbiology, PMB 704, Akure, Ondo State, Nigeria, +234 906 415 6284.

*Corresponding author: waleolas2002@yahoo.com

doi: 10.15414/jmbfs.2018.7.6.566-570

ARTICLE INFO

Received 8. 3. 2018
Revised 19. 3. 2018
Accepted 23. 3. 2018
Published 1. 6. 2018

Regular article

OPEN ACCESS

ABSTRACT

This study was carried out to examine the sanitary quality of overlying waters in an earthen pond containing African catfish (*Clarias gariepinus*) in both wet and dry seasons. Water samples were collected twice weekly at different times of the day (i.e., morning and afternoon) from the earthen pond in Akure, Nigeria in August, September 2016, and February, March 2017 (n=48). Levels of faecal indicator bacteria were determined using membrane filtration method and physicochemical properties (such as temperature, pH, salinity etc.) of the overlying water samples were determined using standard methods. The results revealed that the concentration of *E. coli* in the overlying waters ranged from 0.78 to 1.40 Log_{10} CFU 100 ml^{-1} in morning samples and 0.85 to 1.38 Log_{10} CFU 100 ml^{-1} in afternoon samples while the concentration of faecal coliforms in the overlying waters ranged from 1.30 to 1.86 Log_{10} CFU 100 ml^{-1} in morning samples and 1.26 to 1.88 Log_{10} CFU 100 ml^{-1} in afternoon samples. Faecal coliforms showed the highest prevalence in both seasons compared to *E. coli* and intestinal enterococci and their levels were greater in the wet periods than the dry periods. The findings from this study suggest that the sources of faecal contamination of the overlying waters in the earthen pond originate mainly from non-human sources and the adoption of microbial source tracking techniques would further elucidate the appropriate management and mitigation strategies to control faecal contamination of the overlying waters in the earthen pond especially for human health protection.

Keywords: Faecal indicator bacteria, human health, microbial source tracking, earthen fish pond, overlying waters

INTRODUCTION

Approximately 60% of human population in low-and middle-income countries derive 30% of their annual protein from fish (**Omojowo and Omojasola, 2013**). The advantage of fish as food is as a result of its easy digestibility and high nutritional value (**Abo-Elela *et al.*, 2005**). However, fish are susceptible to a wide variety of microbial pathogens and may be a route of transmission of pathogens to humans when microbially contaminated fish are consumed. The greatest microbial risks are associated with the ingestion of fish raised in overlying waters impacted with faecal matter that may originate from either human or non-human sources. Wastewater discharges in fresh waters and coastal seawaters are the major source of faecal microorganisms that contaminate aquatic lives especially fish (**Shahidul and Tanaka, 2004**). Fish pond may contain bacteria, heavy metals, inorganic minerals and chemicals that are unsuitable for human consumption and studies have demonstrated that the release of untreated or partially treated effluents into water bodies has significant effect on aquatic life as well as human health, especially consumers of seafood (**Naylor *et al.*, 2000**; **Rose *et al.*, 2001**; **Olalemi *et al.*, 2016**).
There are increased interests in the application of quantitative microbial risk assessment (QMRA) to estimate the risk and severity of the illnesses associated with pathogens, their infectivity and population exposed to microbially contaminated surface waters including ponds (**Wuertz *et al.*, 2011**). The total coliform group are microorganisms that can survive and proliferate within the water environment and they include several species of the *Enterobacteriaceae* family. These bacteria are found in the gastrointestinal tract of humans and non-humans. *E. coli* serves as a true classical indicator of faecal pollution, although its survival and persistence in the environment is limited. Intestinal enterococci form a sub-group of the larger faecal streptococci group and are well known for their association to faecal pollution (**Somaratne and Hallas, 2015**). The presence of faecal coliforms in the flesh and gastrointestinal tract of fish demonstrates the level of faecal contamination of their overlying waters. Faecal coliforms may gain access into ponds through direct discharge of faecal wastes from mammals, aquatic animals, birds, agriculture, storm, runoff, human sewage, combined sewer overflow, heavy flood or erosion. Pets, especially dogs, can contribute to faecal contamination of the overlying waters in ponds. Birds such as swans, geese, seagulls and other waterfowl can be a significant source of elevated faecal coliform count in ponds (**Doyle and Erickson, 2006**).
Generally, bacterial pathogens associated with ponds are classified as indigenous and non-indigenous. The non-indigenous contaminate the ponds through point or non-point sources e.g., *E. coli*, *Clostridium botulinum*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens* and *Salmonella*. The indigenous are found naturally in ponds e.g., *Aeromonas* species. Urbanization in cities in most low-income countries has resulted in the concentration of large population in some areas living under poor sanitation conditions (**Olade, 1987**). This invariably has led to increased waste generation with heaps of waste everywhere. During rainfall, some of these wastes are washed into the poor drainage systems and subsequently into nearby rivers and ponds. The changes in nutrient levels and bacteriological properties can directly affect aquatic and human activities when such water is discharged to lakes, streams and rivers. The prevention of excessive input of the nutrients is hardly achievable since most originate from non-point sources. Oxygen depletion is often associated with such excessive input of nutrients and it leads to increased organic matter production in lakes or ponds. The microflora present in ponds may change to pathogenic microorganisms as a result of influx of large number of opportunistic pathogenic organisms from point source pollution (**Njoku *et al.*, 2015**).
This study was aimed at determining the fate, occurrence and survival of bacterial faecal pollution markers in an earthen fish pond in Akure, Nigeria and to examine the effect of seasonality as well as physicochemical characteristics on the levels of the faecal pollution markers. This hazard evaluation is an important step in preventing and managing human waterborne and/or foodborne illnesses (such as gastroenteritis) that may occur as a result of contact with the faecally impacted overlying waters or consumption of *Clarias gariepinus* raised in such waters.

MATERIAL AND METHODS

Sampling site and collection of samples

The sampling site was situated within the Federal University of Technology, Akure, Obakekere campus. The site contains a characteristic swamp, water ways, vast plains with high biodiversity of plants. The earthen pond is affected by fluctuating faecal input from farm animals such as cattle and sheep grazing in around the pond as well as from birds feeding on certain aquatic organisms in the pond. It is also affected by diffuse pollution from runoffs during storm events. Sampling activities were carried out twice weekly over a period of twelve weeks in August, September, 2016 (wet period) and February, March, 2017 (dry period). On each sampling occasion, a grab sample of approximately one litre of overlying water was collected at a depth of about 20 – 30 cm in a pre-sterilised plastic bottle in accordance with standard protocol (**Anon. 2012**). Water samples were collected in the morning within the hours of 8 and 10 am and in the afternoon within the hours of 12 and 3 pm. The overlying water samples were transported to the laboratory in a cool box with ice packs and processed immediately within less than one hour.

Enumeration of *E. coli* in the overlying water samples

The concentrations of *E. coli* in overlying waters were determined using the membrane filtration method (ISO 9308-1) (**Anon. 2014**). The membrane filters were placed on freshly prepared selective media (MLSA and EMB). Agar plates were incubated at 37 ^{o}C for 24 hours, and colonies were counted, and then concentrations were calculated and expressed as colony-forming units (CFU) 100 ml^{-1} of water.

Enumeration of faecal coliforms in the overlying water samples

The concentrations of faecal coliforms in overlying waters were determined using the membrane filtration method (ISO 9308-1) (**Anon. 2014**). The membrane filters were placed on freshly prepared selective media (*m*-FC). Agar plates were incubated at 44 ^{o}C for 24 hours (*m*-FC), and colonies were counted, and then concentrations were calculated and expressed as colony-forming units (CFU) 100 ml^{-1} of water.

Enumeration of intestinal enterococci in the overlying water samples

The concentrations of intestinal enterococci in overlying waters were determined using the membrane filtration method (ISO 7899-2) (**Anon. 2000**). The membrane filters were placed on freshly prepared selective media (*m*-Ent). Agar plates were incubated at 37 ^{o}C for 48 hours (*m*-Ent), and colonies were counted, and then concentrations were calculated and expressed as colony-forming units (CFU) 100 ml^{-1} of water.

Determination of the physicochemical properties of the overlying water samples

The physicochemical properties of the overlying water from the earthen pond were measured using standard methods (**Anon. 2012**). These include temperature (Celsius), pH, electrical conductivity (micro Siemens per centimetre), alkalinity (milligrams per litre), turbidity (nephelometric turbidity units), total dissolved solids (milligrams per litre), dissolved oxygen (milligrams per litre) and salinity (parts per thousand).

Statistical analysis

Data were transformed to $\log_{10}$, then examined using general descriptive statistics and checked for normality using the skewness and kurtosis statistic. Analysis of variance and test of significance using Duncan's new multiple range test were undertaken using Statistical Package for Social Sciences (SPSS) Version 20.0, and all data were subjected to the Pearson's correlation analysis to determine whether there were positive correlations between the concentration of the faecal pollution markers and physicochemical properties of the overlying waters from the earthen pond.

RESULTS AND DISCUSSION

Detection of *E. coli* in the overlying water samples

The mean concentration of *E. coli* in the overlying waters from the earthen pond ranged from 0.78 to 1.40 $\log_{10}$ CFU 100 ml^{-1} for morning samples and 0.85 to 1.38 $\log_{10}$ CFU 100 $ml^{-1}$for afternoon samples. In general, the concentration of *E. coli* in the overlying waters appeared to be higher in samples collected in the morning (54%) compared to those collected in the afternoon (30%) and appeared to be higher during the dry periods compared to the wet periods (Figure 1).

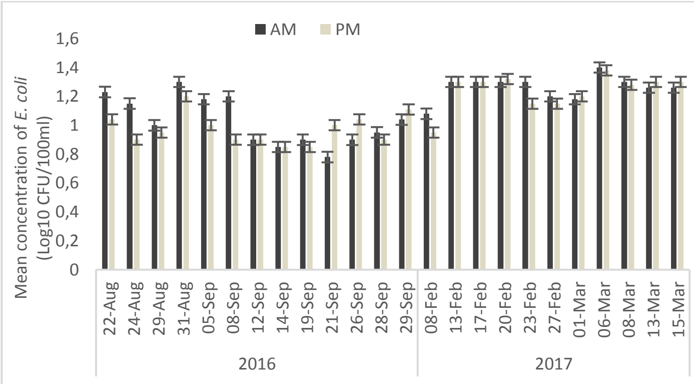

Figure 1 Mean concentration of *E. coli* in water samples collected from the earthen pond over the period of study (n = 48)

Detection of faecal coliforms in the overlying water samples

The mean concentration of faecal coliforms in the overlying waters from the earthen pond ranged from 1.30 to 1.86 $\log_{10}$ CFU 100 ml^{-1} for morning samples and 1.26 to 1.88 $\log_{10}$ CFU 100 $ml^{-1}$for afternoon samples. The concentration of faecal coliforms in the overlying waters appeared to be higher in samples collected in the afternoon (84%) compared to those collected in the morning (16%) and appeared to be higher during the wet periods compared to the dry periods (Figure 2).

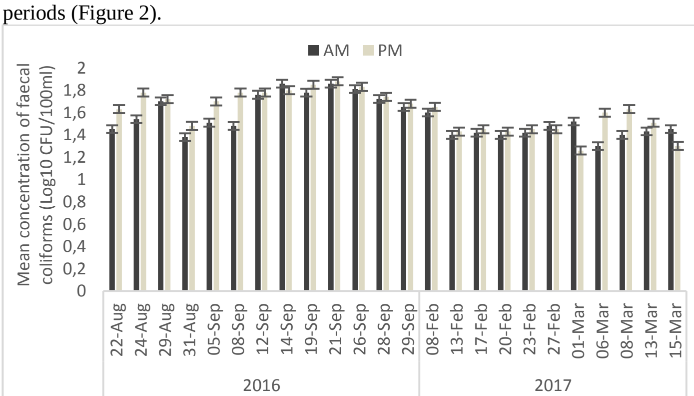

Figure 2 Mean concentration of faecal coliforms in water samples collected from the earthen pond (n = 48)

Detection of intestinal enterococci in the overlying water samples

The mean concentration of intestinal enterococci in the overlying waters from the earthen pond ranged from 1.00 to 1.38 $\log_{10}$ CFU 100 ml^{-1} for morning samples and 0.60 to 1.40 $\log_{10}$ CFU 100 $ml^{-1}$for afternoon samples. The concentration of intestinal enterococci in the overlying waters appeared to be higher in samples collected in the morning (58%) compared to those collected in the afternoon (38%) and appeared to be higher during the wet periods compared to the dry periods (Figure 3).

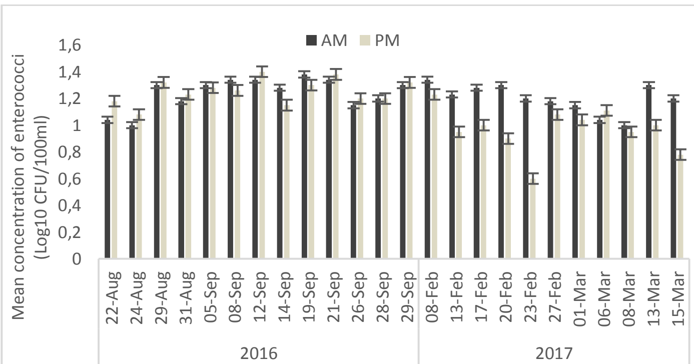

Figure 3 Mean concentration of intestinal enterococci in water samples collected from the earthen pond (n = 48)

Seasonality of faecal indicator bacteria in the overlying water samples

In this study, two seasons were considered, that is, wet (August and September, 2016) and dry (February and March, 2017). The concentration of faecal coliforms and intestinal enterococci were higher in overlying water samples collected during the wet period compared to those collected during the dry period. Contrarily, the concentration of *E. coli* was higher in overlying water samples collected during the dry period compared to those collected during the wet period (Figure 4). Faecal coliforms showed the highest prevalence in both seasons compared to *E. coli* and intestinal enterococci. In addition, there were statistically significant differences in the mean concentrations of *E. coli*, faecal coliforms and intestinal enterococci ($P < 0.05$) in the overlying waters from the earthen pond in morning and afternoon samples during both seasons.

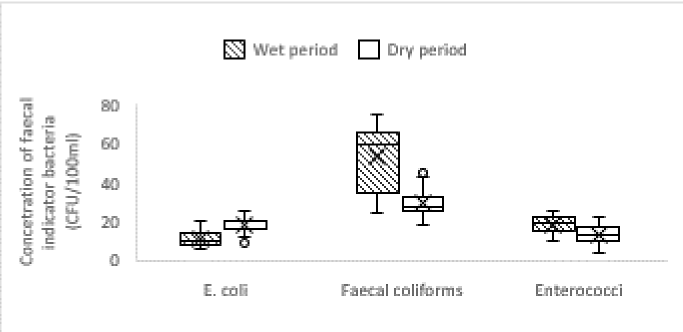

Figure 4 Boxplot of seasonality of faecal indicator bacteria in water samples collected from the earthen pond during wet and period

Physicochemical properties of the overlying water samples

The temperature of the overlying waters from the earthen pond ranged from 20 to 30 ˚C for morning samples and 21 to 30 ˚C for afternoon samples, while the amount of dissolved oxygen ranged from 7.62 to 14.25 mg/l for morning samples and 7.09 to 16.00 mg/l for afternoon samples. The pH values ranged from 6.0 to 8.2 for morning samples and 6.0 to 7.3 for afternoon samples whereas turbidity values ranged from 3.0 to 8.2 NTU for morning samples and 3.0 to 7.4 NTU for afternoon samples. The total dissolved solids ranged from 124 to 220 mg/l for morning samples and 125 to 230 mg/l for afternoon samples, while the values of electrical conductivity ranged from 176 to 360 µS/cm for morning samples and 170 to 380 µS/cm for afternoon samples. Salinity ranged from 130.0 to 142.5 ppt for morning samples and 129.6 to 145.5 ppt for afternoon samples whereas alkalinity ranged from 55 to 98 mg/l for morning samples and 30 to 99 mg/l for afternoon samples (Table 1). In addition, mean values of turbidity, pH, dissolved oxygen and alkalinity were observed to be slightly higher during wet period than dry period, whereas those of temperature, total dissolved solids and salinity were higher during dry period than wet period. However, the mean values of electrical conductivity exhibited no statistical difference in both wet and dry periods.

Relationship between faecal indicator bacteria and physicochemical properties of the overlying water samples

The relationships between the concentrations of *E. coli*, faecal coliforms, intestinal enterococci and those of the physicochemical properties of the overlying waters from the earthen pond were analysed using Pearson's correlation coefficient (Table 2). The concentration of faecal coliforms in the overlying waters showed a positive correlation with the level of turbidity of the waters ($r = 0.51$), whereas the concentration of *E. coli* in the overlying waters showed a negative correlation with turbidity ($r = -0.52$). Similarly, the concentration of *E. coli* in the overlying waters showed a negative correlation with those of faecal coliforms ($r = -0.99$). Furthermore, the values of dissolved oxygen in the overlying waters exhibited negative correlations with those of temperature ($r = -0.61$), salinity ($r = -0.50$) and total dissolved solids ($r = -0.50$), whereas the values of salinity of the overlying waters showed a positive correlation with those of total dissolved solids ($r = 0.89$).

Table 1 Physicochemical characteristics of overlying water samples from the earthen pond over the period of study

Physicochemical parameters	**Morning samples**	**Afternoon samples**
Temperature (˚C)	23.21 ± 2.95 (20.00-30.00)	26.21 ± 2.65 (21.00-30.00)
Dissolved oxygen (mg/l)	10.01 ± 2.48 (7.62-14.25)	10.43 ± 2.88 (7.09-16.00)
pH	6.75 ± 0.66 (6.00-8.20)	6.57 ± 0.47 (6.00-7.30)
Turbidity (NTU)	4.70 ± 1.69 (3.00-8.20)	4.16 ± 1.45 (3.00-7.40)
Total Dissolved Solids (mg/l)	150.50 ± 31.18 (124.00-220.00)	154.58 ± 34.68 (125.00-230.00)
Electrical conductivity $\times 10^2$ (µS/cm)	2.52 ± 0.46 (1.76-3.60)	2.46 ± 0.64 (1.70-3.80)
Salinity (ppt)	133.94 ± 3.67 (130.00-142.50)	134.18 ± 4.48 (129.60-145.50)
Alkalinity (mg/l)	88.88 ± 9.64 (55.00-98.00)	79.92 ± 21.08 (30.00-99.00)

Legend: Values are expressed as Mean ± Standard Deviation (n = 24) (Range: Minimum - Maximum)

Table 2 Significant Pearson's correlation coefficient (r) between faecal indicator bacteria and the physicochemical characteristics of the overlying water samples from the earthen pond over the period of study

	Temp.	**pH**	**SAL**	**ALK**	**TDS**	**DO**	**EC**	**TUR**	***E. coli***	**FC**	**IE**
Temp.	1.00										
pH	-0.32	1.00									
SAL	0.42	-0.24	1.00								
ALK	-0.07	0.15	0.02	1.00							
TDS	0.30	-0.23	**0.89**	-0.15	1.00						
DO	**-0.61**	0.43	**-0.50**	0.23	**-0.50**	1.00					
EC	-0.01	0.21	-0.12	-0.08	-0.07	0.10	1.00				
TUR	0.06	-0.01	0.15	-0.01	0.19	-0.03	0.25	1.00			
E. coli	-0.09	0.13	-0.14	0.08	-0.17	0.38	0.19	**-0.52**	1.00		
FC	0.07	-0.13	0.16	-0.10	0.19	-0.36	-0.18	**0.51**	**-0.99**	1.00	
IE	-0.14	0.23	-0.14	0.16	-0.06	0.28	0.08	0.15	-0.45	0.44	1.00

Legend: Temp. – Temperature; SAL – Salinity; ALK – Alkalinity; TDS – Total dissolved solids; DO – Dissolved solids; EC – Electrical conductivity; TUR – Turbidity; FC – Faecal coliforms; IE – Intestinal enterococci. Bold values signifies positive correlation.

Table 3 Skewness and Kurtosis, standard normal cumulative distribution values based on $\log_{10}$ transformed concentration of faecal indicator bacteria in the overlying waters from the earthen pond over the period of study

Faecal indicator bacteria	Skewness	Kurtosis	Standard Normal Cumulative Distribution (*Z* value)	Normal Distribution
E. coli	-0.572	-0.960	0.903	Yes
Faecal coliforms	0.571	-1.015	0.961	Yes
Intestinal enterococci	-0.745	-0.449	0.910	Yes

This study investigated the fate, occurrence and distribution pattern of *E. coli*, faecal coliforms and intestinal enterococci in overlying waters from an earthen pond containing African catfish (*Clarias gariepinus*) in Akure, Nigeria and examined the effect of seasonality and physicochemical properties of the overlying waters on the levels of the bacterial faecal pollution markers. This hazard evaluation of the sanitary quality of the overlying waters in the earthen pond is an important step in preventing and managing human waterborne and/or foodborne illnesses (such as gastroenteritis) that may occur as a result of contact with the faecally impacted overlying waters or consumption of *Clarias gariepinus* raised in such waters. The standard normal cumulative distribution values showed that levels of *E. coli*, faecal coliforms and intestinal enterococci in the overlying waters were approximately normally distributed throughout the period of study (Table 3). This is similar to the observation of **Eze and Ogbaran** (**2010**) who examined microbiological and physicochemical characteristic of fish pond water in Ughelli, Nigeria.

The mean concentration of *E. coli* in the overlying waters that appeared to be higher in samples collected in the morning compared to those collected in the afternoon may be as a result of direct faecal input from farm animals such as cattle and sheep grazing in around the pond early in the morning as well as from birds feeding on certain aquatic organisms in the pond. It may also be due to the poultry manure fed into the pond to serve as food for the *Clarias gariepinus.* The reduction in the levels of *E. coli* in the afternoon may be as a result of ultraviolet radiations from the sun which have been described as a major factor influencing the inactivation of bacteria of enteric origin in waters (**Chandran and Hatha, 2003, Olalemi *et al*., 2016**). The mean concentration of *E. coli* appeared to be higher during the dry periods compared to the wet periods and this may not be unconnected with levels of turbidity that were lower in dry period than the wet period. In this study, the levels of *E. coli* demonstrated an inverse relationship with turbidity of the overlying waters ($r = -0.52$) i.e., the lower the turbidity during dry periods the higher the levels of *E. coli.*

On the other hand, the mean concentration of faecal coliforms in the overlying waters that appeared to be higher in samples collected in the afternoon compared to those collected in the morning may likely be due to the fact that faecal coliforms consist of several species of the *Enterobacteriaceae* family that grow and proliferate at optimum temperature and this is achieved in the afternoon when temperature rises. The temperature obtained in this study ranged from 20 – 30 ˚C and this is very similar to the observation of **Ntegwu and Edema (2008)** and within the limit that supports fish productivity. The higher values of faecal coliforms during wet period compared to the dry period may be as a result of increased rainfall and increased pollution from both point and diffuse sources that include runoff from agricultural land following storm events, carrying faecal matter into the pond and increasing the level of microbial pathogens (**Wilkes *et al*., 2013**). The level of turbidity in dependent of factors such as the presence of humic substances produced through decomposition of certain organic matter and intensity of suspended soil particles (**Njoku *et al*., 2015**). In this study, faecal coliforms in the overlying waters showed a positive correlation with the level of turbidity of the overlying waters ($r = 0.51$) i.e., the higher the turbidity especially during wet periods the higher the level of faecal coliforms. This observation is in agreement with **Mallin *et al*.** (**2000**) where the authors observed that turbidity correlated positively with levels of enteric bacteria in a system of coastal creeks. Interestingly, the concentration of intestinal enterococci in the overlying waters from the earthen pond were higher in samples collected in the morning compared to those collected in the afternoon and this may likely be as a result of higher rate of faecal contamination of the overlying waters from point sources in the morning. Similar to levels of faecal coliforms, the concentrations of intestinal enterococci were higher during the wet periods compared to the dry periods. This may be due to the slightly positive correlation between intestinal enterococci and faecal coliforms ($r = 0.44$) in the overlying waters from the earthen pond.

In this study, the Pearson's correlation coefficient demonstrated that the concentration of *E. coli* in the overlying waters showed a negative correlation with those of faecal coliforms ($r = -0.99$). Dissolved oxygen is important in aquaculture and reduces as a result of increase in water temperature, respiration and organic matter decomposition by aerobic aquatic organisms (**Eze and Ogbaran, 2010**). Although, the minimum amount of dissolved oxygen for tropical fish should be 5 mg/l (**Saloom and Duncan, 2005**), the high levels of dissolved oxygen obtained in this study may be as a result of photosynthetic activities of primary producers that include a high biodiversity of plants in the earthen pond. The values of dissolved oxygen in the overlying waters from the earthen pond exhibited negative correlations with those of temperature ($r = -0.61$), salinity ($r = -0.50$) and total dissolved solids ($r = -0.50$), whereas the values of salinity of the overlying waters showed a positive correlation with those of total dissolved solids ($r = 0.89$). The values of pH observed in this study is in agreement with many previous studies emphasizing that pH between 6 and 9 are basic requirement for increased fish production (**Ehiagbonare and Ogunrinde, 2010**). **Stome and Thomforde (2003)** had also reported that the desirable range for pond pH is 6.5 – 9.5 and acceptable range is 5.5 -10.0 in order to maintain good pond productivity and fish health. It is interesting to note that the findings from this study is in agreement with **Ntengwe and Edema** (**2008)** where the authors examined the physicochemical and microbiological characteristics of water for fish production using small ponds. Although, the results from this study are in contrast with what is obtainable in surface waters such as streams or rivers where there is continuous flow of water at certain velocity and constant dilution.

CONCLUSION

The findings from this study suggests that the fate, occurrence and survival of bacterial faecal pollution markers in the earthen pond were influenced to a large extent by environmental factors and that seasonality played a major role in the levels of observed faecal pollution markers. Results also suggest that the sources of faecal contamination of the overlying waters in the earthen pond originate mainly from non-human sources and the adoption of microbial source tracking techniques would further elucidate the appropriate management and mitigation strategies to control faecal contamination of the overlying waters in the earthen pond especially for human health protection.

Acknowledgments: The authors are grateful to the Department of Microbiology, School of Science, The Federal University of Technology, Akure, Ondo State, Nigeria for providing a conducive research environment throughout this study.

REFERENCES

Abo-Elela, G. M., Elsersy, N.A., Elshenawy, M. & Abdel-Mawla, E. (2005). Microbial assessment of El-Max fish farm Egypt. *Journal of Aquatic Research*, (1): 171-184.

Anon. (2014) ISO 9308 – *1. Water Quality. Detection and Enumeration of Escherichia coli and Coliform Bacteria – Part 1: Membrane Filtration Method for waters with low bacterial background flora.* Geneva, Switzerland: International Organisation for Standardisation. https://www.iso.org/standard/55832.html

Anon. (2000) ISO 7899 – *2. Water Quality. Detection and Enumeration of Intestinal Enterococci – Part 2: Membrane Filtration Method*. Geneva, Switzerland: International Organisation for Standardisation. https://www.iso.org/standard/14854.html

Anon. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.) APHA/AWWA/WEF: Washington DC, USA.

Chandran, A. & Hatha, A. M. (2003). Survival of *Escherichia coli* in a tropical estuary. *The South Pacific Journal of Natural and Applied Sciences*, *21*(1): 41-46. https://www.doi.org/10.1071/SP03008

Doyle, M. P. & Erickson. M. C. (2006). Closing the door on the faecal coliform assay. *Microbe*, 1: 162-163.

Ehiagbonare, J. E. & Ogunrinde. Y. O. (2010). Physicochemical analysis of fish pond in Okada and its environs. *African Journal of Biotechnology*, 36: 5922-5928. http://www.doi.org/10.5897/AJB09.995

Eze, V. C. & Ogbaran, I. O. (2010). Microbiological and physicochemical characteristic of fish pond water in Ughelli, Delta State, Nigeria. *International Journal of Current Research*, 8: 082-087.

Shahidul, I. M. & Tanaka, M. (2004). Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Marine Pollution Bulletin*, *48*(7-8): 624-649. http://www.doi.org/10.1016/j.marpolbul.2003.12.004

Mallin, M. A., Williams, K. E., Esham, E. C. & Lowe, R. P. (2000). Effect of human development on bacteriological water quality in coastal watersheds. *Ecological applications*, *10*(4): 1047-1056. http://dx.doi.org/10.1890/1051-0761(*2000*)010[1047:EOHDOB]2.0.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N.,Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, 405: 1017-1024. http://dx.doi.org/10.1038/35016500

Njoku, O. E., Agwa, O. K. & Ibiene, A. A. (2015). An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria. *African Journal of Food Science*, *9*(3): 155-162. http://www.doi.org/10.5897/AJFS2014.1208

Ntengwe, F. N. & Edema. M. O. (2008). Physicochemical and microbiological characteristics of water for fish production using small ponds. *Physics and Chemistry of Earth*, 33:701-707. http://www.doi.org/10.1016/j.pce.2008.06.032

Olalemi, A., Purnell, S., Caplin, J., Ebdon, J. & Taylor, H. (2016). The application of phage-based faecal pollution markers to predict the concentration of adenoviruses in mussels (*Mytilus edulis*) and their overlying waters. *Journal of Applied Microbiology*, 121: 1152-1162. https://www.doi.org/10.1111/jam.13222

Omojowo, F. S. & Omojasola, P. F. (2013). Microbiological quality of fresh catfish raised in ponds fertilized with raw and sterilized poultry manures. *American Journal of Research Communication*, 2325-4076.

Rose, J. B., Epstein, P. R., Lipp, E. K., Sherman, B. H., Bernard, S. M. & Patz, J. A. (2001). Climate variability and change in the United States: Potential impacts on water- and foodborne diseases caused by microbiologic agents. *Environmental Health Perspectives*, *109*(2): 211-221.

Saloom, M. E. and Duncan, R. S. (2005). Low dissolved oxygen levels reduce anti-predatory behaviours of the fresh water Clam (*Corbicula fluminea*). *Fresh Water*, 50: 1233-1238. http://www.doi.org/10.1111/j.1365-2427.2005. 01396.x

Somaratne, N. & Hallas, G. (2015). Review of risk status of groundwater supply wells by tracing the source of coliform contamination. *Water*, *7*(7): 3878-3905. http://www.doi.org/10.3390/w7073878

Stone, N. M. and Thormforde, H. K. (2003). Understanding your fish pond water analysis report. University of Arkansas Co-operative Extension Printing Services, 1-4.

Wilkes, G., Brassard, J., Edge, T. A., Gannon, V., Jokinen, C. C., Jones, T. H., Neumann, N., Pintar, K. D. M., Ruecker, N., Schmidt, P. J., Sunohara, M., Topp, E. & Lapen, D. R. (2013). Bacteria, viruses, and parasites in an intermittent stream protected from and exposed to pasturing cattle: Prevalence, densities, and quantitative microbial risk assessment. *Water Research*, *47*(16): 6244-6257. http://www.doi.org/10.1016/j.watres.2013.07.041

Wuertz, S., Bambic, D., McBride, G. & Miller, W. (2011). *Quantification of pathogens and sources of microbial indicators for QMRA in recreational waters*. IWA publishing. pp. 200.