



## FAECAL COLIFORMS (FC) AND FAECAL STREPTOCOCCI (FS) RATIO AS TOOL FOR ASSESSMENT OF WATER CONTAMINATION: A CASE STUDY OF RIVER SOKOTO, NORTHWESTERN NIGERIA

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### ABSTRACT

The United States Environmental Protection Agency (USEPA) set limit of 200 faecal coliforms/100ml for bacterial contamination of surface water has frequently been exceeded due to agricultural runoff as nonpoint pollution. The study evaluated the effects of cattle rearers and or farmers, and cattle rearing on faecal contamination of water from River Sokoto. Water samples from six designated points on River Sokoto were assessed on monthly basis for faecal coliform and faecal streptococci from January to December, 2014 using faecal coliform/faecal streptococci ratio (FC/FS). The six points studied were namely P1, a point 5 metres away from farmland; P2, a point close to farmland; P3, a point close to residents along the riverside; P4, a point on stream drainage immediately from Sokoto Cement factory; P5, a point on the stream close to the river and P6, a point 5 metres away on the river. Very high mean concentrations of FC and FS were recorded at all sampling points with values exceeding surface water standards of 200 faecal coliform/100ml. While the highest mean FC value of 18,525 MPN/100ml (29.1%) was recorded at P3, the least value of 7,592 MPN/100ml (11.9%) was obtained at P2. Mean FS was recorded highest (2,350 MPN/100ml) at P5 (21.8%) and lowest (625 MPN/100ml) at P4 (5.8%). Mean FC/FS ratios of sampled water P1, P5 and P6 were < 4 (3.78, 3.95 and 3.95 respectively) indicating domestic animal contamination. However, P4 had the highest mean FC/FS ratio > 4 (11.53) indicating human contamination; P2 and P3 also had values > 4 (5.66 and 7.34 respectively) also pointing to human contamination. The FC/FS ratio identified domestic animal contamination sources but did not differentiate between domestic animal and human sources of

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contamination. Thus the limitation of its use more as a regulatory tool than a diagnostic tool in identifying contamination sources.

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**Keywords:** Animal contamination, Human contamination, Faecal coliform (FC), Faecal streptococci (FS), Contamination sources, River Sokoto.

### Contribution/ Originality

This study contributes in the existing literature by ascertaining the use of faecal coliforms and faecal streptococci ratio in the determination of sources of contamination of surface water for good water quality.

## 1. INTRODUCTION

While *E. coli* can be useful for predicting the possible presence of faecal contamination in water via spatial/temporal distribution studies [1] it does not provide any indication as to the source of pollution. In order to apply effective remediation practices for water bodies impaired by faecal contamination, the sources must be identified [2]. This has led to much research and investment in recent years into the field of source attribution, a suite of discriminatory methods which have the potential to distinguish host sources [2-6]. Physical condition assessments are also appropriate for assessing pollutant loading to rivers and streams [7].

Faecal coliform (FC), Faecal streptococci (FS), and *Escherichia coli* (*E. coli*) are bacteria living in the intestinal tracts of human and other vertebrates. They are deposited through faecal waste into the environment where they cause contamination of surface water and groundwater resulting in chronic water-borne infections. Water-borne diseases range from mild to severe and some can be deadly. Many of these diseases are transmitted by faecal contact. Water contaminated with faecal wastes are unsafe for contact recreation and drinking [8].

Bacteriological examination of water samples which is usually carried out is to estimate the level of faecal pollution and the presence of other pathogenic organisms that could be hazardous to man and animals. This exercise could be expensive and at the same time laborious. And that is why it could not be a routine practice. Bacteria in the intestines of vertebrates have majorly been used as indicators of faecal pollution. Total coliforms, faecal coliforms, and faecal streptococci have all been used as pollution indicators at various times [9, 10] Pathogenic microorganisms found in non-treated wastewater have the ability to reproduce easily due to the large amount of available nutrients, thereby affecting the environment and presenting a great risk to health [11, 12].

Watershed characteristics, land use management, and the proximity of domestic animals to streams play an important role in the severity of faecal contamination [13]. Cattle grazing increases faecal coliform in agricultural runoff compared with background faecal coliform levels [13-15]. Grazing animals mostly wild, contribute high background counts of faecal coliforms and faecal streptococci to waterways [16]. While health risk from human sewage has been well established [17] the risk associated with domestic, agricultural or wild animal faeces is less clearly defined

[18]. Outside of additional epidemiological studies at water bodies solely impacted by animal sources, a direct approach for monitoring and identifying pathogens in water would be of benefit in safeguarding public health [19, 20]. To properly assess fecal contamination of a site, it is necessary to identify the contamination source.

Geldreich, et al. [21] first suggested the use of an FC to FS ratio as a more valuable informational tool for assessing pollution sources than the use solely of FC densities. Geldreich [22] suggested that the fecal coliform/fecal streptococci ratio (FC/FS) could be used to differentiate between contamination from human (FC/FS > 4), domestic animal (FC/FS between 0.1 and 0.6), and wild animal (FC/FS < 0.1) sources. Mean FC/FS ratio has been used to characterize some sites [23, 24]. The frequency of FC/FS ratios representative of each contamination source has also been used [25]. While the FC/FS ratio is no longer recommended as a stand-alone source tracking method, traditional and/or alternative indicators, employed in tandem with this technique, may provide more useful information [26]. It is, therefore, very important to understand the assumptions and limitations associated with each faecal source tracking tool and its application.

In this vein, the effects of cattle rearing and cattle rearers/farmer on faecal contamination of water from River Sokoto were evaluated to determine the quality of the water for the safety of the users.

### 1.1. Study Area

The study area on River Sokoto is adjacent to Kalambaina industrial area of the metropolis. This area harbours factories such as cement, aluminium, foam, fertilizer and tanning industries. Residents along the bank of the river farm crops such as vegetables and use water from the river to irrigate them. People in this area also rear animals.

## 2. MATERIAL AND METHODS

Water samples from six different points namely P1, P2, P3, P4, P5 and P6 on River Sokoto were analysed on monthly basis for faecal coliform and faecal streptococci from January to December, 2014. Water samples for the analysis were collected in dry heat sterilized 100 ml amber bottles and immediately transported to the laboratory for analyses in an ice-box. All samples were analyzed for concentrations of faecal coliform and faecal streptococcus by the multiple-tube dilution technique using Most Probable Number (MPN) method [27].

In order to do faecal coliform count, ten-fold serial dilutions of water samples were prepared in distilled water. 1ml, 0.1ml and 0.01ml of each dilution were aseptically transferred to quintuplicate of 10ml sterile Lauryl tryptose broth fermentation tubes already containing inverted Durham tubes. Afterwards, the tubes were examined for accumulation of gas in the Durham tubes after 24 to 48 hours of incubation at 35°C. This concludes presumption test for coliform. Subsequently, confirmation test was carried out on all primary fermentation tubes indicating gas accumulation in Durham tubes after 24 to 48 hours. The tubes were gently shaken and one loopful of culture was transferred to a fermentation tube containing 10ml of Brilliant Green lactose broth with inverted

Durham tubes. The tubes were incubated at 35<sup>0</sup>C for 48 hours. Formation of gas in the inverted tubes confirmed coliform group. One loopful of culture from the confirmed test was taken and placed in the EC medium containing inverted Durham tubes and incubated in a water bath at 44.5<sup>0</sup>C for 24 hours. Accumulation of gas in the inverted tubes confirmed the presence of faecal coliforms. [27]

In order to do faecal streptococci count, serial dilutions of water samples were made in distilled water from 10<sup>-1</sup> to 10<sup>-3</sup>. One milliliter (1ml) and 0.1ml of each dilution were aseptically transferred to quintuplicates of 10ml aliquots of sterile Azide dextrose broth and incubated at 35<sup>0</sup>C. The tubes were examined for turbidity in 24 to 48 hours. Tubes showing turbid growth were confirmed by streaking on Aesculin-azide agar and incubated at 35<sup>0</sup>C for 24 hours. All plates having brownish-black colonies with brown halo confirmed the presence of faecal streptococci. Negative catalase test further confirmed faecal streptococci.

Standard tables for computation of Most Probable Number were used to estimate faecal coliform and faecal streptococci and the results were reported as MPN/100ml. The actual values of the bacteria in the water were obtained by multiplying by the dilution factors.

### 3. RESULTS AND DISCUSSION

Industrial effluents, domestic wastes and agricultural runoff majorly constitute channel through which surface water gets contaminated. The water bodies therefore become pathogen laden and such water becomes hazardous to man and animal. However, sewage is treated prior to discharge into streams or rivers. In order to determine the concentrations and ratios of FC and FS in River Sokoto, the industrial area adjacent to the river where farming and animal rearing also being practice was chosen.

It was shown in the results that the mean Faecal coliform (FC) and Faecal Streptococci (FS) counts were extremely high at all sampling sites and above surface water standards of 200 faecal coliforms/100 ml (Table 1; Fig. 1). Various activities such as bathing [28] human defaecation and animal defaecation observed around the sampled area might be responsible for this. Faecal bacteria are normally found in manure deposits, but there is need for a factor such as rainfall to move faecal bacteria through soil into streams and river [29].

Highest mean FC count (18,525 MPN/100ml) was recorded at P3 (29.1%) and lowest (7,592 MPN/100ml) at P2 (11.9%) as shown in Table 1 and Fig 1. Highest mean FS (2,350 MPN/100ml) was similarly obtained at P5 (21.8%) and lowest (625 MPN/100ml) at P4 (5.8%) as shown in Table 1 and Fig 1. High values of FC and FS recorded in this work was in accordance with the work done by Kulshrestha and Sharma [28] and may be as a result of various activities like defaecation (human and animal) at the sampling area. Sampled water from points P1, P5 and P6 were found to have mean FC/FS ratio less than four (3.78, 3.95 and 3.95 respectively) indicating domestic animal contamination (Table 2; Fig 2). Highest mean FC/FS ratio greater than four (11.53) was recorded at P4 which is an indication of human contamination. Stream P4 was highly polluted possibly because of the bad habit of farmers along the stream deaecating on their farmlands. The preliminary survey

of the study area has shown that farmers defecate on their farmlands. Similarly, points P2 and P3 with 5.66 and 7.34 values greater than four also indicated human contamination (Table 2; Fig 2). Conclusively, the FC/FS ratio as a tool identified sources of contamination of domestic animal but did not differentiate between domestic animal and human sources of contamination.

**Table-1.** Different Levels of Mean Concentration and Percentage of FC and FS from six sampling points on River Sokoto

Sampling Points	FC (MPN/100ml)	FS (MPN/100ml)	% FC	% FS
P1	8042	2140	12.6	19.9
P2	7592	1512	11.9	14.0
P3	18525	1997	29.1	18.5
P4	8175	623	12.9	5.8
P5	10650	2350	16.7	21.8
P6	10658	2150	16.8	20.0
Total	63642	10772	100	100

Key

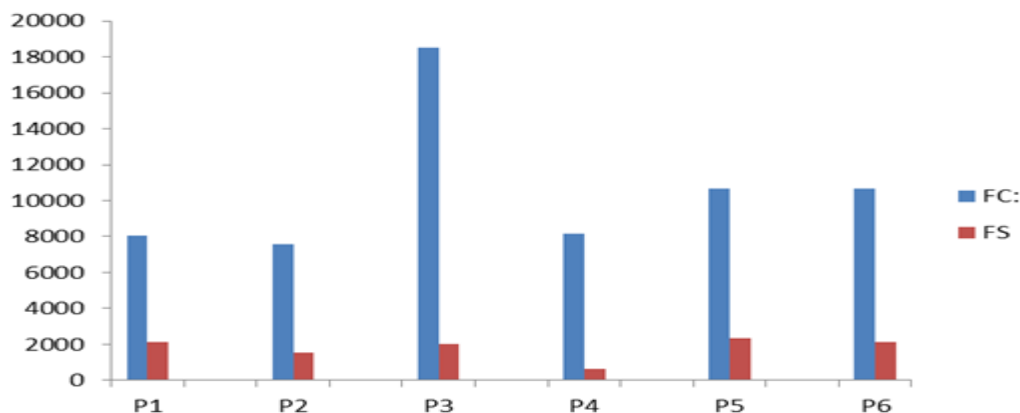
FC = Faecal coliform and FS = Faecal streptococci

**Table-2.** Different Ratios of Faecal Coliform and Faecal Streptococci at six sampling points on River Sokoto on monthly basis

Month	FC/FS Ratio at Sampling Sites					
	P1	P2	P3	P4	P5	P6
January	6.45	4.35	10.83	13.00	5.00	9.68
February	6.21	4.50	11.89	15.00	5.47	10.00
March	6.67	4.74	12.25	18.33	5.44	9.26
April	3.75	8.10	2.83	9.38	3.40	3.62
May	6.03	6.25	8.28	12.50	1.39	1.31
June	1.03	3.78	10.00	7.69	6.15	2.28
July	1.11	3.00	10.00	8.00	6.00	2.10
August	2.50	6.25	8.00	11.25	2.00	1.50
September	2.14	7.33	6.67	10.67	2.50	1.64
October	2.50	6.90	7.14	12.50	3.00	1.80
November	2.75	5.71	5.60	11.00	3.00	2.00
December	4.20	7.00	4.58	9.00	4.00	2.17
Mean	3.78	5.66	7.34	11.53	3.95	3.95

Key

FC = Faecal coliform and FS = Faecal streptococci



**Fig-1.** FC and FS (MPN/100ml) at various sampling points on River Sokoto

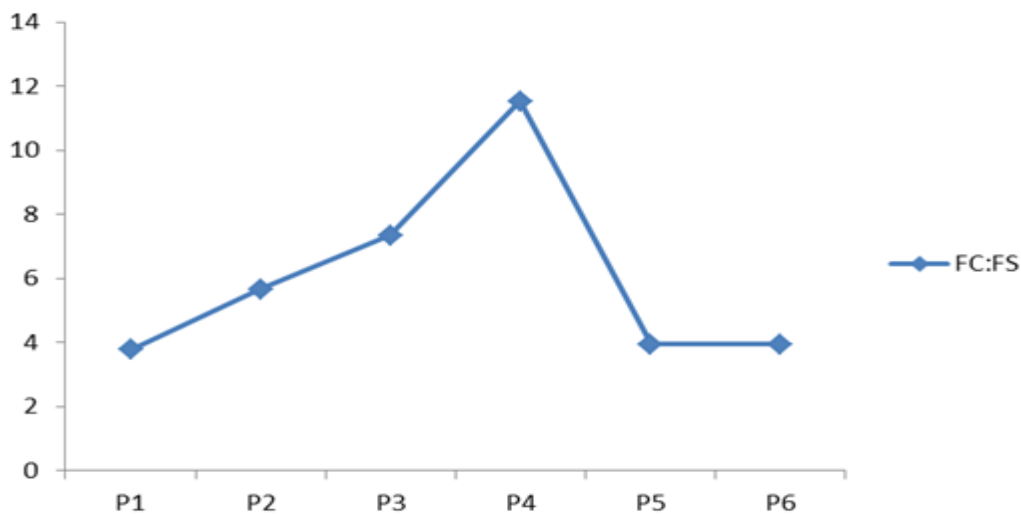


Fig-2. FC: FS Ratio at various sampling points on River Sokoto

#### 4. CONCLUSION

The results of this study, to a large extent showed high concentrations of FC (18,525 MPN/100 ml) and FS (2,350 MPN/100 ml). Very high ratio of FC: FS (11.53) was also determined in the sampled waters of River Sokoto. Thus, inadequate treatment of sewage/effluent will always result in the discharge of FC and FS far in excess of the allowable limits with the FC: FS ratio above 4.0. It should however be emphasized that the FC/FS ratio could mostly be used as a regulatory tool rather than a diagnostic tool in the identification of contamination sources.

#### 5. RECOMMENDATION

It is recommended that further research should be done on this topic to further explore sources of Faecal Coliforms and Faecal Streptococci in our environment to better evaluate the water quality of our streams and rivers.

#### REFERENCES

- [1] E. Geldreich, *Sanitary significance of faecal coliforms in the environment. Water pollution control research series, Publication #WP-20-3*. Cincinnati, OH, USA: Federal Water Pollution Control Administration, US Department of the Interior, 1966.
- [2] US EPA, "Microbial source tracking guide document," United States Environmental Protection Agency. Office of Research and Development, # EPA-600/R-05/064, Washington, D.C, USA, 2005.
- [3] T. Scott, "Use of DNA fingerprinting and novel molecular methods to identify sources of *Escherichia coli* in the environment," PhD Dissertation. University of Florida, Gainesville, 2002.
- [4] T. Scott, J. Rose, T. Jenkins, S. Farrah, and J. Lukasik, "Microbial source tracking: Current methodology and future directions," *Appl. Environ. Microbiol.*, vol. 68, pp. 5796–5803, 2002.
- [5] J. Simpson, J. Santo Domingo, and D. Reasoner, "Microbial source tracking: State of the science," *Environ Sci. Technol.*, vol. 36, pp. 5279–5288, 2002.

- [6] K. Field and M. Samadpour, "Faecal source tracking, the indicator paradigm, and managing water quality," *Wat. Res.*, vol. 41, pp. 3517–3538, 2007.
- [7] K. Abbott, "Guiding remediation of the root river, Racine, WI, USA, through correlation of stream bank condition, river morphology and infrastructure surveys to chemical and microbial source tracking," MS Thesis, University of Surrey, United Kingdom, 2008.
- [8] M. W. McBroom, C. Mingteh, and C. Wells, "Bacteriological water quality of forested and pastured streams receiving land-applied poultry litter," Faculty Publications. Paper No. 8, 2003.
- [9] K. O. Isobe, M. Tarao, M. P. Zakaria, N. H. Chiem, L. Y. Minh, and H. Takada, "Quantitative application of fecal sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong Delta, Vietnam," *Environ Sci. Technol.*, vol. 36, pp. 4497–4507, 2002.
- [10] J. E. Park, T. S. Ahn, H. J. Lee, and Y. O. Lee, "Comparison of total and faecal coliforms as faecal indicator in eutrophicated surface water," *Water Sci. Technol.*, vol. 54, pp. 185 – 190, 2006.
- [11] D. Pusch, D. Y. Oh, S. Wolf, R. Dumke, U. Schröter-Bobsin, M. Höne, I. Röske, and E. Schreier, "Detection of enteric viruses and bacterial indicators in German environmental waters," *Arch. Virol.*, vol. 150, pp. 929-947, 2005.
- [12] K. A. Gilbride, D. Y. Lee, and L. A. Beaudette, "Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control," *J. Microbiol. Method*, vol. 66, pp. 1-20, 2006.
- [13] A. R. Tiedemann, D. A. Higgins, T. M. Quigley, H. R. Sanderson, and C. C. Bohn, "Bacterial water quality responses to four grazing strategies – comparisons with oregon standards," *J. Environ. Qual.*, vol. 17, pp. 492-498, 1988.
- [14] H. L. Gary, S. R. Johnson, and S. L. Ponce, "Cattle grazing impact on surface water quality in a Colorado front range stream," *J. Soil Water Conserv.*, vol. 38, pp. 124-128, 1983.
- [15] A. Bernhard and K. Field, "A PCR assay to discriminate human and ruminant feces on the basis of host differences in bacteroides-Prevotella genes encoding 16S rRNA," *Appl. Environ. Microbiol.*, vol. 66, pp. 4571–4574, 2000.
- [16] V. C. Obuseng, M. Moshoeshe, and F. Nareetsile, "Bile acids as specific faecal pollution indicators in water and sediments," *European Scientific Journal*, vol. 9, pp. 273-286, 2013.
- [17] T. Wade, R. Calderon, E. Sams, M. Beach, K. Brenner, A. Williams, and A. Dufour, "Rapidly measured indicators of water quality are predictive of swimming-associated gastrointestinal illness," *Environ. Health. Perspect.*, vol. 114, pp. 24–28, 2006.
- [18] USEPA, "Proceedings of the Expert's Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria," Washington, D.C., United States Environmental Protection Agency. #EPA-823/ R-07/006, 2007.
- [19] I. Bertrand and J. Schwartzbrod, "Detection and genotyping of *Giardia duodenalis* in wastewater: Relation between assemblages and faecal contamination origin," *Wat. Res.*, vol. 41, pp. 3675–3682, 2007.

- [20] N. Ruecker, S. Braithwaite, E. Topp, T. Edge, D. Lapen, G. Wilkes, W. Robertson, D. Medeiros, C. Sensen, and N. Neumann, "Tracking host sources of *Cryptosporidium* spp. in raw water for improved health risk assessment," *Appl. Environ. Microbiol.*, vol. 73, pp. 3945–3957, 2007.
- [21] E. E. Geldreich, H. F. Clark, and C. B. Huff, "A study of pollution indicators in a waste stabilization pond," *Journal of Water Pollution Control Federation*, vol. 36, pp. 1372-1379, 1964.
- [22] E. E. Geldreich, "Faecal coliform and faecal streptococcus density relationship in waste discharges and receiving waters," *Crit. Rev. Environ. Control*, vol. 6, pp. 349-369, 1976.
- [23] M. D. Jawson, L. F. Elliott, K. E. Saxton, and D. H. Fortier, "The effect of cattle grazing on indicator bacteria in runoff from a Pacific Northwest watershed," *J. Environ. Qual.*, vol. 11, pp. 621-627, 1982.
- [24] S. Sharma, J. Singh, S. Nar, and S. Devi, "Harnessing bacterial Indicators along with physicochemical parameters to assess pollution in the Ganges river," *Journal of Pure and Applied Microbiology*, vol. 7, pp. 1409-1415, 2012.
- [25] W. Weaver, J. Entry, and A. Graves, "Numbers of faecal streptococci and *Escherichia coli* in fresh and dry cattle, horse, and sheep manure," *Canadian J. Microbiol.*, vol. 51, pp. 847–851, 2005.
- [26] O. Savitcheva and S. Okabe, "Alternative indicators of faecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives," *Wat. Res.*, vol. 40, pp. 2463–2476, 2006.
- [27] APHA, *Standard methods for the examination of water and wastewater*, 20th ed. Washington, D.C: American Public Health Association, 1998.
- [28] H. Kulshrestha and S. Sharma, "Impact of mass bathing during Ardhkumbh on water quality status of river Ganga," *J. Environ. Biol.*, vol. 27, pp. 437-440, 2006.
- [29] J. Singh, S. Sharma, S. Nara, and S. Devi, "Harnessing bacterial indicators along with physicochemical parameters to assess pollution in the Ganges River," *J. Pure Appl. Microbio.*, vol. 7, pp. 1409-1415, 2013.

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