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Evaluation of Parasites as Veritable Indicators of Faecal *Escherichia coli* Contamination of Surface Waters: A Case Study of Adada River, Enugu State, Nigeria

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Abstract There is need for alternate quick-search of pathogens' distribution in community water sources, instead of the cumbersome "*Escherichia coli* detection." Parasites were evaluated as possible indicators of faecal contamination of surface waters, using Adada River in Nigeria as case-study. Seventeen parasites of medical importance (in dry season) and 13 (rainy season) isolated from the river (at measured geographical coordinates) were analyzed for their quality and quantity and connected with the distribution of the river's isolated *Escherichia coli*, using Pearson's Correlation Analysis. The 17 parasites consist of: *Taenia* sp, *E. coli, E. histolytica, B coli, Cercaria/miracidia, S mansoni, S haematobium, A. lumbricoides, Giardia* sp, hookworm, T. *trichiura, S. stercoralis*, I. *butschlii*, C. *mesnili*, E. *nana*, B. *hominis and* H. *diminuta;* while the 13 consist of*: Taenia* sp,. *E. coli, E. histolytica, B. coli, S. mansoni, A. lumbricoides, Giardia* sp, hookworm, *T. trichiura*, S. *stercoralis, I. butschlii, C. mesnili and E. vermicularis.* Biological index, using the Pearson's Correlation Analysis, revealed significant correlation relationship of *Escherichia coli* with the presence of *I butschlii* in the dry season), non in rainy season. From the evaluation, potential index analysis indicated that *I. butschlii* could serve as markers for *Escherichia coli* faecal bacteria indicator, and possible index for future monitoring of the potability of such surface waters. The methodology is straight forward, cost effective, less cumbersome than other currently existing approaches.

Keywords: *pearson correlation analysis, Enterococcus, water analysis, Kirby-Bauer Disc Diffusion, Iron, total hardness*

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1. Introduction

According to Amadi *et al*. [1], water is the common name assigned to the liquid state of a naturally occurring hydrogen-oxygen compound with the molecular formula H_2O, chemical structure of H-O-H and IUPAC name of hydrogen/hydroxonium ion (depending on the oxidation state); the solid state is known as ice, while the gaseous state is called steam. It is the most important natural resource, second only to air. Also, of the many substances on earth, it is one of the most important for maintenances of life [2]. Despite occupying more than 75% of the earth surface, it is hardly found naturally in potable form owning to many biological, physical and chemical pollutants [3]. So, it must be treated before consumption, and must be certified potable before consumption. There are standard methods laid down by the World Health Organization (WHO) for this as well as various nationals: United State Environmental Protection agency (USEPA), Nigerian Standard Organization(NSO), National Agency for Food and Drugs Administration and Control (NAFDAC), etc; biologically, it is by detection of *Escherichia coli* as evidence of faecal pollution, absence of which mean potability. The first of such method was the detection of *Bacillus coli*, later renamed the *Escherichia coli* by Castellani and Chalmers [4] in honour of Dr. Escherich, the initiator. But over many decades, this method has been found wanting as a universal indicator due to some limitations. Quest for other mean therefore ensued. Currently, there are six emerging microbiological methods as follows: Fast detection using chromogenic substances, Application of monoclonal and polyclonal antibodies, Immuno-magnetic separation, and Gene sequencing methods, Microarray and Biosensor [5], but all these

methods have the problems of costs: affordability, speed, high-technology, etc. that will definitely not be easy for routine laboratory, and to developing and under-developed countries where more than 65% of the world population reside. Hence, though still undergoing development, they are currently not an acceptable universal indicator of faecal contamination in quest.

Besides, Ashbolt *et al*. [5] pointed out that water sanitary engineers require simple and rapid methods for the detection of faecal indicator bacteria which is oblivion of the cumbersome culture and high-tech. Notwithstanding, it has long been recognized that artificial culture media lead to only a very small fraction (0.01–1%) of the viable bacteria present being detected [6]. Further, these authors agreed with Vivian [7]'s suggestion that using more than one methods of determining the degree of sewage pollution would be prudent and advantageous. And, also particularly in support of Ashbolt *et al [5]*'s suggestion that substances can be used to avoid the need for isolation of pure culture and confirmatory tests, such as the use of faecal sterol biomarkers Therefore, the uses of alternative indicators offer a new way to distinguish sources of faecal contamination and monitor river health, as suggested by Leeming *et al*. [8]; which could be in conjunction with existing microbiological indicators or in isolation. Most importantly, the quest for a universal faecal indicator of human biotic origin as a microbial risk assessment in potable, agricultural or recreational water must most importantly put into consideration, factors like cost, affordability and sustainability. This is in lieu of the fact that it must not only be something within the reach of routine laboratories, but also those of 2nd and 3rd World countries where greater than 65% of the world population resides. That was one of the reasons why for more than two centuries now, the ability to reach a consensus on the matter has been an enigma.

It was on this premises for a cost-effective means, and on the tripod that certain elements, ions, and parasites has been associated with the distribution of certain bacteria and parasites in water [1,9,10] that this project was borne. To the best of our knowledge, such association has never been linked to any faecal bacteria. This project therefore aimed to assess parasites as veritable indicators of faecal *Escherichia coli* contamination of surface water, as a means of qualitative microbial risk assessment factor. This study then brought forth a case of Adada River, used untreated by more than 16 communities of more than one million populations in Nsukka area of Eastern Nigeria. There is need to assess its microbial risk factors, using the river as a case study. The specific objective of this study were to (i) select six sampling sites/stations according to vegetation's cover and river use along Adada River water flow, and use digital-phone-compass to measure the geographical coordinates of the selected sites; (ii) examine Adada River and determine its distribution, types and quantity of parasites of human biotic origin in six stations in rainy and dry seasons; (iii) determine the distribution and quantity of *Escherichia coli* faecal bacteria indicator in the six stations; (iv) determine whether these parasites of human biotic origin can be connected with the distribution of the *Escherichia coli*, using Pearson's Correlation Analysis; (v) if so, such properties connected will be assessed further for true affinity and avidity using Pearson's Possible Paired Correlation Analysis;

2. Null Hypothesis

Ho_1: Correlation does not exist between *Escherichia coli* and any parasite of human biotic origin in the rainy and dry seasons

Ho_2: No correlation between all possible pairs of parasites in the rainy and dry seasons

3. Materials and Methods

3.1. Sampling Site

Water samples were collected in duplicates at six different sites (stations 1- 6) along the Aku bank of the Adada River flow, at about six kilometers from Aku, a village located at Igbo-Etiti Local Government Area in Enugu State of Nigeria on $6^0$40"N and $7^0$18"E on the geographical map ($6^0$42'7"N $7^0$19'56"E on Infinix Hot 7 Smartphone-compass, measured at the Post Office). The sampling areas were selected according to the vegetation's cover and river use as follows:

Station 1 is geographical coordinate: $6^0$42'2"N $7^0$17'19"E (Infinix Hot 7 Smartphone-compass). It was upstream, towards the water source where there is limited human activity; the vegetation was originally rainforest, but in the distant past slightly disturbed by water tanker drivers that created a part to the river from where they were then fetching water they sold to the local communities.

Station 2 is geographical coordinate $6^0$44'20"N $7^0$16'50"E. It was ways downstream from station1, at the beginning of where the river water was diverted for an ongoing Adada River Dam construction; the vegetation is only still slightly virgin, and disturbed by Fulani herdsmen that occasionally graze cattle along the bank of the river, and it is the camping site of the construction workers.

Station 3 is geographical coordinate $6^0$44'25"N $7^0$16'49"E. It was about the foot of the embankment where the Adada River water was diverted for the ongoing construction of the dam, and heavily disturbed by the ongoing construction work, and tanker driver that come to fetch water they sell to the local communities and beyond.

Station 4 is geographical coordinate $6^0$44'17"N $7^0$16'37"E. It was down-stream, a bit from the tail of the dam proper where from far and wide there are human activities, such as washing of clothes, soaking of cassava for fermentation, swimming, picnics, farmland at both banks, and point where Fulani herdsmen occasionally bring their cattle to drink water.

Station 5 is geographical coordinate $6^0$44'13"N $7^0$16'32"E. It was the temporary run-off point downstream for the diverted water flow from the dam, and also heavily disturbed on both banks of the river by heavy human activities, such as farmlands, etc.

Station 6 is geographical coordinate $6^0$44'11"N $7^0$16'29"E. It was a little way downstream from station 5, before a former animal husbandry established by Eastern Nigeria Development Corporation (ENDC/ADP), also where Adada Secondary School [site of the re-proposed satellite Adada Campus of Enugu State University of Science and Technology(ESUT)] students fetches water, bath, wash clothes, swim, fishing, etc.

All the stations environments were formerly typical rainforest, gradually converted as described above into agricultural, grazing, fishing, recreational, and now the N2.6 billion Naira dam in progress. The climate is typical tropical rainforest, with average temperature of 25°C (range18°C – 37°C) and average rainfall of 156.89mmHg.

3.2. Determination of Geographical Coordinates

The digital phone-compass App was downloaded and installed into the "Infinix Hotspot 7" smart phone from the internet. At the precisely stated location or spot, the smart phone was put on and the compass icons clicked on, and waited for the App to booth. As soon as the phone-compass App booths, it brings out the precise geographical coordinate of the spot, this was then read off and recorded.

3.3. Collection of Water for Analysis

At each of the six sampling stations, water samples were collected in duplicates at some distance from the shore with clean pre-sterilized 500-ml bottles with stoppers. The bottles were aseptically opened five centimeters (5cm) below the water surface, rinsed with the first set of water samples, then filled with the required water sample, and the bottle aseptically closed. These were done between 10.00am to 12.00pm (late morning to early afternoon by which human activities have resumed), and done in two different sampling periods, June 13, 2016 (rainy season) and February 27, 2017 (dry season), precisely at the geographical coordinates. The samples were transported to the laboratory under ice and stored at about 4^0C until they were ready for analysis.. A total of 24 water samples were collected (6 stations x duplicates samples = 12 x 2 seasons = 24 total). Total of 26 sample analysis were done (24 water samples plus control x 2 seasons).

3.4. Isolation, Detection, Identification and Enumeration of Parasites

Parasites were isolated using a slightly adjusted Finch, (2008) method. They were microscopically detected and identified in each water samples by the various morphological characteristics of their potentials (ova, cysts, larvae, oocyst and adults) as summarized in Table 1. Stoll counting technique for parasites as described by Cheesbrough [11] for fluid or water specimens was adopted and used, except that normal saline was used in the dilutions.

Table 1. Summary of the descriptive identification of the isolated parasites

Parasites	Descriptive identification
S. stercoralis	Microscopically identified as large, unsheathed, active mobile rhabditiform larva, measuring about 250*u*m x 16um, showing characteristics large bulbed oesophagus, differentiated from hookworm larvae by shorter mouth cavity
Hookworms (identified as ova and larvae)	Ova, which are colourless, thin-shell (which appear as black line around an ovum), oval in shape and about 65 x 40*u*m in diameter, usually segmented with 4-8 cell-stage, and distinguished from the ova of *Trichostrongylus* spp, *Ternidens deminitus*, *S. fuelleborni* and *Oesophagostum* spp; and Larvae, distinguished from *S. stercoralis* larvae by its characteristic deeper buccal cavity.
T. trichiura	Identified by a characteristic yellow-brown, barrel-shaped ovum, about 25–50*u*m in size with colourless protruding mucoid plug at each end.
A. lumbricoides	Identified by decorticated, fertilized and unfertilized eggs: fertilized eggs that were about 50–70*u*m x 30–50*u*m in length and breath, respectively, yellow-brown in colour, oval in shape, and containing a central granular mass covered by a shell with uneven albuminous coat; unfertilized eggs which were darker in colour and contains a central mass with larger granules that is covered by a thinner wall with more albuminous coat, and more elongated (90 x 45*u*m in size) than the fertilized one
S. mansoni	Identified by eggs that were oval in shape, pale yellow-brown in colour, and measuring about 60 – 150*u*m with, at times, fully visualizable internal fully developed miracidium, and with the characteristic single lateral spine.
S. haematobium	Identified by large eggs (145-45*u*m in length and breath, respectively), that are pale yellow-brown in colour and oval in shape, each containing a fully developed miracidium and the characteristic single terminal spine.
Taenia spp	Identified by round eggs of about 30–40*u*m in diameter, containing barely visible onchosphere that is surrounded by thick, brown radially striated wall.
E. vermicularis	identified by colourless eggs measuring about 30–50*u*m that were oval in shape and flattened on one side, and containing barely visualizable larva.
E. histolytica (identified by trophozoites and cysts)	Trophozoites with active unidirectional amoeboid movement, unit nucleus that has barely discernible central karyosome, and measuring about 20–25*u*m in size; and Cysts that were round (10–15*u*m in diameter), containing 1–4 nuclei with barely discernible central karyosome, and having some chromatoid bodies in immature ones, and distinguished from larger *E.* coli (15–30*u*m in size), with 1–8 nuclei, and at times needle-like chromatoid body.
I. butschlii	Identified by small cysts (9-15*u*m in sizes) with only one nucleus that has compact mass of glycogen inclusion and no chromatoid body
G. lamblia (identified by its trophozoites and cysts)	Trophozoites were small, pear-shaped flagellates (12-15 x 5-9*u*m in length and breath, respectively) with rapid tumbling and spinning motions, having some discernible structures like four pairs of flagella, two axonemes, two discernible nuclei, large concave sucking disc located on the ventral surface, one or two curved median bodies; and by Cysts that are also very small (7-12*u*m in diameter) with some discernible internal structures in the saline medium (e.g. four nuclei, remain of flagella, axonemes and median body. Both cysts and trophozoites were carefully differentiated from those of other flagellates of medical importance: *C. mesnili*, *Retortamonas intestinalis*, E. hominis and *Pentatrichomonas hominis* by their trophozoites with single nucleus, fewer flagella, shapes and smaller sized cysts that have not the characteristic appearances of *G. lamblia* (i.e. remains of flagella, four nuclei grouped to one end, and peculiar shape).
C. mesnili	Identified by lemon-shaped cysts that are smaller in size (<8um) than those of other medical important flagellates (*R. intestinalis* = pear-shaped), and containing no remains of internal structure like *G. lamblia.*
B. coli (identified by trophozoites and cysts)	Trophozoites seen as large ciliates (50-200*u*m x 40-70*u*m in length and breath, respectively), with rapidly revolving movement, well discernible macro-nucleus, two contractile vacuoles, discernible cilia beating at the region of the funnel-shaped cytostome when carefully focused; and by the round and thick-walled cysts that are also large (50-60*u*m in diameter) with discernible cilia lining the wall of the cyst
B. .hominis	Identified as small round protozoa (about 15-30*u*m in size), with peripheral cytoplasm, a central vacuole, no discernible nucleus even at x40, and a granule which form a ring around the periphery

3.5. Bacteriological Study

This was done by the "Standard methods of bacteriological analysis of water," after Cheesbrough [11] and as specified by Ashbolt *et al* [5] for thermophilic *Escherichia coli* (and confirmed by molecular tests using 16s rRNA gene.

3.6. Statistical Analysis

Results obtained in the parasitic analysis of the river were summarized in tables. *Pearson's Correlation Relationship* Analysis was used to determine correlation of *E. coli* with the distribution of the isolated parasites. The statistical analysis was done in two bits of the seasons as discernable from Table 2 and Table 3 below. Null hypothesis (H_{o1}) is: Correlation does not exist between *E. coli* and any parasites of human biotic origin in the rainy and dry seasons; Ho_2: No correlation between all possible pairs of parasites in the rainy and dry seasons. First, the Pearson's correlation matrix (Pearson r) was determined; this interprets both signs (+ or -) and magnitude; the closer the values to one, the greater the affinity. Analyzed was further done for the statistical correlation significance (p-value) and tabled below (Table 4 and Table 5). Thus, the p-value of this correlated Pearson's correlation coefficients was ascertained. P-value less than 0.05 imply statistical correlation significance, and therefore reject the hypothesis (H_{o1}), to imply significant specified correlation relationship. Pearson's Possible Paired Correlation Analysis was used to determine correlation of the parasites to each other (Table 5), in the same manner as the correlation analysis.

4. Results

Of all the 17 parasites (Table 2) detected in the dry season and analyzed, *I. butschlii* showed statistically significant positive correlation relationship to the distribution of *E. coli* in the dry seasons (Table 4).

Of all the 13 parasites (Table 3) detected in the rainy season and analyzed, none showed statistically significant positive correlation relationship to the distribution of *E. coli* in the rainy seasons (Table 6).

Result in Table 4 of the significant test of Pearson's Correlation relationship showed that *I butschlii* exhibited significant positive correlation relationship ($r = 0.8783$) with the distribution of *E. coli* in the dry season ($p < 0.05$).

However, surprisingly, *C. mesnili*, *B coli* and *Entamoeba coli* that exhibited very strong significant positive paired correlation relationship to *I butschlii* (Table 5) were not found to have had significant Pearson's Correlation Relationship to *Escherichia coli.*

Result in Table 6 of the significant test of Pearson's Correlation relationship showed that no parasite exhibited significant positive correlation relationship with the distribution of *E. coli* in the rainy season ($p < 0.05$).

Isolated *E. coli* molecular confirmation is with 99% identity to *Escherichia coli* strain JJ1897 complete genome NCBI accession number CP013837).

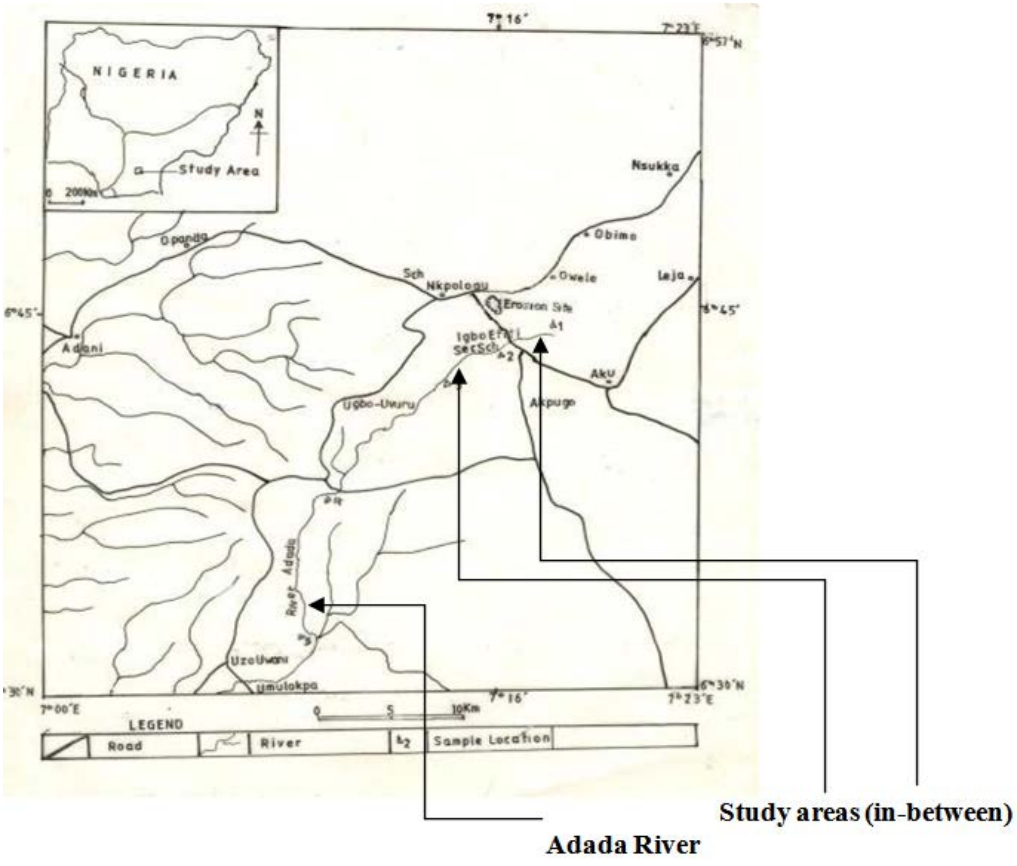

Figure 1. Map of Nigeria showing Enugu State and Adada River and study areas (Source: Nweze, N.K. (2009). Algal diversity of Adada River, Nigeria. I. Chlorophyta (green algae) and Euglenophyta (euglenoids). Available at: https://www.researchgate.net/figure/Map-of-the-study-area-showing-Adada-river-and-sampling-locations_fig1_216676438)

Table 2 showed the distribution of *Escherichia coli* with detected parasites (biological property) in quality and quantity in the six stations (1- 6) in duplicates (A, B) along the Adada River water flow in the dry seasons.

Table 2. *E coli* faecal bacteria indicators' distribution with parasites (biological indices) in Adada River in the dry season

	Stations	**1A**	**1B**	**2A**	**2B**	**3A**	**3B**	**4A**	**4B**	**5A**	**5B**	**6A**	**6B**
Diversity (/ml)	**Abundance/%**												
Taenia sp	$3.35x10^4$/25.5%	$5.0x10^2$	$2.5x10^3$	$1.5x10^3$	$1.5x10^3$	$4.0x10^3$	$4.5x10^3$	0	$2.5x10^3$	$1.0x10^3$	$2.5x10^3$	$4.0x10^3$	$9.0x10^3$
Entamoeba. coli	$1.25x10^4$/9.5%	$5.0x10^2$	0	0	0	$5.0x10^2$	0	$5.0x10^3$	$1.0x10^3$	$3.0x10^3$	0	$2.0x10^3$	$5.0x10^2$
E. histolytica	$1.7x10^4$/12.9%	$1.0x10^3$	0	0	0	$4.5x10^3$	0	$3.5x10^3$	$5.0x10^2$	$2.0x10^3$	0	$5.5x10^3$	0
B. coli	$2.5x10^3$/1.9%	0	0	0	0	0	0	0	0	0	0	$2.5x10^3$	0
Cercaria/Miracidia	$3.0x10^3$/2.3%	0	0	0	0	0	0	0	0	$5.0x10^2$	0	$2.0x10^3$	$5.0x10^2$
S. mansoni	$6.5x10^3$/5.1%	0	$1.0x10^3$			$5.0x10^2$	$5.0x10^2$	$1.5x10^3$	$5.0x10^2$	$1.0x10^3$	0	$5.0x10^2$	$1.0x10^3$
S. haematobium	$7.0x10^3$/5.3%	0	$1.5x10^3$	$5.0x10^2$	$3.0x10^3$	0	0	$5.0x10^2$	0	$5.0x10^2$	$1.0x10^3$	0	0
A. lumbricoides	$5.0x10^3$/3.8%	$5.0x10^2$	$1.0x10^3$	0	0	0	$5.0x10^2$	0	0	0	$1.5x10^3$	0	$1.5x10^3$
Giardia lamblia	$2.3x10^4$/17.5%	0	0	$1.0x10^3$	0	$4.0x10^3$	$3.5x10^3$	0	$2.5x10^3$	$1.5x10^3$	$3.0x10^3$	$6.0x10^3$	$1.5x10^3$
Hookworm	$1.0x10^4$/7.6%	0	$5.0x10^2$	$5.0x10^3$	$5.0x10^2$	0	$1.0x10^3$	$5.0x10^2$	$1.0x10^3$	$5.0x10^2$	0	$5.0x10^2$	$5.0x10^2$
T. trichiura	$5.0x10^2$/0.4%	0	0	0	0	0	0	0	0	0	0	$5.0x10^2$	0
S. stercoralis	$5.0x10^2$/0.4%	0	0	$5.0x10^2$	0	0	0	0	0	0	0	0	0
I. butschlii	$2.0x10^3$/1.5%	0	0	0	$5.0x10^2$	$1.0x10^3$	0	0	0	0	0	$5.0x10^2$	0
C. *mesnili*	$1.0x10^3$/0.8%	0	0	0	0	0	$1.0x10^3$	0	0	0	0	0	0
E. nana	$5.0x10^3$/3.8%	0	$5.0x10^3$	0	0	0	0	0	0	0	0	0	0
B. hominis	$5.0x10^2$/0.4%	0	0	0	0	0	0	0	0	0	0	$5.0x10^2$	0
H. diminuta	$2.0x10^3$/1.5%	0	0	0	0	0	0	0	0	0	0	0	$2.0x10^3$
Total counted	**$1.315x10^5$**	**$2.5x10^3$**	**$1.15x10^4$**	**$8.5x10^4$**	**$5.5x10^3$**	**$1.45x10^4$**	**$1.1x10^4$**	**$1.1x10^4$**	**$8.0x10^3$**	**$1.0x10^4$**	**$8.0x10^3$**	**$2.45x10^4$**	**$1.65x10^4$**
Total/Mean in stations	**$6.575x10^4$**	**$7.0x10^3$**		**$7.0x10^3$**		**$1.275x10^4$**		**$9.5x10^3$**		**$9.0x10^3$**		**$20.5x10^4$**	

Keys: WHO STD = World Health Organization Standard; Mg/ L = milligram/litre; NAD = No Any Data; gm = gram; ml = milliliter; Qty = Quantity; 1 – 6 = Stations; A and B = Duplicates.

Table 3 showed the distribution of *Escherichia coli* with detected parasites (biological property) in quality and quantity in the six stations (1- 6) in duplicates (A, B) along the Adada River water flow in the rainy seasons.

Table 3. *E coli* faecal bacteria indicators' distribution with parasites (biological indices) in Adada River in the rainy season

	Stations	**1A**	**1B**	**2A**	**2B**	**3A**	**3B**	**4A**	**4B**	**5A**	**5B**	**6A**	**6B**
Diversity (/ml)	**Abundance/%**												
Taenia sp	$4.5x10^4$/23.1%	$1.0x10^3$	$8.0x10^3$	$1.0x10^3$	$1.0x10^3$	$1.0x10^4$	$4.0x10^3$	$6.0x10^3$	$2.0x10^3$	$1.0x10^3$	$2.0x10^3$	$4.0x10^3$	$5.0x10^3$
Entamoeba. coli	$6.0x10^3$/3.1%	0	$3.0x10^3$	$1.0x10^3$	0	0	$1.0x10^3$	0	0	0	0	0	$1.0x10^3$
E. histolytica	$6.6x10^4$/33.9%%	$1.1x10^4$	$8.0x10^3$	$5.0x10^3$	$1.4x10^4$	0	$1.5x10^4$	$2.0x10^3$	$2.0x10^3$	$3.0x10^3$	$2.0x10^3$	$4.0x10^3$	0
Balantidium coli	$2.0x10^3$/1.0%	$2.0x10^3$	0	0	0	0	0	0	0	0	0	0	0
Schistosoma. m*ansoni*	$1.1x10^4$/5.6%	$1.0x10^3$	$1.0x10^3$	0	0	0	0	$1.0x10^3$	$1.0x10^3$	$1.0x10^3$	0	$4.0x10^3$	$2.0x10^3$
Ascaris. lumbricoides	$5.0x10^3$/2.6%	0	$1.0x10^3$	$1.0x10^3$	$1.0x10^3$	0	0	0	0	$2.0x10^3$	0	0	0
Giardia sp	$2.3x10^4$/11.8%	0	$2.0x10^3$	$2.0x10^3$	$2.0x10^3$	0	$3.0x10^3$	0	$5.0x10^3$	$5.0x10^3$	$1.0x10^3$	$2.0x10^3$	$1.0x10^3$
Hookworm	$2.5x10^4$/12.8%	0	0	$2.0x10^3$	11000	$4.0x10^3$	$2.0x10^3$	$1.0x10^3$	$2.0x10^3$	$1.0x10^3$	0	$2.0x10^3$	0
Trichuris trichiura	$1.0x10^3$/0.5%	0	0	0	0	0	0	0	$1.0x10^3$	0	0	0	0
Enterobius . *vermicularis*	$1.0x10^3$/0.5%	0	0	0	0	0	0	0	0	0	0	$1.0x10^3$	0
Strongyloides. stercoralis	$3.0x10^3$/1.5%	0	0	0	0	0	0	0	0	0	0	0	$3.0x10^3$
Iodamoeba *butschlii*	$6.0x10^3$/3.1%	0	$6.0x10^3$	0	0	0	0	0	0	0	0	0	0
Chilomastix. mesnili	$1.0x10^3$/0.5%	0	0	0	0	$1.0x10^3$	0	0	0	0	0	0	0
Total counted	**$1.95x10^5$**	**$1.5x10^4$**	**$2.9x10^4$**	**1.2x104**	**.9x10^4**	**$1.5x10^4$**	**$2.5x10^4$**	**$1.0x10^4$**	**$1.3x10^4$**	**$1.3x10^4$**	**$5.0x10^3$**	**$1.7x10^4$**	**$1.2x10^4$**
Total/Mean in stations/ml	**$9.75x10^4$**	**$2.2x10^4$**		**$2.05x10^4$**		**$2.0x10^4$**		**$1.15x10^4$**		**$9.0x10^3$**		**$1.45x10^4$**	

Keys: WHO STD = World Health Organization Standard; Mg/ L = milligram/litre; NAD = No Any Data; gm = gram; ml = milliliter; Qty = Quantity; 1 – 6 = Stations; A and B = Duplicates.

The significant test result in Table 4 showed that the correlation of *I butschlii* to *E. coli* was strongly significant in the dry season, while other parasites were not significantly correlated. Surprising, *C. mesnili*, *B coli* and *E coli* that exhibited very strong significant positive paired correlation relationship to *I butschlii* (Table 5) were not found to have had significant Pearson's Correlation Relationship to *Escherichia coli*.

Table 4. Significant test of Pearson's Correlation Relationship between *E coli* and seventeen parasites in dry season

Variables (/ml or gm)	Pearson r	95% CI	R^2	P value	P value summary	n
E.. coli vs. *Taenia* sp	0.3355	-0.6542 to 0.9016	0.1125	0.5157	ns	6
E. coli vs. *Entamoeba. coli*	-0.3421	-0.903 to 0.6499	0.117	0.5069	ns	6
E. coli vs. *E. histolytica*	0.378	-0.6254 to 0.9103	0.1429	0.4601	ns	6
E. coli vs. *B. coli*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
E. coli vs. Cercaria/miracidia	-0.2449	-0.8813 to 0.7072	0.06	0.6399	ns	6
E. coli vs. *S. mansoni*	-0.06143	-0.8315 to 0.7895	0.003774	0.908	ns	6
E coli vs. *S. haematobium*	-0.43	-0.9204 to 0.5861	0.1849	0.3947	ns	6
E. coli vs. *A. lumbricoides*	-0.2169	-0.8745 to 0.7217	0.04706	0.6797	ns	6
E. coli vs. *Giardia* sp	0.5579	-0.4635 to 0.9427	0.3113	0.25	ns	6
E. coli vs. Hookworm	-0.1706	-0.8627 to 0.744	0.02909	0.7466	ns	6
E. coli vs. *T. trichiura*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
E. coli vs. *S. stercoralis*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
E. coli vs. ***I. butschlii***	**0.8783**	0.2324 to 0.9866	0.7714	0.0213	**ss**	6
E. coli vs. C. *mesnili*		vertical line			n.a	6
E. coli vs. *E. nana*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
E. coli vs. *B. hominis*		Horizontal line			n.a	6
E. coli vs. *H. diminuta*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6

Keys: ns = not significant; ss = strongly significant; n.a = Not applicable.

Result in Table 5 of the significant test of all possible pairs of Pearson Correlation Relationship for the parasites during rainy and dry seasons showed that *C. mesnili*, *B coli* and *E coli* exhibited very strong significant positive paired correlation relationship to *I butschlii.*

Table 5. Significant test of all possible pairs of Pearson Correlation Relationship for the parasites during rainy and dry seasons

RAINY SEASON	
B. coli and *Entamoeba coli*	showed a significant positive paired correlation relationship (r = 0.894).
Giardia sp and *Entamoeba coli*	showed a significant negative paired correlation relationship (r = -0.868).
I. butschlii and Entamoeba coli	**showed a significant positive paired correlation relationship (r = 0.894).**
I. butschlii and B. coli	**showed a significant positive paired correlation relationship (r = 1).**
E. vermicularis and S. *mansoni*	showed a significant positive paired correlation relationship (r = 0.916).
S. stercoralis and *S. mansoni*	showed a significant positive paired correlation relationship (r = 0.916).
S. stercoralis and *E. vermicularis*	showed a significant positive paired correlation relationship (r = 1).
DRY SEASON	
C. *mesnili* and *I. butschlii*	**showed significant positive correlation relationship (r = 0.8783).**
S. mansoni and *Entamoeba. coli*	showed significant positive correlation relationship (r = 0.824).
S. haematobium and *E. histolytica*	showed significant negative correlation relationship (r = -0.9287).
S. haematobium and *S. stercoralis*	showed significant positive correlation relationship (r = 0.86).
S. stercoralis and Hookworm	Hookworm showed significant positive correlation relationship (r = 0.9807).
Giardia sp and *E. histolytica*	showed significant positive correlation relationship (r = 0.8291).
Cercaria/Miracidia and *B. coli*	coli showed significant positive correlation relationship (r = 0.9798).
Cercaria/Miracidia and *T. trichiura*	showed significant positive correlation relationship (r = 0.9798).
Cercaria/Miracidia and *H. diminuta*	showed significant positive correlation relationship (r = 0.9798).

The significant test result shown in Table 6 indicated that there was no significant correlation relationship of *E. coli* with any parasites in the rainy season

Table 6. Significant test of Pearson's Correlation Relationship between *E coli* and thirteen parasites in rainy season

Variables (/ml or gm)	**Pearson r**	**95% CI**	**R^2**	**P value**	**P value summary**	**n**
E. coli vs. *Taenia* sp	-0.1123	-0.8467 to 0.7694	0.01261	0.8323	ns	6
E. coli vs. *Entamoeba. coli*	-0.4324	-0.9208 to 0.5842	0.187	0.3918	ns	6
E. coli vs. *E. histolytica*	-0.03498	-0.8232 to 0.7993	0.001224	0.9475	ns	6
E. coli vs. *B. coli*	-0.2961	-0.8931 to 0.6785	0.08768	0.5688	ns	6
E. coli vs. *S. mansoni*	-0.3841	-0.9115 to 0.621	0.1475	0.4522	ns	6
E. coli vs. *A. lumbricoides*	0.3488	-0.6455 to 0.9044	0.1216	0.4981	ns	6
E. coli vs. *Giardia* sp	0.5548	-0.4671 to 0.9421	0.3078	0.2532	ns	6
E. coli vs. Hookworm	-0.2167	-0.8745 to 0.7218	0.04697	0.68	ns	6
E. coli vs. *T. trichiura*	-0.2961	-0.8931 to 0.6785	0.08768	0.5688	ns	6
E. coli vs. *E. vermicularis*	-0.2961	-0.8931 to 0.6785	0.08768	0.5688	ns	6
E. coli vs. *S. stercoralis*	-0.2961	-0.8931 to 0.6785	0.08768	0.5688	ns	6
E. coli vs. *I. butschlii*	-0.2961	-0.8931 to 0.6785	0.08768	0.5688	ns	6
E. coli vs. *C. mesnili*	0.3203	-0.6638 to 0.8983	0.1026	0.536	ns	6

Keys: ns = not significant; s = significant.

5. Discussion

According to Sures et *al* [12], parasites are attracting increasing interest from parasite ecologists as potential indicators of environmental quality because of the variety of ways in which they respond to anthropogenic pollution. They also suggest how environmental science and parasitology might profit from each other in the near future. Interest on this work aligned with this assertion.

Laboratory analysis of water supplies from Adada River showed that I *butschlii* exhibited significant positive correlation relationship (r = 0.8783 with the distribution of *E. coli* in the dry ($p < 0.05$). This correlation indicated some levels of affinity or relativity that can be extrapolated as indices of affiliations. Therefore, the hypothesis that correlation does not exist between E. *coli* and any parasite of medical importance in the rainy/dry seasons has to be rejected, to imply significant specified correlation relationship of *E coli* with *I butschlii.*

Thus, Pearson's correlation matrix (Pearson r) interprets both signs (+ or -) and magnitude; the closer the values to one, the greater the affinity. The plus sign (+) indicates direct relationship in many senses, such as if either of the comparing factors increases, the other also does; the negative sign (-) indicates inverse relationship and opposite of what the plus sign interprets.

Further, normally for river water analysis of this nature, replicate samples from four stations are usually taken and analyzed in duplicates [1,2,10], but 6 duplicate samples (12 number total) was specially taken in this work for better sample size and statistical significance.

Biological indices in terms of correlation (tag) to bacteria distribution is different dimension from normal standard method of water analysis in the determination of the potability or levels of pollution or degree of contamination of water sample. Perusal of this work indicated that only *I* butschlii showed significant positive correlation to the distribution of *E. coli*. This is in spite of more than 18 other different parasites detected and analyzed in the river, but it must not be regarded as exhaustive due to limitations of this particular research work. Though, numerous correlations were not, however, really expected from the blind search among all isolated parasites of human biotic origin in this river. Selection of the chosen parasites analyzed were simply based on those of medical importance or human biotic origin that can be isolated, and on hope of finding just one, probably more, that can tag *E. coli*, and as such to be a substitute indicator. In something similar to this, according to WHO [13] snail ecologists, for an instance, have tried to correlate snail distribution with physico chemical factors and to discover the range of these factors within which the snail thrives.

There has also been a not-validated similar observation in snail population with organic matters, as a reflex of suspended matters which to an extent also agrees with the view of some other snail ecologists. Snail ecologists had as well found some physical, chemical and environmental correlation to snail distribution. These physical and chemical factors include: temperature, DO, TS, UDS, TDS, pH, conductivity, electrolytes, calcium, organic matters, vegetation and osmotic stress [14,15]. The environmental factors, though not validated, include: rain, season, climatic condition, slow flowing streams and topography. These investigations were similar to this one with *E. coli* (Table 2 and Table 3), except that biological indices (parasites) were rather correlated in this work.

Another near assertion to this fact is from the works not validated which stated that vegetation as a reflex of suspended solids (TSS/UDS) is a positive index of aquatic life in general. However, it was biological indices that were investigated in this work. The only other case in literature where chemical property was related to a biological index was in a work by Simard *et al.*, [16], which could not be validated, whereby nematodes were related mostly to soil chemicals [pH, P, K, etc rather than physical (sand, silt)] parameters. However, for this work, to the best of our knowledge, there was paucity of literature for correlation of biological indices on bacteria or faecal indicators, there was consequently paucity of comparative analysis in that `direction. It is more so because this is a new dimension clearly different from the other six approaches mentioned in the introductory chapter that were trying to replace the use of "detection of *Escherichia coli*" as a way of QMRA.

Why the correlations of *I* butschlii to *E. coli* occurred only in the dry seasons cannot be immediately explained, except that it may be due to seasonal and compositional changes/differences of the river in those periods. This is very likely because, in another work [17,18] in the significant test of all possible pairs of Pearson correlation relationship for physico-chemical properties (Mg and K), had showned that Mg and K exhibit significant positive paired correlation relationship (r = 0.8681) in the rainy season; hence, a sort of co-adaptability. But, surprisingly in this work**,** *C. mesnili*, *B. coli* and *E. coli* that exhibited very strong significant positive paired correlation relationship to *I. butschlii* (Table 5) were not found to have had significant Pearson's Correlation Relationship to *Escherichia coli* (Table 4). Reasons might be related to the limits of experimental errors in this work or the seasonal change.

As for explanation to Pearson's correlations, under normal conditions, biological factors are exposed to a wide range of varying and often interacting environmental factors which produces collective effects on them and it is usually difficult to separate the effect of any one factor from the other [19]. There is, therefore, no immediate explanation for these correlations apart from some parasites' specificity for a particular niche due to either physiological factor, or environmental factor, or need for a special ecological niche [20,21]. For instance, *Plasmodium* spp specificity for the red blood cells' ecological niche is due to its affinity for iron that is best found in the required abundance in the heme proteins in the blood. It however, is also in line with observed speculation that certain physico-chemical index has been found to correlate with some bacteria's ecological niches [9], though never before investigated in faecal bacteria's indicators to the best of our knowledge.

Lastly, as is with this research venture, none of the currently four emerging microbiological methods for qualitative microbial risk assessment (QMRA) of water potability as enumerated above (after Ashbolt *et al.* [5] in the introductory chapter, including the one on the horizon and of immediate future development (microarrays and biosensors) are strictly abiding by Bonde [22]

requirements for an indicator organism. Bonde (1997) outlined (for indicator organism) that: a, it must be present whenever the pathogens concerned are present; b, it must be foreseen only when the presence of pathogenic organisms is an imminent danger; c, it must occur in greater number than the pathogens; d, it must grow readily on relatively simple media; e, it must be more resistant to disinfectants and to aqueous environment than the pathogens; f, it must yield characteristic and simple reactions enabling, as far as possible, an unambiguous identification of the group or species; g, it should preferably be randomly distributed in the sample to be tested, or it should be possible to obtain uniform distribution by simple homogenization procedure; h, its growth in artificial media must be largely independent of any other organism present; that is, the growth indicator bacteria should not be seriously inhibited by the presence of other species. All current approached deviates from these.

Thus, from this evaluation, biological/parasitic index analysis indicated that *I butschli*i in the results could serve as markers for *E. coli*. These may as well serve as indices for future monitoring of the potability of such widely used water source in a community. Further, the method is a straight forward, cost effective, has lower risk exposure and is less cumbersome than all the other currently existing approaches.

6. Recommendation

These studies also underscore the need for adequate environmental management of such an important water resource. Lastly, geographical coordinates should be a paradigm in environmental microbiology; it greatly assists follow-up of precise locations, and it is simple, cost-less, and requires no special technical know-how or training [17,18].

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