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# Evaluation of Physico-Chemical Parameters as Veritable Indicators of Faecal *Escherichia coli* Contamination of Surface Waters

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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." Physical and chemical (physico-chemical) parameters were evaluated as veritable indicators of faecal *Escherichia coli* contamination of surface waters, using Adada River in Nigeria as case-study. Thirty-two (32) physico-chemical parameters were analyzed in the river (at specified geographical coordinates) for their quality and quantity and connected (using Pearson's Correlation Analysis) with the distribution of the river's isolated *Escherichia coli*. The 32 physico-chemical parameters consist of 11 cations, 6 anions, 7 physical properties, 3 properties relating to oxygen and 5 properties relating to anions/cations. Physico-chemical indices from the analysis, revealed very significant positive correlation relationship of *Escherichia coli* with the presence of Mg (Magnesium) and K (Potassium) in the dry and rainy season, respectively. *E. coli* affinity tests (Kirby-Bauer Disc Diffusion) for these metals were also positive. Mg and K also showed significant positive Pearson's possible paired correlation relationship. From this evaluation, potential index analysis indicated that Mg and K could serve as markers for the faecal bacteria indicators, and possible index for future monitoring of the potability of such surface water. The method is straight forward, cost effective, less cumbersome than other currently existing approaches.

**Key words:** Water analysis, Pearson Correlation Analysis, *Escherichia coli*, physical and chemical indicator, Kirby-Bauer Disc Diffusion.

## 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 (International Union of Pure and Applied Chemistry) 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 WHO (World Health Organization) for this as well as various nationals: USEPA (United State Environmental Protection Agency), NSO (Nigerian Standard Organization), NAFDAC (National Agency for Food and Drugs Administration and Control), etc.; biologically, it is by detection of *Escherichia coli* as evidence of faecal pollution, absence of which means 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

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mean therefore ensued. Currently, there are four emerging microbiological methods as follows: fast detection using chromogenic substances, application of monoclonal and polyclonal antibodies, immuno-magnetic separation, and gene sequencing methods [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's [7] suggestion that using more than one method of determining the degree of sewage pollution would be prudent and advantageous. And, also particularly in support of Ashbolt, et al.'s [5] 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 put into consideration, among many factors: 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 reside. 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 premise for a cost-effective means, and on the tripod that certain elements, ions, and parasites have been associated with the distribution of certain bacteria and parasites in water [5, 9, 10] that this project was borne. Such association has never been linked to faecal bacteria. This project therefore aimed to assess physical and chemical (physico-chemical) parameters 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, that is utilized 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) examine Adada River and determine its distribution, quality and quantity of the 32 physico-chemical parameters in specific six stations (of specified geographical coordinates); (ii) to along this, also determine the distribution and quantity of *Escherichia coli* in the six stations; (iii) to then determine whether some physico-chemical factors can be connected with the distribution of detected *Escherichia coli* faecal bacteria indicators, using Pearson's Correlation Analysis; (iv) if so, such properties connected will be assessed further for true affinity and avidity using Kirby-Bauer Disc Diffusion test; (v) such affinity and avidity connected will reaffirm the use of those parameter as marker for the respective faecal bacteria contamination in water.

If physical and chemical indices of faecal bacteria indicators' distribution can be assessed, it will not only serve as a marker for the potability of Adada River, but those of other water resources. Not only could it be a cheaper and simpler approach to qualitative microbial risk assessment, but may be the best affordable.

## 2. Materials and Methods

### *2.1 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°40' N and 7°18' E on the geographical map (6°42′7″ N, 7°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.

2.1.1 Station 1

Station 1 has geographical coordinate: 6°42′2″ N, 7°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.

2.1.2 Station 2

Station 2 has geographical coordinate 6°44′20″ N, 7°16′50″ E. It was ways downstream from station 1, 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.

2.1.3 Station 3

Station 3 has geographical coordinate 6°44′25″ N, 7°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 came to fetch water they sold to the local communities and beyond.

2.1.4 Station 4

Station 4 has geographical coordinate 6°44′17″ N, 7°16′37″ E. It was down-stream, a bit from the tail of the dam proper where from far and wide comes people (i.e. surrounding villagers, students, picnickers, etc) that produce 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.

2.1.5 Station 5

Station 5 has geographical coordinate 6°44′13″ N, 7°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..

2.1.6 Station 6

Station 6 has geographical coordinate 6°44′11″ N, 7°16′29″ E. It was a little way downstream from station 5, before a former animal husbandry established by ENDC/ADP (Eastern Nigeria Development Corporation), also where Adada Secondary School (site of the re-proposed satellite Adada Campus of ESUT (Enugu State University of Science and Technology)) 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 2.6 billion Naira (about $6,842,105.26) dam in progress. The climate is typical tropical rainforest, with average temperature of 25 °C (range 18-37 °C) and average rainfall of 156.89 mm.

### *2.2 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 (5 cm) 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 10.00 am to 12.00 pm (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 °C until they were ready for analysis. However, some physico-chemical properties: odour (manual), taste (manual) and temperature (mercuric thermometer) were done at source. A total of 24 water samples were collected (6 stations × duplicates samples = 12 × 2 seasons = 24 total). Total of 26 sample analysis were done (24 water samples plus control × 2 seasons).

### *2.3 Physical and Chemical Analysis*

Physical and chemical properties were determined for each water sample based on amount of water as specified by protocol for each analysis, performed at PRODA (Project Development Institute) in Emene Industrial Layout, Enugu, Nigeria. Composition and quantity of 11 cations/metals (K, Na, Zn, Ca, Fe, Pb, Mg, Cd, Ni, Cu and Co) were measured by atomic absorption spectrophotometer. BOD (Biochemical Oxygen Demand) and DO (Dissolve Oxygen) were performed by Winkler titration. COD (Chemical Oxygen Demand), $SO_4^{2-}$ and $Cl^-$ were determined by titrimetric method. $CO_3^{2-}$ was measured by complexiometric titration. $PO_4^{3-}$ and $NO_3^-$ were measured by colorimetric method. Composition of $CN^-$ was measured by Liebig-Devieje's method. TH (Total Hardness as Ca or Mg) was done by titration. Total alkalinity and total acidity were done by volumetric/acidimetric analysis. pH was measured with pH meter. Measurement of electrical conductivity was with conductometer. Colour was measured with Lovibond tinctometer. Turbidity was measured with turbidity meter. Temperature was measured with mercuric thermometer. Odour and taste were by manual tasting.

2.3.1 Total UDS (Un-dissolved Solid)/TSS (Total Suspended Solid)

Fifty milliliters (50 mL) of the river water sample was measured out. This was filtered to the last drop with the aid of a weighed Whattman's filter paper; then the filter paper was heated to dryness. The net weight of the residue suspended on the filter paper was taken as the UDS or SS (Suspended Solid); i.e. Total weight of filter paper after filtration - original weight of filter paper before filtration = suspended or UDS/TSS. The result is expressed in mg/L, since the sample was measured out by volume.

2.3.2 TS (Total Solid) and TDS (Total Dissolved Solid)

Fifty milliliters (50 mL) of the river water sample was measured into a weighed 100 mL porcelain dish; both content and porcelain dish were then heated to dryness at a temperature of 103 to 105 °C; and cooled in a dry atmosphere inside a desiccator. The weight of the porcelain dish was checked again. The difference in initial weight of the porcelain dish before the heating and after the heating the water to dryness was taken as the weight of the TS. This was also expressed in mg/L since the sample was originally measured out in volume. TDS = TS – TSS.

### *2.4 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.

2.4.1 Affinity Tests of Mg and K Positive Correlated Physico-Chemical Metals/Cations

The affinity tests were based on the pattern of Kirby-Bauer Disc Diffusion analysis to determine sensitivity. This was done to two different concentrations of the respective statistically positively correlated parameters (i.e. Mg and K). The concentrations used for each were the highest/lowest forms they were found in the Adada River water samples (Tables 1 and 2): Mg (0.02 - 30 mg/L) and K (0.01 - 0.6 mg/L). Sensitivity discs of about 5 mm in diameter were got from perforation of Whattman's filter paper. The discs were sterilized in autoclave, wrapped in aluminum foil, then dried, and immersed in duplicates in each of the prepared two concentrations of the soluble compounds of Mg and K: $MgSO_4$ (Magnesium Sulphate) and KCl (Potassium Chloride). After drying, the discs were impregnated onto prepare sterile nutrient agar cultures of the isolated *E. coli*, and incubated at 37 °C for 24 h. Ofloxacin test discs were used as controls. After the 24 hours' incubation, the plates were read. Discs with clear zones of inhibition (sensitive) mean non-affinity; resistant one means affinity.

*2.5 Statistical Analysis*

Results obtained in the physical and chemical analysis of the river were summarized in tables. Pearson's Correlation Relationship analysis was used to determine correlation of *E. coli* to the distribution of the physical and chemical parameters analyzed. The statistical analysis was done in five bits as follows: parameters relating to: (a) cations/metals (Mg, Na, K, Ca, Ni, Zn, Co, Fe, Pb, Cu and Cd); (b) cations and anions (pH, conductivity, alkalinity, acidity and TH); (c) anions ($CN^-$, $Cl^-$, $NO_3^-$, $SO_4^{2-}$ and $PO_4^{3-}$); (d) oxygen ($O_2$, BOD & COD); and (e) abstracts and physical properties (TS, TDS, SS), as discernable from Tables 1 and 2. Null hypothesis ($H_{o)}$ is: Correlation does not exist between *E. coli* and any physical and chemical parameters 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. Only the parameters relating to cations/metal, where correlations were found, were analyzed further for the statistical correlation significance (*p*-value) and tabled below (Tables 3 and 4). Thus, the *p*-value of this correlated Pearson's correlation coefficients was ascertained. *p* value less than 0.05 implied statistical correlation significance, and therefore rejected the (above) hypothesis, to imply significant specified correlation relationship. Pearson's Paired Correlation Analysis was used to determine correlation of the physico-chemicals to each other (Table 5), in the same manner as the correlation analysis.

## 3. Results and Discussion

*3.1 Results*

Of all the 32 physical and chemical parameters analyzed (Tables 1 and 2), only Mg and K (Tables 3 and 4, respectively) showed statistically significant positive correlation relationship to the distribution of *E. coli* in the dry and rainy seasons, respectively.

Table 1 showed the distribution of *Escherichia coli* with the physical and chemical properties in quality and quantity in the six stations (1-6) in duplicates (A, B) along the Adada River water flow in the rainy season. From Table 1, the Pearson's Correlation statistical analysis was done in five bits as follows: parameters relating to: (a) cations/metals (Mg, Na, K, Ni, Zn, Co and Fe; (b) cations and anions (pH, conductivity, alkalinity and acidity); (c) anions ($Cl^-$, $NO_3^-$, $SO_4^{2-}$ and $PO_4^{3-}$); (d) oxygen ($O_2$, BOD & COD); and (e) abstracts and physical properties (TS, TDS, SS), as discernable from the table above. Only the parameter relating to cation/metal (a) was found correlated, so the statistical significance of the correlation (*p*-value) in this group was further investigated as shown in Table 3.

Table 2 showed the distribution of *Escherichia coli* with the physical and chemical properties in quality and quantity in the six stations (1-6) in duplicates (A, B) along the Adada River water flow in the dry season. From Table 2, the Pearson's Correlation statistical analysis was done in five bits as follows: parameters relating to: (a) cations/metals (Mg, Na, K, Ca, Ni, Zn, Co, Fe, Pb and Cd); (b) cations and anions (pH, conductivity, alkalinity, acidity and TH); (c) anions ($CN^-$,$Cl^-$, $NO_3^-$, $SO_4^{2-}$ and $PO_4^{3-}$); (d) oxygen ($O_2$, BOD & COD); and (e) abstracts and physical properties (TS, TDS, SS), as discernable from Table 2 above. Only the parameter relating to cation/metal (a) was found correlated, so the statistical significance of

**Table 1 Aggregate results of *E. coli* faecal bacteria indicators' distributions with physical and chemical properties in Adada River in the rainy season.**

| Parameter/stations | WHO STD | 1A | 1B | 2A | 2B | 3A | 3B | 4A | 4B | 5A | 5B | 6A | 6B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | 0/100 mL | 0 | 0 | 0 | 0 | $1.02\times10^5$ | 0 | 0 | 0 | $1.92\times10^5$ | 0 | 0 | 0 |
| Mg (mg/L) | ≤30 mg/L | 0.06 | 0.06 | 0.07 | 0.05 | 0.02 | 0 | 0.08 | 0.02 | 0.15 | 0.11 | 0.12 | 0 |
| Na (mg/L) | < 200 | 0.31 | 0.64 | 0.37 | 0.49 | 0.20 | 0.35 | 0.14 | 0.31 | 0 | 0.20 | 0 | 0.14 |
| K (mg/L) | No limit | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0.60 | 0 | 0.01 | 0 |
| $CN^-$ (mg/L) | 0.07 | 0.002 | 0.000 | 0.0004 | 0.0008 | 0.0004 | 0.0004 | 0.00012 | 0.0004 | 0.00012 | 0.00012 | 0.0008 | 0.0008 |
| Ni (mg/L) | 0.02 | 0.03 | 0.15 | 0.41 | 0.17 | 0 | 0.34 | 0.01 | 0.22 | 0.28 | 0.52 | 0 | 0.14 |
| Zn (mg/L) | 3.0 | 0.06 | 0 | 0.09 | 0.02 | 0.04 | 0 | 0.11 | 0.09 | 0.02 | 0 | 0.14 | 0.01 |
| Co (mg/L) | 0.04-0.10 | 0 | 0 | 0 | 0 | 0 | 0 | 0.16 | 0.10 | 0. | 0.01 | 0 | 0.21 |
| Fe (mg/L) | 0 – 0.3 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0.16 | 0.10 | 0 | 0.01 | 0 | 0.21 |
| Cu (mg/L) | 2.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| pH | 6.5-8.5 | 5.44 | 5.08 | 6.17 | 5.90 | 5.06 | 5.63 | 4.66 | 5.15 | 5.56 | 5.63 | 5.63 | 5.23 |
| Conductivity $\times 10^3$ | 0.15-0.5 | 13.08 | 16.64 | 16.25 | 13.88 | 15.23 | 14.85 | 16.76 | 16.20 | 16.63 | 15.06 | 15.68 | 15.58 |
| Alkalinity (mg/L) | 100-200 | 50 | 100 | 100 | 50 | 50 | 100 | 100 | 50 | 50 | 100 | 100 | 80 |
| Dissolved Solid (mg/L) | < 500 | 40 | 220 | 140 | 250 | 70 | 250 | 230 | 170 | 60 | 520 | 110 | 60 |
| SS (mg/L) | 25 | 11.0 | 90 | 330 | 30 | 160 | 40 | 270 | 410 | 330 | 70 | 310 | 120 |
| TS (mg/L) | 500 | 160 | 300 | 480 | 280 | 240 | 300 | 520 | 600 | 400 | 580 | 540 | 220 |
| $Cl^-$ (mg/L) | 0-16 | 0.09 | 0.02 | 0.12 | 0.19 | 0.19 | 0.06 | 0.07 | 0.04 | 0.13 | 0.13 | 0.18 | 0.06 |
| $NO_3^-$ (mg/L) | 50 | 291.2 | 326.6 | 229.8 | 131.2 | 158.8 | 177 | 237.6 | 232.4 | 177.8 | 168.4 | 271.8 | 125 |
| $SO_4^{2-}$ (mg/L) | 250 | 0.53 | 0.23 | 0.81 | 0.82 | 0.65 | 0.47 | 0.53 | 0.32 | 0.65 | 0.23 | 0.49 | 0.58 |
| $PO_4^{3-}$ (mg/L) | 0.03-0.10 | 4.68 | 4.19 | 5.48 | 4.02 | 3.84 | 4.16 | 4.39 | 4.79 | 5.10 | 6.45 | 4.40 | 4.03 |
| DO (mg/L) | 4-5 | 7.11 | 7.74 | 7.64 | 8.01 | 8.46 | 8.42 | 7.43 | 7.45 | 8.62 | 8.60 | 8.48 | 8.60 |
| COD (mg/L) | NAD | 100 | 84 | 80 | 120 | 64 | 92 | 60 | 88 | 78 | 44 | 86 | 124 |
| Acidity (mg/L) | NAD | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 | 500 | 100 |

WHO STD =World Health Organization Standard; DO = Dissolved oxygen; mg/ L = milligram/litre; NAD = No Any Data; 1, 2, 3, 4, 5, and 6 = Stations; A, B = duplicate samples; Zero calcium, copper, lead, cadmium, BOD, odour, taste and colour; Temperature = 27-32 °C; all controls were normal.

**Table 2 Aggregate results of *E. coli* faecal bacteria indicators' distributions with physical and chemical properties in Adada River water in the dry season.**

| Parameters/Stations | WHO STD | 1A | 1B | 2A | 2B | 3A | 3B | 4A | 4B | 5A | 5B | 6A | 6B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | 0/100 mL | 0 | 0 | 0 | 0 | $1.02\times10^5$ | $1.6\times10^4$ | 0 | 0 | 0 | 0 | 0 | 0 |
| Mg (mg/L) | ≤ 30 mg/L | 0.26 | 0.30 | 0.40 | 0.31 | 0 | 0.23 | 0.28 | 0.29 | 0.29 | 0.33 | 0.28 | 0.32 |
| Na (mg/L) | < 200 | 1.31 | 1.07 | 1.43 | 1.49 | 1.24 | 1.24 | 1.11 | 1.17 | 1.23 | 1.25 | 1.47 | 1.37 |
| K (mg/L) | No limit | 0.16 | 0.20 | 0.13 | 0.22 | 0.23 | 0.33 | 0.32 | 0.33 | 0.42 | 0.44 | 0.45 | 0.38 |
| Ca (mg/L) | 13.4-14.0 | 0.02 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0.01 | 0.07 | 0.13 | 0.05 | 0.02 |
| Ni (mg/L) | 0.02 | 0.12 | 0.19 | 0.04 | 0 | 0 | 0.33 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zn (mg/L) | 3.0 | 0 | 0 | 0.02 | 0.05 | 0.07 | 0.07 | 0.09 | 0.05 | 0.09 | 0.18 | 0.07 | 0.08 |
| Co (mg/L) | 0.04-0.10 | 0.07 | 0.14 | 0.13 | 0.14 | 0.13 | 0.15 | 0.03 | 0.15 | 0.01 | 0.17 | 0.05 | 0.18 |
| Fe (mg/L) | 0 - 3.0 | 0 | 0 | 0.03 | 0.15 | 0 | 0 | 0 | 0 | 0 | 0 | 0.10 | 0.35 |
| Pb (mg/L) | 0.01 | 0 | 0 | 0 | 0.1 | 0.26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cd (mg/L) | 0.01 | 0.01 | 0.03 | 0.01 | 0 | 0 | 0 | 0 | 0.01 | 0.06 | 1.35 | 0 | 0.13 |
| Cu (mg/L) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| pH | 6.5-8.5 | 4.97 | 4.86 | 5.11 | 5.10 | 4.95 | 4.92 | 4.83 | 4.82 | 5.20 | 5.19 | 5.30 | 5.30 |
| Conductivity (µs/cm) | 0.15 - 0.5 | 32.50 | 19.28 | 16.19 | 16.77 | 17.82 | 16.99 | 17.66 | 17.79 | 17.33 | 8.83 | 8.65 | 8.96 |
| Alkalinity (mg/L) | 100-200 | 30.00 | 20.00 | 20.50 | 20.00 | 20.50 | 30.00 | 10.00 | 20.00 | 20.50 | 20.50 | 2.00 | 30.00 |
| Acidity (mg/L) | NAD | 10.0 | 20.0 | 10.0 | 20.0 | 20.0 | 30.0 | 20.0 | 20.0 | 20.0 | 20.50 | 10.0 | 20.0 |
| TH (mg/L) | 100-200 | 1.20 | 1.40 | 1.40 | 1.20 | 1.00 | 1.20 | 2.00 | 1.20 | 3.60 | 1.00 | 0 | 0 |
| Dissolved solid (mg/L) | < 500 | 0.01 | 0.01 | 0.01 | 0.03 | 0.02 | 0.05 | 0.02 | 0.05 | 0.02 | 0.02 | 0.01 | 0.02 |
| SS (mg/L) | 25 | 0.01 | 0.01 | 0.01 | 0.02 | 0.03 | 0.02 | 0.03 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 |
| TS (mg/L) | 500 | 0.02 | 0.02 | 0.02 | 0.05 | 0.05 | 0.07 | 0.05 | 0.07 | 0.04 | 0.04 | 0.02 | 0.04 |
| $CN^-$ (mg/L) | 0.07 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Cl^-$ (mg/L) | 0-16 | 75.45 | 171.77 | 40.71 | 112.20 | 11.92 | 11.92 | 43.69 | 7.94 | 13.90 | 11.92 | 50.63 | 26.81 |
| $CO_3^{2-}$ (mg/L) | 0.75 | 2.10 | 1.40 | 2.40 | 3.30 | 2.80 | 2.40 | 2.40 | 2.50 | 1.90 | 1.60 | 3.70 | 2.60 |
| $NO_3^-$ (mg/L) | 50 | 3.70 | 3,30 | 4.20 | 7.20 | 2.80 | 8.00 | 1.06 | 2.00 | 3.80 | 4.80 | 5.00 | 2.00 |
| $SO_4^{2-}$ (mg/L) | 250 | 21.87 | 19.56 | 30.28 | 28.11 | 25.40 | 23.36 | 20.24 | 19.70 | 21.46 | 25.40 | 23.09 | 20.65 |
| $PO_4^{3-}$ (mg/L) | 0.03-0.10 | 0.42 | 0.44 | 0.36 | 0.30 | 0.34 | 0.35 | 0.44 | 0.46 | 0.46 | 0.17 | 0.45 | 0.44 |
| DO (mg/L) | 4-5 | 7.20 | 7.81 | 6.81 | 7.27 | 8.29 | 7.93 | 7.87 | 7.52 | 8.48 | 8.54 | 8.45 | 8.36 |
| BOD (mg/L) | NAD | 1.16 | 1.47 | 0.94 | 0.22 | 1.42 | 2.19 | 1.44 | 1.45 | 1.49 | 1.42 | 2.10 | 1.12 |
| COD (mg/L) | NAD | 2.40 | 6.02 | 0.80 | 4.20 | 0.80 | 4.00 | 0.80 | 3.20 | 2.00 | 3.04 | 2.00 | 0.80 |

WHO STD = World Health Organization Standard; DO = Dissolved oxygen; mg/L = milligram/litre; NAD = No Any Data;1, 2, 3, 4, 5, and 6 = Stations; A, B = Duplicate samples; Zero odour, taste and colour; Temperature = 28-34 °C; all controls were normal.

the correlation ($p$-value) in this group was further investigated as shown in Table 4.

The significance test showed that the correlation of K to *E. coli* was significant, while those of the other cation/metals were not significantly correlated.

Result in Table 3 of the significant test of Pearson's Correlation relationship showed that Mg exhibited significant positive correlation relationship ($r = 0.946$) with the distribution of *E. coli* in the dry season ($p < 0.05$). Also, result in Table 4 of the significant test of Pearson's Correlation relationship showed that K also exhibited significant positive correlation relationship ($r = 0.8144$) with the distribution of *E. coli* in the rainy season ($p < 0.05$).

Table 5 further showed that Mg and K exhibited significant positive paired correlation relationship ($r = 0.8681$) which might be related to their both correlations with the distribution of the *E. coli*.

Results of the affinity tests of positively correlated metals/cations showed that both Mg and K did not show any zone of inhibition (i.e. strong affinity for *E. coli*). All the controls of ofloxacin test discs showed wide zone of inhibitions, indicating non-affinity.

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

### 3.2 Discussion

Laboratory analysis of water supplies from Adada River showed that Mg and K exhibited significant positive correlation relationship ($r = 0.946$ and $r = 0.8144$, respectively) with the distribution of *E. coli* in the dry and rainy seasons, respectively ($p < 0.05$). These correlations indicate high levels of affinity or relativity that can be extrapolated as indices of affiliations. Therefore, the hypothesis that correlation does not

**Table 3 Result of the significant test of Pearson's Correlation relationship between *E. coli* and physical and chemical properties relating to metals/cations in rainy season.**

| Variable/cations (mg/L) | Pearson $r$ | 95% CI | $R^2$ | $p$ value | $p$ value summary | $n$ |
|---|---|---|---|---|---|---|
| *E. coli* vs. Mg | 0.4273 | -0.5882 to 0.9199 | 0.1826 | 0.398 | ns | 6 |
| *E. coli* vs. Na | -0.4267 | -0.9198 to 0.5887 | 0.1821 | 0.3987 | ns | 6 |
| *E. coli* vs. K | 0.8144 | 0.008402 to 0.9789 | 0.6633 | 0.0485 | s | 6 |
| *E. coli* i vs. Ni | 0.7016 | -0.2554 to 0.9642 | 0.4922 | 0.1203 | ns | 6 |
| *E. coli* vs. Zn | -0.74 | -0.9694 to 0.1791 | 0.5477 | 0.0926 | ns | 6 |
| *E. coli* vs. Co | -0.4543 | -0.9249 to 0.5659 | 0.2064 | 0.3654 | ns | 6 |
| *E. coli* vs. Fe | -0.6424 | -0.9557 to 0.3534 | 0.4127 | 0.1689 | ns | 6 |

ns = not significant; s = significant.

**Table 4 Result of the significant test of Pearson's Correlation relationship between *E. coli* and physical and chemical properties relating to metals/cations in dry season.**

| Variables/cations (mg/L) | Pearson $r$ | 95% CI | $R^2$ | $p$ value | $p$ value summary | $n$ |
|---|---|---|---|---|---|---|
| *E. coli* vs. Mg | 0.946 | 0.9942 to 0.5788 | 0.8949 | 0.0043 | ss | 6 |
| *E. coli* vs .Na | -0.1585 | -0.8595 to 0.7494 | 0.02514 | 0.7642 | ns | 6 |
| *E. coli* vs. K | -0.09227 | -0.8409 to 0.7775 | 0.008514 | 0.862 | ns | 6 |
| *E. coli* vs. Ca | -0.3296 | -0.9003 to 0.6579 | 0.1086 | 0.5235 | ns | 6 |
| *E. coli* vs. Ni | 0.6595 | -0.3272 to 0.9582 | 0.4349 | 0.1542 | ns | 6 |
| *E. coli* vs. Zn | 0.06336 | -0.7888 to 0.8321 | 0.004015 | 0.9051 | ns | 6 |
| *E. coli* vs. Co | 0.6231 | -0.3813 to 0.9528 | 0.3882 | 0.1863 | ns | 6 |
| *E. coli* vs. Fe | -0.28 | -0.8894 to 0.6879 | 0.0784 | 0.591 | ns | 6 |
| *E. coli* vs. Cd | -0.2324 | -0.8783 to 0.7138 | 0.054 | 0.6577 | ns | 6 |

ns = not significant; ss = strongly significant. The significant test showed that the correlation of Mg to *E. coli* was strongly significant, while those of the other cation/metals were not significantly correlated.

**Table 5 Significant test of all possible pairs of Pearson correlation relationship for physical and chemical properties relating to metals/cations during dry and rainy season.**

| | |
|---|---|
| Dry season | |
| Pb and Mg | exhibit significant negative correlation relationship ($r$ = -0.946). |
| Zn and K | exhibit significant positive correlation relationship ($r$ = 0.8752). |
| Cd and Ca | exhibit significant positive correlation relationship ($r$ = 0.9703). |
| Rainy season | |
| K and Mg | exhibit significant positive correlation relationship ($r$ =0.8681). |
| Co and Zn | exhibit significant positive correlation relationship ($r$ =0.8862). |
| Fe and Co | exhibit significant positive correlation relationship ($r$ =0.8326). |

K and Mg exhibited significant positive paired correlation to each other, both were as well found to have had very significant Pearson's paired correlation relationship to *E. coli.*

exist between *E. coli* and any physical and chemical parameters in the rainy/dry seasons has to be rejected, to imply significant specified correlation relationship of *E. coli* with Mg and K. Similar observation had been noted by Amadi, et al. [12] in *Enterococcus* sp. whereby there was high statistical significant correlation between it and iron (Fe); so also negative significantly correlation relationship had been noted.

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, 12], but 6 duplicate samples (12 number total) were specially taken in this work for better sample size and statistical significance.

Physical and chemical indices in terms of correlation (tag) to bacteria distribution are different dimension from normal standards 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 apparently only Mg and K showed significant positive correlation to the distribution of *E. coli*. in spite of more than 30 other different physico-chemical parameters 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. Selection of the chosen parameters analyzed were simply based on the most common surface water physico-chemicals, 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 not validated include: rain, season, climatic condition, slow flowing streams and topography. Almost all these parameters were similar to the investigations with *E. coli* (Tables 1 and 2), but none except Mg and K produce correlations.

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, none of the physical parameters (TS, TSS and TDS) investigated in this work showed any correlation (Tables 1-4).The only other case in literature where chemical property was related to a biological index was in a work by Simard, et al. [16], whose reference could not be validated, whereby nematodes were related mostly to soil chemicals (pH, P, K, etc.) rather than physical (sand, silt) parameters. None of the chemical properties mentioned, which were also investigated in this work (Tables 1-4), showed any correlation. Because in this work, which is a new dimension as earlier indicated, there was paucity of literature for correlation of physical and chemical and 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 four approaches mentioned in the introductory chapter that are trying to replace the use of "detection of *Escherichia coli*" as a way of QMRA (Qualitative Microbial Risk Assessment).

Why the correlations of Mg and K to *E. coli* occurred separately in the rainy and 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, furtherance to the work, the avidity tests showed that both Mg and K have affinity for *E. coli*; since the faecal bacterium was not resistant to both metals in the Kirby-Bauer Disc Diffusion test used for the avidity test. Likewise, it must be further so, because the significant test of all possible pairs of Pearson correlation relationship for physico-chemical properties relating to metals/cations during dry and rainy season (Table 5), showed that Mg and K exhibited significant positive paired correlation relationship ($r$ = 0.8681) in the rainy season; hence, a sort of co-adaptability.

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 [17]. There is, therefore, no immediate explanation for these correlations apart from some parasites' specificity for a particular niche due to either physiological need, or environmental factor, or need for a special ecological niche [18, 19]. 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.

Lastly, as is with this research venture, none of the currently four emerging microbiological methods for 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 [20] requirements for an indicator organism. Bonde [20] 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 approaches deviate from these.

# 4. Conclusions and Recommendations

*4.1 Conclusions*

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

*4.2 Recommendations*

These studies also underscore the need for adequate environmental management of such an important water resource. Lastly, for future follow-ups, geographical coordinates of work-place should be a paradigm on works in environmental microbiology.

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