**Evaluation of Bacterial contamination in drinking water at White Nile State, Sudan during 2022-2023**

**Abstract**

The ecosystems in Sudan are divided into aquatic and terrestrial habitats. The biodiversity including description of status of ecosystems and populations. This study aimed to evaluate of Bacterial contamination in drinking water at White Nile State (Kosti and Rabak Cities) during February 2022 and October 2023. Five random sample of drinking water were collected from each City. The quantity was 250 ml and were collected in 250 ml clean and aseptic plastic container. This test was conducted in the Microbiology Laboratory, Faculty of Engineering and Technology, University of Gezira. The technique used was the direct pour and spread plate count method, using 1.0 ml of the drinking water and agar media selective to coliform bacteria. The results showed that, the quality standard for this bacterium was zero in both cities during the whole study periods. The contamination with *E. coli* observed to be gradually increased each season. Rabak drinking water was significantly contaminated with *E. coli* more than Kosti. The quality evaluation for the drinking water, whatever the city, should be routine work, and the pollution sources should be detected and treated immediately.

**Keywords:** Biodiversity, aquatic fauna, rainwater, White Nile State.

**1. Introduction**

“According to the WHO, waterborne diseases account for an estimated 3.6% of the total global burden of disease, and cause about 1.5 million human deaths annually. The WHO estimates that 58% of that burden, or 842,000 deaths per year, is attributable to a lack of safe drinking water supply, sanitation and hygiene” [1].

The bacterial population in the collected drinking water samples will be enumerated in the Standard Plate Count Agar plates by Standard Plate Count (SPC) method. The Drinking water sample were usually serially diluted to determine the bacterial population on sterile Petri-plates containing Standard Plate Count Agar for the growth of bacterial colonies. The numbers of bacterial colonies in the Standard Plate Count Agar plates were counted and calculated as number of colonies counted/Amount of sample taken × Dilution factor [2].

“The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern. *Escherichia coli* and *Pseudomonas aeruginosa* are commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage. Indicator organisms are used because even when a person is infected with a more pathogenic bacteria, they will still be excreting many million times more indicator organisms than pathogens. Analysis is usually performed using culture, biochemical and sometimes optical methods. When indicator organisms' levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (where suspected) using specific culture methods or molecular biology” [3].

“The most reliable methods are direct plate count method and membrane filtration method. mEndo Agar is used in the membrane filtration while VRBA Agar is used in the direct plate count method. VRBA stands for violet red bile agar. A media that contains bile salts which promotes the growth of gram negative and has inhibitory characteristic to gram positive although not complete inhibitory. These media contain lactose which is usually fermented by lactose fermenting bacteria producing colonies that can be identified and characterized. Lactose fermenting produce colored colonies while non lactose fermenting produces colorless ones. Because the analysis is always based on a very small sample taken from a very large volume of water, all methods rely on statistical principles” [4,14].

**2. Materials and Methods**

**2.1 The study area (Kosti and Rabak Cities)**

White Nile State (WNS) is one of the 18 states of Sudan that located the southwestern part of Sudan, between 32o 20'– 30o 13' N and 28o 22'– 13o 27' E. The State is divided into 9 localities. The area is located across three different climatic zones; savannah with low and high rainfall and flood plans.

**2.2 Study design**

A cross-sectional study, conducted during Feb. 2022 and Oct 2023.

**2.3 Sampling of drinking water**

Samples were taken from five (5) random locations that are selected within Kosti city and another five random locations from Rabak city. Samples were taken from sources peoples usually used it for drink. The quantity was 250 ml for each, and it was kept in cleaned plastic container in aseptic conditions as was suggested by CAWST [5].

**2.4 Microbiological tests**

This test was conducted in the Microbiology Laboratory, Faculty of Engineering and Technology, University of Gezira. The technique used was the direct pour and spread plate count method, using 1.0 ml of the drinking water and membrane lauryl sulphate agar media which is selective to coliform bacteria. This media is composed of 92.2 g of the medium: (40 g) casein peptone, lactose (30 g), agar (15 g), yeast extract (6 g), sodium lauryl sulfate (1.0 g), and phenol red (0.2 g) in one liter of distilled water, and the mixture was dissolved by heating with frequent agitation for one minute, then sterilized in autoclave at 121ºC for 15 minutes, cooled to 45-50ºC, mixed well and dispensed into Petri-dishes as was described by APHA [6]. The target microorganism was the bacterium *E. coli*. After inoculation of water sample into the media, the Petri dishes were incubated at 37oC. The time taken to obtain results, was 48 hours, after which the number of the observed colonies (pale yellow in color) on the agar plate were counted.

**2.5 Data analysis**

The obtained data were subjected to suitable statistical tools (simple descriptive statistics, and least significant differences) so as to evaluate the quality characteristics of White Nile State (Kosti and Rabak cities) drinking water. Excel-2010 was used to analyze the obtained data.

**3. Results**

The test for *E. coli* contamination within Kosti and Rabak drinking water samples collected during (Feb. 2022; Table 1) showed means of 60.8 + 37.8 and 124 + 23.2 colonies/plate, respectively. It is clear that, Rabak drinking water was significantly (referring to mean + standard error) contaminated with *E. coli* bacteria more than Kosti samples. The same was noticed during (Oct. 2022; Table 2), (Feb. 2023; Table 3) and (Oct. 2023; Table 4). The quality standard for this bacterium was zero in both Cities during the whole study periods.

It was also noticed that, the contamination with *E. coli* gradually increased each season as a result of absence of contamination treatment and may be as a result of the war situation in Sudan, that threated directly and indirectly the White Nile State and dispensed the treatment effort.

**Table (1)** No. of *E. coli* colonies/plate within Kosti and Rabak drinking water (Feb. 2022)

|  |  |  |
| --- | --- | --- |
| **Replicate** | **Kosti samples** | **Rabak samples** |
| **Rep-1** | 8 | 76 |
| **Rep-2** | 8 | 64 |
| **Rep-3** | 24 | 164 |
| **Rep-4** | 56 | 136 |
| **Rep-5** | 208 | 180 |
| **Mean+SE** | 60.8 + 37.8 | 124.0 + 23.2 |

**Table (2)** No. of *E. coli* colonies/plate within Kosti and Rabak drinking water (October 2022)

|  |  |  |
| --- | --- | --- |
| **Replicate** | **Kosti samples** | **Rabak samples** |
| **Rep-1** | 40 | 64 |
| **Rep-2** | 68 | 16 |
| **Rep-3** | 88 | 400 |
| **Rep-4** | 24 | 92 |
| **Rep-5** | 48 | 224 |
| **Mean+SE** | 53.6 + 11.1 | 159.2 + 69.4 |

**Table (3)** No. of *E. coli* colonies/plate within Kosti and Rabak drinking water (Feb. 2023)

|  |  |  |
| --- | --- | --- |
| **Replicate** | **Kosti samples** | **Rabak samples** |
| **Rep-1** | 92 | 228 |
| **Rep-2** | 168 | 184 |
| **Rep-3** | 180 | 140 |
| **Rep-4** | 144 | 140 |
| **Rep-5** | 84 | 284 |
| **Mean+SE** | 133.6 + 19.5 | 195.2 + 27.5 |

**Table (4)** No. of *E. coli* colonies/plate within Kosti and Rabak drinking water (Oct. 2023)

|  |  |  |
| --- | --- | --- |
| **Replicate** | **Kosti samples** | **Rabak samples** |
| **Rep-1** | 182 | 164 |
| **Rep-2** | 104 | 216 |
| **Rep-3** | 158 | 360 |
| **Rep-4** | 176 | 220 |
| **Rep-5** | 228 | 220 |
| **Mean+SE** | 169.6 + 20.1 | 236.0 + 32.7 |

**4. Discussion**

The test for *E. coli* colonies within Kosti and Rabak drinking water samples collected during all study period showed significant differences between them, also the contamination of the drinking water increased gradually throughout the study periods and sites. This reflects the increase in the organic waste and decrease in treatment of pollutants in both Kosti and Rabak cities during the study period. WHO standards of contamination (colonies/plate) of drinking water with *E. coli* accepted only 0 colony.

“Drinking water is an important constituent for all types of living beings. Groundwater is one of the most valuable natural resources, which supports human health, economic development and ecological diversity. Groundwater resource assessment of a region involves a detailed study of the sub-surface water, including geology and hydrogeology, monitoring and production of well data. The water quality guidelines provide a Limit Value for each parameter for drinking water. The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life. It is necessary to know details about different physico-chemical parameters such as color, temperature, acidity, hardness, pH, sulphate, chloride, alkalinity used for testing of water quality. Heavy metals like Pb, Cr, Fe, Hg etc. are of special concern because they produce water or chronic poisoning in aquatic animals” [7].

“With a wide variety of tests available, researchers and practitioners have expressed difficulties in selecting the most appropriate test(s) for a particular budget, application and setting. The searched for available fecal indicator bacteria tests and collated this information was studied. From total of 44 tests, 18 of which yield a presence/absence result and 26 of which provide enumeration of bacterial concentration. The suitability of each test was also assessed and catalogued. The cost per test was found to vary from $0.60 to $7.50, though it is likely to be small of the overall costs of testing. This will be of value to organizations responsible for monitoring national water quality, water service providers, researchers and policy makers in selecting water quality tests appropriate for a given setting and application”[8]. “This article reflected the importance and cost of the tests of microbial drinking water tests. Water pollution is a major global problem that has been the leading cause of morbidity and mortality. The microbiological assessments were performed. Out of 100 samples, 48% of samples were found to be contaminated with *E. coli* was the predominant strain among the coliforms. Out of 100 samples, 48% of samples were found to be contaminated with total coliform. Microorganisms survive in bottled water as they have many nutrients required for the microorganism in ionic form. Surveillance is lacking by the license‐providing organizations followed by governmental organizations” [9].

*“E. coli* is a coliform bacterium known to be gram-negative, facultative anaerobic (can survive outside the body for a limited amount of time), and got a rod-shaped. It is commonly found in the lower intestine of warm-blooded organisms as part of the normal microbiota of the gut, and most of its strains are harmless or even beneficial to humans (some strains benefit their hosts by producing vitamin K2 or by preventing the colonization of the intestine by pathogenic bacteria), but some serotypes are pathogenic and are responsible for food contamination incidents.  *E. coli* is generally growing massively in fresh fecal matter under aerobic conditions, so it was found in the environment where fecal matter is found. The fecal–oral transmission is the major route through which it can cause disease. It is a potential indicator organism to test the environmental samples for fecal contamination” [10] [11] [12] [13].

**5. Conclusions**

The contamination with *E. coli* observed to be gradually increased each season. Rabak drinking water was significantly contaminated with *E. coli* more than Kosti. The quality evaluation for the drinking water, whatever the city, should be routine work, and the pollution sources should be detected and treated immediately.

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