

Microbiological and Organic Pollutants Characteristics of Umuosoko Stream in Ikwuano Local Government Area, Abia State, Nigeria

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Abstract

The microbiological and organic pollutants characteristics of Umuosoko stream in Ikwuano Local Government Area, Abia State, Nigeria were carried out. Twenty water samples were collected from five stations of the stream. The pour plate technique was employed for the isolation of microorganisms. Colonial morphology, Gram staining and biochemical tests were used for identification and characterization. The statistical analyses used were analysis of variance and standard deviation. The mean total aerobic plate count ranged from $5.30 \pm 0.01 \text{Log}_{10}\text{cfu/mL}$ to $5.90 \pm 3.0 \text{Log}_{10}\text{cfu/mL}$ while the mean coliform count was $5.0 \pm 0.50 \text{MPN/100mL}$ to $20.0 \pm 4.0 \text{MPN/100mL}$. The *Salmonella* and *Shigella* and *Vibrio cholerae* mean counts were $0 \pm 0.00 \text{Log}_{10}\text{cfu/mL}$ respectively. The mean fungal count ranged from $2.45 \pm 0.02 \text{Log}_{10}\text{cfu/mL}$ to $2.77 \pm 0.20 \text{Log}_{10}\text{cfu/mL}$. The microorganisms isolated were *Escherichia coli*, *Enterobacter* species, *Staphylococcus aureus*, *Proteus* species, *Streptococcus* species, *Bacillus* species, *Pseudomonas aeruginosa*, *Lactobacillus* species, *Saccharomyces* species; *Kluyveromyces* ranges species and *Aspergillus* species. The values of the organic pollutants tested were as follows: pH, 6 to 9, temperature, 29°C to 31°C , colour, 6TCU to 7TCU, conductivity, $200\mu\text{S/cm}$ to $330\mu\text{S/cm}$, turbidity, 0.01NTU to 0.1NTU, total hardness, 0.2mg/L to 0.4mg/L, total dissolved solid, 360 mg/L to 620mg/L, total suspended solid, 40mg/L to 120mg/L, dissolved oxygen, 4.0mg/L to 5.5mg/L, biochemical oxygen demand, 2.8mg/L - 3.8mg/L, salinity, 0.1ppt to 0.3ppt, nitrate, 21 mg/L to 103mg/L, phosphate, 0.67mg/L to 1.52mg/L, sulphate, 200mg/L to 260mg/L, calcium, 18.7mg/L to 47.0mg/L, while the taste was unobjectionable. The microbiological and nitrate results showed that the stream water is contaminated and must be treated before consumption.

Key words: Microbiological, organic pollutants, characteristics, Umuosoko, streams

1.0 Introduction

Water is essential for life. Man needs water for various other purposes apart from drinking and body functions. The other purposes include its use in transportation, waste disposal and hydroelectric system. About 80% of the earth surface is covered by water. This may be grouped into natural and artificial water. Natural water includes atmospheric water (rain, hail snow), surface water (stream, ponds, lakes, rivers, oceans) and groundwater (spring, well, underground stream). Artificial water on the other hand includes ponds. Surface can be contaminated by some impurities like dusts, smokes or gases, ions from the atmosphere when rain is falling. However, groundwater is subjected to contamination as a result of shallowness, improper construction and proximity to toilet facilities, sewers refuse, dump site and human activities around them. Water may contain dissolved and suspended impurities, chemicals impurities and microorganisms. Many of these microorganisms when present in large quantities and for prolonged period of time can cause health problem (Onyeagba and Isu, 2003; Shelton, 2003; WHO, 2004). There is a strong relationship existing between water, health and disease causation (Ajewole, 2005). Water is a very essential element of human nature, yet a very dangerous element in the spread of disease. A recent study of water related and water borne disease are in one way or the other caused by water (Medema et al., 2003). Water that is free of disease producing microorganisms and chemical substance dangerous to health is referred to as potable water. The consequence of urbanization and industrialization leads to contamination of water.

Water pollution is caused by the mixing of the sewage water, toxic chemicals and industrial effluent along with the drinking water (Uriah and Izuagbe, 1998). Two categories of the sources of surface water pollution based on their origin are point and non-point sources. The contaminants that enter a waterway through a discrete conveyance,

such as a pipe or ditch are called point source pollution. The non-point source pollution is the diffuse contamination that does not originate from a single discrete source. The cumulative effect of small amounts of contaminants gathered from a large area is as a result of non-point source pollution (USGS, 1998; EPA, 2005). Industrial effluents are the most dangerous sources of water pollution. Dyes, salts cyanides, suspended solids, oils and greases are organic pollutants contained in industrial effluents. The monomethyl contained in industrial effluents causes brain damage when it enters human body with water. The drinking of such water mixed with industrial effluents causes direct death (Barunde, 2005).

The contamination of water has been associated with sewage and sewage effluent. It has been generally accepted that surface water contains more harmful microorganisms compared to other sources of water including groundwater and rainwater (Oyebode, 2005). Groundwater sources which include deep well, borehole and spring are not a very safer form of groundwater and are not acceptable for potable water production for commercial purposes. This is because the general mode of sewage disposal is by the use of septic tanks and pit latrine in Nigeria (EPA, 2002). Consequently, high degree of sewage dumps, pesticides from agricultural activities, many practices with domestic wastewater may be sources of bacteria and other organisms capable of producing disease in man and animals including livestock. Other sources include livestock manure and wastewater from municipals, schools, feedlots and swamps (Cheesbrough, 2000). Most of the important microbial pathogens which are likely to be present in polluted or contaminated water include *Salmonella typhi*, *Salmonella paratyphi*, *Shigella* species, *Vibrio* species, *Staphylococcus aureus*, *Campylobacter* species, *Pseudomonas aeruginosa* which cause gastrointestinal tract infections.

Consequently, a number of cases of water-borne diseases have been seen to be the cause of many health hazards. The demand and pollution level of water requires the basic monitoring on the water quality (WHO, 2004; EPA, 2002). Hence, there is need to ascertain the physical, chemical and microbiological quality of this stream to ascertain whether it safe for human consumption. This is justified because it serves as drinking water and use for other domestic activities in Umuosoko and also because water may not be adjudged potable by appearance or taste.

The aim of this study is to determine the microbiological and organic pollutants characteristics of the Umuosoko stream in Ikwuano Local Government Area of Abia State

2.0 Materials and Methods

2.1 Study Area

The study area is Umuosoko, which is a community in Ikwuano L.G.A. of Abia State, Nigeria. The Umuosoko stream is an all season stream. It serves the domestic and recreational needs of the people for the purposes of drinking, washing, bathing and other activities. This segment of stream receives discharges of contaminated water combined-sewer overflows and incompletely disinfected wastewater. Umuosoko stream is the main source of water for the three villages which make up the Umuosoko community. Umuosoko stream is 2km away from the village.

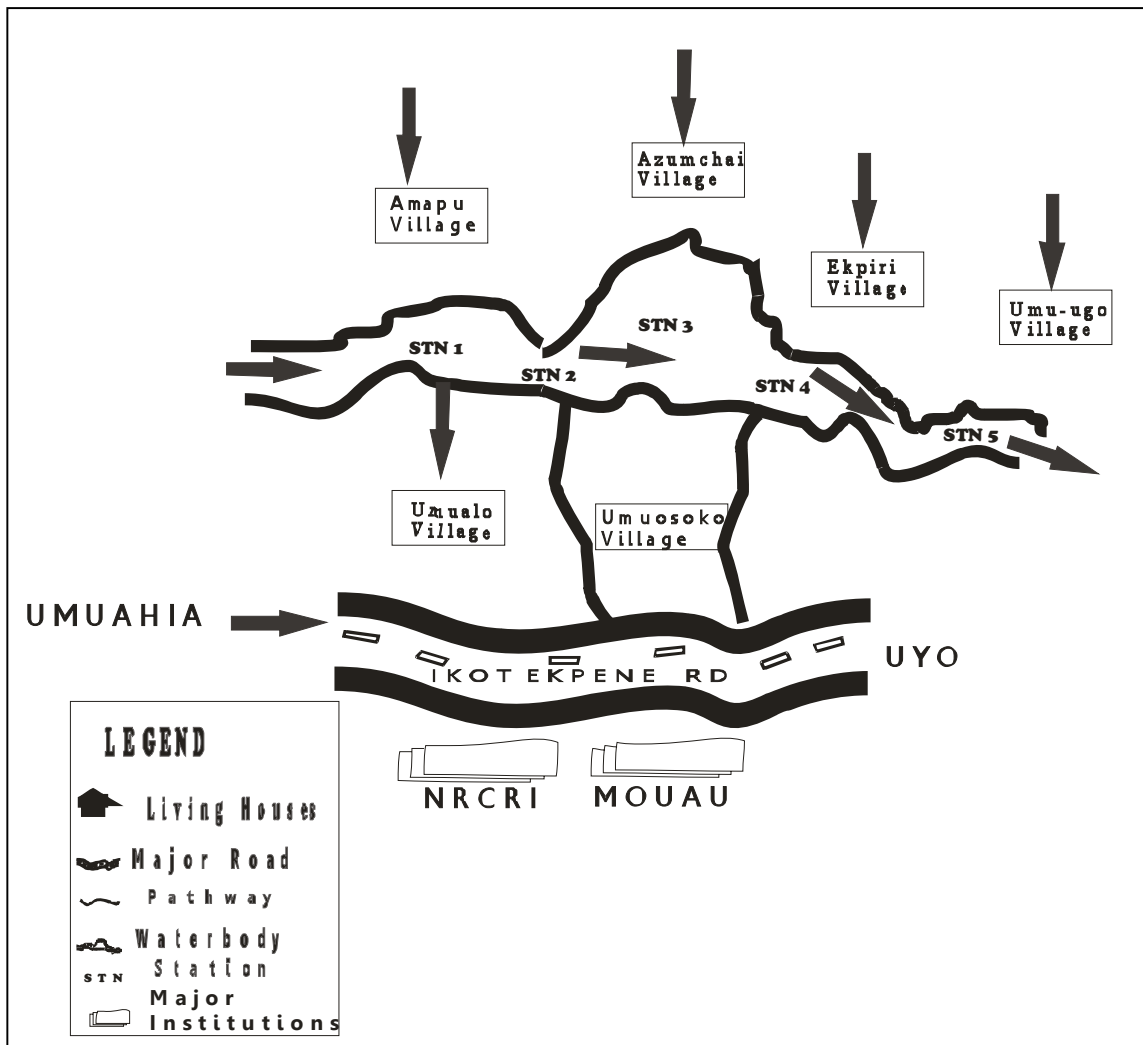


Fig. 1: Sampling stations on the Umuosoko Stream.

(Source: MOWH, 2002)

2.2 Collection of the Stream Water Samples

The bottles that were used for the water sampling have a capacity of at least 200mL. They were fitted with screw caps. The cap and neck of bottle were protected from contamination by using a suitable cover of thin aluminum foil. Silicon rubber liners that will withstand repeated sterilization was used inside screw caps. After being sterilized, the bottles were not opened until the collection of samples.

The stream water samples were collected from different stations along the stream. Station 1 is the spring which is the water fall while stations 2 – 4 comprise the main body of the stream where human activities take place. The samples for the microbial counts were collected in sterile plastic containers, which were previously sterilized with 70% alcohol and rinsed with sterile distilled water. At the river side, the containers were rinsed twice with the river water before being used to collect the samples. Samples for dissolved oxygen (DO) and biochemical oxygen demand (BOD) were collected with clean brown bottles. The samples for the other physiochemical parameters were collected with 500ml sterile plastic containers. They were transported to the laboratory in an ice packed cooler and immediately analyzed on reaching the laboratory.

2.3 Chemical Reagents

Chemical reagents used in the study were of analytical grade and were products of Hach Company, Colorado, USA; BDH Chemicals, Poole's, England and Sigma Chemical Company, St. Louis Missouri, USA. The microbiological media used were products of Oxoid and Difco Laboratories England. They were nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification and for stock culture; Sabouraud dextrose agar used for the isolation of fungi, *Salmonella-Shigella* agar for the isolation of *Salmonella* and *Shigella*, thiosulphate citrate bile sucrose agar for the isolation of *Vibrio cholerae* and MacConkey broth for coliform counts.

2.4 Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the stream water samples were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined using pour plate technique. Then the molten nutrient agar, Sabouraud dextrose agar, *Salmonella-Shigella* agar and thiosulphate citrate bile sucrose agar at 45°C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi, *Salmonella-Shigella*, *Vibrio cholerae* respectively. They were swirled to mix and colony counts were taken after incubating the plates at 30°C for 48h and preserved by sub culturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

2.5 Enumeration of Coliforms

The coliforms were estimated using the Most Probable Number techniques (multiple tube fermentation technique) as described by Cheesbrough (2005).

2.6 Characterization and Identification of Bacterial and Fungal Isolates

Bacterial isolates were characterized and identified after studying the Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, oxidative/fermentation (O/F), utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges-Proskaur reaction and urease production. The tests were performed according to the methods of (Ogbulie et al., 1998; Fawole and Oso, 1998; Cheesbrough (2005; Adeoye, 2007; Agwung-Fobellah and Kemajou, 2007; Ochei and Kolhatkar, 2007). Microbial identification was performed using the keys provided in the Bergey and Holt, 1994

Fungal isolates were examined macroscopically and microscopically using the needle mouth technique. Their identification was performed according to the scheme of Barnett and Hunter (1972) and Larone (1986).

2.7 Physicochemical Parameters

A number of physicochemical parameters of the stream water samples were determined. They included temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), total suspended solids (TSS), turbidity and alkalinity. Others were nitrate, phosphate, sulphate, biochemical oxygen demand (BOD). The pH was measured in-situ using Hach pH meter (Model EC10); temperature and total dissolved solids were measured *in-situ* using Hach conductivity meter (Model CO150). The dissolved oxygen was also measured in-situ using Hach DO meter (Model DO175). Sulphate was determined using Barium chloride (Turbidimetric) method. Nitrate was determined using Cadmium reduction method. Alkalinity and phosphate were measured using potentiometric titration and Ascorbic acid methods respectively. Biochemical oxygen demand was determined using Azide modification method. All analyses were in accordance with Eaton and Franson, (2005).

3.0 Results

The results of the Microbiological and Organic pollutants of the stream water samples are shown in Tables 1-3.

Table 1 shows the mean counts of microorganisms isolated from the stream water samples. The mean total aerobic plate count ranged from $5.30 \pm 0.01 \text{ Log}_{10} \text{cfu/mL}$ to $5.90 \pm 0.30 \text{ Log}_{10} \text{cfu/mL}$ while the mean coliform count was $5.0 \pm 0.50 \text{ MPN/100mL}$ to $20.0 \pm 3.0 \text{ MPN/100mL}$. The *Salmonella* and *Shigella* and *Vibrio cholerae* mean counts were $0 \pm 0.00 \text{ Log}_{10} \text{cfu/mL}$ respectively. The mean fungal count ranged from $2.45 \pm 0.02 \text{ Log}_{10} \text{cfu/mL}$ to $2.77 \pm 0.20 \text{ Log}_{10} \text{cfu/mL}$. The ANOVA, $P < 0.05$ showed that there was significant difference in the mean counts for the total aerobic plate count, coliform count and fungal count among the stations while the ANOVA, $P > 0.05$ showed that there was no significant difference in the mean counts for the *Salmonella-Shigella* count and *Vibrio cholerae* count among the stations.

The microorganisms isolated and their percentage occurrences are shown in Table 2. For bacterial isolates; *Escherichia coli* ranges from 6.7% to 25%; *Enterobacter* species, 6.7% to 18.8%; *Staphylococcus aureus* 6.3% to

26.7%; *Proteus* species, 4.2% to 26.7%; *Streptococcus* species, 8.1% to 13.3%; *Bacillus* species, 0% to 16.7%; *Pseudomonas aeruginosa*, 6.7% to 12.5% and *Lactobacillus* species, 12.5% to 16.7%. For the fungal isolates, *Saccharomyces* species ranges from 0% to 100%, *Kluyveromyces* species, 0% to 23.1%, while *Aspergillus* species ranges from 0% to 25%.

Table 3 shows the values of the organic pollutants of the stream water sample. They ranged as follows: pH, 6 - 9, temperature, 29°C to 31°C, colour, 6TCU to 7TCU, conductivity, 200µS/cm to 330µS/cm, turbidity, 0.01NTU to 0.1NTU, total hardness, 0.2mg/L to 0.4mg/L, total dissolved solid, 360 mg/L to 620mg/L, total suspended solid, 40mg/L to 120mg/L, dissolved oxygen, 4.0mg/L to 5.5mg/L, biochemical oxygen demand, 2.8mg/L - 3.8mg/L, salinity, 0.1ppt to 0.3ppt, nitrate, 21 mg/L to 103mg/L, phosphate, 0.67mg/L to 1.52mg/L, sulphate, 200mg/L to 260mg/L, calcium, 18.7mg/L to 47.0mg/L while the taste is unobjectionable. The ANOVA, $P < 0.05$ showed that there was significant difference in the values conductivity, total dissolved solids, total suspended solids, nitrate, sulphate, calcium and sodium among the stations. However, the ANOVA, $P > 0.05$ showed that there was no significant difference in the values of other parameters among the stations.

It was observed that the bacterial counts obtained from the stream water samples were high especially the coliform count which was above the WHO limit for drinking water. The physicochemical parameters determined were within the WHO limit except nitrate, which should be checked to avoid nitrate poisoning and possible eutrophication of the stream. The isolation of *Escherichia coli* should be of public health concern to avoid the spread of gastroenteritis among consumers of the stream water especially children.

4.0 Discussion

The quality of drinking water has been decreased during this century due to discharge of waste water into water sources as well as environmental pollutants (Liu and Jones, 1995). The high microbial counts recorded may be as a result of human activities and indiscriminate waste disposal which contribute to contamination of the stream thereby increasing the microbial population.

The bacteria and fungi identified were *Escherichia coli*, *Enterobacter* species, *Staphylococcus aureus*, *Streptococcus* species, *Bacillus* species, *Proteus* species, *Lactobacillus* species, *Pseudomonas aeruginosa*, *Saccharomyces* species, *Kluyveromyces* species and *Aspergillus* species. These organisms have been variously implicated in gastrointestinal disorders such as diarrhea, upper respiratory infection and other associated symptoms (Prescott et al., 2002).

The presence of the indicator bacteria such *Escherichia coli*, *Enterobacter* species is undesirable in drinking water and signifies the faecal contamination of the stream water body and also the presence of other enteric pathogens. This is of a major health importance and calls for remedial attention in such water bodies. Moreover *Escherichia coli* is known to cause many enteric diseases such as traveler's diarrhea and other forms of diarrhea (Pandey et al., 1999; Szweczyk et al., 2000).

The most common aetiological agent of septic arthritis is *Staphylococcus aureus* which is also a normal flora of the body and mucous membrane (Ellen and Sydney, 1990; Eze et al., 2008). The consumer is at risk of acquiring food borne diseases. *Staphylococcus aureus* is the major cause of staphylococcal food poisoning. The poisoning is characterized by diarrhea and vomiting (Singleton, 1995; Frazier and Westhoff, 2004; Eze et al., 2008). *Bacillus* species are Gram positive aerobic spore-formers.

Most members of the genus *Bacillus* are saprophytes prevalent in soil, air and on vegetations, which attributes the presence of the organism in the stream water (Thomas, 1994; Brooks et al., 2004; Abdo, 2005; Eze et al., 2008).

The presence of opportunistic pathogens such as *Proteus* species, *Lactobacillus* species and *Pseudomonas aeruginosa* in the water is as a result of discharges from infected immuno-compromised individuals who bath in the stream. Many of these are considered opportunistic microorganisms responsible for up to 50% for nosocomial infection (Sloat and Ziel, 1991). The presence of *Saccharomyces* species and *Kluyveromyces* species may be as a result of the citrus and juicy plant fruits that surround the stream thereby providing source for these organisms (Tayel, 2002).

The organic pollutant parameters tested with the exception of nitrate and conductivity were within the permissible limits of the WHO, (2004) standards.

The essential nutrients, nitrate and phosphate have been observed to enter the aquatic habitat through urban sewage effluent discharge. This may be in the form of treated or untreated sewage, agricultural activities, especially

animal wastes and fertilizer and settle in the sediment thereby increasing the levels (Kiely, 1998). The pollution enters as a point source or carried from diffuse sources in their catchments. It has also been observed that both nitrogen and phosphorus are highly particle reactive and most of them when discharged into water bodies are deposited in bottom sediments incorporated into organic matter. Here bacteria decompose organic matter, through oxygen and sulphate reduction, liberating nitrogen and phosphorus to pore water and overlying water (Kiely, 1998; Parry, 2002; WHO, 2004).

Conductivity measures the ability of aqueous solution to carry electric current solution of most inorganic compounds and more abundant ions have higher conductivity (Eaton and Franson, 2005). The increase in electric conductivity values at Station 2 reflects the strong effect of effluent inflow and human activities in this area.

The fluctuation in the temperature of the water depends mainly on the climatic conditions, sampling times, the number of sunshine hours. This can also be affected by specific characteristics of water environment such as turbidity, wind force, plant cover and humidity (Tayel, 2002). The increased temperature is often associated with low dissolved oxygen content (Eze and Okpokwasili, 2008)

Dissolved oxygen is a very important factor to the aquatic organisms, because it affects their biological process, respiration of animal and oxidation of the organic matter in water and sediments. Complex organic substances are converted to simple dissolved inorganic salts which could be utilized by microorganisms (Okbah and Tayel, 1999). It showed that the Umuosoko water was oxygenated in all the stations.

The minimum value of BOD was at station 1 and 2 and this may be due to the treated water discharged in these areas which decreases the bacterial load in water from this area. The maximal BOD concentration recorded at station 4 could be related to high bacterial load in the water due to high organic matter discharged into the stream from waste water and environmental pollutants (Sabae and Rabeih, 2007).

Hydrogen ion concentration (pH) controls all the aquatic chemical and biological processes. Changes in pH values beyond the optimum range may affect microbial physiology (Hassamin, 2006). The pH values of the different station revealed that the Umuosoko stream was on the neutral side during the study period, which means that it was not affected by the temperature and also resulted in the high bacterial load (Abdo, 2005).

Turbidity of the water may affect the fish and other aquatic organisms mostly due to higher obstruction (Izonfuo and Bariweni, 2001). Eze and Okpokwasili, 2008, stated that many organisms smother in prolonged conditions of very high turbidity by a clogging of their respiratory mechanisms.

Chlorination is the most common method of ensuring microbiological safety in water supply. This can cause the death of most microorganisms in sufficient dose within 30 minutes. Furthermore, since most table and odour producing compounds are organic, chlorine treatment reduces or eliminates them (Cheesbrough, 2000).

Conclusion

It is not possible to assess the potability of any water supply by a single laboratory examination. This does not rule out constant examination of this stream used in the Umuosoko Community. In order to ensure the provision of potable water, procedure whereby water can be examined to determine its microbiological and physicochemical qualities should be carried out. and there should be constant inspection of this Umuosoko stream. Therefore, the usefulness of water for drinking and use for domestic activities underlines its potability. Human activities and indiscriminate waste disposal contributes to the contamination of water. The stream water should therefore be adequately treated before consumption. Subsequent and consistent surveillance and monitoring of the stream should be taken up by the appropriate local authorities to ensure the maintenance of a good drinking water quality. There should be an effective health education programme for the community to enlighten them on the effects of drinking contaminated water to human health. All these together with the monitoring of human activities in the area will proffer a sustainable solution to the problem of good drinking water from Umuosoko stream.

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Table 1: Mean Counts of Microorganisms isolated from the Stream Water Sample

Station	Log ₁₀ cfu/mL				
	TAPC	SSC	VCC	FC	CC(MPN/100mL)
1	5.30 ± 0.01	0 ± 0.00	0 ± 0.00	2.45 ± 0.02	5 ± 0.50
2	5.60 ± 0.03	0 ± 0.00	0 ± 0.00	2.56 ± 0.01	16 ± 1.0
3	5.75 ± 0.10	0 ± 0.00	0 ± 0.00	2.51 ± 0.03	20 ± 3.0
4	5.78 ± 0.20	0 ± 0.00	0 ± 0.00	2.60 ± 0.10	18 ± 2.0
5	5.90 ± 0.30	0 ± 0.00	0 ± 0.00	2.77 ± 0.20	17 ± 1.0

Legend: TAPC = Total aerobic plate count; CC = Coliform count; SSC = *Salmonella-Shigella* count; VCC = *Vibrio cholerae* count; FC = Fungal count.

Table 2: Microorganisms isolated and their Percentage Occurrence

Organisms	Station 1	Station 2	Station 3	Station 4	Station 5
Bacteria					
<i>Escherichia coli</i>	1(6.7%)	7 (20.0%)	8 (21.6%)	5 (20.8%)	4 (25.0%)
<i>Enterobacter</i> species	1 (6.7%)	5 (14.3%)	4 (10.8%)	2(8.3%)	3 (18.8%)
<i>Staphylococcus aureus</i>	4 (26.7%)	6 (17.1%)	6 (16.2%)	4 (16.7%)	1 (6.3%)
<i>Proteus</i> species	4 (26.7%)	3 (8.6%)	3 (8.1%)	1 (4.2%)	1 (6.3%)
<i>Streptococcus</i> species	2 (13.3%)	3 (8.6%)	3(8.1%)	2 (8.3%)	2 (12.5%)
<i>Bacillus</i> species	0 (0%)	2 (5.7%)	4 (10.8%)	4 (16.7%)	1 (6.3%)
<i>Pseudomonas</i> species	1 (6.7%)	3 (8.6%)	4 (10.8%)	2 (8.3%)	2 (12.5%)
<i>Lactobacillus</i> species	2 (13.3%)	5 (14.3%)	5 (13.5%)	4 (16.7%)	2 (12.5%)
Fungi					
<i>Saccharomyces</i> species	0 (0%)	5 (71.4%)	7 (53.8%)	3 (100%)	3 (75.0%)
<i>Kluyveromyces</i> species	0 (0%)	1(14.3%)	3(23.1%)	0 (0%)	0 (0%)
<i>Aspergillus</i> species	0 (0%)	1(14.3%)	3(23.1%)	0 (0%)	1(25.0%)

Table 3: Mean values of the organic pollutants of the stream water samples

Parameter	Station 1	Station 2	Station 3	Station 4	Station 5	WHO limit
pH	6.90	6.90	6.90	6.90	6.90	6.5-8.5
Temperature (^o C)	29	31	30	29	29	12-28
Taste	Unobjectionable					
Colour (TCU)	6	6	7	7	7	15
Conductivity (μ S/cm)	280	255	200	330	260	250
Turbidity (NTU)	0.01	0.01	0.02	0.1	0.1	0.5
Total hardness (mg/L)	0.3	0.3	0.4	0.2	0.3	0 - 5
Total dissolved solid (mg/L)	360	620	580	480	420	1000
Total suspended solids (mg/L)	40	80	120	60	40	500
Dissolved oxygen (mg/L)	4.1	4.0	4.9	5.5	4.9	
Biochemical oxygen demand (mg/L)	2.8	2.8	3.1	3.8	3.2	No limit
Salinity (ppt)	0.1	0.2	0.3	0.1	0.1	No limit
Nitrate (mg/L)	27	27	28	103	89	10
Phosphate (mg/L)	0.67	1.52	1.10	0.95	0.95	
Sulphate (mg/L)	200	251	260	209	220	400
Calcium (mg/L)	18.7	19.0	23.0	47.0	27.0	100
Sodium (mg/L)	29	39	43	33	33	200

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