**The Microbial load of Ujiogba River, Edo Central Senatorial District, Edo State, Nigeria**

Enerijiofi, K.E.[1*], Ekhaise, F.O.[2] and Olatunji, E.O.[1]
[1]Department of Biological Sciences, College of Basic and Applied Sciences,
Samuel Adegboyega University, Ogwa, Edo State
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State
[*]Corresponding author: kingsmore1luv@gmail.com, +2348034553580

**Abstract**
Water is a universal solvent, needed to sustain life. This study revealed the extent of microbial contamination in Ujiogba River, Esan West Local Government Area of Edo State. Forty – two water samples from three sampling points along the course of the river were collected and analysed from January to April, 2012, to determine the river's microbial load. Seven bacterial isolates identified include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* sp., *Salmonella* sp., *Shigella* sp., *Streptococcus faecalis*, *Klebsiella pneumoniae*. The fungal isolates included *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp. and *Rhizopus* sp. The bacterial counts ranged from 21 to 96 x$10^2$cfu/ml, with the highest frequency of occurrence in *Escherichia coli* (25.81%) and least in *Salmonella* sp. (4.84%). The microbial load of water collected downstream was significantly different from the other sampling points. The pH and temperature ranged between 6.8±0.28 to 7.7±1.64 and 28±1.63°C to 35± 2.16°C, respectively. The water was contaminated and did not meet the minimum standard requirement of drinking water as prescribed by the World Health Organisation and United States Environmental and Protection Agency. The microorganisms isolated are capable of causing a lot of waterborne diseases. There is need for continuous monitoring so as to improve water quality and safeguard consumers from the impending public health diseases outbreak.

**Keywords:** Microbial load, *Escherichia coli*, human activities, public health, drinking water

**Introduction**
Water occupies about 70% of the earth's surface, yet it's one of the scarcest commodities especially in the developing countries of the world. They offer a number of benefits and services to man and the environment, but are increasingly being polluted and threatened on a global scale by anthropogenic activities (Agbabiaka and Oyeyiola, 2012; Oliveira *et al*., 2006). A large proportion of the rural population in the developing world take water from natural sources (like rivers) directly for drinking (Akaninwor*et al*., 2007). Some 40% of the world's population in over 80 countries are affected by serious water shortages. In other countries, water is available but too expensive to use because more accessible resources have already been depleted and new sources cost much more to treat to an acceptable standard (Mason, 2002).
Among the serious environmental problems are waste accumulation and lack of adequate and safe water supply. Water pollution is basically rendering the water unfit for human consumption and recreational purposes. Pollution of water should be seen as a gross misuse of an essential but scarce resource (Bour, 2003; Mason, 2002). Indiscriminate and uncontrolled discharges of wastes into rivers impact negatively on its ecosystems and human health (Nwachukwu and Otukunefor, 2006). Rivers have a limited absorptive capacity for sewage and fertilizer from farmland and if this limit is exceeded, the proliferation of bacteria, algae and plant life will consume all the oxygen dissolved in water leading to eutrophication (Agbabiaka and Oyeyiola, 2012).
Microbial contamination by human or animal excreta is the most common reason for water to be considered unsafe for drinking because of the high probability of the presence of pathogenic organisms. In addition, high concentrations of bacteria and nitrates discharged into water can occur from animal husbandry operations like grazing and this can result in health hazards to man due to the presence of pathogens (Obasohan *et al*., 2010).
The use of normal intestinal organisms as indicators of faecal pollution is universally accepted for monitoring and assessing the microbiological safety of water supplies (Dissanayake *et al*., 2004). For example, the coliform group of enteric bacteria which include *Escherichia coli*, *Klebsiella* sp. and *Enterobacter* species are used as water purity indicators (Chao *et al*., 2004; Grant *et al*., 2002). Their presence in drinking water may not cause illness but indicate the presence of disease causing organisms (Nwachukwu and Otokunefor, 2006).
In recent times, environmentalists have become increasingly concerned about the pollution of surface waters (Obasohan *et al*., 2010). The World Health Organization (WHO) estimated that 80% of ill health especially in developing countries stems from lack of safe drinking water (WHO, 2003). Cholera epidemics caused by *Vibrio cholerae* 01, isolated from municipal taps and wells, have been reported from different parts of India, Nigeria and Zimbabwe (Sur *et al*., 2006). Outbreaks of typhoid fever and dysentery were linked to unsanitary mixing of some water supplies and sewage (Uzoigwe and Agwa, 2012). Children are generally vulnerable to intestinal pathogens and it has been reported that 1.1 million children die every year due to diarrhoea disease (Steiner *et al*., 2006; Uzoigwe and Agwa, 2012). For instance, 3,000 Malawian children were infected with diarrhoea in 2005 and 1000 victims died (Pritchard *et al*., 2007).

Ujiogba is a rural community with a population of about 21,156 (Federal republic of Nigeria Official Gazettee, 1991) and its river serves communities such as Obazagbon, Ogwa, Ebelle and Okalo. This water source is used for washing clothes, cars, bikes, bathing and cattle grazing as well as indiscriminate dumping of faecal waste. Unfortunately, water is one the scarcest basic amenities of life in Esan land, Edo Central Senatorial District of Edo State including Ujiogba. This study determined the microbial load of the Ujiogba River and assessed the water quality in comparison to the standards recommended by the WHO and United States Environmental Protection Agency (USEPA).

**Materials and Methods**

**Study area**

Ujiogba River is located on latitude 6°32'N and longitude 6° 10'E and flows from Ugbegun through Ujiogba to Ugun. It's a freshwater, free-flowing during the rainy season and slow moving during the dry season. It is surrounded by economic trees and acts as the natural boundary between Ujiogba and Obazagbon communities in Esan West and Uhunmwode Local Government Areas of Edo State, respectively.

**Sample collection**

Water samples for microbiological analysis were collected directly from three different sampling points designated 1, 2 and 3 along the course of the river with screw capped plastic containers in the early hours of the morning (7.00 to 10.00am). The samples were immediately transported to the laboratory in ice packed coolers where analysis was initiated within 2 hours of collection, following the method of Agbabiaka and Oyeyiola (2012). Sampling point 1 (Downstream) was the point of entry into the river, 2 (Midstream) was 10m from 1 while sampling point 3 (Upstream) was 20m from sampling point 1. The water samples were collected in triplicates from each sampling points.

**Microbiological procedures**

**Isolation and Identification of total culturable heterotrophic bacterial isolates**

The spread plate method was used. Ten-fold serial dilution of each water sample was prepared aseptically in sterile physiological saline up to $10^{-3}$ and 0.1 ml aliquot of each dilution was plated on dried nutrient agar plates in triplicate. All incubations were conducted at 37°C for 24hrs under aerobic conditions and the colonies were counted. The number of colony-forming units per ml (cfu/ml) was calculated by multiplying the number of colonies with the dilution factor. Bacterial isolates were sub-cultured until pure cultures were obtained before being characterized and identified using the Manual of Determinative Bacteriology (Holt *et al*., 1994).

**Isolation and Identification of total coliforms/faecal coliforms**

The multiple tube fermentation method also known as the most probable number (MPN) was used to obtain the total coliforms and tests were performed using three test tube sets to enumerate faecal coliform. All positive tubes from the MPN procedures were sub-cultured on Levine's EMB agar plates in triplicate and incubated at 37°C for 24 hrs.

**Isolation and Identification of *Salmonella/Shigella* species**

The *Salmonella*/*Shigella* agar (SSA) was prepared according to the manufacturer's direction and 0.1 ml aliquot of each water sample was transferred onto the surface of the dried sterilized SSA plates. The plates were inoculated in triplicate and incubated at 37°C for 24 to 48 hrs. Upon incubation, pure cultures were obtained by sub-culturing onto freshly prepared SSA plates and pure colonies were identified using biochemical reactions.

**Isolation and Identification of Fungal isolates**

The fungi counts were determined as described by A.P.H.A. (2002). Potato dextrose agar (PDA) was used for the isolation of fungi using pour plate method and serial dilution technique. The plates were incubated at 25°C for 48 to 72 hrs. The fungal species were sub-cultured to obtain pure cultures. They were characterized and identified according to the method of Fawole and Oso (2001).

**Determination of pH and temperature**

The method of Ujowundu *et al*. (2011) was used to determine the pH and temperature using the digital precision model S-25C pH meter and a thermometer respectively.

**Statistical analysis**

The Chi – square goodness of fit test adopted from Ogbeibu (2005) was used to test for significant differences in the counts obtained for microorganisms in the water samples from the different sampling points at $p < 0.05$ level of significance.

**Results**

The bacteria and fungi count from Downstream were highest, while the Upstream had the least counts (see Table 1). On one hand, *Escherichia coli* was the most frequent isolate at all the three sampling points with 24.00 x $10^2$cfu/ml, 15.00 x $10^2$cfu/ml and 9 x $10^2$cfu/ml estimated from Downstream, Midstream and Upstream, respectively. On the other hand, *Salmonella* sp. was the least at Downstream, Midstream and with 6 x $10^2$cfu/ml, 3 x $10^2$cfu/ml, respectively (Table 2a). However, there was no count for observed *Salmonella* spp. at Upstream. For fungal isolates, *Penicillium* sp. was the highest at Downstream and Upstream with 13x $10^2$cfu/ml and 7x $10^2$cfu/ml respectively, but *Aspergillus* sp. was the highest at Midstream with 11x $10^2$cfu/ml. *Rhizopus* sp. had the least count at all the three sampling point (Table 2b). The pH ranged from 6.8±0.28 to 7.7±1.64 while the temperature ranged while from 28 ±1.68 to 35 ±2.16°C (Table 3).

**Table 1: Total bacterial and fungal count in water samples, n x $10^2$ (cfu/ml)**

| Isolate | Downstream | Midstream | Upstream |
|---|---|---|---|
| Bacteria | 96.00 | 59.00 | 31.00 |
| Fungi | 30.00 | 25.00 | 21.00 |

**Table 2a: Mean specific bacterial and frequency of occurrence in water samples, n x $10^2$ (cfu/ml)**

| Bacterial isolate | Downstream | Midstream | Upstream | Frequencyof occurrence n= 62 (%) |
|---|---|---|---|---|
| *Escherichia coli* | 24 | 15 | 9 | 25.81 |
| *Staphylococcus aureus* | 17 | 13 | 6 | 19.35 |
| *Pseudomonas* sp. | 13 | 8 | 6 | 14.52 |
| *Salmonella* sp. | 6 | 3 | 0 | 4.84 |
| *Shigella* sp. | 8 | 5 | 2 | 8.06 |
| *Streptococcus faecalis* | 18 | 10 | 5 | 17.74 |
| *Klebsiella pneumoniae* | 10 | 5 | 3 | 9.68 |

**Table 2b: Mean specific fungal counts, n x $10^2$ (cfu/ml)**

| Fungal isolate | Downstream | Midstream | Upstream |
|---|---|---|---|
| *Penicillium* sp. | 13.00 | 9.00 | 7.00 |
| *Aspergillus* sp. | 9.00 | 11.00 | 5.00 |
| *Fusarium* sp. | 6.00 | 3.00 | 5.00 |
| *Rhizopus* sp. | 2.00 | 2.00 | 4.00 |

**Table 3: Temperature (°C) and pH values of water samples (Mean ±SE).**

| Sampling points | Temperature | pH |
|---|---|---|
| **Downstream** | 35±2.16 | 7.7±1.64 |
| **Midstream** | 28±1.63 | 6.8±0.57 |
| **Upstream** | 29±2.16 | 6.8 ±0.28 |

Data represent mean± standard deviation of three triplicates

**Discussion**

Water meant for human consumption should be safe, acceptable and must be free from all pathogenic organisms. This study examined the level of microbial contamination of Ujiogba River. The water quality was poor due to high counts of bacterial indicators. Similar results have been reported by Pritchard *et al*. (2007), on

studies carried out in different rural communities of South Africa. The presence of bacterial pollution indicators and pathogenic bacteria groups in the water samples was as a result of high anthropogenic activities. The bacteria load was highest at Downstream, followed by Midstream then Upstream. The high bacteria count at Downstream was not surprising because a lot of domestic activities such as contamination through surface runoff during rains, indiscriminate excreta and urine disposal, washing of cars, bathing and washing of clothes take place at this point.

The total bacterial load in this study ranged from 21 to 96 x $10^2$cfu/ml, which was significantly higher than the WHO standard of <100 cfu/ml for total heterotrophic bacterial in portable water supplies (WHO, 2006). Thus, water from Ujiogba River is a far cry from the universally accepted minimum standard. In addition, water samples in which coliforms are detected should be considered unacceptable for drinking as they are regarded as the principal indicators of water pollution (USEPA, 2003).

The seven bacterial isolates identified in this study are similar to those mentioned in other reports (Akaninwor*et al*. 2007; Aluyi *et al*. 2006; Agbabiaka and Oyeyiola, 2012; Uzoigwe and Agwa, 2012). However, the slight variations in the types of bacteria identified in this study and those of other researchers may in part be attributable to factors such as media, time, period of transportation, method of sampling, temperature and pH (Akortha *et al*., 2011).

The fungal genera identified in this study, were similar to the findings of Agbabiaka and Oyeyiola, (2012) at Foma River, Illorin, Kwara state, Nigeria. Health problems associated with these organisms can cause test and odour problems thereby affecting the aesthetic properties of water (Akaninwor *et al*., 2007).

*Escherichia coli* had the highest frequency of occurrence among the bacterial isolates with (25.81%), followed by *Staphylococcus aureus* (19.35%) and *Streptococcus faecalis* (17.74%).The bacteria and fungi isolated in this study are capable of causing a lot of waterborne diseases such as typhoid fever, dysentery, acute gastroenteritis and urinary tract infections in humans and animals (Orji *et al*., 2006). *Staphylococcus aureus* is a major cause of food poisoning and suppresses the host immune system. Furthermore, most of the fungi especially *Aspergillus* sp. are known for Aflatoxin production. Aflatoxin is primarily responsible for the destruction of the liver by inducing fatty acid metamorphosis of the liver cells. This leads to decrease in Ribosomal Nucleic acid (RNA) polymerase activity with subsequent impairment in the synthesis of nucleic acid (Uraih, 2004). It also weakens the body and is associated with inhibition of libido in males and ovulation in females (Ibeh, 1998).

The temperature range of water samples agree with the reports of Ojokoh and Adetuyi (2002) as well as Agbabiaka and Oyeyiola (2012), where they reported temperature ranges of 29-30˚C and 22-31˚C, respectively. The temperature falls within the mesophilic range, which optimally supports the growth and proliferation of microorganisms. The pH range of 6.8± 0.28 to 7.7±1.64 reported in this study was near neutrality and favoured bacterial growth over fungal. The range was similar to that reported by Uzoigwe and Agwa (2012) on microbiological quality of water (5.67-6.84) collected from boreholes sited near refuse dumpsites.

**Conclusion**

The Ujiogba River plays host to a lot of microorganisms which are capable of compromising human health due to a host of diseases that they cause. In Edo Central Senatorial District (Esan Land), underground water is not easily accessible because the Senatorial district is located on a plateau. If one of the targets of the Millennium development goals (MDGs) is to half the population of people without sustainable access to safe drinking water, the government should rise to its social responsibility by providing these rural dwellers with portable water.

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