IJST EXPLORE International Journal of Science and Technology
CENTRE OF PROFESSIONAL RESEARCH PUBLICATIONS
ISSN 2049-7318

International Journal of Science and Technology Volume 1 No. 11, November, 2012

Microbiological Assessment of Ero and Ureje Dams in Ekiti State, Southwest, Nigeria

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ABSTRACT

Heterotrophic studies of bacteria and fungi organisms were carried out on water samples from Ureje and Ero dams.

Problem statement: Water from Ureje and Ero dams is used for the municipal water supply for drinking and for agricultural purposes. The level of contamination of bacteria and fungi organisms has not been ascertained.

Approach: Heterotrophic studies of bacteria and fungi organisms were therefore carried out on water samples from the Ureje and Ero dams.

Results: The results of the water analyses show that Ureje dam has 140 x 10^5 bacteria count while the Ero dam has 2.8 x 10^5 bacteria count as their minimum counts respectively. Their maximum counts are 150 x 10^5 and 3.0 x 10^5 and averagely 150 x10^5 and 2.6 x10^5 respectively. The water is contaminated as a result of an anthropogenic effect of man and animal wastes. The Ureje and Ero dams have fungi count of 3.0 x 10^2 and 1.0 x 10^2 as minimum counts and maximum counts of 4.0 x 10^2 and 2.0 x 10^2 with average counts of 3.5 x 10^2 and 1.5 x10^2 respectively. The fungi counts indicate that the water is contaminated through agricultural activities around the dams. The probable number of coliform [PNC] and E coli organisms [EC] per 100mls of water samples from Ureje dam is 75 coliform per 100ml of water and 15 E-coli per 100ml of water while Ero dam has 23 coliform per 100ml of water and 9 E.coli per 100mls of water. The World Health Organization [WHO 2002] recommends a standard of 0/100ml of both E coli and coliform for drinking water**.**

Conclusion: The water from the two dams is contaminated based on the recommended standards. The possible isolates are bacteria [E coli sp, klebsiella sp., trichoderma sp.and pseudomonas sp.] and fungi [aspergillus sp.,].These indicate that the water in the dams is equally contaminated.

Keywords: *Ureje, Ero, Dam, Heterotrophic, Bacteria, Fungi*

1. INTRODUCTION

Ureje and Ero dams are located in Ekiti State [Fig. 1]. They lie within the South western Basement Complex of Nigeria [Jones and Hockey, 1964].

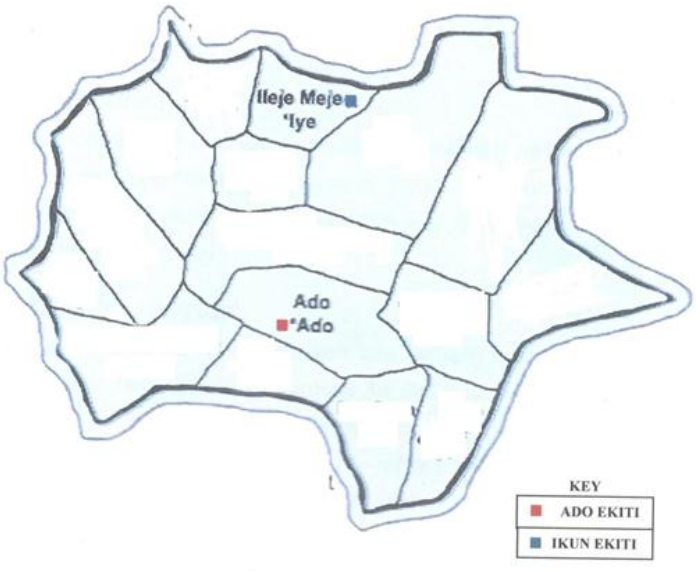

Figure 1: Map of Ekiti State showing Ureje Dam in Ado Ekiti and Ero Dam in Iye Ekiti

The Basement complex consists of migmatite gneiss complex, schist [metasediments], older granites and the late intrusives. Disease causing micro-organisms of human beings, plants, and animals constitute the greater part of the microbial population in our immediate environment [Pelczer et al, 1986]. Heterotrophic organisms such as lactobacilli that have elaborate requirements for specific nutrient [i.e. vitamins] and other growth promoting substances are called fastitudious heterotrophs. Fungi of heterotrophic species fall within eukaryotic organisms that are of great practical and scientific interest because of their microscopic cellular dimension and they require organic compounds for nutrition. To carry out survey on water for specific pathogens can be very difficult and cumbersome, since pathogens do not last long in water.

Ureje and Ero dams are studied for their heterotrophic micro-organism and coliform organisms. Precisely, water from the dams is used for municipal water supply. Generally vast majority of the water from the stream, lake, dams or aquifer is neither potable nor toxic. Water quality is a complex subject partly because water is a complex medium intrinsically tied to the geology and ecology of the area. Water that is commonly used for municipal purposes must be free of contaminants. Contaminants that are found in untreated water include micro organisms such as virus and bacteria [coliforms e.g.

Escherichia coli.]. Coliforms are commonly used as indicator of water pollution. Heterotrophic micro-organisms mostly require one or more organic nutrients to grow and synthesize their cells.

Harry et al, [1980] state that some heterotrophic micro-organisms have sufficient synthetic ability to form all of the amino acid, vitamins and other compounds essential for a living cell from relatively simple materials such as inorganic nitrogen salts provided that they have a source of energy and carbon. WHO, [2002] states that feacal coliform such as E coli are bacteria whose presence indicates that the water is contaminated with human or animal wastes. Disease – causing microbes [pathogens] in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms. These pathogens may pose a special health risk for infants, young children and people with severely compromised immune systems.

Most bacteriological analysis of water is focused on detecting the indicator organisms of fecal contamination [coliform organism]. The presence of E coli [coliform] in the water sample shows that the water is contaminated through human feaces and that will make the water unsafe for drinking. Coliform is an enteric organism which can survive for a long time in water. It is a gram negative organism and the cells are rod like [Bartram et al., 2003]. This coliform can ferment sugar [lactose] to produce gas and acid. Although *P. aeruginosa* can be significant in certain settings such as health care facilities, there is no evidence that normal uses of drinking-water supplies are a source of infection in the general population [WHO, 2002]. However, *Pseudomonas aeruginosa* is sensitive to disinfection and entry into distribution systems can be minimized by adequate disinfection. Brochardt et al., [2003] stated that *Pseudomonas aeruginosa* is detected by HPC, which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms.

2. MATERIALS AND METHODS

TOTAL HETEROTROPHIC BACTERIA COUNT [THB]

Two water samples were collected from Ureje and Ero dams using sterile sample bottles. These samples were carefully brought to the laboratory for microbial analyses. Serial dilution of the water samples was done six times in a set of test tubes, each test tube containing 99ml of distilled water. One milliliter of each dilution was plated out in duplicates into sterile Petri dishes. The prepared and sterilized MacConkey agar and Nutrient agar were introduced into the Petri dishes using pour plate method. The Petri dishes were shaked gently and allowed to stand so that the agar will solidify.

The Petri dishes were incubated in inverted form at 35^{o}C for 48 hours. This allows for the isolation of only aerobes and facultative bacteria. The plates were observed for growth and colonies were later counted. The count was multiplied by the dilution factor and the product expressed as colony forming unit i.e. [CFU] per mililitre of the original sample. The values of bacteria obtained after counting and multiplying with the dilution factor was less than 300 CFU.

TOTAL HETEROTROPHIC FUNGAL COUNT [THF]

The malt extract was used in this fungal count. The medium was prepared according to the procedure of Premalatha [2001] and was sterilized in the autoclave for 15 minutes at 121^{o}C. One mililitre of each dilution of the water samples from Ero dam and Ureje dam was plated out in duplicates into sterile petri dishes. The prepared malt extract agar of 10mls in molten form was poured into the petri dishes holding 1 mililitre of water sample using pour plate method. The petri dishes were allowed to stand so that the agar will solidify. The plates were labeled accordingly and incubated at 30^{o}C for 5 days. The developed colonies were observed and counted while the isolates were identified using methylene blue stain.

PRESUMPTIVE TEST

Presumptive test was carried out on the water samples, in order to know the most probable number of coliform in the two dams. This is done by preparing double strength of the lactose broth. The double strength was poured into three test tubes with Durham tubes. These tubes were labeled according to the amount of water that was dispensed [i.e. 10ml]. The single strength lactose broth was poured into six test tubes with Durham tubes. These tubes were incubated at 35^{o}C for 24hours in order to observe the gas and acid production. The most probable number [MPN] was determined using the MPN Table 5.

COMFIRMATORY TEST

The positive tubes from the presumptive tests [i.e. tubes showing gas and acid] were sub- cultured on Eosin methylene blue agar and macConkey agar and incubated at 35^{o}C for 24hours in order to examine the colonies. Those colonies that had greenish metallic sheen were picked for staining. The gram staining of organism was carried out to check for gram positive or gram negative. Gram negative is an indication of the presence of E-coli.

COMPLETION TEST

These colonies that had greenish metallic sheen were picked and sub cultured into Brilliant green lactose Bile Broth [BGLBB]. The tubes of BGLBB that had Durham tubes in inverted form were further incubated at 37^{o}C for 24hours. The production of gas after incubation is also an evidence of the presence of E-coli.

GRAM STAINING

This was done by preparing slide of the organisms. The film on the slide was flooded with crystal violet stain and left for a minute. The excess was removed, Gram iodine was applied and left to stand for a minute. The excess was removed using

swift running tap. Ethanol was applied for only 30 seconds, swift running water was used to remove the excess saframin that was applied last. The slide was dried and viewed under the microscope using X100 object [oil immersion].

SAMPLE STAINING OF FUNGI ISOLATES

The fungi isolates were picked carefully and placed in a drop of methylene blue stain on a slide. This was covered with a cover slip. The prepared slide was viewed under the microscope using X4, X10 and X40 objectives for proper identification of fungi. Under the microscope, the arrangement of the conidia was seen clearly and the fungi isolates identified as Aspergillus Species [Fig. 2]

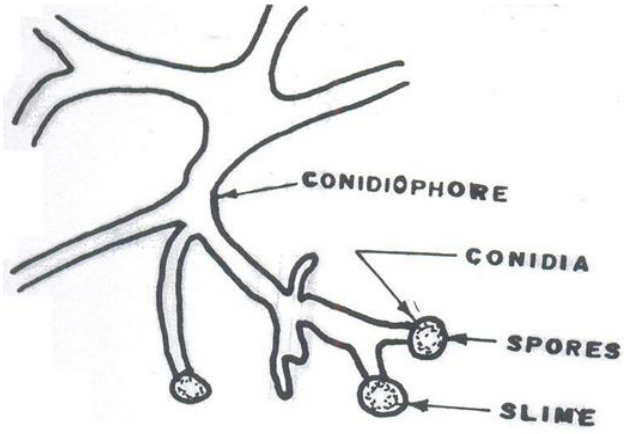

Fig 2: Microscopic and morphologic appearance of Bacteria isotes

Biochemical Test

Biochemical test [sugar fermentation, catalase, citrate utilization, indole, methyl red and vogues prousker] were carried out to enhance identification of the bacteria isolates [Fig. 3].

3. RESULTS AND DISCUSSION

Table 1 shows the result of heterotrophic bacterial counts [HBC] [cfu/ml]. Ero dam had count of $2.6x10^5$ and Ureje had count of $150x10^5$. The low count of $2.6x10^5$ [cfu/ml] of Ero dam may be attributed to the low level of human activities around the dam, while the high count of $150x10^5$ [cfu/ml] at Ureje dam can be attributed to the erosion within the bank of the dam which is very active and through which bacteria can be washed off into the water.

Table: 1 Total heterotrophic Bacterial count [CFU/ml] of water samples from Ero and Ureje dams

Sampling Points	Heterotrophic Bacterial Count		[Cfu/ml]
	Minimum	Maximum	Mean
Ero Dam Ureje Dam	$2.8 x10^5$ $140 x10^5$	$3.0 x 10^5$ $160 x10^5$	$2.6 x10^5$ $150 x10^5$

Ureje dam is within Ado Ekiti metropolis with high population density that can contribute to the high count of bacteria compared to Ero dam that is one kilometer away from the town. WHO [2002] specified that heterotrophic plate count must not be more than 500 bacterial colonies per milliliter which confirms the high contamination level of the two dams. Although, heterotrophic plate count [HPC] has no health effects, it is an analytical method used to measure the variety of bacteria and fungi that are common in water. The lower their concentration in drinking water the better the water system is maintained.

Table 2 shows the result of heterotrophic fungal count [HFC]. Ero dam had fungal count of $1.5x10^2$ [cfu/ml] and Ureje dam had the count of $3.5x10^2$ [cfu/ml].The result also indicates that Ureje dam has high fungal

Table: 2 Total Heterotrophic Fungal Count [Cfu/ml] of Water Samples from Ero and Ureje dams

Sampling Points	Heterotrophic Fungal Count		[Cfu/ml]
	Minimum	Maximum	Mean
Ero Dam Ureje Dam	$1.0x10^2$ $3.0x10^2$	$2.0x10^2$ $4.0x10^2$	$1.5x10^2$ $3.5x10^2$

This can be attributed to the anthropogenic action of dumping dried grasses, leaves of plants and stalks of eaten maize into drainages which eventually find their ways into the dams. Most fungi that are pathogenic occur through accidental contact with environmental sources such as soil, water and dust. The mold aspergillus flavus synthesize a potentially lethal poison called aflatoxin which is the cause of a disease in domestic animals that have eaten grains infested with mold and also a cause of liver cancer in humans [Kathleen, 2005]. The probable number of coliform bacilli and Escherichia coli per 100ml of the water samples are shown in Table 3.

Table: 3 Probable Number of coliform bacilli and E.coli Per 100ml of the water samples

Sample	coliform/100ml	E.coli /100ml
Ero dam Ureje dam	23 75	9 15

Ero dam had coliform bacilli of 23/100ml and E. coli of 9/100ml, while Ureje dam had coliform bacilli of 75/100ml and E. coli of 15/100ml. The high probable number of coliform and E. coli from Ureje water dam shows that human and animal activities are higher at Ureje than Ero dam. The result of Biochemical test and Grams staining reactions are shown in Table 4.

Table 4 : Biochemical/gram staining results.

										Sugar Fermentation						
Isolate Code	Cell Shape	Gram Reaction	Spore Production	Catalase	TSI Reaction	SIM Reaction	Citrate Utilization	MR	VP	Glucose	Maltose	Mandgol	Sucrose	Lactose	O-F OR H &L	Nitrate Reduction
A1	LR	-	-	+	YG NC NC	---	-	+	+	YG	MC	NC	NC	NC	F	+
A2	SR	-	-	+++	NC NC NC	-+-	+	-	-	NC	NC	NC	NC	NC	OX	+
B1	MLR	-	-	++	Y NC NC	---	-	+	-	Y	NC	NC	NC	NC	F	+
B2	SR	-	-	+++	NC NC NC	-+-	+	-	-	NC	NC	NC	NC	NC	OX	+
				LEGEND												
				SR - Short rod			Y-acid production only						F - Fermentative			
				MLR - Medium long rod			YG - Acid and gas production						OX - Oxidative			
				LR - Long rod			NC - No change									

Table 5 : The possible isolates

Isolated Bacterial	Isolated Fungi
Klebsiella edwardsii	Aspergillus funmigatus
pseudomonas aerginosa	aspergillus flavus
klebsiella pneumoniae	aspergillus Niger
pseudomonas aerginosa	

The biochemical results in Fig. 2 indicate that the isolates A^1 and A^2 from Ero dam were identified as Klebsiella sp and Pseudomonas Species. The isolates B^1 and B^2 from Ureje dam were identified as Klebsiella, Pseudomonas and E. coli [Table 5]. Fungi isolates from the two Dams were identified as Aspergillus Species. The morphology of the fungi isolates are shown in Fig. 3.

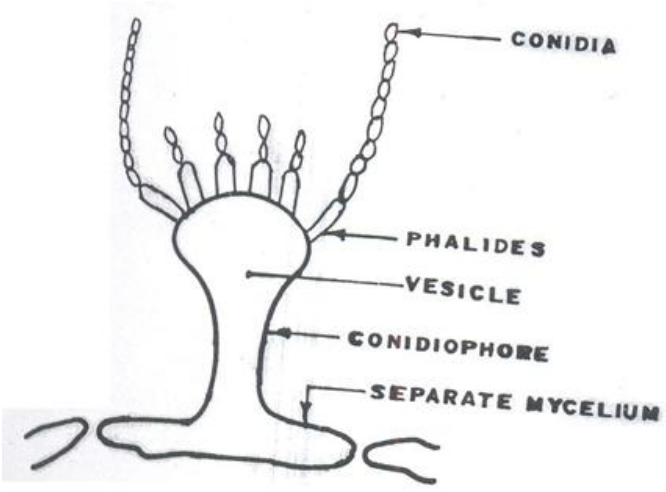

Figure 3: Microscopic and Morphologic Appearance of Fungi Isolates

The presence of Escherichia coli in these dams is related to the type and the number of contamination sources in them. Also, the presence of pseudomonas aeruginosa being an opportunistic pathogenic micro organism is undesirable in recreation waters and in drinking water and it does not conform to WHO [2002] standards.

WHO [2002] reveals that any water intended for drinking should contain feacal and Total coliform count of 0 respectively in any 100ml. Table 4 shows E coli among the possible isolates that are closely related to feacal contamination resulting mainly from wastes dumped and washed by erosion into the dam within the city. The isolates klebsiella Sp. are also found in the respiratory tracts of the chronic lung infections with which the species are associated. No more than 5.0% of samples may be total coliform positive in a month.

4. CONCLUSION AND RECOMMENDATION

This study shows that the population of THB and THF in the two dams does not meet the standard of drinking water set by WHO 2002. The maximum counts of THB in Ureje and Ero dams are 150 x 10^5 and 3.0 x 10^5 per ml and 4.0 x 10^2 and 2.0 x 10^2 per ml for the THF respectively as against maximum 500 THB per ml set by WHO 2002. The study also reveals that the probable number of coliform and E coli organisms in the dams is above the recommended standard of O organisms per 100mls of water as set by WHO 2002. The occurrence and presence of these organisms in the water of the dams show microbiological contamination. The water therefore needs to be treated by chlorination and boiled if it should be taken for drinking by man to avoid the water borne diseases such as typhoid, diarrhea and cholera.

It is recommended that the government at local and state levels should put in place bill boards at various strategic locations near the two dams warning the public against the dangers inherent in using the water for drinking or domestic purposes. Monitoring of the dams and the adjoining streams and rivers should be given special priority to avoid an indiscriminate dumping of refuse and human wastes by people living near the banks of the dams streams and rivers.

REFERENCES

[1] Bartram J (2003) Heterotrophic *plate counts and drinking-water safety: T he significance of HPCs for water quality and human health.* WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.

[2] Borchardt M.A, Stemper M. E, Standridge J. H., (2003) *Aeromonas* isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis. *Emerging Infectious Diseases*, 9:224–228.

[3] Jones H. A., and Hockey R, D., [1964] The Geology of part of S. W. Nigeria Geological Surv. Bulletin 31, 87p Kathleen, P .T [2005] Foundation of microbiology ,5th edition.

[4] Harry W. S. [1980]. Microbes in action, Laboratory manual , pp. 65

[5] Pelczar, M.J, Krieg, R N and Chan, E.C.S [1986]. Microbiology. Tata McGraw-Hill ed., 1993. Pp 100 – 105

[6] Premalatha, K., 2001 Studies on heterotrophic bacteria and on human pathogens

[7] V.Cholerae and V. Parahaemolyticus] from Upper estuary, Porto-Novo south India, Indian. M. Phill. Theisis, Annamalai University. India.

[8] WHO (2002). *Aeromonas*. In: *Guidelines for drinking-water quality*, 2nd ed.

[9] *Addendum: Microbiological agents in drinking water.* Geneva,World Health Organization.