

**MICROBIOLOGICAL AND PHYSICO-CHEMICAL ASSESSMENT OF SAFETY OF  
AGUA LAKE IN ESAN CENTRAL LOCAL GOVERNMENT AREA OF EDO STATE**Adewoyin H. O.\*<sup>1</sup>, Deji S. A.<sup>2</sup> and Olaniran Olarinde<sup>3</sup><sup>1</sup>Dept. of Environmental Health Sciences, School of Public Health, University of Medical Sciences, Ondo City, Ondo State.<sup>2</sup>Dept. of Community Medicine, College of Medicine, Ekiti State University, Ado Ekiti.<sup>3</sup>Dept. of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile Ife, Osun State.**\*Corresponding Author: Adewoyin H. O.**

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**ABSTRACT**

Microbiology and Physico-chemical analyses of Agua Lake in Esan Central Local Government Area of Edo State were carried out using standard procedures. The multiple table method was used in the analysis for indicator organisms; total coliform bacilli, *Escherichia coli* and faecal streptococci count. The heterotrophic bacteria count was high  $1.7 \times 10^9$  cfu/ml –  $8.2 \times 10^{10}$  cfu/ml. The total fungal count at room temperature (28°C for 72 hours) was  $2 \times 10^8$  cfu/ml-  $9 \times 10^9$  cf/ml. The total coliform count was 25-425 cells/100ml, *E.coli*, count was 2-17 cells/100ml and faecal streptococci ratio was greater than 4 in all the water samples. The bacteria isolates included; *Escherichia coli*, *Streptococcus faecalis*, *Klebsiella nterogenes*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*. The fungi isolates were *Penicillium* spp, *Aspergillus niger* and *Candida albicans*. Though, the result of physico-chemical analysis varied, some were not within the W.H.O recommended limit for drinking water. Based on Microbiological and physico-chemical results, Agua lake water is not fit for consumption prior to treatment especially in the rainy season.

**KEYWORDS:** Assessment, safety, Season, Coliform, Gastroenteritis, Indicator.**INTRODUCTION**

Water is a critical component of public health, and failure to supply safe water will place a heavy burden to humanity. (Boe-Hansen, 2001)<sup>[1]</sup> Inadequate water supply is still one of the major challenges in developing countries. The Joint Monitoring Programme (JMP) for Water Supply and Sanitation, implemented by the World Health Organisation (WHO) and UNICEF, reports that 783 million people in the world (11% of the total population) have no access to safe water, 84% of whom live in rural areas. About 187 million people use surface water for drinking purposes; 94% of them are rural inhabitants and they are concentrated in sub-Saharan Africa.<sup>[2]</sup> The provision of clean drinking water, especially in developing countries like Nigeria, has always been a major challenge (Raji and Ibrahim, 2011).<sup>[2]</sup>

Water is one of the best-known ionizing agents because most substances are somewhat soluble in water. It is frequently called the universal solvent because of its capacity to dissolve numerous substances in large amounts, pure water rarely occurs in nature. The evaluation of potable water supplies for coliform bacteria is important in determining the sanitary quality and also to establish the safety of drinking water. Clean water

improves life, predominantly in the deterrence of the spread of disease-causing microorganisms. The provision of clean and safe water is undeniably paramount to human health, hence given priority concern by the United Nations (UN) Sustainable Development Goal (SDG) number 6.<sup>[4]</sup> This goal, among other targets, is set to achieve universal and equitable access to safe and affordable drinking water for all by 2030.<sup>[4]</sup> An increasing number of countries are confronting water stress that affects more than two billion people globally. Water is vital for human existence, on a daily basis, many people experience great difficulties in accessing safe and affordable drinking water with particular reference to developing countries and; there exists an undisputable link between human health and drinking water quality. Therefore, there arises the need to continuously embark on drinking water quality assessments and monitoring (Eseigbe et al., 2018).<sup>[4]</sup>

Conformation with microbiological standards is of special interest because of the capacity of water to spread waterborne disease. The objective of this study is to determine the microbiological and physico-chemical quality of Agua Lake water.

Water quality of any specific area or specific source can be assessed using physical, chemical and biological parameters. The values of these parameters are harmful to human health if they occurred more than the defined limits.

## MATERIALS AND METHODS

Samples were collected at four different hand-dung holes 5cm below the water surface into pre-sterilized two-litre plastic containers between the hours of 10:00 a.m. and 11:00 a.m. on each sampling day and stored on an ice pack. All samples were labelled immediately after they were collected and transported to the laboratory for analysis within six hours of collection (maximum transit time four hours, maximum process time-two hours)

### Microbiological Analysis

Solid and liquid media were prepared according to the manufacturer's specifications. Nutrient agar was used for heterotrophic plate count. MacConkey broth and MacConkey agar were used for the isolation of bacteria from the water samples. Sabouraud dextrose agar (SDA) which had been fortified with antibiotic (chloramphenicol, 0.05ml) was used for fungal enumeration.

The sterility of each batch of test medium was confirmed by incubating two uninoculated tubes and plates along with the inoculated tests. The pure cultures of the bacterial and fungal isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the isolates.

Detection and enumeration of faecal coliform involved three-stage test procedures: Presumptive, Confirmed and Completed tests. The most probable number (MPN) counts were by the multiple tube fermentation technique. The most probable number method was suitable for the examination of turbid water containing a small number of indicator bacteria. Media such as Lauryl tryptose broth,

lactose broth and tryptone broth which inhibited the growth of gram-positive bacteria and permitted the detection of lactose fermenters were used for presumptive and confirmed tests. The completed test was undertaken by culturing from positive tubes of the confirmed test onto MacConkey and nutrient agar.

A heavy inoculum from the multiple tube showing a positive reaction was sub cultured into glucose azide broth (Hannay and Norton, 1947) The confirmation test was carried out by subculturing on MacConkey agar. The most probable number of streptococci was determined from MacCrady's probability table.

### Physico-chemical Analysis

The temperature and pH were measured in situ with calibrated thermocouple and pH meter respectively. Water samples for dissolved oxygen (DO) were fixed at the field with Winkler's solutions (A and B), while samples for biological oxygen demand (BOD) were collected into dark reagent bottles (250 ml). These were incubated for five days before fixing for DO<sub>5</sub> titration. Specific methods employed for the analyses of the following were: Colour- Lovibond colour disc; Phosphate-Ascorbic acid reduction; Chloride – Mercurimetric titration (ASTM, 1982); Iron – Phenanthroline colourimetric method; Heavy metals such as mercury and lead - Atomic absorption spectrophotometer and turbidity-Nephelometric.

## RESULTS

Agua lake water samples contained high numbers of heterotrophic plate count ( $1.7 \times 10^9$  -  $8.2 \times 10^{10}$  cfu/ml), Total coliform count (25 -425 cells/100ml), Fungal count ( $2 \times 10^8$  -  $9 \times 10^9$  cfu/ml), E coli count (2-17 cells/100ml) and the Streptococci count (4 -7 cells/100ml). The presence of faecal streptococci in addition to the coliform especially *E coli* further confirmed the faecal nature of the contaminants in Agua lake.

**Table 6: Shows the ratio of faecal coliform to faecal Streptococci which were all greater than four.**

**Table 1: Heterotrophic plate count/total viable count in agua lake water samples (cfu/ml).**

SAMPLE	APRIL	MAY	JUNE	JULY	AUGUST
1	$5.7 \times 10^9$	$1.1 \times 10^9$	$3.5 \times 10^{10}$	$8.2 \times 10^{10}$	$8.2 \times 10^{10}$
2	$4.9 \times 10^9$	$8.7 \times 10^9$	$2.9 \times 10^{10}$	$8.0 \times 10^{10}$	$7.4 \times 10^{10}$
3	$3.6 \times 10^9$	$7.0 \times 10^9$	$2.8 \times 10^{10}$	$7.9 \times 10^{10}$	$7.1 \times 10^{10}$
4	$1.7 \times 10^9$	$7.1 \times 10^9$	$2.3 \times 10^{10}$	$7.5 \times 10^{10}$	$6.5 \times 10^{10}$

**Table 2: Fungal count in Agua lake water samples (cfu/ml).**

SAMPLE	APRIL	MAY	JUNE	JULY	AUGUST
1	$3.0 \times 10^8$	$4.0 \times 10^8$	$8.0 \times 10^9$	$5.0 \times 10^9$	$6.0 \times 10^9$
2	$5.0 \times 10^8$	$2.0 \times 10^8$	$6.0 \times 10^9$	$4.0 \times 10^9$	$8.0 \times 10^9$
3	$3.0 \times 10^8$	$3.0 \times 10^8$	$9.0 \times 10^9$	$6.0 \times 10^9$	$7.0 \times 10^9$
4	$4.0 \times 10^8$	$3.0 \times 10^8$	$4.0 \times 10^9$	$7.0 \times 10^9$	$9.0 \times 10^9$

**Table 3: Total coliform (cells/100ml) of water from aqua lake.**

SAMPLE	APRIL	MAY	JUNE	JULY	AUGUST
1	55	250	110	175	55
2	25	425	120	275	95
3	25	350	85	425	275
4	40	425	150	200	150

**Table 4: The mean concentration of total coliform count (cells/100ml) of water samples from aqua lake.**

MONTH	MEAN + SEM	P VALUE
APRIL	36.25 + 7.18	P<0.05
MAY	362.50 + 41.45	P<0.05
JUNE	116.25 + 13.44	P<0.05
JULY	268.75 + 56.25	P<0.05
AUGUST	143.75 + 47.88	P<0.0510

F=11.317, P value is 0.0002, considered extremely significant

**Table 5: Monthly variation of *E.coli* in Aqua lake water samples (cells/100ml).**

SAMPLE	APRIL	MAY	JUNE	JULY	AUGUST
1	7	6	17	17	12
2	2	8	2	14	14
3	6	4	2	12	9
4	2	2	4	9	10

**Table 6: The mean concentration of *E coli* count (cells/100ml) water samples from aqua lake.**

MONTH	MEAN + SEM	P VALUE
APRIL	4.25 + 1.31	P<0.05
MAY	5.00 + 1.29	P<0.05
JUNE	4.25 + 1.65	P<0.05
JULY	13.00 + 1.6	P<0.05
AUGUST	11.25 + 1.10	P<0.05

F=8.794, P value is 0.0007, considered extremely significant.

**Table 7: Streptococci count of water samples (cells/100ml).**

SAMPLE	APRIL	MAY	JUNE	JULY	AUGUST
1	4	6	4	6	5
2	4	4	6	7	7
3	6	4	4	5	4
4	5	5	4	7	4

**Table 8: The mean concentration of streptococci count (cells/100ml) of water samples from aqua lake.**

MONTH	MEAN + SEM	P VALUE
APRIL	4.75 + 0.41	P<0.05
MAY	4.57 + 0.47	P<0.05
JUNE	4.50 + 0.50	P<0.05
JULY	6.25 + 0.47	P<0.05
AUGUST	5.00 + 0.70	P<0.05

F=1.674, P value is 0.2081, considered not significant.

**Table 9: Ratio of faecal coliform to Faecal Streptococci in Aqua lake water samples.**

SAMPLE	COLIFORM	STREPTOCOCCI	RATIO	REMARK	
	1	55	4	13.8	>4
	2	25	4	6.3	>4
	3	25	6	4.2	>4
	4	40	5	8.0	>4
	1	250	6	41.7	>4
	2	425	4	106.3	>4
	3	350	4	87.5	>4

	4	425	5	85	>4
	1	110	4	27.5	>4
	2	120	6	20	>4
	3	85	4	21.3	>4
	4	150	4	37.5	>4
	1	175	8	21.9	>4
	2	275	7	39.3	>4
	3	425	5	85.0	>4
	4	200	7	28.6	>4
	1	55	5	11.0	>4
	2	95	7	13.6	>4
	3	275	4	68.8	>4
	4	150	4	37.5	>4

**Table 10: The mean concentration of physico-chemical properties of Agua lake water samples (all units in ppm except otherwise stated).**

MEAN + SEM						
PARAMETER	APRIL	MAY	JUNE	JULY	AUGUST	P VALUE
ODOUR	OBJ	OBJ	OBJ	OBJ	OBJ	
COLOUR (TCU)	5.12 + 0.12	5.00 + 0.00	5.12 + 0.12	5.00 + 0.00	5.12 + 0.12	0.7362 (NOT SIG.)
TEMPERATURE(°C)	26.50 + 0.20	26.25 + 0.14	26.37 + 0.23	26.25 + 0.14	26.37 + 0.12	0.8400 (NOT SIG)
pH	7.03 + 0.01	7.10 + 0.012	7.28 + 0.36	7.27 + 0.03	7.27 + 0.050	<0.0001 (EXT SIG)
BOD (PPM)	4.50 + 0.32	4.45 + 0.30	2.13 + 0.17	3.05 + 0.17	3.14 + 0.11	<0.0001 (EXT.SIG)
DO (PPM)	30.95 + 1.75	39.80 + 1.65	40.40 + 0.98	40.61 + 1.48	42.20 + 0.51	0.56538 (NOT SIG)
TURBIDITY (NTU)	50.32 + 16.66	35.12 + 15.22	40.25 + 19.34	42.25 + 18.95	38.75 + 16.60	0.9777 (NOT SIG)
CONDUCTIVITY(US/CM)	41.00 + 4.93	32.25 + 3.42	44.25 + 3.80	47.25 + 4.75	42.75 + 5.79	0.3260 (NOT SIG)
CHLORIDE (PPM)	45.52 + 3.80	41.95 + 0.65	45.52 + 3.77	45.20 + 1.64	46.55 + 1.91	0.7834 (NOT SIG)
PHOSPHATE (PPM)	6.87 + 2.47	5.747 + 2.42	6.98 + 3.12	8.82 + 2.40	7.60 + 2.47	0.9401 (NOT SIG)
NITRATE (PPM)	31.80 + 12.96	26.39 + 6.64	41.00 + 8.21	41.64 + 7.70	42.98 + 6.31	0.6073 (NOT SIG)
IRON (PPM)	1.19 + 0.63	1.012 + 4.04	0.79 + 0.06	0.94 + 0.09	0.79 + 0.07	0.9171 (NOT SIG)
MERCURY(PPM)	ND	ND	ND	ND	ND	
LEAD (PPM)	ND	ND	ND	ND	ND	

Result represent the MEAN + SEM of five estimations. Not sig =Not Significant.

Ext Sig =Extremely Significant.

ND =Not Detected.

°C =Degree Celcius

OBJ =Objectionable.

TCU =True colour unit.

## DISCUSSION

The study revealed a high microbial count which was an indication of the presence of high organic matter and dissolved salts in the water which are usually a common feature of natural and untreated water. Abednego *et al.* (2013) recorded a high number of total coliform counts exceeding the WHO permissible limit.<sup>[5]</sup> The presence of coliform especially *E coli* has been used as an indicator of faecal pollution of water (Cheema *et al.*, 2018; Choudhury *et al.*, 2016).<sup>[6]</sup> Their presence, therefore, at Agua Lake indicated faecal pollution.

The results obtained in Tables 4 and 6 were considered extremely significant (P<0.05) while Table 8 shows that difference was not statistically significant (P>0.05).

The presence of faecal streptococci in addition to the coliform especially *E coli* further confirmed the faecal nature of the contaminants in Agua Lake.

Therefore, the source of contamination can be ascribed to human sources.

The physico-chemical properties of the water samples from Agua Lake Irrua did not comply with the standard limit for potable water. BOD measures the amount of oxygen utilized by microorganisms such as bacteria oxidize organic matter available within the water (Aniyikaiya *et al.*, 2019).<sup>[7]</sup> The results obtained in Table 10 showed that the BOD and the pH were extremely significant (P<0.05) while other parameters results obtained were not significant (P>0.05). However, mercury and lead were not detected.

The most serious Public health risk associated with drinking water supplies is microbial contamination. The presence of microorganisms poses a threat to human health because of their potential in causing human diseases.<sup>[8]</sup>

The high count could also be due to the study period, human activities, leachates from waste dumps, runoff from farmlands and the stagnancy of the water in the various "wells" (the sampling stations) of Agua Lake.<sup>[9]</sup>

The physico-chemical properties of the water samples from Agua Lake did not comply with the standard limit for potable water in relation to WHO and SON guidelines for drinking water quality.<sup>[9]</sup>

In conclusion, considering the cost and vital role of good water in our daily activities, appropriate and decent water supply can never be overemphasized in our rural communities. This requires the relevant participation of various agencies and the federal government by Prompting health education programmes to explain the importance of clean water and the relationship which exists between water and health. The provision of alternative sources of water is strongly recommended and frequent examinations of water should be employed, to ensure the appropriate distribution of clean pipe-borne water in our rural communities. Minimizing the prevalence of parasitic fungi, viral and bacterial infections in society relies considerably on the availability of potable water as this constitutes one of the major sources of infection.

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