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ENVIRONMENTAL IMPACT OF MICROBES ON AWBA DAM; ITS EFFECT ON ECOTOURISM

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ABSTRACT: *Recreational use of water is often given inadequate consideration and care. This is of particular concern as the recreational use of water is becoming popular in Nigeria. Many of these are increasingly contaminated by domestic sewage and industrial effluents. This study is therefore relevant in assessing the environmental impact of microbes on ecotourism in Awba dam. A total of nine water (n=9) and soil (n= 9) samples were collected at entry, middle and the end of the Awba dam for heavy metal analysis and microbial assay. Samples were assessed for heavy metals using an official procedure and atomic absorption spectrophotometry. Total aerobic plate count, Isolation and characterization of strains was done using standard methods. For enumeration of E. coliO157:H7, colonies were characterized using standard methods. The direct slide agglutination technique was utilized for serology. The presumptive E. coli isolates were subjected to agglutination tests with specific E. coli O157:H7 . For the antibiotic sensitivity test, the Bauer-Kirby disc diffusion method was used to test the sensitivity of the isolates. Statistical analysis of ANOVA was used and Duncan multiple range test was used to separate the means. All the values obtained for the total aerobic count and total coliform count for soil and water were higher than EPA recommended value for recreational waters. For the antibiotic Sensitivity Profile, isolates from Awba dam showed the highest sensitivity (16.17mm) to ciprofloxacin while lowest was with Augmentine (8.25mm).Furthermore the isolate from the control point showed highest sensitivity to CPR and NIT (14mm) and least for AUG (5mm) Generally, E.coliO157:H7 isolates were highly sensitive to Oflatoxin and Ciproflaxin(93.3%) while the isolate was completely resistant to Ampicilin and Cefuroxime. The presence of E.coliO157:H7 in the dam can make the dam unfit for recreational activities and also for the community household chores, if not well treated . The University management should device means of controlling waste water that enters into the dam by providing alternate channels of discharge .This will reduce the growth and spread of the microbes in the soil and water of the dam.*

KEYWORDS: Total Coliform Count, Heavy Metals, Physico-Chemical Parameters, E.coli O157:H7.

INTRODUCTION

Fresh water ecosystem provides the very basis for human health, ecological sustainability, economic and homeland security. Fresh water is one of mankind most precious commodity. The increasing scarcity of clean fresh water through population growth and development, droughts, contamination and other factors places greater demands on the very foundation of society. This can also affect recreation activities such as sport fishing. According to Timothy (2002), water- based tourism relates to any touristic activity undertaken in or in relation to water resources, such as lakes, dams, canals, creeks, streams, rivers, water ways, marine coastal zones, seas, oceans and ice-associated areas. Water-based and water enhanced recreational

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activities are often less detrimental to water quantity and quality compared to many other human uses of water like agriculture, industry etc. (Long,2012).Water-based tourism activities include boating, sailing, motor boating, swimming, skiing, fishing etc. Lakes all over the world are used as resource for ecotourism, natural tourism, leisure tourism and conference tourism and are attracting millions of tourists.

The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. (AWWA research foundation, 1993).

Various research work has been done on Awba dam, some of which include the work of Chuwuka *et al.*, (2008) who worked on the influence of persistent presence of water hyacinth on specific physicochemical properties of Awba dam. Yeside (1995) also studied the physico-chemical parameters of Awba dam. Olagbemide (1992) carried out limnological study of Awba dam.However, nothing has been done to assess the impact of microbes on the ecotourism potentials of the dam.

This study is aimed at determining the microbial load of the dam and the antibiotic sensitivity profile of the isolate *E. coli*O157:H7.

MATERIALS AND METHODS

Brief history of the study area

The University of Ibadan is the first University in the sub-sahara region of West Africa. It is with a total land area of 12.07km^2 which is on latitude (7°27’3’’N, 3°53’30’’E) and longitude 3053N and 3054E, at an altitude of 185m above sea level. The dam was constructed in 1974 and expanded to its size in 1971 by damming the Awba dam stream at a point where it flowed through natural valley. The dam is about 8.5m high, 110m long with a crest of about 12.2m. The reservoir has a maximum length of about 700m and a maximum depth of 5.5m with a surface area of about 6ha (Ugwumba and Ugwumba, 1993). Throughout the year, the water remains relatively constant, while excess water is made to spill way. The water of the lake is still with occasional multi-directional water movement due to wind effect. Wind action in the reservoir is minimal in the dry season and the high temperature at this period result in thermal stratification of the water.

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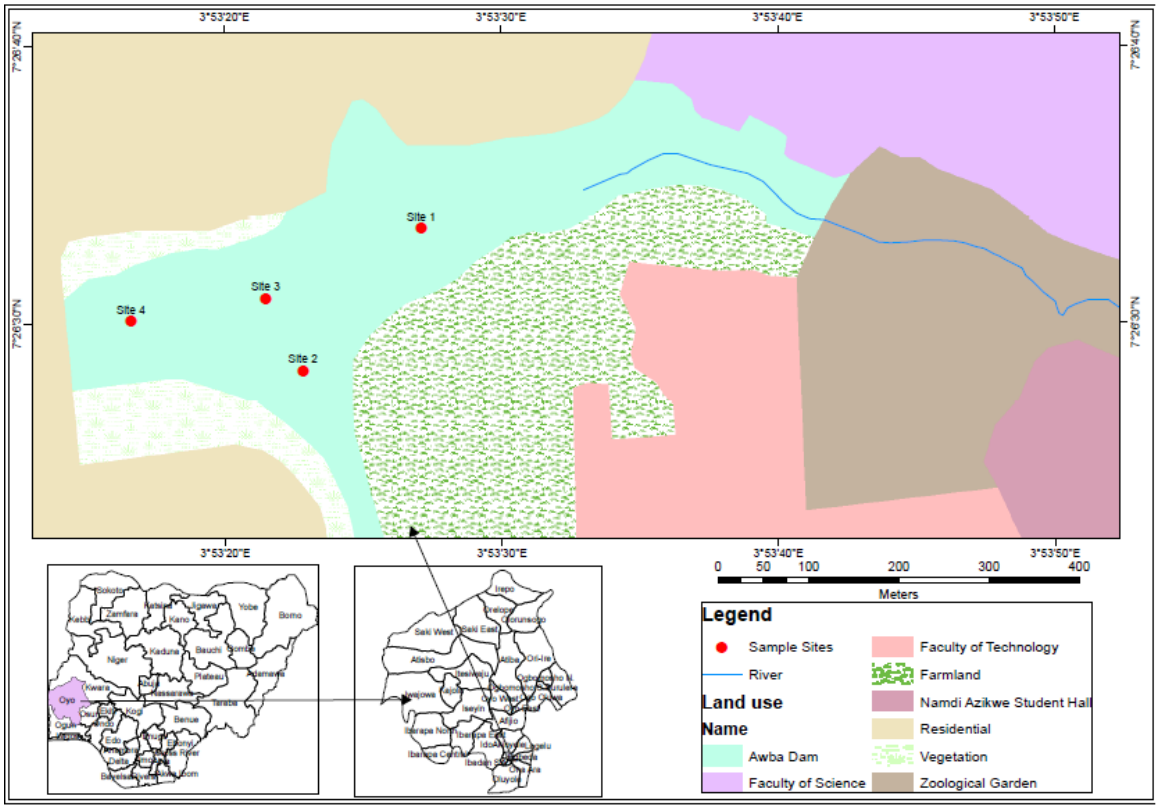

Plate1. Location of sample sites on Awba dam

Sampling zones

Awba dam was divided into 3 sampling zones; Entry point(site 1), Middle point(site 2) and End point(site 4) of the dam. The river flowing through Emmanuel College which discharges into the dam was used as the control. All samples were taken in replicates.

Heavy metals determination

Sampling procedure

Treatment of sample containers and glassware

All sample containers used for the collection of water samples were thoroughly washed and cleaned prior to sample collection in order to prevent contaminations of any sort. The 1L plastic containers were thoroughly washed with soapy water and rinsed copiously with a lot of tap water. They were thereafter thoroughly rinsed with a lot of distilled water. They were then air-dried and kept for sample collection.

Sample collection

Sample containers were rinsed thoroughly with the sample water before sample collection after which the sample containers were tightly capped and labeled appropriately. 500mL water samples were collected from 3 different points (entry, middle, end) of the dam by 9.00am in triplicates. The 9 water samples collected from different designated points were pulled together to form 3 composite samples. The sample of water that served as the control was taken at the upstream (the stream that serves the dam with water) in triplicate. The samples were preserved

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with 2mL of concentrated nitric acid per liter immediately after collection. The procedure above was carried out both for wet and dry season**.**

Procedure

Digestion of water sample

50ml of the water sample, collected in triplicate was transferred into a 100ml beaker and 5ml of concentrated nitric acid was carefully added. The solution was heated on a water bath for a few hours in order to concentrate the solution to about 10ml. The resulting concentrate was allowed to cool and then filtered into a 25ml volumetric flask.

The beaker was rinsed into the filtered paper with little quantity of distilled water and the solution was made up to mark. The filtrate was then transferred into a plastic vial for instrumental analysis.It involves the aspiration of the aqueous solution of the sample into air-acetylene flame. A reagent blank was prepared and analyzed following the same procedure with distilled water in the stead of sample.

Copper was detected at wavelength 324.7µm while Zinc was detected at wavelength 213.9 µm with both having slit width 0.7mm and the limit of detection 0.005mg/l. Mn was detected at wavelength 279.5 µm with slit width 0.7mm and its limit of detection is 0.3mm.Fe was detected at wavelength 248.5 was detected at wavelength 213.9 µm with slit width 0.2mm.Its limit of detection is 0.05mg/l.

Microbial Assay Of Water And Soil From Awba Dam

Sample collection for microbial assay

A total of nine water (n=9) and soil (n= 9) samples were collected at entry, middle and the end of the dam. Samples were collected into sterile universal bottles under aseptic conditions in triplicates from each point, stored on ice packs and immediately taken to the laboratory for analysis. Water and soil samples from the upstream (the stream that serves the dam with water) were taken in triplicate and served as control.

Total aerobic plate count, Isolation and characterization of strains

At each sampling,10ml of water or 10g of soil were withdrawn aseptically. Serial dilutions were made in sterile 0.1% peptone water and appropriate dilutions were surface plated on sorbitol MacConkey agar plates for the enumeration of E. coli O157: H7 , and on Nutrient agar plates for the enumeration of total aerobic plate count. All of the plates were incubated at 37^{o}C for 24 h. Colonies on each agar plate were enumerated using a colony counter (Model 3325, Leica Queber: Dark field, USA).

For enumeration of *E. coli*O157:H7, at least one colourless discrete colony from each of the sorbitol MacConkey agar plates was subcultured trice for purification. Colonies were characterized using standard methods according to Barrow and Feltham, 1993. The direct slide agglutination technique was utilized for serology. The presumptive *E. coli* isolates were subjected to agglutination tests with specific *E. coli* O157:H7 (Difco^{TM,} UK).

Antibiotic sensitivity test

The Bauer-Kirby disc diffusion method of Bauer *et al*., 1966 was used to test the sensitivity of the isolates. According to the manufacturer's specification, nutrient agar (LAB M, Lancashire,

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UK) was prepared and dispensed in petri dishes. Then 10^8 CFU of a 24 h broth culture of the test cultures were inoculated into each of the solidified agar plates in replicates and gently spread. Plates were allowed to dry after which the antibiotic sensitivity discs (Gram negative for *E. coli*) were placed onto the petri dishes followed by incubation of the preparation at 37°C for 24 h. Zone of inhibition (mm) around each antibiotic indicated sensitivity of the organism present in the culture to that antibiotic. Zones of inhibition was interpreted in millimeter scale as prescribed by Adetunji and Adegoke (2008) and resistance pattern in percentage was done according to Adewoye and Lateef (2004).The antibiotic sensitivity disc contained : CAZ: Ceftazidimine (30µg), CRX: Cefuroxime (30µg), GEN: Gentamicin (10µg), CPR: Ciprofloxacin (5µg), OFL: Ofloxacin(5µg), AUG: Amoxycilin Clavulanate (30µg), NIT: Nitrofurantoin (30µg), AMP: Ampicilin (10µg) (Abtek Biological Ltd., England).

Statistical Analysis

The result of the microbial analysis were presented as mean± standard deviation. Analysis of Variance (ANOVA) was used to compare the means of the heavy metals and the microbial samples obtained from the sampling sites. Duncan multiple comparism of mean were used to measure similarities of sampling stations. The package used for statistical analysis is SPSS 20. Chart and figures were schemed using Microsoft excel (2007) functions.

RESULTS

Heavy metals

Zinc

The values of Zinc ranged from 0.00 to 0.17mg/l. The highest mean was recorded in wet season (0.05±0.65mg/l), while the lowest mean was recorded in dry season (0.02±0.27mg/l).

During the rainy season, Site 2 had the highest mean (0.07±0.01mg/l) while control had the lowest mean (0.24±0.001mg/l). There is no significant difference between control and sample site, however, there is significant difference within control and also within site at $p \leq 0.05$.

During dry season, Site 1 had the highest mean (0.17±0.14mg/l) while control had the lowest mean of 0. There is no significant difference between Test and Control, however, there is significant difference within control and within sample site.

Copper

The value of Copper ranged from (0.00 to 0.08mg/l). The highest was recorded in December (dry season) and the lowest was recorded in October. Copper was high in dry season than in rainy season, but not significant at $p \leq 0.05$. Test had the highest mean while control had the lowest mean during raining season as shown in fig. 1 while site 2 had the highest mean while control had the lowest mean during wet season.

Iron

The value of Iron ranged from 0.01mg/l to 0.62mg/l. The highest value was recorded in December (dry season) and the lowest value was recorded in October (wet season) as shown in fig. 1.This was higher in dry season than in rainy season but not significant at $p \leq 0.05$.

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During wet season, Test had the highest mean while control had the lowest mean . Site 2 had the highest mean while control had the lowest mean as shown in fig.2.

During dry season, Test was higher than control. Site1 had the highest mean while control had the lowest mean as shown in fig. 3.

Manganese

The value of manganese ranged from 0.04 to 9.60mg/l. The highest value was recorded in December (dry season) and the lowest value recorded in October (wet season) as shown in fig.1. Manganese was higher in dry season than in wet season and was also significant at $p \leq 0.05$.

As shown in fig. 2, at wet season, Control was higher than Test. During dry season, Test was higher than control as shown in fig. 3. Site 4 had the highest mean while control had the lowest mean.

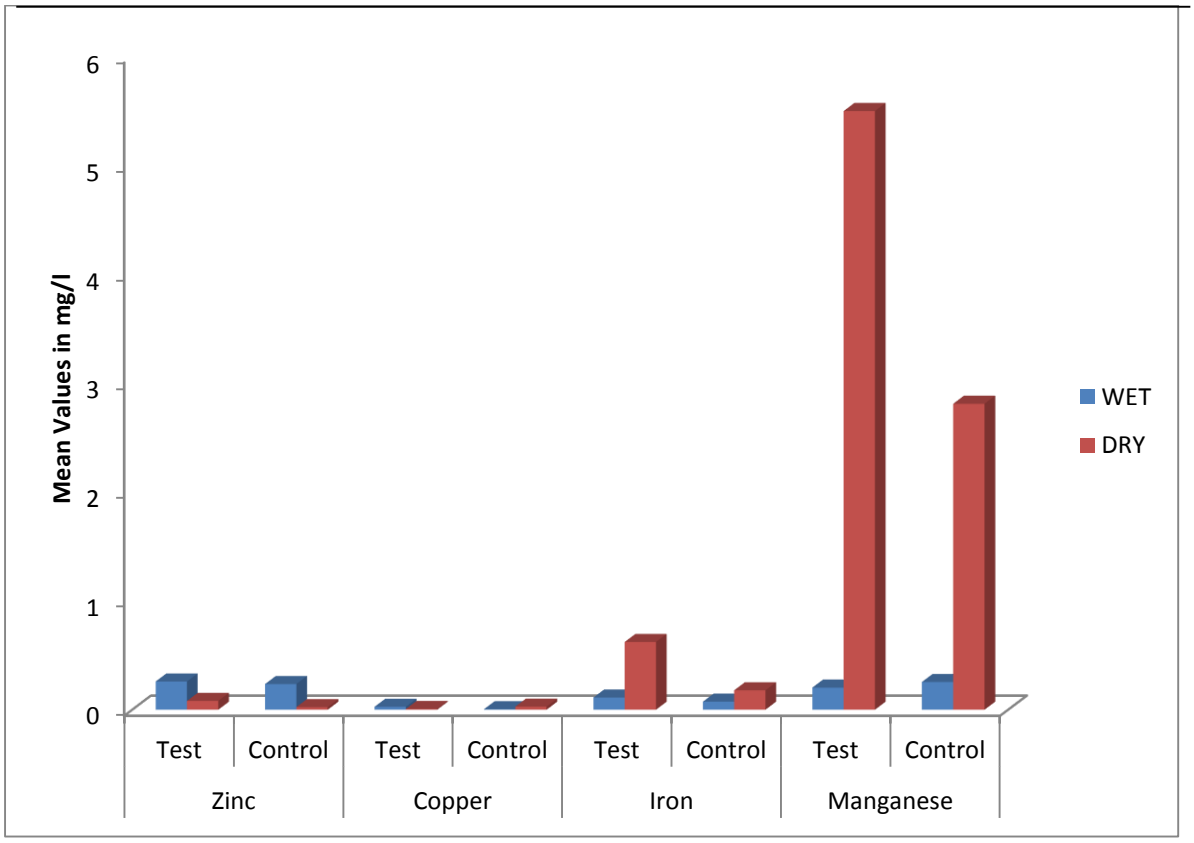

Fig. 1: Seasonal variation in heavy metals of Test and Control during study period

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WET SEASON

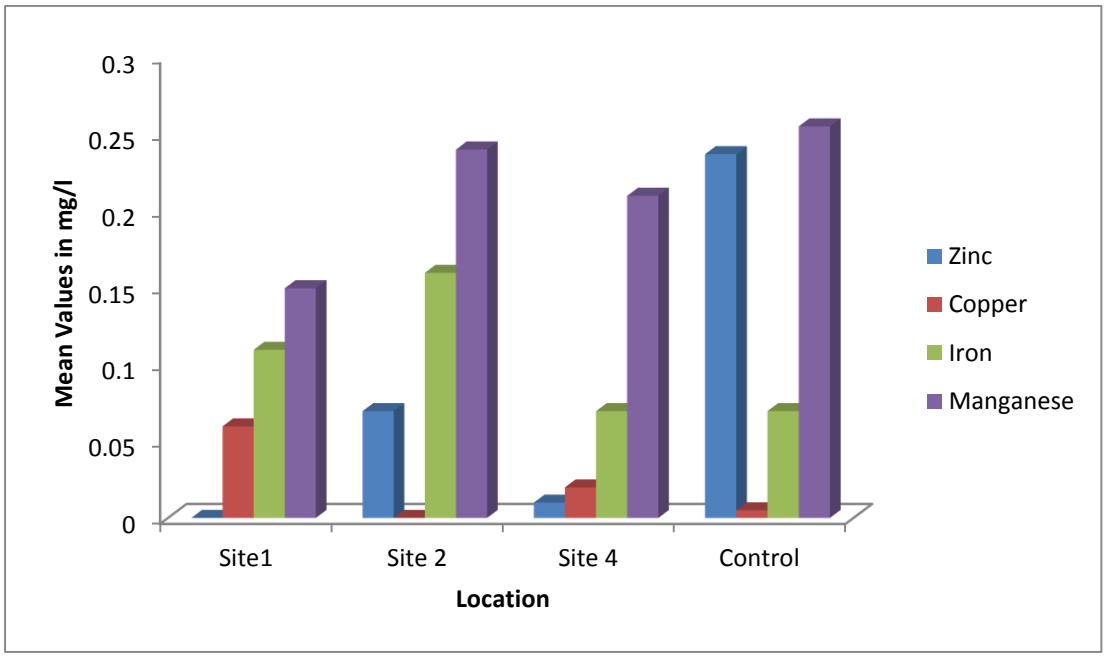

Fig.2: Spatial variation in heavy metals of Test and Control along sampling stations during wet season study period

DRY SEASON

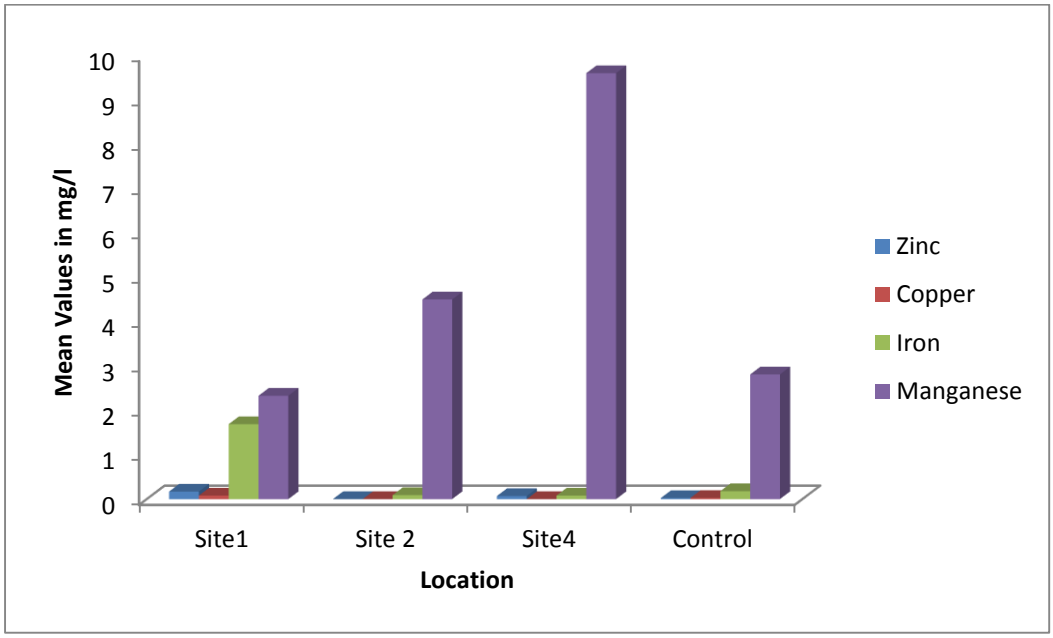

Fig.3: Spatial variation in heavy metals of Test and Control along sample station during Dry season study period

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Bacteriological examination result

Bacteria load along Test and Control site

Bacteriological analysis of the water and soil samples obtained from Test and control site had high bacteria counts for *E. coli*. Mean log cfu/g for soil and log cfu/ml for water of the counts obtained are given in fig. 4 and fig. 5.

Total aerobic count for water was higher in Test 9.81±0.1logcfu/ml than control 9.62±0.14log cfu/ml and was significant at $p \leq 0.05$. Total aerobic count for soil was higher at Test 9.68±0.15log cfu/g than control 9.32±0.3log cfu/g and significant at $p \leq 0.05$.

Total Coliform Count for water at Test was higher 9.61±0.183log cfu/ml than control 9.5± 0.2log cfu/ml but not significant at $p \leq 0.05$. Total Coliform Count for soil was higher in Test 9.48±0.18log cfu/g than control 9.43±0.5log cfu/g.

As shown in fig. 5, for Test, Total Aerobic Count for water was higher at the entry point 9.88log cfu/ml while end and middle point had the least mean of 9.78log cfu/ml. Total Aerobic Count for soil was higher at the middle 9.76log cfu/g, while end point had the lowest mean of 9.64log cfu/g.

Total Coliform Count for water was higher at the entry point 9.64, while middle had the lowest mean of 9.57log cfu/ml. Total Coliform Count for soil was higher at the entry 9.52log cfu/ml while end point had the lowest 9.42 log cfu/ml. As shown in fig.5, for control, Total Aerobic Count for water was higher at entry point 9.76 log cfu/ml while end point had the least 9.48 log cfu/ml. Total Aerobic Count for soil was higher at end point 9.67 log cfu/g and lower at entry and middle point at 9.15log cfu/g. Total Coliform Count for water was higher at entry point 9.77 log cfu/ml while end point had the lowest 9.39log cfu/ml. Total Coliform Count for soil was higher at end point 9.79log cfu/g while entry point had the least 8.85 log cfu/g.

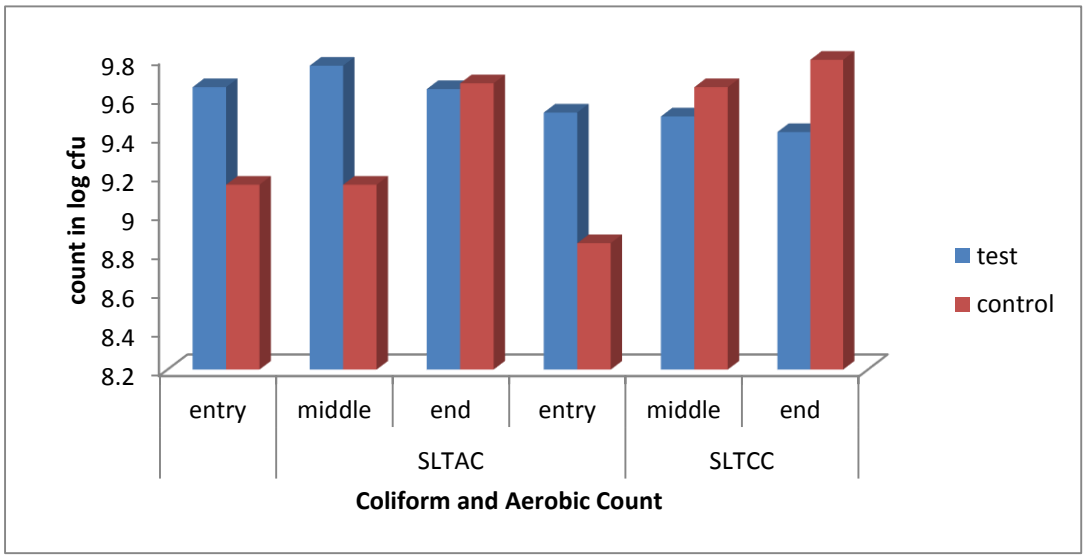

Figure 4: Spatial variation in soil TAC and TCC at entry, middle and end of Test and Control

KEY: SLTAC=SOIL TOTAL AEROBIC COUNT
SLTCC=SOIL TOTAL COLIFORM COUNT

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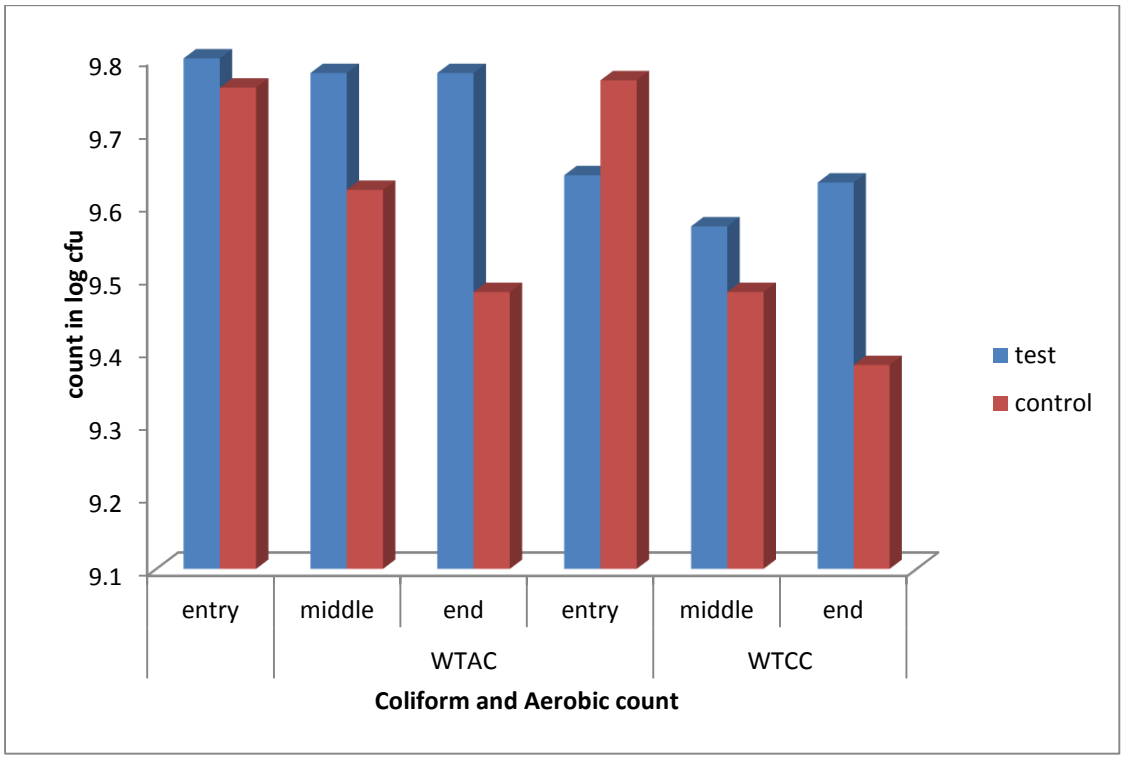

Figure 5: Spatial variation in water TAC and TCC at entry, middle and end of Test and Control

KEY: WTAC=WATER TOTAL AEROBIC COUNT;

WTCC=WATER TOTAL COLIFORM COUNT

Bacteria identification

Escherichia coli O157:H7

Twelve isolates of *Escherichia coli*O157:H7 were obtained in this study**.** Isolate were from all sampling points vis: Middle point water(MW), End point water(EW), Entry point water(ETW), Middle point soil(MS), End point soil(ES), Entry point soil(ETS), Middle point water for control(MWC), Middle point soil for control(MSC), Entry point water for control(ETWC), Entry point soil for control(ETSC), End point soil for control(ESC), End point water control(EWC).

Antibiotic Sensitivity Profile

Isolates from Awba dam showed the highest sensitivity (16.17mm) to ciprofloxacin while lowest was with Augmentine (8.25mm).Furthermore, the isolate from the control point showed highest sensitivity to CPR and NIT (14mm) and least for AUG (5mm) (Fig. 6).

The resistance pattern in isolates were: AMP and CRX (100%); AUG (60%); NIT (40%); GEN (18.3); CPR and OFL (6.7%). (Fig. 7) .

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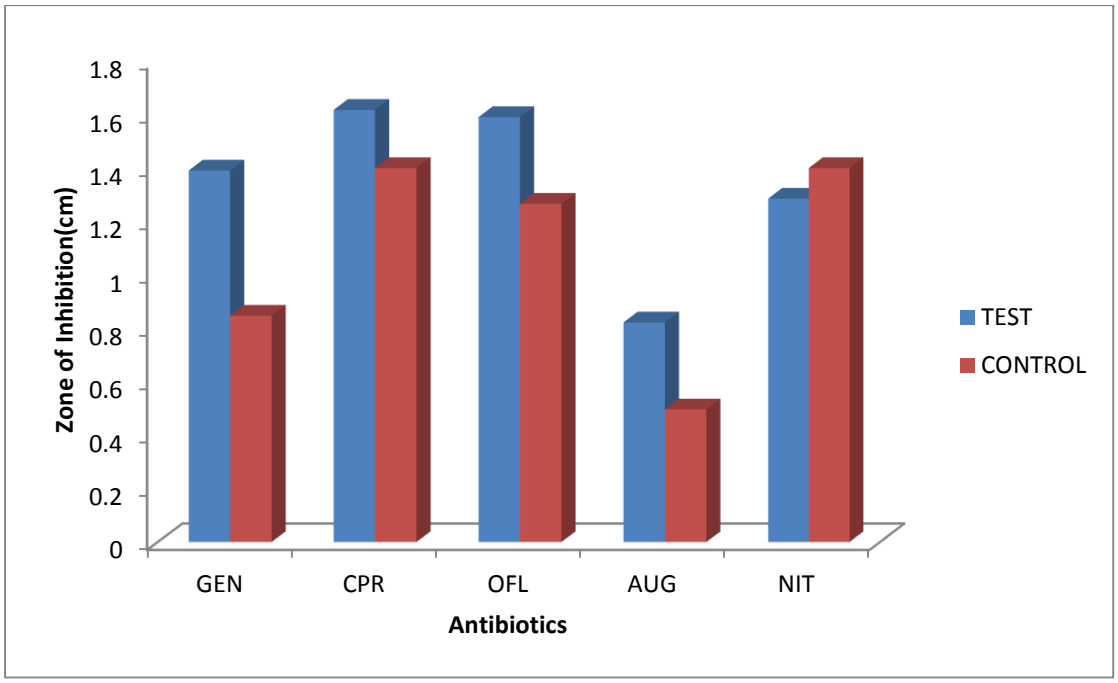

Fig. 6: Spatial variation in *E.coli* sensitivity to Antibiotics of Awba dam and Control

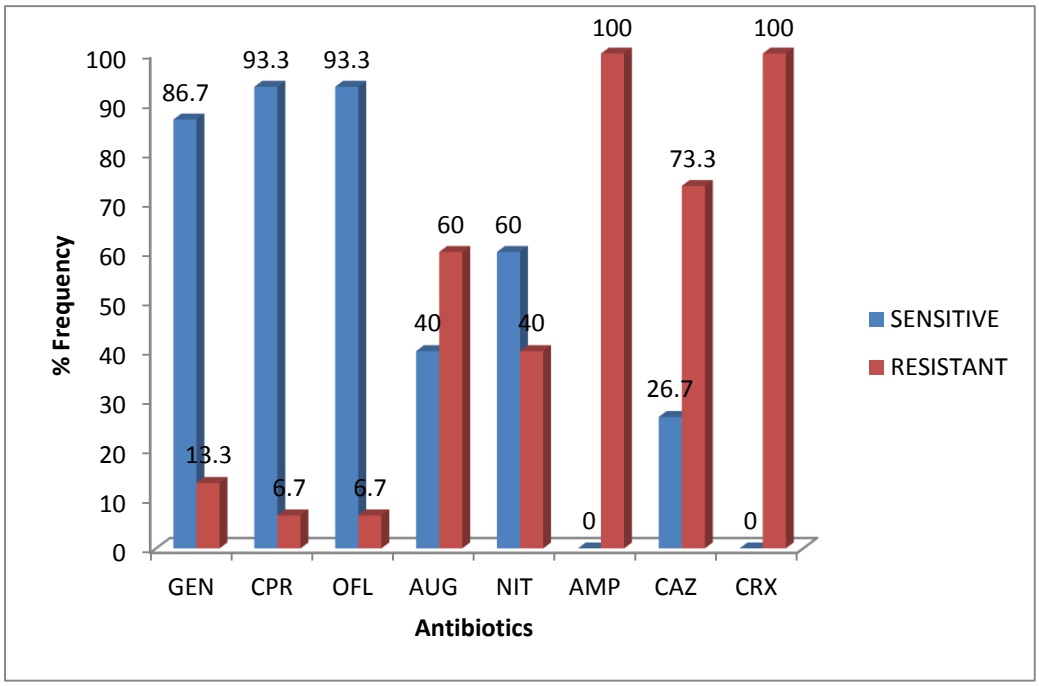

Fig.7: Spatial variations of % frequency sensitivity and resistance of *E.coli* on Gram –ve Antibiotic Sensitivity Discs.

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DISCUSSION

Recreational water quality criteria are used to assess the safety of water to be used for swimming and other water-sport activities. The primary concern is to protect human health by preventing water pollution from faecal materials or from contamination by microorganisms that could cause gastro-intestinal illness, ear, eye or skin infections. Criteria are therefore usually set for indicators of faecal pollution, such as faecal coliforms and pathogens. There has been a considerable amount of research in recent years into the development of other indicators of microbiological pollution including viruses that could affect swimmers.

The ranges recorded for heavy metals level during the study period includes Zinc (0.00 – 0.17 mg/l), Copper (0.00 – 0.08mg/l), Iron (0.01 – 0.625mg/l), Manganese (0.038 – 9.6 mg/l).

Zinc is an essential trace element that can cause symptoms of deficiency and can be toxic when exposures exceed physiological needs. In the acceptable range, zinc, which is necessary for various metabolic processes, embryonic development, cellular differentiation and cell proliferation, provides the substrates for expression of the genetic potential of the individual, i.e., optimum growth, health, reproduction and development. Higher Zinc concentrations can be attributed to anthropogenic contamination, although natural processes (both abiotic and biotic) can contribute to localized high Zinc concentrations.

The guidelines value for Zn in drinking water is given as 5.0 mg/L by WHO(Olutona *et al*.,2012).The value obtained in this research lies within the acceptable range while it is higher than the NESREA limit (0.03mg/l) for aquatic life.

Iron ranged from 0.01- 0.65mg/l. The iron range fell within EPA, 1976 recommended limit of 1.0mg/l for fresh water aquatic life. Iron is an essential trace element required by both plants and animals. In some waters, it may be a limiting factor for the growth of algae and other plants. The ferrous or bivalent ($^{Fe++}$) and the ferric or trivalent irons (Fe^{+++}), are the primarily forms of concern in the aquatic environment.

Manganese ranged from 0.038-9.6mg/l. The tolerance value reported for manganese for aquatic life range from 1.5 – 1000mg/l, thus, manganese is not considered to be a problem in fresh water. Manganese is one of the most abundant metals in Earth's crust, usually occurring with iron. It is a component of over 100 minerals but is not found naturally in its pure(elemental) form (ATSDR, 2000). Manganese is an element essential to the proper functioning of both humans and animals, as it is required for the functioning of many cellular enzymes (e.g. manganese superoxide dismutase, pyruvate carboxylase) and can serve to activate many others (e.g. kinases, decarboxylases, transferases, hydrolases) (IPCS, 2002). Manganese can exist in 11 oxidative states; the most environmentally and biologically important manganese compounds are those that

contain Mn2+, Mn4+ or Mn7+ (USEPA, 1994).

It is of note that these metals are higher in dry season when compared with wet season. This may be due to dilution by rainfall during the wet season.

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MICROBIAL ANALYSIS

Microbial assay: Isolation of *E. coli* O157:H7

High coliform counts (log cfu/ml) for water and (log cfu/g) for sediment was obtained from water and soil sample at Awba dam compared to Control site. The average counts for water and sediment samples far exceeded the International standards. This study validates the presence of this strains in both the water and sediment sample of the dam. The presence of *E.coli*O157:H7 in Awba dam is of public health significance. Faecal contamination of Awba dam include: human sewage like municipal waste, animal waste(domestic and wildlife), leaking septic tank, since sewage water from residential buildings and student`s hostels flow into the dam are possible reasons for this. *E.coli* O157:H7 is an enterohemorragic strain which produces a Shiga-like toxin and causes a bloody diarrhoea, which can be especially severe in children and the elderly (Riley *et al.,* 1983). If left untreated, the infection may lead in some patients to hemolytic uremic syndrome, a form of kidney damage (Boyce *et al.,* 1995). Similar to findings in this present study, population based study of sporadic *E.coli* O157:H7 infection found a statistically significant association between swimming in outdoor pools and lakes and *E.coli* O157:H7 illness (Parry *et al*., 1998).Since Awba dam is used for fishing, occurrence of any toxigenic strains of *E.coli* in the water might have direct impact on the food chain.

Antibiotics Sensitivity Profiling

Isolates from Awba dam showed the highest sensitivity (16.17mm) to ciprofloxacin while lowest was with Augmentine (8.25mm). Furthermore the isolate from the control point showed highest sensitivity to CPR and NIT (14mm) and least for AUG (5mm) (Fig. 6).The resistance pattern in isolates were: AMP and CRX (100%); AUG (60%); NIT (40%); GEN (18.3); CPR and OFL (6.7%). (Fig. 7)

The highest sensitivity to CPR and complete resistance to AMP and CRX of *E.coli* O157:H7 and resistance CPR and OFL is of importance. Similarly high resistance levels were also observed by Arpin *et al*., 1992; Charpentier *et al.*, 1995. Issa *et al.*, 2011 also reported 100% resistance of *E.coli* to Ampicilin. The sensitivity demonstrated by E.coli 0157:H7 to Ciprofloxacin and Ofloxacin is similar to that observed by earlier researchers (Cardosso *et al.,* 2006; Soomro *et al.,* 2010; Adetunji and Ishola, 2011a). Ciprofloxacin is a fluoroquinolone antimicrobial that is increasingly and successfully used for the treatment of septicaemic salmonellosis in humans (Soomro *et al.*,2010).

Decreased sensitivity of *E.coli* O157:H7 to Ampicilin(AMP) and Ceftazidime(CAZ) is due to the selection of beta-lactamase producing strains. β- lactamases continues to be the leading cause of resistance to β- latase antibiotics among gram positive bacteria. Typically, they derive from genes plasmid-encoded β- lactamases for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β- lactamases. TEM-1 is the most common plasmid-mediated β-lactamase of Ampicilin resistant *E.coli*(Paterson *et al.,* 1999; Rice, 1999). The Sentry study has reported an increase from one year to another of the number of beta-lactamase producing strains, their % been 19.5% in 2004 (Fritsche *et al.,* 2005).

The sensitivity of *E.coli* strains to Ciprofloxacin (CPR) is comparable with the data from literature, about 50% (Gums,2005; Livadarium *et al.,* 2006). Strains of *E.coli* are sensitive to third generation of aminoglycosides and to fourth generation of Cephalosporins and to Carbapenerns.

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The exaggerated use of antibiotics has led to the selection of new strains of bacteria that are resistant to antibiotics, a situation which is found in the case of *Escherchia coli* strains.

CONCLUSION

Bacteriological assay conducted indicates higher count than stipulated by Environmental Protection Agency (EPA). Several biochemical tests revealed that Awba dam is contaminated by the presence of *E.coli* O157:H7 which is an indicator organism, thus can make Awba dam unfit for swimming while other recreational activities can still be done, as reports have it of cases of transmission of the organism to humans from recreational waters.

The discovery that pathogenic organisms are sensitive to CPR and Ofloxacin (OFL) and completely resistant to CRX and AMP suggests that there is a possibility of the emergence and circulation of multidrug resistant super- bacteria which might be of serious economic and public concerns.

RECOMMENDATIONS

1. From the report of this study, it is evident that the presence and significant abundance of *E.coli* O157:H7 in the dam is dangerous for human health. It is therefore recommended that other recreational activities, apart from swimming should obtain for Awba dam.
2. The University management should device means of controlling waste water that enters into the dam by providing other channels of discharging these.
3. Water quality objectives for multipurpose uses of water should be set at a level that provides for the protection of the most sensitive use of this water body. These are for human consumption, fish rearing and for recreation.

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