EFFECT OF STORAGE CONTAINERS ON THE BACTERIOLOGICALQUALITY OF WATER FROM DIFFERENT SOURCES

*Osuji M.J. **and Odu,** N. N.[2]
department of Microbiology, Federal University Of Technology Oiuerri Imo State
[2]Department of Microbiology, University Of Port Harcourt, Rivers State

Abstract: This research work was therefore carried out to assess the effect of different containers on the quality of different water samples and the effect of long storage time on the bacterial load (bioload) of the water samples. Spread plate count technique was adopted using Nutrient, Salmonella/Shigella, MacConkey and Thiosulphite Citrate bile sucrose agar. Calabash, Glass, Metal and Plastic containers were used in the storage of rainwater, river water and tap water. In rain water stored in calabash, the total heterotrophic bioload decreased from 9.21ogcfu/ml to 5.31ogcfu/ml. For the river water stored in glass container, the bioload decreased from 7.01ogcfu/ml to 5.01ogcfu/ml. In other containers, there were such decreases. Several factors were noticed to be responsible for the bioload changes. The factors include; the toxins and acids produced as secondary metabolites during the stationary phase, temperature variation, nutrient depletion, and pH. Plastic container recorded the highest bioload decrease. People are advised to use plastic containers for water storage and they should filter the water before using so as to remove the biofilms formed at the wall of the containers due to death of bacteria when toxins are produced and nutrient depleted.

Key words: Bioload, bacteriological, biofilms, metabolites, nutrient, coliform.

Inrroduction

In most areas of the world, water is a scarce commodity. Water is also the most abundant substance on the earth surface and is used as a universal solvent (Melvin *et al* 1991). Because it is scarce, there is the tendency to store it for future use when ever it is found because many cannot boast of getting it the next time. But in areas where water is abundant, it is oftentimes taken for granted.

*Corresponding author:
malachyosuji760@gmail.com *Osuji M.I(1)

Water has to be potable before it can be granted fit for human use (Diersing 2009). When it is not handled properly, it has the ability to harbour pathogenic microorganisms (CWA 303). Contaminants that may be in untreated water include microorganisms such as viruses and bacteria; inorganic contaminants such as salts and metals; pesticides and herbicides; organic chemical contaminants from industrial processes and petroleum use; and radioactive contaminants (Water SA 2006). Water quality depends on the local geology and ecosystem, as well as

Nigerian Journal of Microbiology 2015, 29: 3148-3158
Published online at www.nsmjournal.org

human uses such as sewage dispersion, industrial pollution, use of water bodies as a heat sink, and overuse which may lower the level of the waters (EPA 2002). The common sources of water available to man include rain, surface and underground water (Okafor 1985, Beiger et al 1992 and Connel 1996). According to Okoro (1986), for the past 15years, the problem of providing good drinking water for people in the cities and villages of developing countries has been of great concern to WHO and most government. Even in some areas where surface water is available, if it is improperly handled or constructed in the case of shallow well; it can be responsible for the outbreak of diseases by most enteropathogenic organisms like E.coli, Salmonella, shigella, pseudomonas aeuruginosa and klebsiella which will multiply if nutrient are available (Okpokwasili and Akujobi 1996; Nnochiri 1990).

Tap water that was identified as the best source of drinking water is now being contaminated through burst pipe, unhygienic nature of worker (Nnodim 2000). In the United States, the U.S. Environmental Protection Agency (EPA) limits the amounts of certain contaminants in tap water provided by public water systems (EPA 2006). The Safe Drinking Water Act authorizes EPA to issue two types of standards: *primary standards* regulate substances that potentially affect human health, and *secondary standards* prescribe aesthetic qualities, those that affect taste, odor, or appearance (EPA 2002). These contaminants have led to suffering of some consumers from diseases like intestinal tract diseases and diarrhoea. These diseases which are indicative of poor water quality call for monitoring of the few potable water sources to determine their public health quality (Ogbulie *et al* 1998). Nester *et al* 2004 recommended that to regulate the level of water portability, standards have to be set. This act or standard include the US safe drinking water act of 1974, amended in 1986, 1996 and 2002. This act gave the environment protection Agency the authority to set drinking water standards in order to control the level of contamination of drinking water (EPA 2002). This research work was therefore carried out to asses the effect of different containers on the quality of different water samples and the effect of long storage time on the bacterial load (Bioload) of the water samples.

2.0.0 MATERIALS AND METHODS

2.1.0 Water samples from the three different sources were collected using three different sterile plastic containers of 10-liter capacity, which were sterilized by rinsing thoroughly with hot water first and followed by 76% ethanol.

2.2.0 WATER SOURCES AND TIME OF SAMPLE COLLECTION

The three water sources used in this research work include (i) rain water (from zinc house) (ii) river water (Otamiri river) (iii) tap water (ISEPA prefab owerri). Because this research is weather dependent, the three samples were collected on the 23/06/2010 the day rain fell within 45 minutes interval and transported to the laboratory.

2.3.0 SAMPLE COLLECTION MODE AND STORAGE PERIOD

As stated above, three sterilized 10-litre containers were used for

the collection of the sample each for a sample. The samples and the containers were wrapped in a sanitary waterproof bag to avoid contamination during transportation. After the day 1 analysis on 23/06/2010, the samples were subsequently analyzed weekly. Storage of sample lasted for 14 weeks.

2.4.0 Portion of the three samples was analysed to check for different parameters (a) Temperature (b) pH and (c) Conductivity. The pH of the water sample was measured using the pH/Temp meter by Suntex TS2. Thus the sensitive electrode was dipped into the water.–The meter was switched on and it shows the pH value of the sample. The same meter was also used for the determination of temperature. For conductivity, meter by Suntex SC-120 was used like that of pH.

2.5.0 BACTERIOLOGICAL ANALYSIS

The water samples were analysed using spread plate and other bacteriological techniques. The following media were used.

(1) Nutrient agar by LAB M was used for the enumeration of total Heterotrophic Count. (2) Thiosulphite citrate bile sucrose agar by Merk for the enumeration of total *Vibrio* count. (3) SS agar by Biotech for the enumeration of total *Salmonella/Shigella* count and (4) MacConkey agar by Oxiod used for the enumeration of total Coliform. For each of the estimations, the water samples were serially diluted in 9ml of sterile distilled water up to ten dilutions and a 0.1ml from each tube plated out by spread plate method. These were done at time interval of weeks 1 - 14

3.0.0 ISOLATION, PRESERVATION AND IDENTIFICATION OF PURE COLONIES

ISOLATION

Six identical isolates were collected from each plate of nutrient agar while four were collected from the three different selective media used. The colonies formed on the four media (TCBS, SSA, NA and MAC) were isolated by several sub-culturing onto fresh media until pure colonies were obtained.

PRESERVATION

Agar slants were prepared and were used in the preservation of the pure isolates. Streaking method as described by Ogbulie *et al* (1998) was used to ensure purity of the isolates.

IDENTIFICATION

Before the test was done, the isolates were first sub cultured on nutrient agar. The following tests were done as described by Ogbulie *et al* (1998). These include (1) Gram staining test (2) Motility test (3) Catalase test (4) Methyl red test and (5) Oxidase test.

TABLE 1. : IDENTIFICATION RESULTS OF ISOLATES

Colony characteristics	Gram staining	Catalase test	Motility test	Methyl test	Oxidase test	Suspected microbial isolates
Yellow isolate in NA	+ve cocci	+ve	-ve	-ve	+ve	*Staph aureus*
Green isolate in TCBS	-ve rod	-ve	+ve	-ve	+ve	*V.parahaemolyticus*
Pink isolate in SSA	-ve	+ve	-ve	-ve	-ve	*Shigella sp*
Pink isolate in MAC	-ve	+ve	+ve	+ve	-ve	*E.coli*
Yellow isolate TCBS	-ve	Ve	+ve	-ve	+ve	*V.cholera*
Black isolate in SSA	-ve	+ve	+ve	+ve	+ve	*Salmonella*

4.0.0 ESTIMATION OF CHANGES IN BACTERIAL LOAD OF DIFFERENT WATER SAMPLES STORED IN DIFFERRENT CONTAINERS

The changes of bacteriological population of different water samples stored in different containers were estimated using the formulae.

Change in population (Δ in pop) = population at time (t) -- population at time (0)

Percentage change in population (% Δ in pop) =

$$\frac{\text{Change in Bioload}}{\text{Population at time (0)}} \times \frac{100}{1}$$

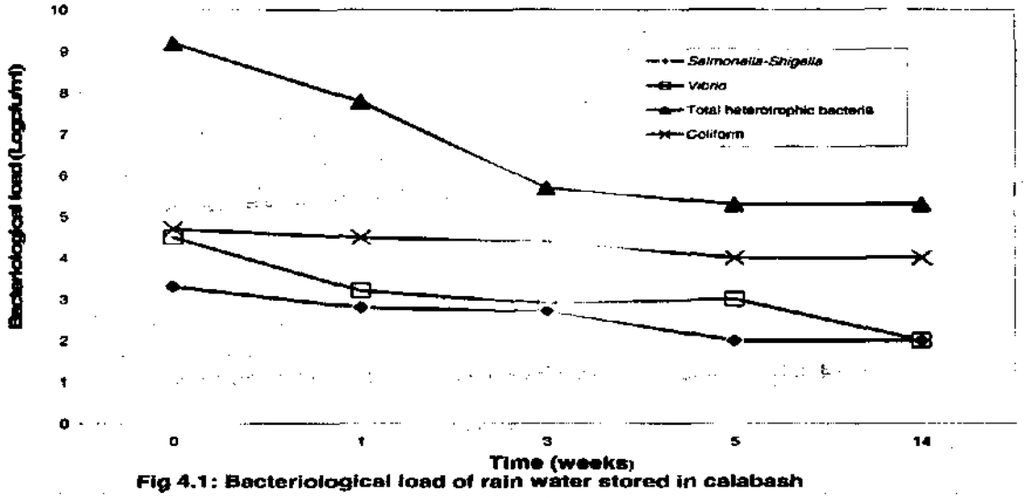

Fig 4.1: Bacteriological load of rain water stored in calabash

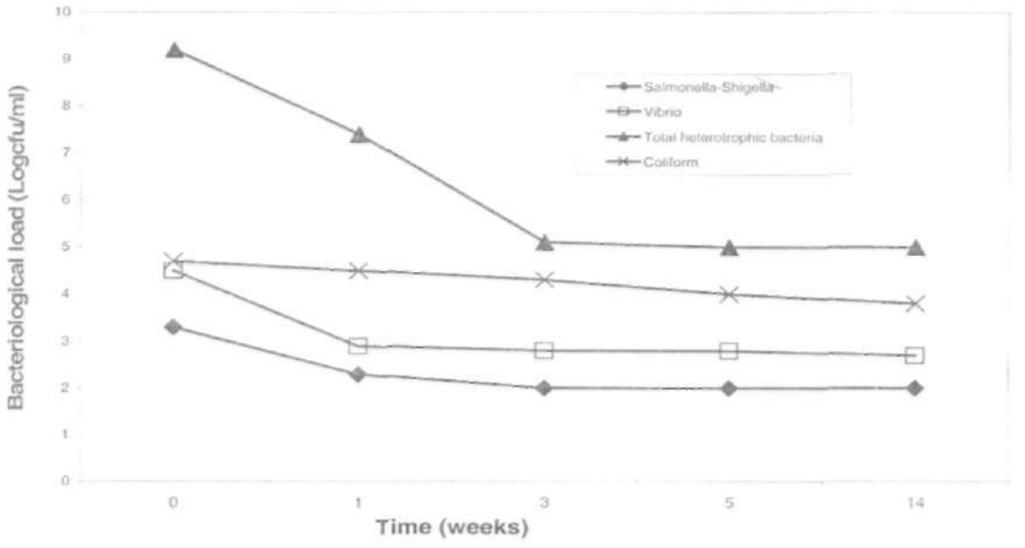

Fig 4.2: Bacteriological load of rain water stored in bottle

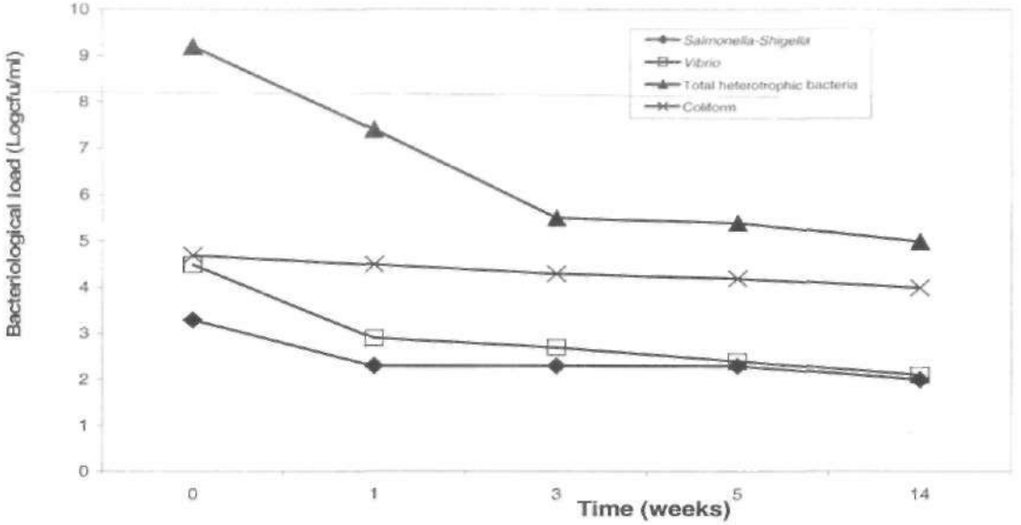

Fig 4.3: Bacteriological load of rain water stored in metal container

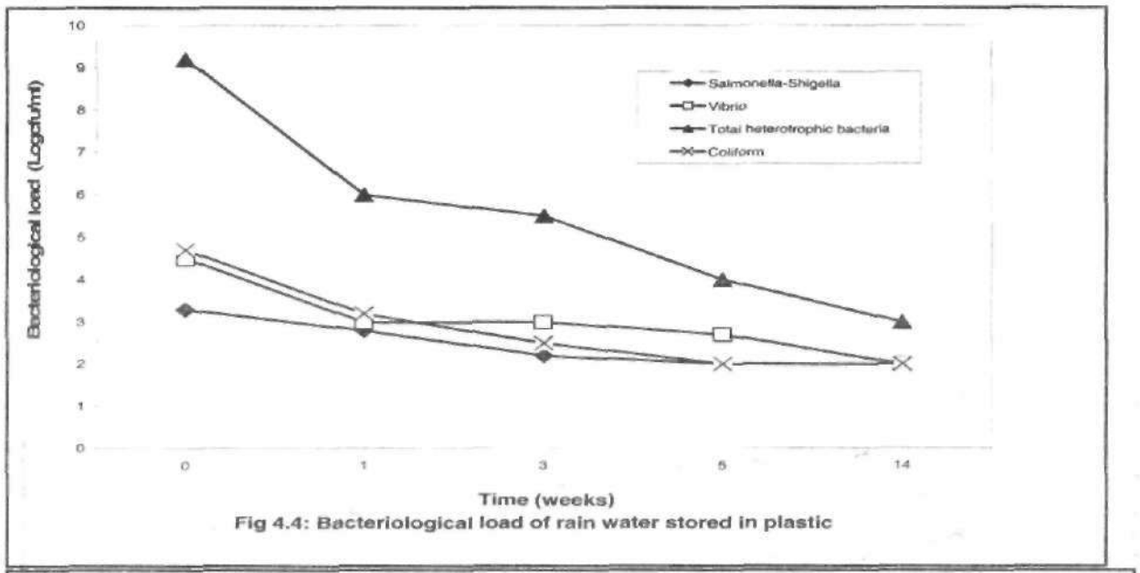

Fig 4.4: Bacteriological load of rain water stored in plastic

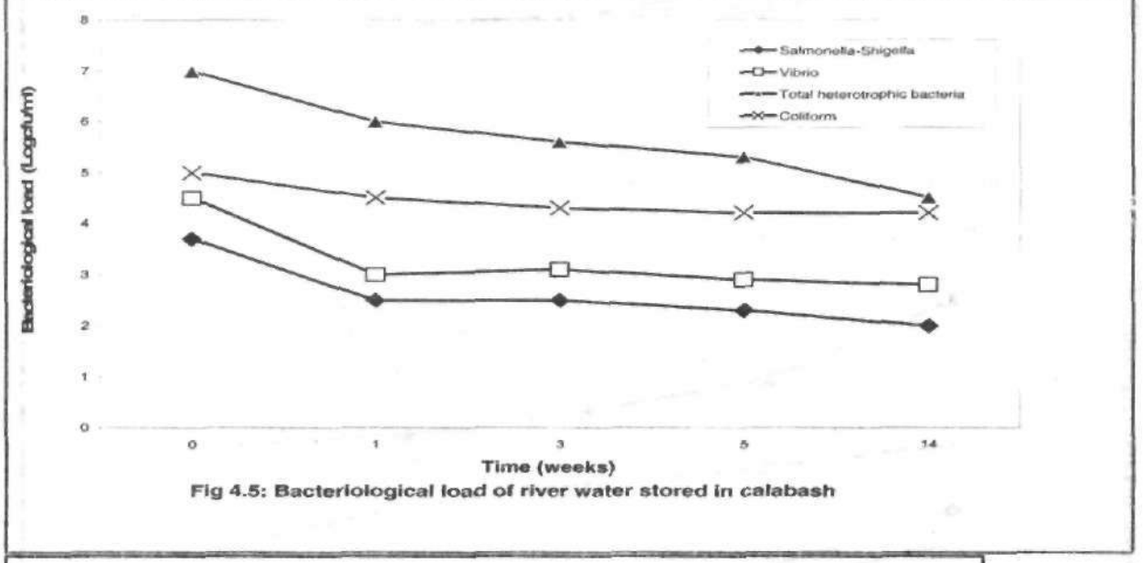

Fig 4.5: Bacteriological load of river water stored in calabash

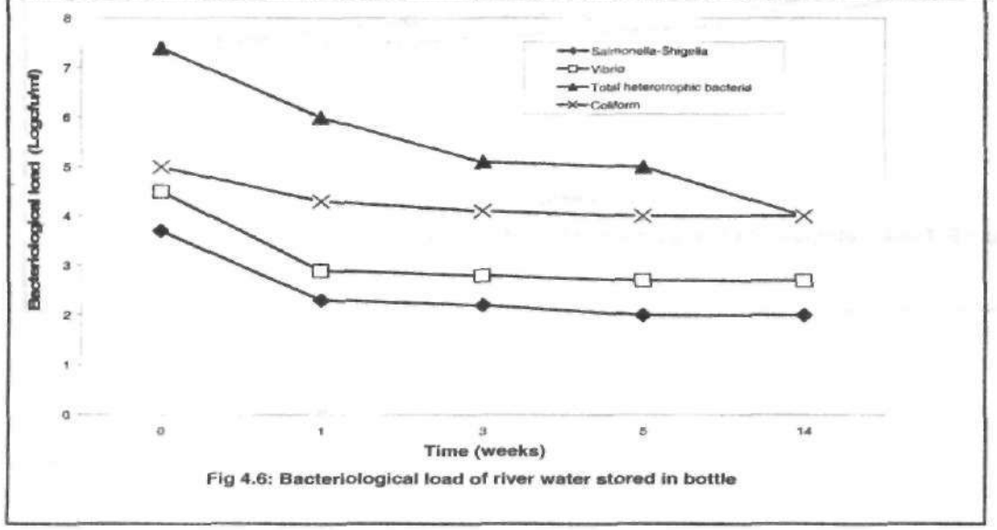

Fig 4.6: Bacteriological load of river water stored in bottle

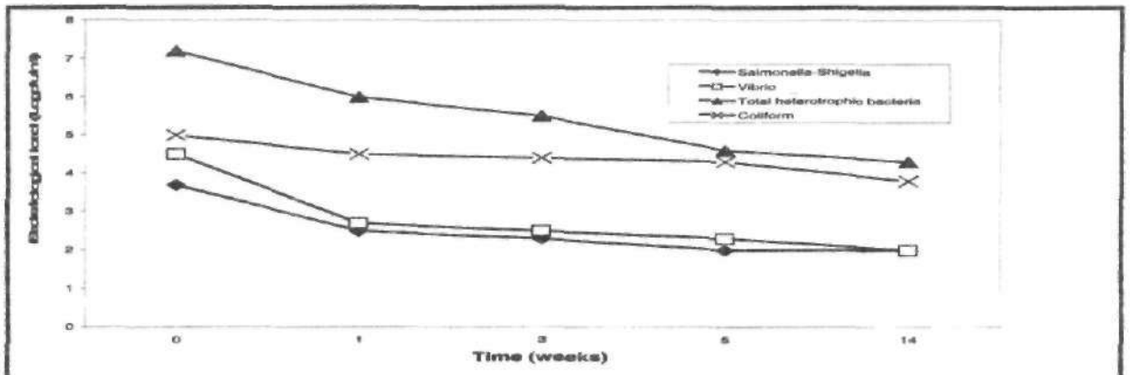

Fig 4.7: Bacteriological load of river water stored in metal

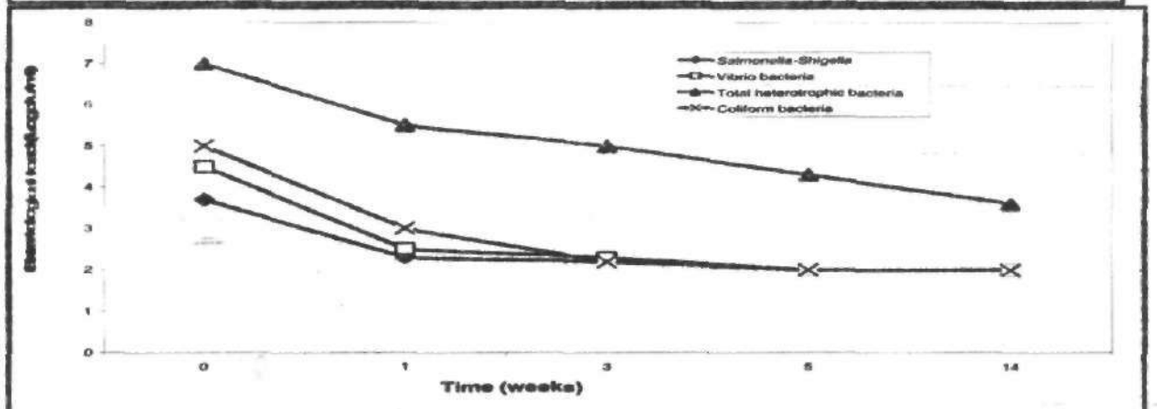

Fig 4.8: Bacteriological load of river water stored in plastic

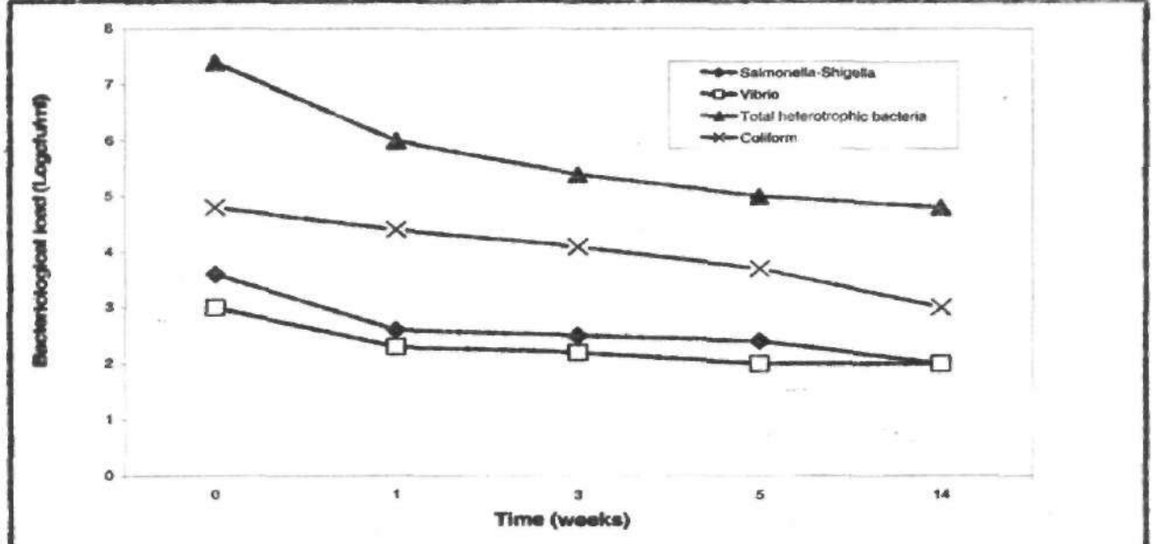

Fig 4.9: Bacteriological load of tap water storred in calabash

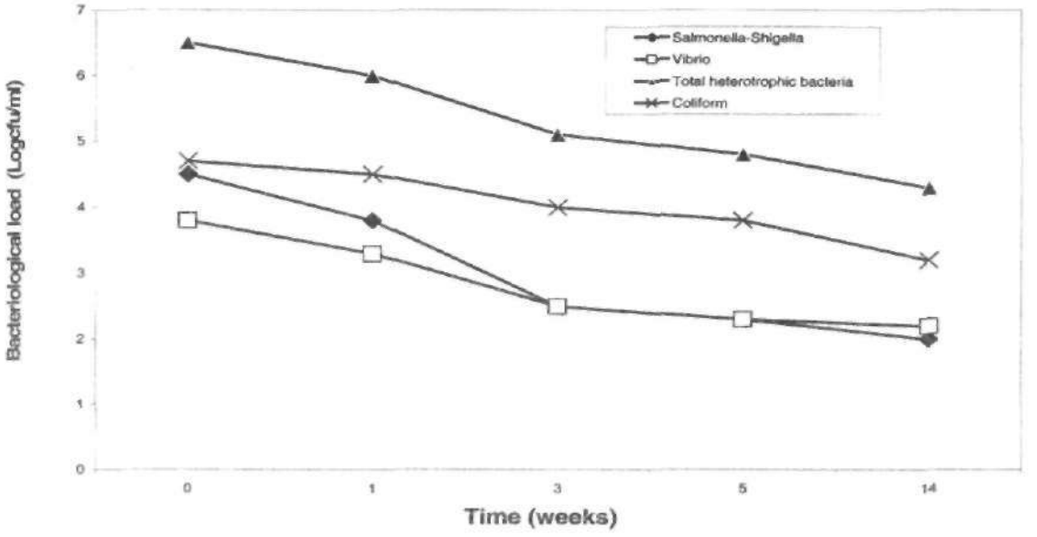

Fig 4.10: Bacteriological load of tap water stored in bottle

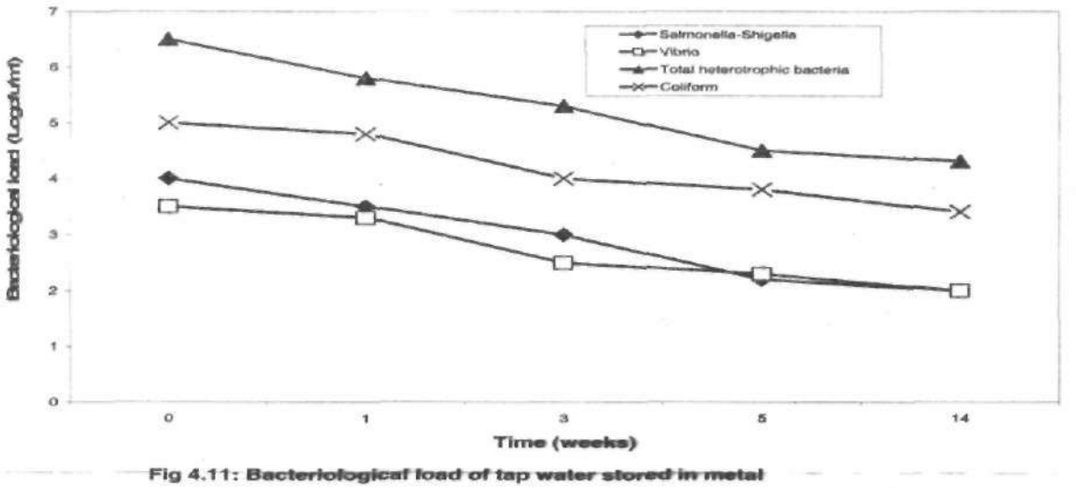

Fig 4.11: Bacteriological load of tap water stored in metal

5.0.0 DISCUSSION OF RESULTS

In this research work, the length of time cells remain in each storage interval varies depending on the species. This has explained why we have different curves in the growth pertain as shown in the charts 4.1- 4.12. Apart from the toxins and nutrient depleted condition that led to reduction in bioload, other factors were noticed to have affected the growth of bacterial cells.

It was surprising to see that *E.coli* could be found in the tap water which comes from the public water supply. This explains why people still fall sick after drinking from the municipal water supply. This could be as a result of busting of water pipes which were not fixed on time or untreated water (EPA 2006).

This research work was done in a batch culture system. All the containers have perfect and tight cork and were never opened to fresh air

and water at any point in time. To this effect, nutrients were not introduced into the stored water sample throughout the incubation periods. At the same time, products were not removed throughout the incubation periods. Under this batch culture system, bacterial load increased in number in a predictable fashion and then eventually declines (Nester *et al* 2004). Several factor affected microbes isolated in this work; and they include

(i) Metabolites synthesized during the exponential phase serve as food to some microbes
(ii) Toxins produced which killed some microbes that cannot tolerate them
(iii) Biofilms formed which on its own protects some microbes from the toxins
(iv) The nucleic acid of some dead microbes serves as nutrient some bacteria (Nester *et al* 2004).

The factors affecting the growth of bacteria in fresh water stored in different containers, usually glass, were studied in order to reconcile the different results which have been obtained by previous workers (EPA 2002). Growth occurred in two sites in the body of the water and at the surface of the container and was affected by the constituents of the container (Taylor and Collins 2004). Bacteria in a flowing water like Otamiri experience continuous culture system. When the water was fetched and stored; the system changes automatically to a batch culture system.

According to Taylor and Collins (2004), bacteria invariably grew on the sides of the container and were presumably dependent for their multiplication on having a site of attachment; thus the increase in the count per unit volume which occurred when glass were vigorously shaken was greater in small bottles than in large glass, and was due to removal of some of the cells attached to the walls. Soluble chemical substances in the walls of containers retarded bacterial growth. As bacterial load increases at the initial time, available nutrients and level of oxygen begin to deplete.

For water bacteria especially aerobes, the nutrient that becomes limiting is usually oxygen (Geo *et al* 2001). Apart from the depletion of nutrient and oxygen due to high bacterial load at the start of the storage, other factors are responsible for the low bioload recorded towards the end of the storage period. Berg *et al* (1998,1991) and Nester *at al* (2004) said that as the cell's surroundings change, cells begin to synthesize different enzymes and proteins, which give rise to new metabolites (product of cell's body reaction), e.g. antibiotics and toxins. One astonishing thing that happens at the late log phase is that it serves as a transition phase to the stationary phase where the secondary metabolites are produced.

It is not possible in this research work to conclude that each week corresponds to a bacterial growth phase. Also it is difficult to give a sharp boundary between phases as regards to the storage time shown in the chart. Nester *et al* (2004) also noted that as cells die due to the secondary metabolites, they would release their peptide and nucleic acid that will serve as food and energy to those cells that will survive the harsh condition.

Glass and plastic recorded a high reduction in bioload of the stored water, but plastic recorded higher bioload reduction than glass. Other factors include the pH and the temperature. It was noticed that as the pH enters the acidic region (9.00 at week 0 to 5.00 at week 14), bacterial load reduces. The complexity of water quality as a subject is reflected in the many types of measurements of water quality indicators. Some of the simple measurements listed below can be made on-site — temperature, pH, dissolved oxygen, conductivity, Oxygen Reduction potential (ORP)— in direct contact with the water source in question The same was for temperature as it lowers (EPA 2006). Bacterial isolates or strain found in this work dependent solely on the following

(i) Nature of human activities going on within the Otamiri river site.
(ii) Source of contamination
(iii) Nature of contaminant
(iv) Level and nature of water treatment

6.0.0 CONCLUSION AND RECOMMENDATIONS

The batch culture system is a good method of (1) reducing water bacterial load.

(2) Starving the bacterial cells. When the cells are confined in this stored condition, they will finish the available oxygen and nutrients. Though there is survival of the fittest, some cells will still remain. This explains why people could store water for so long and microorganism could still be found in the water.

Since run offs into water bodies and broken pipes serve as source of microorganisms to the water; proper refuse disposal, and other sanitary techniques (sanitary landfill) should be devised by government. This could be done through the environment protection agencies to control contamination of natural water. Also governments should not waste time in fixing broken pipes to prevent entrance of pathogens.

People should also be educated on the need to use plastic containers for water storage since it recorded the lowest bacterial load at the end of the storage time. People should also filter their water so as to get rid of the biofilms formed at the wall of the containers and debris of bacteria that died due to toxins, acids and other harmful secondary metabolites produced during the stationary phase of growth as well as nutrient depleted environment.

REFERENCES

Berge C.C; Allen MJ and Smith B.B (1991). National field Evaluation Of a defined substrate method for the simultaneous Enumeration of total Coliform and *E.coli* from Water: Comparison with the standard multiple tube Fermentation method:Collaborative method. *Journal of Association of Official AnalyticalChemistry* **74**:526-529

Clean Water Act, Section 303(d), 33 U.S.C. § 1313; Section 305(b), 33 U.S.C. § 1315(b); Section 314, 33 U.S.C. § 1324

Diersing, Nancy (May 2009). "Water Quality: Frequently Asked Questions". *PDA*. NOAA. http://floridakeys.noaa.gov/pdfs/wqfaq.pdf. Retrieved 2009-08-24.

Hodgson K, Manus L. A drinking water quality framework for South

Africa. Water SA. 2006;32(5):673-678 [1].

Geo,R.G;Janet,S.B and Morse K.I.(2001)Enteric Gram Negative Rods. In: *Medical microbiology*. 21st edition.Lange Medical book/Me Graw- hill Pp225-228

Harvey H.W. (1992) on changes taking place in seawater during storage. Journal of marine Biology association United Kingdom 25:225

Melvin, D.J;David OJ;John,T.N and James,L.W(1991)World of Chemistry. Sanders college publisher London pp22

Nester, E. W; Anderson, D.G;Robert,C.E; Pearsall, N. N and Nester M.T (2004) Bacterial Disease of the Upper Alimentary system: In *Microbiology, a Human perspective.* 4th edition. McGraw-Hill Comp. New York;pp 616-617

Ogbulie J. N; Uwaezuoke, J. C and Ogiehqr,S.I (1998) Water Quality Microbiology In *Introductory Microbiology practical*. Springfield Publisher Nig.pp 114-115.

Okafor, N (1985) *Aquatic and waste microbiology.* 1st Edition. Fourth Dimension Publisher Enugu.pp 1-3,107-126.

Taylor C.B. and Collins V.G. (2004) Development of Bacteria in water stored in Glass containers. *Freshwater biological association.* Way castle Ambleside Westmorland.

United States Environmental Protection Agency (EPA). Washington, DC. "Water Quality Standards Review and Revision."2006.

U.S. EPA. Washington, DC."Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms." Document No. EPA-821-R-02-012. October 2002.