Research Article

Earth & Environmental Science Research & Reviews

Environmental Risk Assessment of Hospital Wastewater in Federal Medical Centre (Fmc), Umuahia, Nigeria

Nwakanma C* and Obi O

Department of Environmental Management and Toxicology, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

***Corresponding author**
Nwakanma C, Department of Environmental Management and Toxicology, College of Natural Resources and Environmental Management Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, E-mail:dr.nwakanmac@gmail.com

Submitted: 03 Aug 2018; **Accepted**: 22 Aug 2018; **Published**: 20 Sep 2018

Abstract
The study on environmental risk assessment of hospital wastewater at Federal Medical Center (FMC), Umuahia, Nigeria was carried. To this end, the microbial load and heavy metal analyses of the hospital wastewater were determined using established procedures. The wastewater samples were collected directly from the outlet of different wards (Surgical, Children and Emergency wards), with pre-cleaned sterile and dried containers. The result show that they were significant varieties in the bacteria and fungi load of the hospital waste water from the different wards. The bacteria load was on the range of 192×10^7 cfm/ml to 275×10^7 cfm/ml (1.92×10^7 cfm/ml to 2.75×10^7 cfm/ml) while the fungi load ranged from 1.3×10^3 to 4.0×10^3 cfm/ml as shown in the result, the total aerobic viable bacteria count (TVC) was highest 275×10^7 in the wastewater for children ward and lowest, 192×10^7 in the wastewater from the surgery ward. The concentration of lead was in the range 0.02mg/l to 0.09mg/l while cadmium concentrations varies between 0.02 to 0.15mg/l, chromium and copper were in the range of 0.00 to 0.03 and 0.07 to 0.2mg/l respectively, while zinc was found to be between 0.07 to 0.09mg/l and mercury was 0.02mg/l. The result shows that the concentration of the different wards varied significantly ($p<0.05$). In general, the physicochemical properties of the hospital wastewater samples show that, their disposal into the environmental will impact significantly on the environment. It may be rewarding to treat this wastewater prior to discharge into the environment.

Keywords: Micro-organisms, Federal Medical Centre, Hospital waste, Risk Assessment

Introduction
Environmental risk assessment (ERA) is a scientific activity which assesses the potential effect of pollutants on the environmental and as well as on human health. Environmental risk assessment provides a systematic procedure for predicting potential risk to human health or the environment. It further predicts within, there may be risk of adverse effects on the environmental caused by pollutants. Hospitals generate considerable amount of medical waste each year and this attributed to advances in medical services Gaulam et al., and Ekhaise and Omavwoya, observed that hospital waste water has similar quality to municipal waste water in all water treatment plants effluents due to their inefficient removal in conventional systems [1-3]. Again, according to Jolibois and Gnarbet, observed that hospital waste water includes a variety of micro-contaminants that are chemicals, heavy metals, disinfectants and some specific detergents resulting from diagnosis laboratory, research activities and medicine excrement by patients and as such hospitals wastewater may have adverse effect and impact on the environmental and human health [4]. Also, observed that the discharged of hospital effluents into the natural environment represent a significant contribution to the general contamination of the aquatic environment. The research further reported that investigations in developed countries recorded the increase of viruses and pathogenic bacteria in hospital wastewater; Other research reports also showed the increase of many different pollutants in hospital waste water including molecules from un used and excreted non–metabolisable pharmaceuticals, antibiotic resistant enteric bacteria radioisotopes [5,6]. It was also observed that one of these pollutants, particularly residues of drugs and compound of organo chlorinated, leave treatment plants with little or no degradation however, it was reported that the third world (developing) countries where poverty is high, the circulation of pathogenic germs within the population is made by intermediaries among which water plays a major part. In view of this, it appears that dangers or risk generated by hospital waste water to human health in those countries are much of microbial nature than chemical. In consideration of the above, this project was designed to study the quality characteristics (biological and physico-chemical) of hospital wastewater from Federal Medical Centre, Umuahia, Abia State, as the biggest and most visited specialized hospital complex in Abia State. As one way of identifying some environmental stressors released by the hospital. The aim of the study is to carry out the assessment of hospital wastewater in FMC, Abia State.

Material and Methods
The study was conducted in the Federal Medical Center (FMC) Umuahia, Abia State Capital. Umuahia has population of 359,230 according to the 2006 Nigeria census. The hospital has ten wards,

two medical wards, on each for male and female, the paediatrics wards, two theatres, one obstetric and other for general surgery. Other areas of the hospital include antenatal clinics, x-ray departments, pharmacy and medical laboratory units, Medical records and statistics among others.

The wastewater samples were collected directly into clean sterilized sample bottles. The collected samples were put in a modified atmospheric package (MAP) and taken to the laboratory without delay. Samples covered wastewater from different hospital wards (Surgical, Children and Emergency wards). This was done for three different Mondays 4/07/2017, 11/07/2017, and 18/07/2017 at about 9am and were designated with (a, b, c) respectively. The wastewater samples were subjected to laboratory analysis to determine their physico-chemical and biological properties. The following methods were used.

Five different culture media were used for this work and they were prepared according to the isolation. A common selective and differential growth medium. The MSA powder was weighed and prepared at a concentration of 111g to 1000ml according to manufacturer's guide. It was autoclaved for 15minutes at 121˚C and cooled to about 45˚C before 20ml was dispersed into each petri dish. It contains the high concentration of salt that makes it selective for gram positive bacterium staphylococci (and Micrococci) because it contains a level of NaCl.

A selective and differential culture medium designed to selectively isolate gram negative and enteric bacilli bacteria and differentiate them based on lactose fermentation. By utilizing the lactose available in the medium lactose positive bacteria will produce acid that will lower the pH of the agar below 6.8 which results in the appearance of pink colonies.

The agar powder was weighed and prepared at a concentration of 48.5g to 1000ml according to the manufacturers guide. It was autoclaved for 15 minutes at 121˚C and allowed to cool to 45˚C and 20mls was dispersed into each petri dish.

A selective and different medium used to isolate salmonella and shigella from faeces, urine, fresh and canned foods. It inhibits the growth of gram positive organisms. The medium was weighed and prepared at a concentration of 63g to 1000ml according to the manufacturers guide, it was boiled and allowed to cool to 45˚C before dispersed into petri dishes. It was observed for growth after 24hours. This culture medium is used for the isolation and differentiation of gram negative bacilli the medium is primarily used for detection and confirmation of coliforms. 37.5g of the medium was weighed and dissolved in one litre of distilled water. It was autoclaved for 15minutes at 121˚C and allowed to cool before dispersing into petri dishes each. It was observed for growth after 24 hours.

A general purpose medium used for the cultivation of a wide variety of microbes. It is frequently used for the cultivation and purification of cultures. It provides amino acids, minerals and other nutrients used by a wide variety of bacteria for growth. It contains agar as a solidifying agent, 28g of the medium was weighed and dissolved in 1000ml of water. It was autoclaved at 121˚C for 15minutes and allowed to cool to 45˚C. it was then dispensed into petri dishes 20mls each inverted and allowed to stand for 24 hours.

Heavy metals in hospitals wastewater were determined by atomic absorption spectrophotometer (AAS) following a double acid wet digestion method as described by (APHA, 1995). A measured volume (10ml) was treated with the combination of concentrated nitric acid (HNO3) and concentrated per chloric acid (HCl04) at high temperature. The resulting digest was diluted to a specific volume and filtered through Whitman no 42 filter paper to obtain the extract that was used for AAS assay. Ace the test, heavy metals run in turns in the instrument using the appropriate hollow cathode lamp at their respective wavelengths.

The direct culture method APHA accordingly, a unit volume 10ml of a well-mixed sample were diluted in a 10fold in series as shown below.

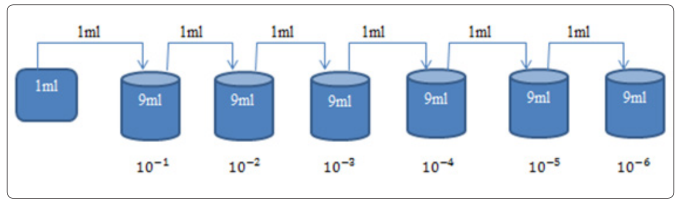

Figure 1: 10-fold serial dilution

Innocula of 1.0ml were collected aseptically from the 4th and 6th diluent and cultured by pour plate technique and incubated at 37˚C for 24 to 48 hours [7]. The number of colonies in the culture plate were counted with the aid of an electronic colony counter. The formula below was used to calculate the total viable count (TVC) expressed in colony forming units (CFU) per ml.

This was determined using the multiple tube fermentation technique and were expressed as the most probable number (MPN) of coliforms per 100ml of samples [7].

Each sample were cultured in 15 test tubes comprising of 10 tubes of single strength broth and 5 tubes of double strength broth. The first tubes of single strength broth were inoculated with 0.1ml sample while the second 5tubes was inoculated with 1.0ml each and each of the double strength broth test tubes was inoculated with 10 ml of samples. After inoculation, the number of tubes showing positive results in each of the groups were counted and use was made of the standard MPN probability test to estimate the number of coliforms in the test sample.

This entails the determination of different types of bacteria species present in the sample. This was done by isolation characterization and identification of the bacteria in the waste water samples.

This involved subculture technique. In this regard the culture plate (as in the determination of total viable count) were examined for the presence of distinct colonies. From such distinct colonies, innocula was collected and aseptically transferred to a fresh solid sterile agar plate and incubated at 37˚C for 24 to 48 hours. The sub culture was examined for uniformity as mark of purity. The pure cultures were used for characterization and subsequent identification.

Isolated and characterized bacteria were identified based on their matching characteristics with existing taxa in standard manuals. On this regard, the recorded characteristics of the isolates was compared with what is available in standard manuals. The Bergys manual of determinative bacteriology for the identification of medical bacteria were used for comparism which share the same characteristics when matched with the ones in the manuals was identified accordingly. Obtained data on the occurrence of bacteria isolates of the wastewater samples from the different hospital wards of FMC and the levels of the different heavy metals in the samples and their physicochemical characteristics was subjected to statistical analysis of variance (ANOVA) to determine the level of differences between them. The statistical package for social sciences (SPSS) version was used for the analysis.

Results and Discussion

Table 1: Microbial Load of Hospital Wastewater

Sample	Bacteria load×10^7	Fungi Load×10^3
Surgical	214.33 ± 12.74	3.33 ± 1.16
Children	275.00 ± 8.72	1.33 ± 0.58
Emergency	192.33 ± 10.01	4.00 ± 1.41

The values show the mean of triplicate analysis of ± standard deviation; figure with different superscript down the column are significantly different (P<0.05)

This table shows the microbial loads of the test hospital wastewater collected from three different wards in Federal Medical Centre (FMC) Umuahia. The result show that they were significant varieties in the bacteria and fungi load of the hospital waste water from the different wards. The bacteria load was on the range of 192×10^7 cfm/ml to 275×10^7 cfm/ml (1.92×10^7 cfm/ml to 2.75×10^7 cfm/ml) while the fungi load ranged from 1.3×10^3 to 4.0×10^3 cfm/ml as shown in the result, the total aerobic viable bacteria count (TVC) was highest 275×10^7 in the wastewater for children ward and lowest, 192×10^7 in the wastewater from the surgery ward. The variation between the bacteria and fungi loads of the wastewater from the different wards, were of significant different (P<0.05). Generally, the results appear to be in line with findings of previous researchers to the subject matter. reported bacteria counts of 228×10^9, 137×10^6 and 144×10^9 cfm/ml in hospital waste from the surgical ward, baby's ward and medical ward respectively in a hospital in Enugu, South East Nigeria. Also the author recorded higher bacteria load than fungi as is the case with project report findings. The high bacteria load of the hospital waste reflects the potentials of the wastewater pollutant when discharged into the environment [8]. On the other hand, there are possibilities of the microbes assisting in degradation of solids thereby giving lower sludge production [9]. Notwithstanding, it is not just the microbial loads as needed that matters in the assessments of hospital wastewater but the types of microbes. Some microbes may be pathogenic while others may help by playing beneficial roles (when discharged into the environment) such as nutrient recycling [10].

Table 2: Occurrence of Microbial Isolates in Hospital Wastewater

Samples	Staphylococcus spp	E.coli spp	Salmonella spp	Proteus spp	Bacillus spp	Pseudomonas spp	Streptococcus spp	Klebsiella spp	Candida spp	Aspergillus spp
Surgical A	+	+	-	-	-	+	-	-	-	-
B	+	+	+	-	+	+	-	-	-	-
C	+	+	-	-	-	+	-	-	-	-
	100% (3/3)	100% (3/3)	33.3% (1/3)	0% (0/3)	33.3% (1/3)	100% (3/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
Children A	+	+	+	-	-	+	+	-	-	-
B	+	+	+	-	+	+	+	+	-	-
C	+	+	-	-	-	+	+	-	-	-
	100% (3/3)	100% (3/3)	67.7% (1/3)	67.7% (0/3)	33.3% (1/3)	100% (3/3)	100% (3/3)	33.3% (1/3)	0% (0/3)	0% (0/3)
Emergency a	+	+	-	-	+	+	+	-	-	-
b	+	+	-	+	+	-	+	-	+	-
c	+	+	+	-	-	+	-	+	+	+
	100% (3/3)	100% (3/3)	33.3% (1/3)	33.3% (1/3)	67.7% (2/3)	67.7% (2/3)	67.7% (2/3)	33.3% (1/3)	67.7% (2/3)	33.3% (1/3)
Total no	9	9	9	9	9	9	9	9	9	9
No of +ve	9	9	4	1	4	8	5	2	2	1
% of occurance	100% (9/9)	100% (9/9)	44.4% (4/9)	11.1% (1/9)	44.4% (4/9)	88.9% (8/9)	55.6% (5/9)	22.2% (2/9)	22.2% (2/9)	11.1% (1/9)

Keywords: a = First sample from each ward; b= Second sample from each ward; c=Third sample from each ward.

The values show the mean of triplicate analysis of ± standard deviation; figure with different superscript down the column are significantly different (P<0.05).

This table shows the types of micro-organisms isolated from the different hospital wastewater samples. The result shows the presence of up to eight bacteria species and two species of fungi in all. However, there were significant variation in their relative occurrences in the wastewater from the different hospital wards. The isolated micro-organisms included some established pathogens as well as some non-pathogens. The bacteria isolates included some species of staphylococcus, streptococcus, and bacillus, as gram positive bacteria as well as salmonella, klebsiellea, Escherichia, proteus and pseudomonas as gram negative bacteria. These organisms contain some species which pose harm to public health and as such are pathogenic [11]. The frequency of occurrence of the bacteria isolates shows that there was variation in the prevalence of the different organism in the sample from different hospital wards. E. coli and staphylococcus aureus were present in all the samples and both had 100% occurrence in the test samples. Bacillus subtilis had the least occurrence of 11.1% being present in one out of the 3 samples (emergency ward wastewater). Also, it was observed that the occurrence of the isolates was highest in the emergency ward wastewater sample and least in the surgical and wastewater. The presence of the variety of bacteria isolates means there is a potential risk to the recipient environmental water or the soil. Again, it is observed that the isolates found in the hospital wastewater matched with the findings of previous research work [12].

It was also observed that the occurrence of fungi was very low and selective. Candida albicans and aspergillus species occurred only in the emergency wards. The low occurrence of the fungi is indicative of the fact that the effluent wastewater will not be conducive to the growth of the fungi. In general, there were many types of micro-organisms in the hospital wastewater and this was attributed to the diverse forms of disease brought in by patients and accordingly captured in the wastewater.

Table 3: Heavy Metals in Hospital Wastewater

SAMPLE	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Copper (Cu)	Zinc (Zn)	Mercury (Hg)
FEPA LIMITS	<1	<1	<1	<1	<1	0.05
Surgical	b	c	C	A	A	B
	0.09 ± 0.01	0.15 ± 0.00	0.03 ± 0.00	0.20 ± 0.12	0.08 ± 0.00	0.02 ± 0.00
Children	a	B	A	A	A	A
	0.02 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.09 ± 0.04	0.09 ± 0.04	0.00 ± 0.00
Emergency	a	A	B	A	A	b
	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.02 ± 0.00

Keywords: a = First sample from each ward; b= Second sample from each ward; c= Third sample from each ward.

The values show the mean of triplicate analysis of ± standard deviation; figure with different superscript down the column are significantly different ($P<0.05$).

The table shows the heavy metal content of the hospital waste water samples. Obtained results show that there were many heavy metals in the wastewater including lead, cadmium, chromium, copper, zinc and mercury but at varying concentrations. The concentration of lead was in the range 0.02mg/l to 0.09mg/l while cadmium concentrations varies between 0.02 to 0.15mg/l, chromium and copper were in the range of 0.00 to 0.03 and 0.07 to 0.2mg/l respectively, while zinc was found to be between 0.07 to 0.09mg/l and mercury was 0.02mg/l. The result shows that the concentration of the different wards varied with significant from difference ($p<0.05$). Lead was higher in surgical ward wastewater (0.09mg/l) and least (0.02mg/l) and least in children ward wastewater. Also, cadmium, chromium and copper were higher in the surgical ward. The level of heavy metals in the wastewater implies that the discharge of the wastewater into the environment possess danger as the metals are not biodegradable but ratio can accumulate in the food chain such as vegetable and groundwater [13]. Nelson and Campbell observed that pilling up other metal in the environment may lead to their accumulation to toxic levels [14]. In all, however, these metals were found to be in the wastewater at levels which fall within the permissible level by World Health Organisation (WHO) and Federal Environmental and Protection Agencies (FEPA).

Table 4: Physicochemical Parameters of Hospital Wastewater

SAMPLE	TS	COD	TDS	pH	Temp	DO	BOD
FEPA LIMITS	2500mg/l	15-50	50mg/l	6-9	20˚C	10-50	50mg/l
Surgical	a	a	b	c	a	b	B
	666.67 ± 125.83	426.67 ± 92.37	266.67 ± 28.87	10.00 ± 0.00	32.00 ± 0.00	21.67 ± 0.12	16.97 ± 0.15
Children	b	a	c	a	b	C	c
	258.33 ± 175.59	480.00 ± 160.00	700.00 ± 86.60	9.57 ± 0.06	37.33 ± 0.57	58.80 ± 1.58	40.60 ± 1.65
Emergency	A	A	A	b	b	a	A
	533.33 ± 28.87	426.67 ± 92.37	133.33 ± 28.87	9.87 ± 0.06	36.67 ± 0.57	19.13 ± 0.15	14.40 ± 0.36

Keywords: a = First sample from each ward; b= Second sample from each ward; c= Third sample from each ward.TS= Total Solids;COD= Chemical Oxygen Demand; TDS= Total Dissolved Oxygen;FEPA= Federal Environmental Protection Agency; DO = Dissolved Oxygen; BOD= Biological Oxygen Demand

The values show the mean of triplicate analysis of ± standard deviation; figure with different superscript down the column are significantly different (P<0.05).

This table shows the physicochemical characteristics of the test hospital wastewater. The result show that the hospital wastewater had pH in the alkaline range having values between 9.47 and 10.0, while the temperature at the time of sampling was between 32.00˚C (surgical ward wastewater) and 37.33˚C ± 0.57. The above result showed that the wastewaters failed tolerable pH level (WHO and FEPA). pH of water is a critical factor that affects its other qualities including the level of dissolved oxygen (DO) as well as the type of micro-organisms that thrive well in the water.

The total solids (TS) in the wastewater was in the range of 258.3mg/l to 666.7mg/l and was the highest in the surgical ward sample and least in the children ward samples and was within permissible limit of FEPA (2500mg/l).

The DO and BOD were high having ranges of 19.13 to 58.80 and 14.40 to 40.60mg/l respectively. Both the DO and BOD were highest in the children ward. These two parameters are widely used to characterize the organic content of wastewater as it measures the amount of dissolved oxygen in the water. From the result, it was observed that only the children ward failed the acceptable criteria for wastewater, 10-50mg/l according to the FEPA standards.

The chemical oxygen demand, (COD) was in the range of 426.67 to 480.00 and was also highest in the children ward wastewater sample. From the obtained figures, the COD/BOD ratio will be in range of 29.6 to 12.0 which is considerably high.

Again, the BOD is low, 14.40 – 40.60 mg/l and was within the permissible limit of 50mg/l.

In general, the physicochemical properties of the hospital wastewater samples show that, their disposal into the environmental will impact significantly on the environment. It may be rewarding to treat this wastewater prior to discharge into the environment.

It was observed that hospital wastewater has significant influence on the environment. The microbial load as well as the high concentrations of the physicochemical parameters suggests that the activities of hospital wastewater in the environment is a major health and environmental threat, which therefore, calls for a proper regulatory system on disposal of the hospital wastewater into the environment and also, it would be rewarding if the hospital wastewater is treated prior discharge into the environment [15-53].

References

1. Venio N, Tuhkaren T, Kronberg L (2007) Elimination of Pharmaceuticals Sewage Treatment Plants in Finland. Water Research 41: 1001-1012
2. Guatam AK, Kumar S, Sabumon PC (2007) Preliminary Study of Physicochemical Treatment Options for Hospital Wastewater. Journal of Environmental Management 83: 298-306
3. Ekhaise FO, Omavwoya BP (2008) Influence of Waste Water Discharged from University of Benin Teaching Hospital (UBTH) On Its Receiving Environment. Idosi Publications American Euro-Asian Journal of Agricultural and Environmental Science 4: 484-488
4. Jolibois B, Gnarbet M (2007) Hospital Waste Water Geno-Toxicity. Animals of Occupation Hygiene 50: 189-196.
5. Halling-Sorensen B, Nielsen N, Lanzky PF, Ingersler F, Holten-Lutzhoft HC, Jorgenson Se (1999) Occcurence, Fate and Effects of Pharmaceutical Substances in Environment. A Review: Chemosphere 36: 357-393
6. Kummerer K (2001) Drugs in the Environment: Emission of Drugs, Diagnostic Aids and Disinfectants in Wastewater by Hospitals in Relations to other Sources. A Review: Chemosphere 45: 957-969
7. Cheesbrough M (2000) District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press 64-70
8. Hsa Aluyi, Fo Ekhaise, Dm Adelusi (2006) Effect of Human Activities and Oil Pollution on the Microbiological and Physiological Quality of Udu River, Warri, Nigeria. Journal of Applied of Sciences 6: 1214-1219.
9. Ward-Paige CA, Risk MJ, Sherwood OA, Jaap WC (2005) Clinoid Sponge Surveys Florida reefs tract suggest Land-based Nutrient Input. Mar. Pollut. Bull 51: 570-570.
10. Kris M (2007) Waste water Pollution in China. Available from http: www.dbc.uci/wsustain/suscoasts/krismin.html. Assessed 16/06/2008.
11. Onyeleke SB, Istifanus N (2009) The Microbiological Effects of Hospital Wastes on The Environment. African Journal of Biotechnology 8: 1253-1257.
12. Giroletti E Lodola L (1993) ISPRA COURSES: Wastewater Treatment and Management. Medical Waste Treatment 1-8.
13. Hussein H, Farag S, Kandil K And Moawad, H (2005)Tolerance and Uptake of Heavy Metals by Pseudomonads. Process biochemistry 40: 955-961.
14. Nelson WO, Campbell PGC (1991) the effect of acidification on the geochemistry of Al, Cd, Pb and Hg in freshwater environments; a literature review. Environ. Pollut 71: 91-130.
15. Aarestrupfm, Ahrens P, Madsen M, Pallesen Lv, Poulsen Rl, Westh H (1996) Glycopeptides Susceptibility Among Danish Enterococcus Faecium and Enterococcus Faecalis Isolates of Human and Animal Origin. Antimicrobial Agents and Chemotherapy 40: 1938-1940.
16. Agbor A (2007) Chemicals Make Thane Creek the Worst Polluted Water Body Daily DNA Mumbai India Outline. Http://Www.Dnaindia.Com/Mumbai/Report.
17. American Public Health Association (2001) Standard Methods for the Examination of Water and Wastewater, 23rd Edition. Washington D.C.
18. American Public Health Association (2000) Standard Methods for The Examination of Water and Wastewater American Water Works Association and Water Pollution Control Federation 20th Edition.
19. Williams & wilkins, Batimore (1957) Bergey's Manual of Determinative Bacteriology.9th edition, ISBN 0-68300603-7.
20. Bendz D (2005) Occurrence and Fate of Pharmaceutically Active Compounds in the Environment, a Case Study: Hoje, Sweden. Journal of Hazardous Materials 122: 195-204.
21. Chikere Cb, Okpokasili Gc, Ichiakoro (2000) Characterization of Hydrocarbon Utilizing Bacteria in Tropical Marine Sediments. African Journal of Biotechnology 8: 2541-2544.
22. Dwaf (1996) Waste Management Series. Minimum Requirements for Handling, Classification and Disposal of Hazardous Waste. Second Edition 1998. Department of Water Affairs and Forestry (DWAF) Pretoria.
23. Egemen (2000)Environment and Water Pollution Ege University,

Faculty of Fisheries. Zmir, Turkey.
24. Emmanuel (2001) Fate of Glutaraldehyde in Hospital Wastewater and Combined Effects of Glutaraldehyde and Surfactants On Aquatic Organisms. Environment International 31:399-406.
25. Grabow WK, Very A, Uys M, Villiers JC (1997) Evaluation of the Application of Bacteriophages as Indicator of Water Quality. South Africa Water Research Commission Final Report 540/1/98.
26. Luca guardabassi, Andreas petersen, John E. Olsen, Anders Dalsgaard (2008)Antibiotic Resistant in Acinotobacteria Sap Isolated from Sewers Receiving Effluent from a Hospital and Pharmaceutical Plants. Applied and Environmental Microbiology 64: 3499-3502
27. Fuggle RF (1983) Nature and Ethics of Environmental Concerns. In Environmental Concerns in South Africa.
28. Hafiza BS (2001) Characteristics of Industrial Wastewater Ert417/4. Wastewater Treatment in Bioprocess Industry.
29. Hong Konghussein H (2003) Hospital Authority. Sars Precautions in Hospitals Tolerance and Uptake of Heavy Metals by Pseudomonas. Process Biochemistry 40:955-961.
30. Heberer T (2002) Occurrence, Fate, and Removal of Pharmaceutical Residues in the Aquatic Environment: A Review of Recent Research Data. Toxicology Letters 131: 5-17.
31. Jones O, Voulvoulis N, Lester JN (2004) Potential Ecological and Human Health Risk Associated with the Presence of Pharmaceutically Active Compounds in the Aquatic Environment. Critical Revision Toxicology 34: 335-350.
32. Jorgensen S E, Halling-Sorensen B (2000) Drugs in The Environment. Chemosphere 40: 691-699.
33. Kobayashi Y (2006) Removal Characteristics of Human Antibiotics During Wastewater Treatment in Japan. Occurrence and Removal of Municipal and Hospital, Wastewaters in Greece. Journal of Hazardous Materials 179: 804- 817.
34. Kummerer K (2000) Biodegradability of Some Antibiotics, Elimination of the Genotoxicity and Affection of Wastewater Bacteria in a Simple Test. Chemosphere 40: 701-710.
35. Langumir D (1997) Aqueous Environmental Chemistry Prentice Hall, Inc. New Jersey.
36. Lokande RS, Singare PU, Pimple DS (2011) Quantification Study of Toxic Heavy Metals Pollutants in Sediment Samples Collected from Kasardi River Flowing Along the Taloja Industrial Area of Mumbai, India. The New York Science Journal 4: 66-71
37. Lowe Ksn, Rothe J, Tomaras K, Dejong K, Tujolke M, Et Al. (2007)Influent Constituent Characteristics of Modern Waste stream from Single Sources Literature Review. Water Environmental Research Foundation 1-89.
38. Mahnik S, Lenz K (2007) Fate of Fluorouracil, Doxorubicin, Epirubicin, and Daunorubicin in Hospital Wastewater and Their Elimination by Activated Sludge and Treatment in a Membrane-Bio-Reactor, Chemosphere.
39. Marcos VS (2007) Wastewater Characteristics, Treatment and Disposal. Biological Wastewater Treatment Series 1:1
40. Mcmillian Gk (1994) pH Measurement and Control 2nd Ed Instrument Society of America.
41. Metcalf X, Eddy X (2003) Wastewater Engineering: Treatment and Reuse. In: Wastewater Engineering, Treatment, Disposal and Reuse. Techobanoglous G, Burton FL, Stensel HD (eds), Tata McGraw-Hill Publishing Company Limited, 4th Edition. New Delhi, India.
42. National Guideline and Standards for Industrial Effluents (1991) Gaseous Emissions and Hazardous Waste Management in Nigeria. Federal Environmental Protection Agency (FEPA), Lagos, Nigeria.
43. Paillard D, Dubois V, Thiebaut R, Nathier F, Hogland E, Caumette P, And Quentine C (2005) Occurrence of Listeria spp. In effluents of French Urban Wastewater Plants. Applied Environmental Microbiology 71: 7562-7566.
44. Payment P, Franco E (1993). Clostridium Perfingens and somatic Coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoa cysts. Appl. Environ. Microbial
45. Pontius F (2007) A Balanced Approach to Regulating Endocrine Disrupting Chemicals in Drinking Water. Inside EPA Risk Policy Report, October 15 Pg. 32-35 Publishing London Pp. 308-327
46. Purdom C E (1994) Estrogenic Effects of Effluents from Sewage Treatment Works. Chemical Ecology 8: 275-285.
47. Schwarzenbach RP, Gschwend P M, Imboden D M (2004) Environmental Organic Chemistry. Wiley Hoboken New Jersey, USA.
48. Smith VH, Tilman GD, Nekol JC (1999) Eutrophication: Impacts of Excess Nutrient Inputs On Freshwater, Marine and Terrestrial Ecosystems. Environmental Pollution100:179-196.
49. Tsai C T, Lai Y C (2008) Study of Bio-aerosol Emissions Form Aeration Treatment Facilities of Hospital Wastewater. Journal of Aerosol Science 29 (1-2); 248
50. United States Environmental Protection Agency (USEPA) (1994) Chemical Summary for Chlorine. Office of Pollution Prevention and Toxics. Cincinnati (Oh)USEPA: EPA 7
51. Vogelsang C, Grung M, Jantsch TG, Tollefsen KE, Liltved H (2006) Occurrence and Removal of Selected Organic Micro pollutants at Mechanical, Chemical and Advanced Wastewater Treatment Plants in Norway. Water Research 40: 3559-3570.
52. Water Environmental Federation (2007) Characterization and Sampling of Wastewater 17-23.
53. World Health Organization Permissible Limits (2007) Environmental Health Criteria, 2nd Edition No. 54. Geneva, Switzerland