JASEM ISSN 1119-8362

Full-text Available Online at
www.ajol.info and
www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *Sept.* 2016
Vol. 20 (3) 724-732

Cytogenotoxicity Screening of Untreated Hospital Wastewaters Using the *Allium cepa* Test.

*** [1]BABATUNDE, BB; VINCENT-AKPU, IF; AIWERIOGHENE, ABED-NEGO OSAYANDE**

Environmental Biology Unit, Department of Animal and Environmental Biology,
Faculty of Science, University of Port Harcourt
**Correspondence author: bolaji.babatunde@uniport.edu.ng*

ABSTRACT: Cytogenetoxicity of University Teaching Hospital (UTH) wastewaters was investigated using the *Allium cepa* assay. Heterogeneous samples of untreated wastewaters from four sections of the hospital comprising the laboratories, mortuary, laundry and the kitchen were collected twice daily for six months and designated as complex mixtures. Physico-chemical parameters of the wastewaters were determined in accordance with standard methods. Onions root growth inhibition test was used to assess the toxic status of the wastewaters, while cytogenotoxicity was measured by microscopic investigation of the chromosomal aberrations. Onion bulbs were exposed to 1%, 5%, 10% 25% and 50% concentrations of the effluent samples in the dark for 72 hours before measuring the root lengths of the onion bulbs. Results of BOD, COD, TSS and pH showed levels above the maximum permissible limits for discharge of hospital effluent into the environment. There was inhibition of root growth of *A. cepa* in a concentration dependent pattern compared to control. An effective concentration EC_{50} of 8.20% and 9.1% was deduced for the 1^{st} and 2^{nd} samples respectively. Various morphological defects of the onions roots were also observed. Cytological analysis of root tips after 48 hrs exposure to the different concentrations showed reduction in frequency of mitosis in the meristematic zones of the root tips. Various types of structural chromosomal aberrations and micronucleus were induced in the treated cells. The University Teaching Hospital wastewaters samples are believed to contain potent toxic substances that provoked the cytogenotoxic responses herein and should be treated before discharge into the environment. ©JASEM

http://dx.doi.org/10.4314/jasem.v20i3.27

Key Words: *Cytogenotoxicity screening, hospital wastewaters, Allium test*

The hospital is an integral part of human existence as it provides health care services to all and sundry. However, hospitals represent an incontestable source of many chemical compounds including disinfectants, detergents, and diagnostic agents such as X-Ray, laboratory reagents, morgue chemicals and un-metabolized antibiotics and other drugs by inmates. These chemicals represent the complex mixture usually contained in hospital wastewaters which is more often than not released directly into our environment with little or no knowledge whether they may have an impact on the environment and human health (Kummerer, 2001). Indeed, some of the substances found in hospital wastewaters are genotoxic and are suspected to be a possible cause of the cancers observed in the last decades (Jolibois and Guerbet, 2005).

Despite the growing concern over hospital waste management, scant attention has been paid to wastewater generated from hospitals, medical research laboratories, and health care institutions. Hospitals consume a significant amount of water in a day, ranging from 400 to 1200 L $day^{-1}bed^{-1}$ and generate equally significant amounts of wastewater loaded with pathogenic microorganisms, pharmaceutical wastes, heavy metals, toxic chemicals, radioactive elements and infectious materials containing HIV, Hepatitis virus etc. This wastewater if left untreated, could lead to outbreaks of communicable and infectious diseases, diarrhoea epidemics, water contamination, radioactive pollution and a host of other ecological and public health complications such as antibiotic resistance among wild pathogens (Guardabassi *et al.,* 2000) and feminization in fish exposed to birth control medications (Jobling *et al.,* 1998). Non target organisms including man become victims of the salvation others have enjoyed through the health care; the irony becomes save one, kill two.

Hospital wastes could be dangerous to the ecological balance and public health; they could pollute both underground and surface water including water supply sources. Despite these potential threats, literature is not replete on the ecological risk

**Correspondence author: bolaji.babatunde@uniport.edu.ng*

evaluation of hospital effluents. However, it is known that hospital effluents generally show levels of physico-chemical parameters such as pH, total suspended solids (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD) above regulatory limits (Ravikant *et al.,* 2006).

Toxicity, cytotoxicty and genotoxicity of hospital effluents have also been evaluated in a few bioassays with positive results including the Allium test employed in this present study.
The *Allium cepa assay* is a highly sensitive bioassay, simple to carry out, cheap and widely acceptable for evaluating complex mixtures such as hospital effluents (Fiskesjo, and Levan 1993; Rank, and Nielson, 1993).

It is evident from the few works that hospital effluents as a complex mixture are clearly cytotoxic to biological systems or potentially genotoxic, which is defined as the capacity to interfere with gene expression of cells (Fracasso *et al.,* 1993). Residues associated with patient excretions may also contain several products with mutagenic activity (Kummerer, 2001). Ortolan and Ayub, (2007) evaluated the possible presence of cytotoxic (that is, cell direct toxicity) and genotoxic activities in the effluents of a typically large general hospital, in *de Porto Alegre*, Brazil. They reported that the hospital generated approximately 27,000 m³ of effluents a year; and inpatients effluents had more genotoxic effects in the umuC test than laboratory effluents. Giuliani *et al.,* (1996) also used the umuC test for the analysis of the polluting load generated by a hospital with 1400 beds in Zurich, Switzerland. The authors reported that considerable amounts of genotoxic substances were released into the environment through hospital effluents. Thus, these seem to present a potential environmental risk. The mutagenicity value of hospital effluents has been shown using Ames and Hamster Cell tests on hospital wastewater (Hartmann *et al.,* 2001). According to Emmanuel *et al.,* (2001), hospital wastewater showed a high toxicity as determined using the Daphnia and Luminescent Bacteria tests. This may be due to the presence of organohalogen compounds (OHCs) resulting from the disinfectants in hospital effluents (Emmanuel *et al.,* 2002). OHCs result from reactions of oxide-reduction between organic matter and the disinfectants. OHC are mostly lipophilic, persistent, and toxic.

Antibiotics which form a good percentage of hospital effluents such as Ofloxacin was found to be genotoxic, and sulfamethoxazole, Ofloxacin and Limcomycin were mutagenic in Ames test and SOS Chromotest (Hartmann *et al.,* 2001). Monarca *et al.,* (2002); Olaitan *et al.,* (2015) and Jos *et al.,* (2003) investigated the potential toxicity and genotoxicity of Pharmaceutically active compounds and reported that they were found to induce chromosomal aberrations in the merismatic cells of Allium *cepa* and inhibited its root growth and cell division.

Nigeria is one of the developing nations confronted with associated developmental problems of rapid industrialization and urbanization, exponential growth of human populations, and serious environmental pollution crisis as a result of low environmental awareness, lack of sanitary facilities, potable water, non-compliance to regulatory standards by industrialists and other wastes generators. The problem is complicated by non-existence of waste management facilities, irregular monitoring actions by relevant authorities due to lack of infrastructure and supporting legislation, competition for the nation's limited economic resources (Odiete,1999). Consequently, our environment, especially the aquatic ecosystem is laden with synthetic and foreign substances making water unsuitable for human consumption and habitation by aquatic biota. Most teaching hospitals in Nigeria have no functional wastewater treatment facility and therefore discharge wastewaters they generate directly into the environment. Poor management of medical waste exposes medical staff, waste-handling workers and the surrounding communities to infections, toxic effect and injuries; this situation poses a serious health problem in most developing countries of the world (Coker *et al.,* 1999).

Hospital wastewater samples are very often different in nature eliciting different effects on biological systems. The aim of the paper was to investigate the potential toxicity and genotoxicity of an untreated hospital wastewaters obtained from a University Teaching Hospital in the Niger Delta Region of Nigeria using the Onion Bulbs *Allium cepa* Technique.

MATERIALS AND METHODS

Study Area: Wastewater samples were collected from University Port Harcourt Teaching Hospital, which is one of the biggest hospitals in Rivers State and occupies an estimated 100-109 hectares of land and houses about 500 hospital beds. It is located in Choba, Obio/Akpor Local Government Area of Rivers State (Latitude N 4^{o} 53' 35.13" and Longitude E 6^{o} 55' 45.50" and 18 m above sea level).

Wastewater Sampling and Analysis: Heterogeneous samples of wastewaters exiting the kitchen unit, laundry unit, staining laboratory and general laboratory for the morgue were collected twice daily for six months. The samples were mixed together to maintain its heterogeneous nature. This was designated as COMPLEX MIXTURE (UTH1, 2,3,4,5,6,7,8,9…………360) and delivered into 10 liter plastic containers with screw caps and transported the same day to the Animal and Environmental Biology, Laboratory at University of Port Harcourt. The samples were stored at 4^0C if not used the same day. The physico-chemical properties of samples were analyzed according to ASTM, (1994) method.

Allium cepa assay**:** The experiments were conducted as described by Fiskesjo (1997) with minor modification (Babatunde and Bakare, 2006). Equal sized bulbs (1.5$\pm$ 2cm in diameter) of commercial variety of *Allium cepa* (diploid 2n = 16) where obtained from Mile three Market, Port Harcourt, Rivers State. The onion bulbs were sun-dried for more than two weeks before start of experiment (Bakare, 2002; Babatunde and Bakare, 2006). In order to replace bulbs that may dry up or be destroyed by mould, 200 onion bulbs were obtained (twice the number needed for each set of experiment). Bulbs were easily stored at variations of room temperature (25$\pm$ 1^oC).

Just before use, the outer scales of the bulbs were carefully removed and the brownish bottom plates scraped away without destroying the root primordial. The peeled bulbs were washed in water to remove impurities and placed on a filter paper to drain.

Toxicity of samples to onion root growth" An estimate of the degree of toxicity was provided by expressing the mean root growth for each treatment in percentage of the control (negative control – tap water) in which root growth was assumed to be maximum (100%). The six months sampling exercise was categorized into two, first three months and second three months for the purpose of comparison.

Solutions of five concentrations (1%, 5%, 10%, 25% and 50%) were prepared for each sample using tap water which also served as a negative control. The municipal water was allowed to stand under sunlight for about 24 hrs to get rid of any impurity that might alter results. The peeled and washed onion bulbs, twelve for each concentration were randomly selected and placed on glass beakers filled with the test liquid UTH effluent and grown at room temperature in the dark to avoid effect from light. Test solutions were changed every 24 hrs for 72 hrs. Two onion bulbs with the most poorly grown roots from each concentration were set aside after 48 hrs and their root tips cut and fixed in 3:1 v/v glacial acetic acid solution and stored at 4^oC for cytological analysis. The experiment was terminated after 72 hours and the remaining 10 bulbs from each concentration of the test samples were set aside for measurement of individual root length using a ruler. From the values obtained, percentage root growth inhibition in relation to control and the EC_{50} (effective concentration at which root growth amounted to 50% inhibition compared to control) was determined by probit. The effects of samples on the morphology of growing roots, mitotic index and chromosomes were also examined and photographs taken.

RESULTS AND DISCUSSIONS

The results of the physical and chemical characteristics of the UTH Effluent are presented in Table 1. The values obtained from the analysis were compared with the Quality standards for effluent discharge by FEPA 1991. Temperature of the complex mixture (28.0^{o}C) fell within the permissible range by FEPA, the pH value of 7.61 was slightly alkaline. Also the sulphate value of 68.8 mg/l and nitrate value of 1.77 mg/l were well below the permissible limit by FEPA, (1991). Dissolved oxygen of 3.0 mg/L was below the permissible limit by FEPA (5 mg/L minimum) and the Biochemical Oxygen Demand of the effluent (320 mg/l) was far above the stipulated standard of 50 mg/L, similarly the Chemical Oxygen Demand 274 mg/l for the effluent was above that of standard set by FEPA 80 mg/L. The Total Dissolved Solid and Total Suspended Solid of the effluent samples were 180 mg/L and 120 mg/L respectively compared to 30 mg/L stipulated standard. According to Ravikant *et al.,* 2006), physico-chemical characteristics of hospital effluents usually deviate from the norm as shown in the present analysis. Such deviations are capable of altering the overall water quality of the recipient ecosystem with consequences on reproductive and behavioural ecology of aquatic organisms residing there. The results of the second three months were generally in the same range as the first three months not varying statistically at $P > 0.05$.

Toxic effects of UTHE on A. cepa root growth: Table 2 shows the summary of effect of UTH samples on the root growth of *A. cepa*. The percentage root growth of treated onion bulbs in relation to the negative control decreased with increased concentration of UTHE samples. The highest percentage root growth of 92.5% and 89.1 % was recorded in 1% concentration in the first and second samples respectively while the least percentage root growth of 18.9% and 17.2 % was recorded at 50% concentration during the first and second samples respectively. 33.3% of the bulbs in 25% concentration had no root growth and 41.7% of 50% concentration had no root growth. Mean root lengths decreased with increase concentration of UTH effluent recording the longest and shortest roots at control and 50 % concentration respectively (Fig 2 and Table 2). Root lengths of treated samples were statistically different from those in control indicating probable presence of substance inhibiting onion root growth in the samples. This was confirmed by percentage inhibition of root growth by UTHE samples which were strictly concentration dependent as illustrated in Fig 2 showing the highest root growth inhibition at 50 % concentration. The effective concentration EC_{50} at which root growth inhibition amounted to 50 % of control was 8.3 % and 9.1 % in the first and second samples respectively. Relative to the highest concentration tested in this study, the EC_{50} shows weak toxicity but the concentrations tested generally showed high positive correlation value of 0.95 with mitotic inhibition at the cellular level.

Mitotic index showing proportion of diving cells decreased with increase concentration of UTH effluent samples Table 3. This showed that the UTH effluent samples were capable of inhibiting mitosis during cell division and this can have far reaching consequences on overall growth and development of the organism. Aberrant cells showing anaphase and metaphase disruptions, sticky chromosomes, chromosome lags, micronucleus and pulverized cells were recorded at higher concentrations and only few of such observations in control (Table 3 and plate 1 a-f). Chromosome lags and dead cells were recorded at all treated concentrations. Proportion of aberrant cells in treated samples compared with control showed significant levels at P<0.05 Table 3. Besides root growth inhibition, mitotic inhibition and chromosomal aberrations induced by the samples, there were other macroscopic observations such as brownish root tips in most of the onion bulbs, coiled roots, spiral root etc. The *Allium cepa* test is sensitive, simple, inexpensive to perform and widely used as reconnaissance assay to alert scientists of potential toxicity and genotoxicity of environmental contaminants. It has been used by other researchers to investigate hospital effluents with similar results obtained in the present study (Paz *et al.,* 2006; Bagatini *et al.,* 2009). The *Allium cepa* test has also been used to evaluate the genotoxicity of a hospital effluent in Santa Maria, Rio Grande do Sul State, Brazil. During the study, similar abberant cells observed in the present study such as chromosomal disruptions, anaphasic bridges, and micronuclei during telophase were observed, indicating environmental toxicity risk (Ortolan and Ayub, 2007). In hospitals a large variety of substances are in use for medical purposes such as diagnostics and research. After application, diagnostic agents, disinfectants and excreted non-metabolized pharmaceuticals by patients reach the wastewater. Indeed, some of the substances found in wastewaters have been reported to be genotoxic and are suspected to be a possible cause of the cancers observed in the last decades (Preeti *et al.,* 2009). The present study has therefore revealed the potential of UTH wastewater to cause cytogenooxic effects in the *Allium cepa* indicating that organisms including humans may suffer cancer if exposed to it.

One the primary endpoints of toxicological assays generally investigate the ability of a toxicant to interact with the DNA. Since DNA is similar in configuration in all living cells, it is believed that effects elicited in this research can be recorded for any other living cells including those of humans exposed to UTH wastewater. Several researchers have also opined that substances present in hospital wastewaters have genotoxic potential and may cause damage to human and ecologic health (Giuliani *et al.,*; 1996, Hartmann *et al.,* 2001; Kummerer, 2001; Jolibois and Guerbet, 2005; Ortolan and Ayub, 2007).) . Emmanuel *et al.,* 2001 reported similar cytogenotoxic results of hospital effluent using the Daphnia and Luminescent Bacteria test while Hartmann *et al.,* (2001) reported mutagenicity of hospital effluent in the Ames and Hamster tests. Hospital effluents are loaded with pathogenic microorganisms, partially metabolized pharmaceutical substances, radioactive elements, and other toxic substances. Such effluents if not treated properly can damage the natural environment and create a biological imbalance. The University of Port Harcourt Teaching Hospital with inmate capacity of 500 beds is a fairly large hospital and the volume and quality of wastewater generated and discharged into the environment without treatment may pose great health risk for human and other receiving ecosystems as suggested by results of the cytogenotoxicity assessment in the present study.

Table 1: Physico-chemical characteristics of UTH effluent samples compared with standards for effluent discharge.

Parameters	Standards FEPA 1991	UTHE Analysis 1st three Months	UTHE Analysis 2nd three Months
Temperature ^{O}C	< 40	28.0	29.5
DO mg/L	5. 0	3.0	3.1
BOD mg/L	50	320	300
PH	6.5-7.5	7.61	8.2
COD mg/L	80	274	265.5
TDS mg/L	30	180	195.5
TSS mg/L	30	120	105.5
Nitrate mg/L	10	1.77	1.85
Sulphate mg/L	200-400	68.8	65

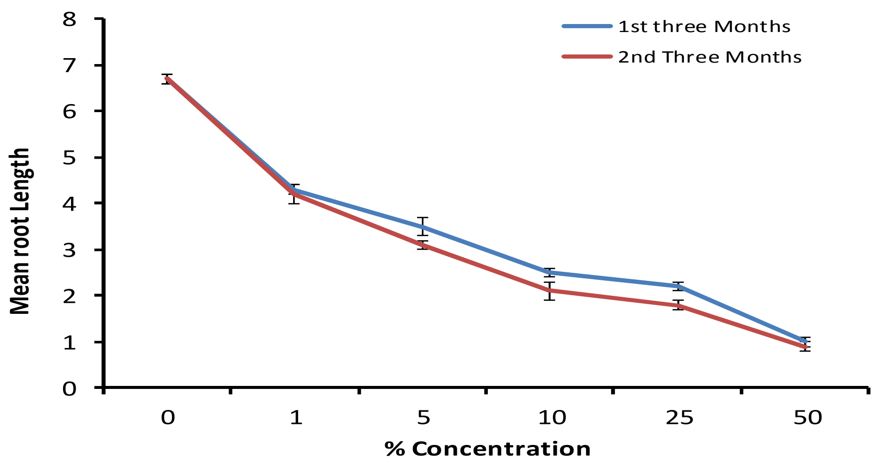

Fig 1: Mean±SE of root length against percentage concentration of the hospital effluent

Table 2: Results from UTH wastewater samples on root growth of *A.cepa* after three and six months

Concentration	First Three Months				Second Three Months			
	Average root length (range)	Mean±SE	Growth in % of Control	95% confidence Limit	Average root length (range)	Mean±SE	Growth in % of Control	95% confidence Limit
Negative Control	4.3-7.2	6.7-0.1		0.39	4.3-7.1	6.7±0.1		
1	3.2-5.3	4.3-0.1	92.5	0.37	3.2-5.6	4.2±0.2	89.1	0.40
5	3.1-4.7	3.5-0.2	85.1	0.38	2.6-4.1	3.1±0.1	72.1	0.39
10	2.9-3.4	2.5-0.1	43.6	0.36	2.1-2.8	2.1±0.2	55.2	0.52
25	2.1-3.2	2.2-0.1	25.2	0.38	1.7-2.1	1.8±0.1	31.4	0.45
50	0.1-1.3	0.9-0.1	18.9	0.54	0.9-1.1	0.9±0.1	17.2	0.25
		EC_{50} 8.3%				EC_{50} 9.1%		

∗ = statistically significant (P<0.05)

Table 3: Results of the cytological testing of UTH effluents samples

% Concentration	Mitotic Index ± SE	Number of Classified cells				Proportion of Aberrant cells in Total counted cells (4000)
		Anaphase Bridge	Sticky chromosomes	Chromosome Lags	Pulverised cells	
Negative Control	45.1±0.1	None	None	None	None	0.02
1	23.1± 0.1	None	None	1	4	1.01*
5	16.9± 0.2	2	None	1	3	2.05*
10	12.8± 0.2	3	2	3	5	2.18*
25	09.0± 0.1	2	3	2	8	2.21*
50	05.2± 0.2	2	6	4	11	2.56*

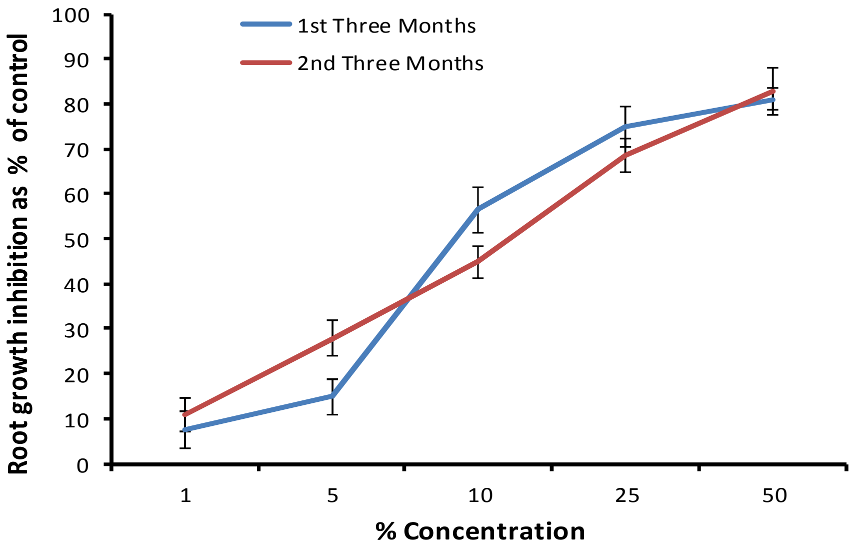

Fig 3: *A. cepa* root growth inhibition by UTH effluent expressed as percentage of control.

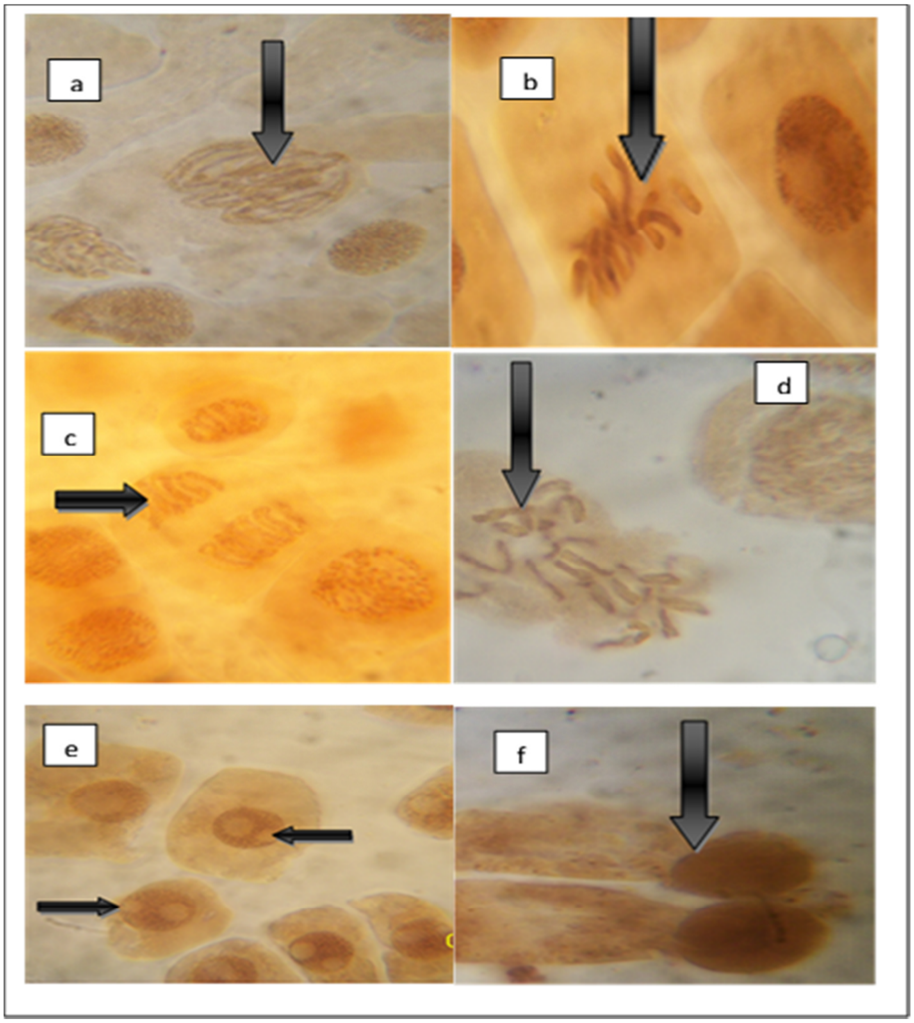

Plate 1: (a) normal late prophase (b) normal metaphase (c) normal anaphase (d) disrupted anaphase (e) micronucleus (f) pulverized cells

Conclusion: The importance of hospitals and more importantly teaching hospitals cannot be overemphasized, quality health, wellbeing and research about health matters are evident benefits of these institutions. However, like many other such necessary service providers, hospitals generate a huge amount of solid and liquid wastes which are often times discharged into the environment untreated. Most of these wastes have been reported to cause hazardous effects on ecologic and human health. Results of the present study are positive for cell toxicity and cytogenotoxicity and agree with similar biological assays by other authors elsewhere in the world and therefore calls for more strict regulatory enforcement and policy review on hospital effluent discharges into our environment. Biomonitoring such as this should be fairly regular activity to forestall public health disasters and avert them in good time.

REFERENCES

ASTM. (1994). Standards Practice for conducting early seedling growth tests 1. America Society for Testing and material Designation. E 1598-1994; 1493-1499.

Bakare, A.A. (2002). In vivo mutagenic and acute effects of leachate from three waste dump sites in South-West Nigeria. Ph.D Thesis, Department of zoology, University of Ibadan, Ibadan, Nigeria.

Babatunde, B.B and Bakare, A.A (2006). Toxicity and Genotoxicity of Agbara Industrial effluents, evaluated using the Allium test. *Pollution Research*, 25 (2) 1-5

Bagatini, M. D., Vasconcelos T. G., Laughinghouse H. D IV., Martins, A. F and Tedesco S. B. (2009). Biomonitoring Hospital Effluents by the Allium cepa L. Test. *Bulletin of Environmental Contamination and Toxicology* 82, (5): 590-592 DOI:10.1007/s00128-009-9666-z

Coker, A.O., Sangodogin, A Y., Ogunlowo, O.O. (1999). Managing hospital wastes in Nigeria, proc. Of the 24th WEDC Con., Islamabad, Pakistan. 70-72.Deloffre-Bonnamour, N., 1995. Wastes rejects from health establishments:liquid effluents to solid wastes. Master's Thesis, University Claude Bernard-Lyon1, Institut Universitaire Professionnalise Department of Environment and Eco development, Lyon, 75pp.

Emmanuel, E., Blanchard, J. M., Keck, G., Perrodin, Y. 2001). Chemical, biological and ecotoxicolgical characterization of hospital effluents. *Dechets sciences et Techniques, revue francophone d' ecologie industrielle*, No 22 – emetrimestre, 31-33.

Federal Environmental Protection Agency (FEPA) (1991). SI8 National Environmental Protection (Effluent limitation) regulation for all categories of industries. *FEPA Monograph* 2

Fiskesjo, G. and Levan A. (1993). Evaluation of the first Tanmeic Chemicals in the Allium Test. *ATLA* 21: 139-149.

Fiskesjo, G. (1997). Allium test for screening chemicals; evaluation of cytological parameters. In: Wang W., Gorsuch J.W, Hughes JS, editors. Plants for Environmental Studies. New York: *Lewis Publishers*; p.308-33

Fracasso, M. E., Barba, A., Tessari, G., Gasperini, S., Brunello, F., (1993). Urinary mutagenic activity after different immunosuppressive protocols in renal transplant patients. *Mutation Research*. 319, 279-283.

Goldstein B.D. (1993). Issues in environmental toxicology Relevant to hazardous waste. Hazardous waste conference New Jersey.

Guardabassi, L., Dijkshoorn, L., olsen J.E., Collard,J.M., and Dalsgaard, A (2000). Distribution of tetracycline resistance determinants A and E and transfer in vitro of tetracycline resistance in clinical and aquatic Acinetobacter strains. *Journal of Medical Microbiology*. 49:929-936

Giuliani, F., Koller,T., Würgler, F. E., Widmer, R. M., 1996. Detection of genotoxic activity in native hospital waste water by the umuC Test. *Mut. Res.* 368, 49-57.

Hartmann A, Kiskinis E, Fjaellman A, Suter W (2001). Influence of cytotoxicity and compound precipitation on test results in the alkaline comet assay. *Mutat. Res*. 497:199-212.

Jobling, S., Nolan,M., Tyler, C.R.,Brighty, G., Sumpter, J.P (1998). Wildspread sexual disruption in wild fish. *Environmental Science Technology* 32:2498

Jolibois, B., Guerbet, M., (2005). Hospital Wastewater genotoxicity. Annals of occupational Hygiene Advance Access Published online on September 5, 2005. *Annals of Occupational Hygiene* doi: 10, 1093/annhyg/mei 051.

Jos, A; Repetto, G; Rios, J.C; Hazen,M.J; Molero, M.L; der Peso,A; Salguero, M; Fernadez-Freire,P; Perez-Martin, J.M; Camean, A (2003). Ecotoxicological evaluation of carbamazepine using six different model systems with 18 end points.

Kummerer, K., 2001. Drugs in the environment: Emission of Drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere* 45, 957 – 969.

Monarca,S.; Richardson,S.D., Ragazzo,P., Zerbini, I. and Alberti, A. (2002). Mutagenic and Disfectantion by- products in surface drinking water disfected with Peracetic acid, Environ. *Toxicol. And Chemistry*, 21(2) 309-318.

Odiete, W.O.(1999). Industrial Pollution in: Environmental physiology of Animal and Pollution. Published by Diversified Resources Ltd, Lagos, Nigeria. 157-219

Olaitan Olatunde James, Sulola Ebenezer Oluwaleye, Adejumo E. Olufunmilayo and

Omoloye Adesina Adebiyi 2015 Cytotoxic Effects and Genotoxic Screening of Pharmaceutical Effluents using Onion Bulbs (Allium cepa L.) Journal of Advances in Biology & Biotechnology. 2(1): 51-58.

Ortolan Maria da Graça Silva; Ayub Marco Antônio Záchia. (2007) Cytotoxicity and genotoxicity of untreated hospital effluents Brazilian Archives of *Biology and Technology.* 50 (4) pp10

Paz M, Muzio H, Mendelson A, Magdaleno A, Tornello NB, Moretton J (2006) Evaluation of genotoxicity of Buenos Aires city hospital wastewater samples. J Braz Soc Ecotoxicol 1(1):1–6Preeti Gupta, N. Mathur, P. Bhatnagar, P. Nagar and S. Srivastava (2009). Genotoxicity evaluation of hospital wastewaters *Ecotoxicology and Environmental Safety* Volume 72, Issue 7, October 2009, Pages 1925-1932

Rank, J. and Nielson, M.H (1993). A modified Allium test as tool in the screening of the genotoxicity of complex mixtures. Heriditas 118: 49-53.

Ravikant, Vikrant Chitnis, S P Jaiswal, D S Chitnis, Kamlakar Vaidya, (2006). Effluent Treatment Plant: Why and How. *Journal of the Academy of Hospital Administration*, **14** (1) 1 – 6

World Health Organization (1985) – Guidelines for drinking Water Quality, Geneva Conference Records 3.