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Bacteriology and Genotoxicity Assessment of a University Wastewater

Okunola A. Alabi*[1], Jesugbamila Olowu[1], Ronand. C. Anaba[1] and Olutayo S. Shokunbi[2]

[1]*Department of Biosciences and Biotechnology, School of Science and Technology, Babcock University, Ikeja, Lagos, Nigeria*
[2]*Department of Biochemistry, Benjamin Carson School of Medicine, Babcock University, Ikeja, Lagos, Nigeria*

ABSTRACT

Higher Education Institution represents an incontestable release source of many chemical compounds in their wastewaters, and which may have an impact on the environment and human health. To study the toxicity and the risk associated with these releases, microbiological and biological tests (such as genotoxicity tests) can be used. Bacteriology of wastewater from a University in Nigeria was carried out. Genotoxicity of the wastewater using mouse sperm morphology assay and physico-chemical analysis were also carried out. Mice were given 0.5ml of 1, 5, 10, 25 and 50 % concentrations of the sample per day for five consecutive days by intraperitoneal injection. Each dose group comprised five mice, and a 5-week post-treatment period was utilized. The sample's bacterial count was 2.73 × 10^7 c.f.u./ml with evidence of faecal contamination with MPN of >1800. Organisms isolated include: Escherichia coli, Proteus vulgaris, Bacillus pumilis, Corynebacterium polisum, C. diptheriae and Enterobacter aerogenes. Physico-chemical analysis of the test sample shows that it contained constituents that are capable of inducing mutation in biological system. The data showed that the test mixtures induced a dose-dependent, statistically significant increase ($P < 0.05$) in the number of sperm with abnormal morphology. This is relevant in environmental waste management, and for the assessment of the hazardous effects of the chemicals in University wastewater.

Keywords: Genotoxicity, wastewater, faecal coliform, antibiotics, Nigeria.

INTRODUCTION

In spite of the fact that the problem of waste management is a very urgent issue for every community around the world, higher education institutions still produce large quantities of waste that is duly monitored by only few of them. In Universities, waste is generated from the following activities: office/administrative activities; laboratory experiment (which produces chemical wastes); demolition, construction and refurbishment of buildings; ground maintenance; maintenance of a transport fleet and parking facilities; catering and hotel services; on-campus residential accommodation; students' union shop; social and catering outlets etc. The waste thus generated ends up

in the environment: in water (ground water, streams, rivers, lakes, drinking water and the sea); in or on land (where soluble or particulate compounds may wash or leach into groundwater); and in the air (as vapors, dust or gases which may settle on land or sea or may dissolve in rainwater). Materials from laboratories, workshops, catering and launderettes are termed "trade effluent" and are mixed, in the university's drainage system with effluent from the residences and the toilets.

Higher education institutions represent an incontestable release source of many chemical compounds in the aquatic environment due to laboratory activities, medicinal and residential excretion into wastewater [1]. However, knowledge about the university wastewater toxicity is scarce and should be studied. Indeed, university wastewater may have an impact on the environment and human health. Water genotoxicity studies are of interest because epidemiologic investigations have shown a link between genotoxic drinking water intake and a rise in cancer [2,3,4].

There are only few studies dealing with municipal wastewater genotoxicity in eukaryotic system, and according to the literature available to us, there is no record found on the genotoxicity of university wastewater. Thus in the present study, bacteriology and genotoxicity of wastewater from a university in Nigeria was investigated. The genotoxicity of the wastewater was studied using mouse sperm morphology assay, after physico-chemical and microbiological analyses have been carried out. Sperm morphology assay provides a direct and effective way of identifying chemical agents that induce spermatogenic damage in man. Animal sperm tests, such as the mouse sperm morphology test, may be used to identify the toxic components of a complex mixture [5,6,7,8]. This test is advantageous because it is very sensitive to mammalian germ-cell mutagens and therefore identifies germ-cell mutagens [9].

MATERIALS AND METHODS

Wastewater collection

Wastewater was collected prior to disposal from a university in Nigeria. It was collected in May at the end of the academic session (comprising of two semesters of about four months each) of the university. The effluent was collected from four major discharge points from the university (which contains all the wastewater generated from all arms of the University) into the surrounding environment and then pooled together to form a composite sample. This was transported to the laboratory and kept at 4^{o}C throughout the period of this study.

Physico-chemical characterization

The wastewater was analyzed for a number of standard physical and chemical properties in accordance with standard analytic methods [10]. The constituents analysed include biochemical oxygen demand (BOD), chemical oxygen demand (COD), chloride, sulphide, ammonia, nitrate and phosphate, total dissolved solids (TDS) and conductivity. Heavy metals analysed include lead (Pb), cadmium (Cd), chromium (Cr), manganese (Mn), arsenic (As), copper Cu), zinc (Zn), iron (Fe) and nickel (Ni). Briefly, 50 ml of each of the samples was digested using 1N concentrated nitric acid. The digested samples were analyzed in duplicate, using a Buck Scientific® Flame Atomic Absorption Spectrophotometer 205.

Bacteriological study

Isolation of microorganisms

The total colony count of bacteria was done by pour plate method using nutrient agar (oxoid). 0.2ml of an appropriate dilution of the serially diluted effluent was used for inoculation of the plates in duplicate and incubated for 24-48hrs at 37^{o}C. The total colony count was determined as described by Nwachukwu [11]. Buchanan and Gibbons [12] taxonomic schemes and description were used to screen and identify the colonies at the end of the incubation.

Faecal coliform test and isolation of *E. coli*

Detection of faecal coliforms and determination of most probable number (MPN) of coliform bacilli was carried out as described by Fawole et al. [13] and Bakare et al. [14]. 0.1, 1 and 10ml of each sample were used to inoculate the lactose broth in five replicates. Tubes were incubated at 37^{o}C for 48hrs and the MPN was determined according to standard methods [10]. Production of gas and acid was taken as positive indication for the detection of faecal coliform bacteria. MacConkey broth was used to culture tubes showing positive results and was incubated at 37^{o}C

for 48hrs before it was plated on Eosine Methylene Blue (EMB) agar and incubated. Colonies grown on EMB plates were selected and finally identified on the basis of morphological, cultural and biochemical characteristics for the isolation of *E. coli*.

Antibiotic sensitivity test
Disc diffusion method was used to test for the antibiotic sensitivity of the bacterial isolates as described by Prescott et al. [14]. The plates were incubated at 37^{o}C for 48hrs after which zones of inhibition were examined according to Chortyk et al. [16].

Laboratory animals
Male swiss albino mice (26-31g) obtained from the Nigeria institute of Medical research (NIMR), Lagos, Nigeria was used for this study. The mice were 12-14 weeks old. They were quarantined in a pathogen-free, well ventilated room to enable them acclimatize to the environment for about 2 weeks. Only mice of ≥14 weeks were treated and tested. Supply of food and water was uninterrupted. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Ethic regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments [17].

Sperm morphology assay
Single intraperitoneal (IP) injection of 0.5ml of each concentration of the test effluent was administered daily for five consecutive days. Five (5) concentrations of 1, 5, 10, 25 and 50 % of the effluent sample together with positive (cyclophosphamide) and negative (distilled water) controls were considered. Five mice were treated for each concentration and a 5 week post-treatment period was considered. This is because spermatogenesis in mice takes about 35days to complete [18]. At 5weeks from the first injection, the mice were sacrificed by cervical dislocation and their caudal epididymes were surgically removed. Sperm smears were prepared from the epididymes as previously described [19,20]. For each mouse, 800 sperm cells were assessed for morphological abnormalities of the sperm cell according to the criteria of Wyrobek and Bruce [5].

Statistical analysis
The difference between the negative control, positive control and the individual dose groups were analyzed by means of the two-tailed student's t-test of significance at the $P<0.05$ level and Dunnett's t-test. The results were reported as mean ± standard error.

RESULTS

Table 1 shows the physico-chemical parameters determined in the university wastewater. The pH value was slightly acidic (5.67). The values obtained for some of the parameters in the effluent were much higher compared with the maximum allowable levels in water by standard organizations [21,22,23]. Heavy metals such as Zn, Cu, Mn, Al, Fe, Ni, Cd and Cr were present in the wastewater in significant concentrations.

The effluent contained large number of bacteria (2.73×10^7 c.f.u/ml), and coliform bacilli with high MPN (1800) as set by coliform index [24]. Bacteria isolated in the wastewater include strains of *Escherichia coli*, *Proteus vulgaris*, *Bacillus pumilis*, *Corynebacterium polisum*, *C. diphtheria*, *Acinetobacter anitratus* and *Enterobacter aerogenes*. The presence of *E. coli* in the tubes indicates a faecal contamination of the effluent. The antibiotic sensitivity test was conducted on three strains of three of the bacteria (which are the most notorious in the vicinity under investigation) to ascertain the level of resistance. Table 2 shows the resistance of bacterial isolates against individual antibiotics. There is a high level of antibiotic resistance with *P. vulgaris* being resistant to the highest number of antibiotics.

Analysis of sperm-head abnormalities was made at 5 weeks after first injection of the effluent. Table 3 shows the effect of the different concentrations of the wastewater on the sperm morphology. Negative control showed 2.70% abnormality while the positive control induced 28.42% abnormal sperm cells, which is a statistically significant ($p<0.05$) increase in abnormal sperm heads as compared to the negative control. The 1, 5, 10, 25 and 50 % concentrations of the effluent induced 5.8, 8.0, 18.8, 28.1 and 30.06 % abnormalities respectively. The induction of

morphological aberration in sperm morphology by the wastewater was concentration-dependent and statistically significant ($p<0.05$) at all concentrations. Abnormal sperm morphology such as, sperm with no hook, knobbed hook, folded sperm cells, amorphous head and pin head were among the observed aberrations in the exposed mice (Fig. 1).

DISCUSSION

The indiscriminate disposal of wastewater into the environment in developing countries makes the generation of large quantities of university wastewater of major health concern. In this study, we examined the bacteriological and genotoxic potential of a university wastewater in Nigeria, along with the physico-chemical and heavy metal analysis. The effluent contained large number of bacteria (2.73×10^7 c.f.u/ml). Organisms isolated from the effluent have been associated with varieties of diseases in man. *E. coli* causes diarrhea, urinary tract and kidney infections and peritonitis septiceamia. *Acinetobacter anitratus* causes meningitis, peritonitis and sinusitis [25,26]. *Proteus vulgaris* has been known to cause urinary tract and wound infections while *Corynebacterium diphtheria* causes diphtheria. The isolation of these organisms is of great concern because this domestic wastewater was collected at the point of discharge into a nearby river, which may not only serve as a source of drinking water to the immediate community but also as a source of food (i.e through fishing). The result of the sensitivity test among the isolates showed a high level of antibiotic resistance, with *P. vulgaris* being resistant to the highest number of antibiotics. Previous work on bacterial resistance to antibiotics has suggested that high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment [27,28,29,30]. This is very possible in universities, where abuse of drug is on the increase. In developing countries, drugs are available to the public on the counter and this has encouraged self-medication that has further increased the prevalence of drug-resistant strains.

The induction of morphological aberration in sperm morphology by the wastewater was seen to be concentration-dependent and statistically significant ($p<0.05$) at all concentrations. No specific abnormality occurred significantly higher than others in the observed result. The observed abnormalities indicate that the effluent constituents had an effect on sperm which had arisen in treated spermatogonial cells. They might have caused damages to the pre-meiotic stages of spermatogenesis, since DNA synthesis occurs before the pre-meiotic phase and no further DNA synthesis occurs throughout spermatogenesis in the cell cycle [31,32]. Moreover, the nuclei of the mammalian gamete resulting from spermiogenesis are usually very homogenous, normally stable and have a marked strain-specific structural definition. Therefore, any abnormalities observed in the sperm heads presumably occurred during spermatogenesis since once the sperm head develops its shape, it's extremely stable. The test sample may therefore be active in inducing sperm-head abnormalities and may be genotoxic. This conclusion is reached because the criteria for a positive response were satisfied, there was an evidence of a concentration-dependent increase in the number of aberrant sperm cells and there was an increase in abnormal sperm morphology which is at least double the negative control in all the treatment levels.

Most of the constituents are known toxicants. Heavy metal analysis of the effluent showed the presence of Zn, Cu, Mn, Al, Fe, Ni, Cd and Cr at various concentrations (Table 1). Individually some of the constituent elements are known mutagens and carcinogens. The observed genotoxic effect of the effluent might have also been as a result of a synergistic reaction of the chemical combination of these metals which might be more destructive than the individual effect. Studies have shown that Cd, Cu and Fe induced reactive oxygen species in eukaryotic systems [33,34]. Exposure of mice to Zn results in single strand breaks in DNA as measured by the comet assay [35]. Ni is known to produce highly selective damage to heterochromatin [36]. Wise et al. [37] had also reported that hexavalent Cr induced chromosomal aberrations, micronuclei and single strand breaks in mammalian cells. Heavy metals have the potential to induce mutations and cancer in living cells [38]. Cd, Cu, As and Fe produce free radicals and when present in an unbound form, it produces reactive oxygen species (ROS) that can cause DNA, protein and lipid damage [34,39,40].

This report is in accordance with previous reports on genotoxic hazards of domestic wastes [14,41,42]. Currently, there is only a small database on the use of mammalian cell assays with complex mixtures. This report however is one of the few reports on the use of a mammalian *in vivo* assay to evaluate the possible genotoxic effect of municipal effluents.

Table 1: Result of the physico-chemical analysis of a university wastewater

Parameters*	Effluent sample	FEPA[a]	USEPA[b]
pH	5.67	6-9	6.5-8.5
COD[c]	130. 04	50	410
BOD[d]	55.13	50	-
TDS[e]	570	2000	500
Salinity	410	-	-
Alkalinity	37	250	20
Hardness	90	-	0.75
Chloride	1320	-	250
Nitrate	42.3	20	10
Phosphate	10.0	5.0	-
NH_3	0.05	0.01	0.02
Cr	0.02	0.05	0.10
Cu	2.22	0.01	1.0
Fe	5.45	0.3	0.30
Mn	0.26	0.05	0.05
Ni	0.01	0.05	-
Cd	0.12	0.01	0.005
Zn	23.10	5.0	5.0

**All values are in mg/L except pH and salinity (ppt.) [a]FEPA: Federal Environmental Protection Agency (2001).*
[b]USEPA: United States Environmental Protection Agency (1989).
[c]COD: Chemical oxygen demand.
[d]BOD: Biochemical oxygen demand.
[e]TDS: Total dissolved

Table 2: Resistance of bacterial isolates against individual antibiotics.[a]

	Bacterial isolates		
[a]Antibiotics	Ec	Pv	Bp
Amx	4(80)	ND	5(100)
Aug	4(80)	2(40)	4(80)
Ofl	5(100)	1(20)	3(60)
Tet	1(20)	2(40)	2(40)
Nal	3(60)	ND	3(60)
Gen	ND	ND	ND
Cot	1(20)	4(80)	ND
Nit	2(40)	4(80)	4(80

[a] No of resistance isolates, (%) in parenthesis; ND, not detected; Ec (E. coli), Pv (P. vulgaris) and Bp (B. pumilis)

Table 3: Summary of morphologically abnormal sperm-head induced by different concentrations of university effluent in mice after 5 weeks exposure

Concentrations (%)	Number of Animals used	Number of Sperms counted	Number of abnormal sperms	% Frequency of abnormality
Distilled water	7	4000	108	2.70
1	7	4000	232	5.80
5	7	4000	320	8.00
10	7	4000	752	18.80
25	7	4000	1124	28.10
50	7	4000	1202	30.06
Cyclophosphamide (20mg/kgbwt)	7	4000	1137	28.42

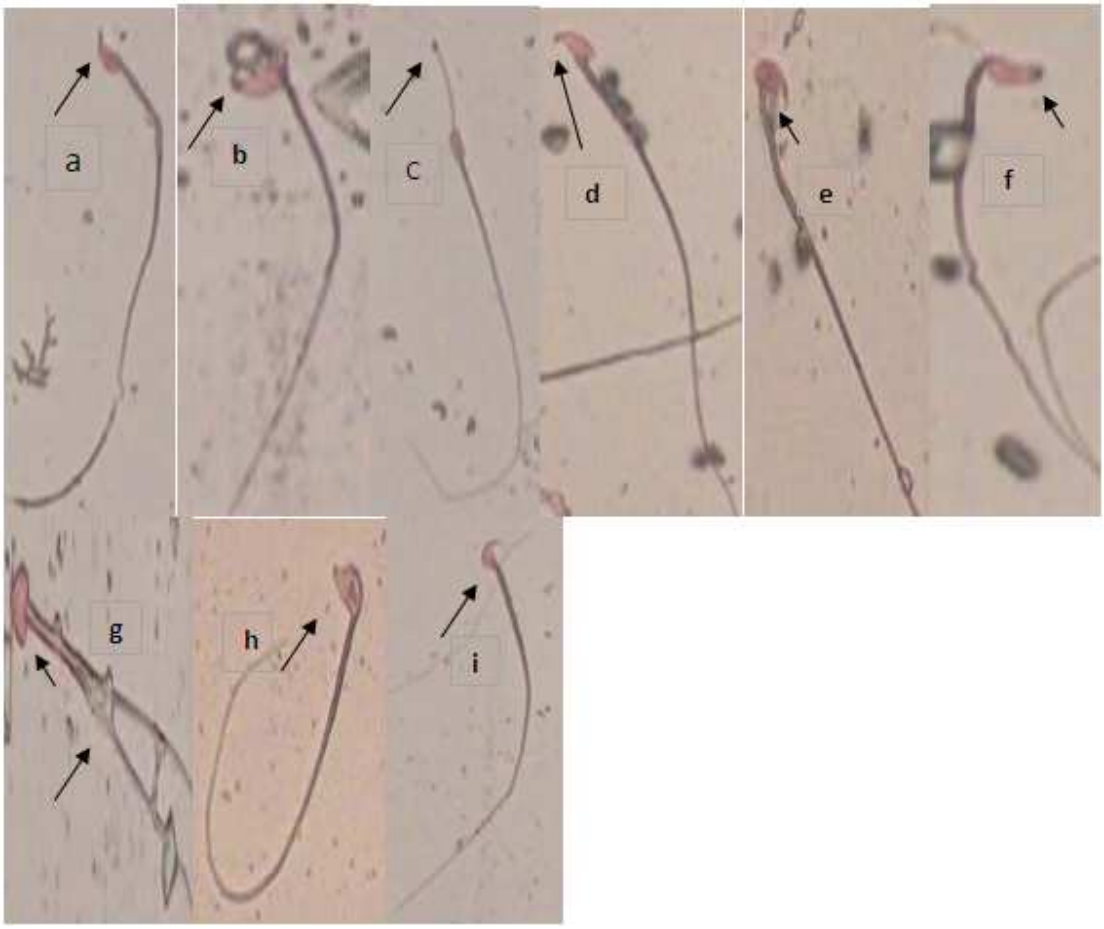

Fig. 1. Abnormal sperm cells induced in mice exposed to different concentrations of the University effluent (a) hook at wrong angle, (b) folded sperm, (c) pin head, (d) very short hook, (e) wrong tail attachment, (f) No hook, (g) double tailed sperm with amorphous head, (h) amorphous head (i) normal sperm cell. Magnification 800x.

CONCLUSION

Our findings may be significant for Nigeria and other countries where large volumes of wastes are generated from higher institutions with very few universities efficiently monitoring their wastes. It should be noted that waste generated from most universities in Nigeria ends up in the environment and most especially in water bodies (groundwater, stream, rivers, lakes and sea). The result of this study showed that there are substances in the test effluent that are capable of inducing genotoxic effects in mouse germ cells. This is relevant to human health because the toxicological target is DNA, which exists in all cellular forms [43].

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