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Research Article

Genetic and systemic toxicity induced in *Allium cepa* and *Mus musculus* by a tropical freshwater dam in Ibadan, Nigeria

Adekunle A. Bakare[1]*, Bernard O. Uzoibiam[2], Ayodeji O. Oteyola[1], Opeoluwa M. Fadoju[1], Olusegun I. Ogunsuyi[1], Adeyinka M. Gbadebo[2], Omonike C. Bakare[3] and Chibuisi G. Alimba[1,4]

[1]*Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria*
[2]*Environmental Biology Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Biological Sciences, Lead City University, Ibadan, Nigeria*
[4]*Leibniz Research Centre for Working Environment and Human Factors (IfADo), Technical University of Dortmund, 44139 Dortmund, Germany*

ABSTRACT

Awba Dam reservoir is an important source of domestic water supply in the University of Ibadan, Nigeria. It receives both point and non-point sources of untreated sewage and wastewaters from student and staff residences, research laboratories and surrounding farmlands. Toxicological evidence on the pollution status of the dam are on the increase. In this study, we investigated the cytotoxic and genotoxic effects of Awba Dam water using the *Allium cepa* and murine sperm morphology assays. We also evaluated systemic toxicity in mice using alterations in haematological, serum hepatic biochemical and histopathological parameters. Bi-monthly composite water samples were collected from the Dam from June – September, 2017. Each sample was used in the *A. cepa* root growth inhibition and chromosome aberration test, and was administered as drinking water to groups of male Swiss albino mice for 35, 70 and 105 days. Tap water from underground source was used as negative control. There was significant ($p<0.05$) mitotic inhibition and induction of different types of chromosome aberrations in *A. cepa*. At the exposure periods in mice, there was significant ($p<0.05$) increase in frequencies of abnormal sperm cells, alterations in haematological parameters and significant ($p<0.05$) increase in the levels of alanine aminotransferase and aspartate aminotransferase when compared with the corresponding negative controls. Histopathological alterations, including degeneration, congestions and inflammations were observed in sections of liver and kidney. Pb, Cd, Ni and Fe in the water samples contributed to the observed toxicity. Awba Dam water contains constituents that induced genetic and systemic damage in mice and *A. cepa*. This is of public health importance to resident biota, and other organisms including man along the food chain.

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*** E-mail:** adebakar19@yahoo.com, adekunle.bakare@ui.edu.ng

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1. INTRODUCTION

The existence of living organisms requires water. This valued resource is increasingly being threatened by pollution from diverse anthropogenic activities and human population growth [1]. Water pollution is a major global problem which requires continuous evaluation and revision of water resource policy at all levels. It has been suggested that water pollution is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily [2]. Vörösmarty et al. [3] reported that 80% of the world's population is exposed to high level of threat due to water insecurity.

In many African countries, a considerable population growth has taken place in recent years, accompanied by a steep increase in urbanization, industrial and agricultural land use. This has led to tremendous increase in discharge of a wide diversity of pollutants into water bodies. Heavy metals, dyes, pharmaceuticals, pesticides, fluoride, phenols, insecticides and detergents are some of the contaminants entering these water bodies; and causing undesirable effects on the different components of the aquatic environment and on fisheries [4].

In Nigeria, like many other developing nations, environmental laws are poorly enforced and the rapid industrial development, agricultural activities, urbanization among others, have resulted in the discharge of a wide range of environmental contaminants into the aquatic ecosystem. Hence, high concentrations of environmental contaminants such as phthalates [5,6], PCBs [7], PAHs [8], toxic metals [9] and PBDEs [10] had been reported in inland waters in Nigeria.

Awba dam reservoir in the University of Ibadan, a small man-made lake with a storage capacity of 227 million liters of water and a treatment rate of 68 thousand liters per day [11], was constructed to serve as reservoir for domestic water supply to the University community. When newly dammed in the 70's, Awba Reservoir had a very high diverse and abundant ecosystem comprising of microscopic and macroscopic flora and fauna [11,12]. However, the water quality and diverse biodiversity of Awba dam have degraded over time due to high levels of contaminants. For instance, Adeogun and Fafioye [13] reported that the quality and quantity of benthic macroinvertebrates had reduced when compared with previous reports from the reservoir. Tyokumbur and Okorie [14] similarly observed a decrease in species richness of planktons in one station (receiving sewage effluent discharge from the University hostels) compared to another station (not receiving effluent). Anago et al. [9] investigated the plankton richness of the reservoir, and observed thirty six taxa of planktons with the presence of pollution indicator species such as *Microcystis, Phacus, Oscillatoria, Surirella, Closterium, Aphanocapsa, Anabaena* and *Euglena* suggesting that the reservoir was polluted. Recently, Adeogun et al. [15] observed the occurrence of intersex with elevated expressions of vitellogenin and zona radiata protein in 34.8% of male Tilapia species examined from the Dam. This observation correlated with significant high concentrations of As, Cd, Pb, Hg and Ni, monobutyltin cation, 4-iso-nonylphenol and PCB congeners (138, 153 and 180) (endocrine disruptors) determined in sediments collected from the reservoir.

Considering that the reservoir is currently in use as a source of water supply for domestic and research purposes in the University community, there is need to examine the potential toxicity of water from the reservoir in eukaryotic systems using plant and animal models. Thus far, there is no information on the cytotoxicity and genotoxicity assessments of water from Awba Dam. Higher plants have been extensively used as test organisms for the detection of genotoxic substances in the environment. *Allium cepa* is one of the plants which has been used in different studies; it is an efficient test organism for environmental monitoring, especially in contaminated aquatic environments [16,17,18]. Similarly, the murine sperm morphology assay provides a direct measure of the quality of sperm produced in chemically treated animals. It is a very sensitive test to detect mammalian germ cell mutagens [19,20]. Hence, in this study, we used the *Allium cepa* chromosome aberration and murine sperm morphology assays to evaluate the potential somatic and germ line cytogenotoxicity of water from Awba Dam reservoir. We also investigated systemic toxicity induced by the water in mice for adequate understanding of the possible mechanisms of DNA and organ toxicity.

2. MATERIALS AND METHODS

2.1 Study site

Awba Dam reservoir is an enclosed watershed within the University of Ibadan, Southwestern Nigeria at an altitude of 185 m above sea level and lies within Latitude 7^o 26' to 7^o 28' N and Longitude 3^o 35' to 3^o 54' E (Figure 1). The dam was constructed in 1964 by damming Awba stream at a point where it flowed through a natural valley and expanded to its present size in 1971. It is a small man-made lake with a surface area of 0.06 km^2, a maximum depth of 5.5 m, a storage capacity of 227 million liters of water and a treatment rate of 68 thousand liters per day [11]. The dam presently serves as the second source of domestic water supply to the water treatment plant for the University community. It receives untreated effluents from staff and students' residences, zoological garden, experimental wastewater from Faculties of Science and Technology laboratories and from non-point sources due to erosion, and leached chemicals from the surrounding farmlands [15].

2.2 Water sample collection and heavy metal analysis

Eleven sampling points were randomly chosen within the reservoir to give a comprehensive representation of the physical and chemical condition of the whole dam. Water samples were collected from the selected sample sites on the reservoir every two weeks (twice in a month) into a pre-cleaned 25 L plastic container to make a composite sample. The samples were collected in the morning at 7 – 8 am with the aid of a canoe and the sampling was carried out from 6th of June to 18th of September, 2017 (samples were tagged as - week 1, 3, 5, 7, 9, 11, 14 and 16). The first composite water sample collected each month along with the control sample (tap water from the Department of Zoology, University of Ibadan, Nigeria), were analysed for selected heavy metals: cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), nickel (Ni) and zinc (Zn) in accordance with APHA [21] method using Perkin Elmer Atomic Absorption Spectrophotometer (AAS-200).

2.3 Biological materials

Onion (*Allium cepa*; 2n = 16) and male Swiss albino mice (*Mus musculus*) were utilised. Onion bulbs of equal-size obtained commercially at Bodija Market, Ibadan, Nigeria were used for this study. About four times the total number of bulbs needed for the experiment was acquired and sun-dried for 2 weeks before the commencement of the experiment. This served to replace any bulb that may dry up, rot or be damaged by mould [16]. These were then used to evaluate the cytogenotoxic potentials of the Awba Dam water samples using root growth inhibition and induction of chromosomal aberration as the assay end points.

Figure 1. Location of the sampling site; Awba Dam reservoir in the University of Ibadan, Nigeria [15].

Male mice (9 - 10 weeks old) obtained from the animal breeding unit of the Department of Physiology were acclimatized for three weeks in the animal house of the Department of Zoology, until they attain the age of 12 - 13 weeks old. They were housed in plastic cages, and given food (Ladokun pelleted feed®) and Awba Dam water as their drinking water (during the experiment) *ad libitum*. They were cared for according to standard guidelines [22]. Ethical approval was obtained from the Animal Care and Use in Research Ethics Committee of the University of Ibadan (UI-ACUREC/17/0078).

2.4 *Allium cepa* assay

Twelve onion bulbs were grown on each bimonthly water samples. The outer dry, brown scales of the bulbs and the bottom plates (dead roots) were carefully removed, leaving the ring of the primordial roots intact. They were put in tap water for cleaning and to prevent the primordial roots from drying up. These bulbs were later placed directly on the sample in 100 mL beakers at 27±2° C in the dark. Bulbs grown in underground borehole water served as the negative control while those grown in 10 ppm lead nitrate served as the positive control. The experiment was performed as a semi-static exposure test and the test solutions were replaced every 24 hours with fresh samples [23,24].

Cytogenotoxicity assay: At 48 hours of exposure, two onion bulbs were harvested; 1 – 2 cm to the root tip of each root on each bulb was cut and fixed in methanol:glacial acetic acid (3:1, v/v) for 24 hours for analysis of chromosome aberration. The roots were hydrolysed with 1N HCl at 60°C for 5 minutes. They were then rinsed in distilled water three times. Two roots were used for each slide preparation. They were teased on glass slides and stained with acetocarmine for 10 minutes. Excess stain was removed with filter paper, a cover slip carefully lowered onto each slide and tapped gently to exclude air bubbles. The cover slip was sealed on the slide with finger nail polish [25]. Six slides were prepared for each sample and control, out of which four were used for microscopic analysis (1000 cells were scored per slide) at ×1000 with oil immersion. The occurrence and frequency of aberrant cells were examined in all the stages of cell division and percentage aberrations were determined relative to the total number of dividing cells. The mitotic index (MI) was determined by counting the number of dividing cells per test sample and the controls relative to the total number of cells scored.

Root growth inhibition test: At 72 hours, the length of the roots of 10 bulbs from the test samples and controls were measured (in cm) with a ruler. From the average root lengths of onion grown on test samples and the control, the percentage root growth inhibition in relation to the negative control was determined [16], and the EC_{50} was also estimated. The effect of each bimonthly samples on the morphology of growing roots was examined.

2.5 Sperm morphology assay in mice

Thirty six (36) male Swiss albino mice (12 – 13 weeks old) were divided into nine groups: 4 mice in each of the negative control (underground water), the experimental groups, and the positive control (Cyclophosphamide; 20 mg/kg body weight IP). Each of the experimental groups was exposed via drinking of Awba Dam water for 35, 70 and 105 days (that is 5, 10 and 15 weeks respectively) with their corresponding negative and positive controls. Induction of sperm abnormalities was done in accordance with standard procedures [19,20,26]. Spermatogenesis in mice lasts for 34.5 days [27], hence, the likelihood of newly formed spermatozoa being exposed to the water sample. The mice were sacrificed by cervical dislocation at the end of 5th, 10th and 15th weeks and caudal epididymis were surgically excised from the exposed animals, minced in a mixture of normal saline and 1% Eosin stain (9:1 v/v) for 45 minutes. Thereafter, slides were prepared, air-dried and scored microscopically at x1000 (with oil immersion) for sperm morphological abnormalities according to Wyrobek and Bruce [28] and Bakare *et al.* [20]. Six slides were prepared from each mouse and four of the slides at 250 sperm cells/slide were scored.

2.6 Analysis of haematological parameters and serum clinical biochemicals

At post exposure, blood was collected through the retro orbital sinus in mice using the micro-haematocrit capillary tubes into the plane and EDTA bottles respectively. Blood collected into the EDTA bottles were immediately used to determine the level of haematological parameters such as red blood cells (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), platelet count (PLT) and WBC differentials [lymphocytes (LYM), neutrophils (NEUT), monocyte (MON) and eosinophils (EOS) [29].

Blood collected into the plane bottles were centrifuged at 3000 rpm for 10 minutes and serum collected for biochemical analysis. Hepatocellular injury markers: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed in the serum according to the methods of Reitman and Frankel (1957) using Randox diagnostic kit (Randox Laboratories Ltd, County Antrim, United Kingdom). All assays were done in duplicates.

2.7 Organ indices and histopathology

The liver, kidney and testes of the exposed mice were excised, rinsed in normal saline to remove blood clots and blotted dry with Whatman filter paper. Portions of the liver, kidney and testes were fixed and analyzed for histopathological lesions according to Alimba et al. [30].

2.8 Statistical analysis

Data were presented as mean ± SE. Graphpad Prism 5 and Microsoft Excel® 2013 packages were used to analyse the data. The percentage root growth relative to the negative control was determined. Student's t-test was used to analyse the difference in the frequencies of chromosomal aberrations (CA) and mitotic index (MI) in the exposed and control samples. Differences in the Frequency (%) of sperm abnormalities, haematological parameters, organ indices, serum biochemical tests were analyzed using One-way ANOVA with Dunnett post-hoc multiple Test at $p < 0.05$.

3. RESULTS

3.1 Physical characteristics and heavy metal analysis of Awba Dam water

Heavy metal analysis of the water samples and negative control are presented in table 1. All the analyzed heavy metals were below national and international (NESREA, SON and USEPA) permissible limit for drinking water quality; except for cadmium (in June), lead (in September), nickel (from June to August) and iron (in September) that were higher than the permissible limits.

3.2 Macroscopic effect of Awba Dam water samples on root growth in *Allium cepa*

Table 2 shows the macroscopic effects of ADW on the root growth of *A. cepa*. The negative control roots were straight, whitish in colour and displayed good growth. The roots of bulbs grown on Awba Dam water samples showed diverse morphologies as compared to the negative control; some were long and scattered, while others were short and scanty; and they all appeared milky-white in colour. Root growth was maximum in the control (100%). The samples of weeks 1 and 5 caused more root growth than in the negative control. Compared to the negative control, there was a significant poor root growth on ADW samples of weeks 7, 9, 11, and 16. The EC_{50} was however indeterminate.

Table 1. Heavy metals analyzed in Awba Dam water collected from June – September, 2017

S/N	Heavy Metals (mg/L)	NC (range)	Awba Dam				USEPA	NESREA	SON
			June	July	August	September			
1	Cadmium	ND – 0.03	0.01	0	0	0	0.005	0.2	0.003
2	Lead	ND – 0.23	0	0	0.02	0.29	0.015	0.05	0.01
3	Copper	ND	0	0	0	0	1.3	0.05	1
4	Nickel	ND – 0.07	0.05	0.06	0.06	0	-	-	0.02
5	Zinc	ND – 0.07	0.05	0.07	0	0.03	5	-	3
6	Iron	ND – 0.684	0.005	0.014	0.039	0.578	0.3	-	0.3

ND – Not detected, NC – Negative Control (underground water)
USEPA [73] – US Environmental Protection Agency.
http://water.epa.gov/drink/contaminants/index.cfm#List
NESREA – National Environmental Standards and Regulation Enforcement Agency [74]. SON – Standard Organisation of Nigeria [75]. Nigeria Standard for Drinking Water Quality.

Table 2. Summary of root growth inhibitory effect of Awba Dam water samples in *Allium cepa*

Month of sampling	Samples	Mean root length (cm)	SD	Growth in % of negative control	% root growth inhibition
June	NC	4.041	0.74		
	W1	4.103	0.53	101.5	-1.5
	NC	4.682	0.69		
	W3	4.321	0.36	92.29	7.71
July	NC	4.370	0.35		
	W5	4.483	0.70	102.59	-2.59
	NC	4.859	0.84		
	W7	3.963**	0.66	81.56	18.44
August	NC	4.903	0.79		
	W9	3.895**	0.75	79.44	20.56
	NC	4.776	0.47		
	W11	4.230*	0.65	88.57	11.43
September	NC	4.879	0.26		
	W14	4.388	0.26	89.94	10.06
	NC	5.111	0.64		
	W16	3.924**	1.08	76.78	23.22
	PC	0.992***	0.26	21.09	78.9

** $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$: Significantly different from the negative control (NC)*
PC – positive control.

3.3 Mitotic inhibition and chromosomal aberration observed in *A. cepa*

The water samples of weeks 9 and 16 caused a significant (p<0.05) decrease in mitotic index in cells of onion root tips, while the rest were not significantly (p>0.05) different from the negative control (Table 3). There were more dividing cells in root tips of onion grown on weeks 1 and 3 AD water samples. All the water samples induced significant (p<0.05) increase in chromosomal aberrations (Table 3). The positive control showed significant (p<0.05) decrease in mitotic index and induced significant (p<0.05) increase in chromosomal aberrations as compared to the negative control. The aberrations observed are sticky chromosomes, disturbed spindle, distributed metaphase, anaphase bridges, polar deviation, non-disjunction at anaphase, lag/vagrant chromosomes, C-mitosis and binucleate (Figure 2).

3.4 Abnormal sperm morphology assay in mice

The tested water samples induced time dependent significant (p<0.05) increase in abnormal sperm morphology of 11.18, 13.43 and 33.97 % compared to the negative control (1.75, 3.3 and 4.5%) at the 35, 70 and 105 days exposure period, respectively (Table 4). The observed abnormalities include knobbed, wrong tail attachment, short hook, no hook, long hook, double head, two tails, three tails, folded and banana head (Figure 3). Knobbed-head sperm cell was the most predominant while sperm cell

Table 3. Mitotic inhibition and chromosomal Aberrations (CA) observed in *A. cepa* exposed to Awba Dam water.

Sample	No of Dividing Cells	Mitotic Index	Mitotic Inhibition	Prophase	Metaphase	Anaphase	Telophase	Total Aberrant Cells	Frequency of Aberrant Cells (%) based on Total Cells Scored (4000)	Frequency of Aberrant Cells (%) based on No of Dividing Cells
NC(Wk1)	534	13.35	0	277	90	98	69	30	0.75	5.618
AD(Wk1)	620	15.5	-16.11	312	88	120	100	153	3.825	24.68*
NC(Wk3)	608	15.2	0	315	114	66	113	40	1	6.579
AD(Wk3)	666	16.65	-9.54	321	142	88	115	194	4.85	29.13*
NC(Wk 5)	653	16.33	0	359	103	86	105	36	0.9	5.51
AD(Wk 5)	537	13.43	17.76	283	73	77	104	125	3.125	23.28*
NC(Wk7)	515	12.88	0	264	76	62	113	19	0.475	3.69
AD(Wk7)	447	11.18	13.20	244	63	81	59	113	2.825	25.28*
NC(Wk9)	468	11.7	0	259	49	74	86	9	0.225	1.92
AD(Wk9)	289	7.23	38.25*	117	72	44	56	105	2.625	36.33***
NC(Wk11)	448	11.2	0	234	61	64	89	6	0.15	1.34
AD(Wk11)	388	9.7	13.39	170	71	81	66	114	2.85	29.38**
NC(Wk14)	457	11.43	0	241	64	72	80	6	0.15	1.31
AD(Wk14)	415	10.38	9.19	174	98	60	83	138	3.45	33.25**
NC(Wk16)	600	15	0	367	88	66	79	8	0.2	1.33
AD(Wk16)	374	9.35	37.67*	185	65	68	56	103	2.575	27.54**
PC	340	8.5	43.33**	158	81	51	50	111	2.775	32.65**

NC – Negative control, AD - Awba Dam, PC – Positive control (10 ppm lead nitrate)
*Values are significantly different from negative control at * = p<0.05 and ** = p<0.01.*

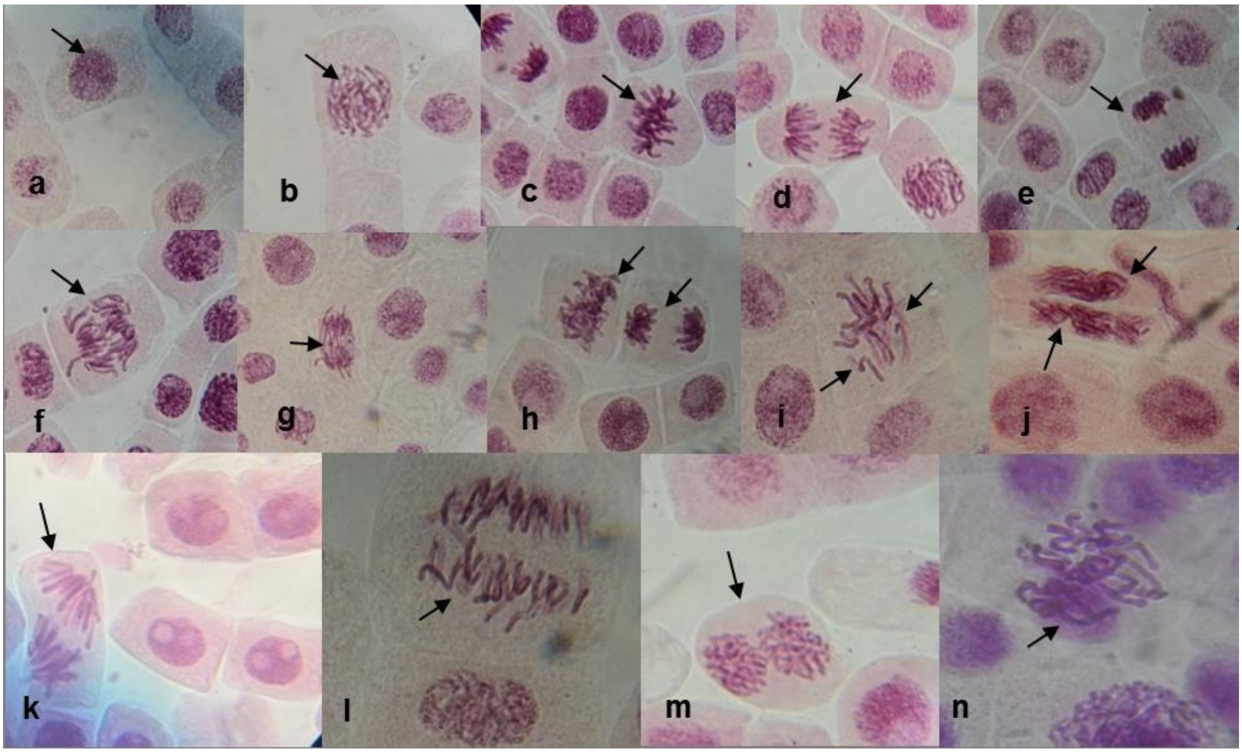

Figure 2. Chromosomal aberrations observed in *Allium cepa* root tip cells exposed to Awba dam water (a–e) Normal cells at Interphase (a), prophase (b), metaphase (c), anaphase (d) and telophase (e); (f, g) Bridges (f) and non-disjunction at anaphase (g); (h) stickiness at metaphase and anaphase; (I, j) Spindle disturbance at metaphase; vagrant chromosomes (i), and Twisted chromosomes (j); (k) polar deviations at anaphase; (l) spindle disturbance at anaphase; (m) binucleate and (n) C-mitosis. (×1000).

Table 4. Summary of the different types of morphological aberrations observed in the sperm of mice exposed to Awba Dam water sample for 35, 70 and 105 days

	Knob bed	Wrong tail attachment	Short hook	No hook	Amorphous head	Long hook	Double head	Double tail	Triple tail	Folded	Banana	No of sperm counted	Total aberration & %Freq.
35-day exposure													
NC	16	0	14	16	15	9	0	0	0	0	0	4000	70
% occurrence	22.9	0	20	22.9	21.4	12.9	0	0	0	0	0		1.75
ADW sample	159	0	16	70	170	15	3	5	1	4	4	4000	447
% occurrence	35.6	0	3.6	15.7	38.0	3.4	0.7	1.1	0.2	0.90	0.90		11.18***
PC	324	198	192	72	246	18	5	5	0	7	34	4000	1101
% occurrence	29.4	18.0	17.4	6.5	22.3	1.6	0.5	0.5	0	0.6	3.1		36.70***
70-day exposure													
NC	38	23	37	13	13	3	0	0	0	0	5	4000	132
% occurrence	24.2	17.2	28.0	9.8	9.8	2.3	0	0	0	0	3.8		3.30
ADW sample	161	50	29	23	77	23	3	7	0	1	29	4000	403
% occurrence	40.0	12.4	7.2	5.7	19.1	5.7	0.7	1.7	0	0.2	7.1		13.43**
PC	254	106	158	112	159	14	7	3	0	4	35	4000	852
% occurrence	29.8	12.4	18.5	13.1	18.7	1.6	0.8	0.4	0	0.5	4.1		42.6***
105-day exposure													
NC	38	36	33	11	24	6	0	1	0	1	28	4000	178
% occurrence	21.3	20.2	18.5	6.2	13.5	3.4	0	0.6	0	0.6	15.7		4.45
ADW sample	302	104	113	66	255	44	14	14	0	10	97	4000	1019
% occurrence	29.6	10.2	11.1	6.5	25.0	4.3	1.4	1.4	0	1.0	9.5		33.97*
PC	302	264	244	40	301	50	63	8	0	24	100	4000	1396
% occurrence	21.6	18.9	17.5	2.9	21.6	3.6	4.5	0.6	0	1.7	7.2		46.53**

*NC – negative control (underground water), ADW – Awba Dam water, PC – positive control (20 mg/kg bw cyclophosphamide). Values are significantly different from negative control at * = P<0.05; ** = P<0.01; *** = P<0.001*

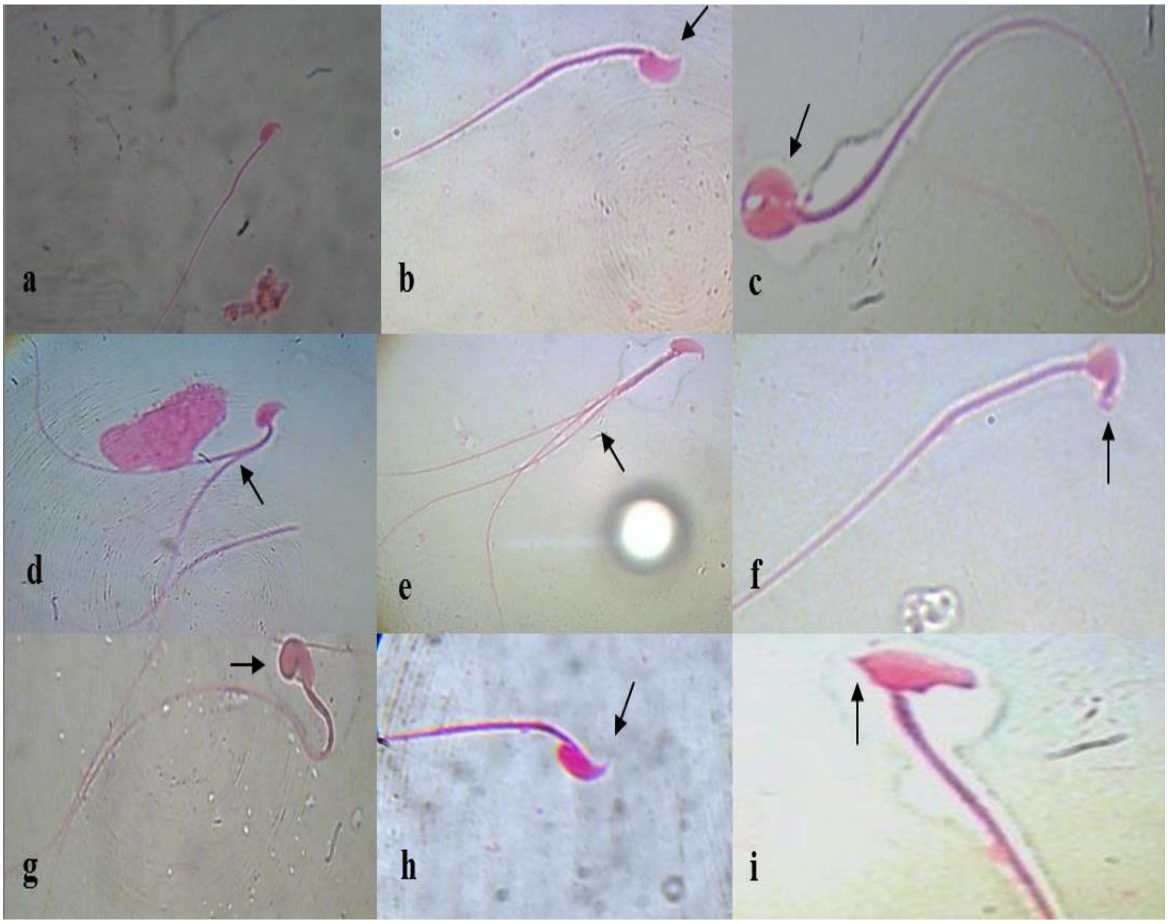

Figure 3. Abnormal sperm cells induced in mice exposed to ADW sample. (a) normal sperm cells (b) no hook (c) two heads (d) two tails (e) three tails (f) knobbed head (g) amorphous head (h) banana head (i) wrong tail attachment, x1000.

with triple tails was the least. The positive control also significantly induced 36.70, 42.60 and 46.53 % sperm abnormalities respectively.

3.5 Haematological parameters

Mice exposure to the tested water sample showed increase in the PCV, Hb and RBC at the exposure periods: 35-day and 70-day, but a reduction in these parameters was observed in the 105-day of exposure (Table 5). The WBC and platelet counts decreased according to exposure period, however only platelet count was significantly different from the corresponding negative control only at the 105-day exposure period. Lymphocyte count increased only at the 105-day exposure period. Neutrophils count increase during all exposure period, while MCH, MCHC and MCH decreased.

3.6 Serum biochemical analysis

The water samples induced 1.8-fold and 1.5-fold significant ($p<0.001$) increase in the AST activities in mice serum after 35 and 70 days exposure periods respectively, and 1.2-fold decrease at the 105-day exposure period (Figure 4). The samples also induced 1.6-fold increase, 1.1 fold significant ($p<0.05$) decrease and 4.0 fold significant ($p<0.001$) increase in serum ALT levels at the three exposure periods respectively (Figure 5).

3.7 Histopathological assessment of the kidney, liver and testes

Microscopic examination of the kidney, liver and testis from the negative control group showed the normal cellular architecture of the nephrons, hepatocytes and seminiferous tubules respectively, while in ADW water treated groups at the exposure periods, there were disruption of these cellular architecture (Figure 6).

Histopathological lesions observed in the nephrons and hepatocytes include foci of cloudy swelling (oedema), degeneration of tubular epithelial cells, mild congestion of interstitial blood vessels and tubules containing intraluminal pinkish materials (likely to be tubular casts), multiple foci of aggregates of mononuclear inflammatory cells especially around the portal tracts, random foci of single-cell hepatocellular necrosis, moderate congestion of hepatic sinusoids and veins and megalocytes (hepatocytes with extremely large nuclei). No histopathological alteration was observed in the testes of mice exposed to the water samples compared with the negative control (Figure 6).

Table 5. Alterations in haematological parameters in mice exposed to Awba Dam water sample for 35, 70, and 105 days.

	35 days			70 days			105 days		
	NC	ADW	PC	NC	ADW	PC	NC	ADW	PC
PCV (%)	30.00±2.739	34.75±3.497	35.67±3.480	32.00±3.189	36.33±3.844	22.50±0.5000	27.25±2.462	25.33±3.756	30.67±0.8819
Hb (g)	9.925±0.9420	11.40±1.267	11.63±1.189	10.40±1.153	10.90±1.320	7.000±0.0	8.775±0.7771	8.167±1.317	9.933±0.2667
RBC (%)	4.773±0.4914	6.630±0.7389	5.710±0.7484	5.265±0.6291	5.930±0.7251	3.235±0.01500	4.253±0.3963	4.230±0.6149	4.920±0.2957
WBC (%)	4675±613.9	3750±225.5	6067±581.2	5613±619.3	4867±543.4	3725±1575	4913±1131	3500±1474	7283±1146
PLT	116750±1833	102250±2626	189333±6143	156250±4159	145333±4139	120500±7500	176250±9612	86333±16974**	154667±137*
LYM	69.50±1.555	67.00±1.958	68.33±3.180	68.75±1.377	66.00±2.082	63.00±0.0	66.00±1.958	67.33±2.603	65.00±3.000
NEUT	27.00±1.732	28.75±28.75	27.67±4.096	27.50±1.190	30.33±1.667	34.00±0.0*	29.75±2.056	30.00±2.082	30.67±3.333
MON	2.250±0.4787	2.250±0.2500	2.000±0.5774	1.500±0.2887	2.667±0.3333	2.000±0.0	1.750±0.4787	1.333±0.3333	2.000±0.0
EOS	1.250±0.4787	2.000±0.4082	2.000±0.5774	2.250±0.4787	1.000±0.5774	1.000±0.0	2.500±0.2887	1.333±0.8819	2.333±0.3333
MCHC (pg^{-1})	0.331±0.0031	0.327±0.0035	0.326±0.0015	0.324±0.0046	0.320±0.0044	0.311±0.0069	0.322±0.0031	0.321±0.013	0.324±0.003
MCV (fl)	6.321±0.1527	6.249±0.2016	6.299±0.2049	6.132±0.1517	6.150±0.1042*	6.955±0.1223*	6.412±0.059	5.986±0.0461	6.259±0.2215
MCH (pg)	2.089±0.0540	2.042±0.0501	2.052±0.0583	1.984±0.0279	2.012±0.0474	2.164±0.01003*	2.066±0.0187	1.920±0.0649	2.028±0.0720

*NC – negative control (borehole tap water); ADW – Awba Dam water sample; PC – positive control (20 mg/kg bw cyclophosphamide). PCV- Packed cell volume; Hb- Haemoglobin; RBC – Red blood cell; WBC – White blood cell; PLT – platelets; LYM – Lymphocyte; NEUT- Neutrophils; MON- Monocyte; EOS – Eosinophils; MCHC – Mean corpuscular haemoglobin concentration; MCV – mean cell volume; MCH – Mean corpuscular haemoglobin. Values are significantly different from negative control at * = P<0.05; ** = P<0.01; *** = P<0.001.*

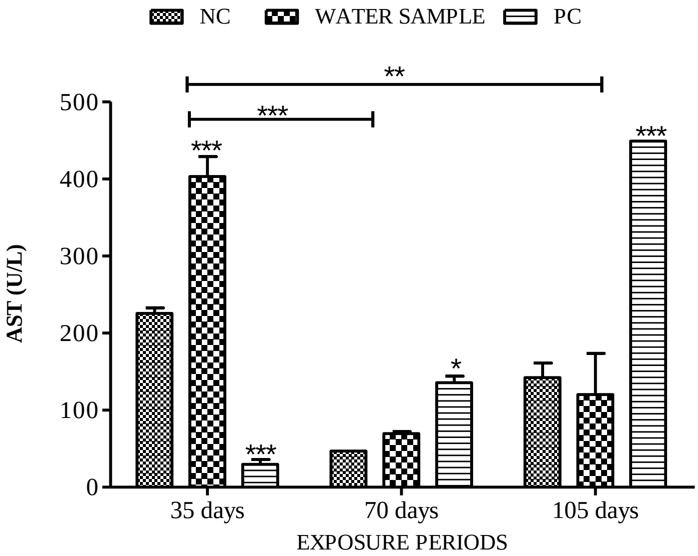

Figure 4. Serum AST activities induced by ADW sample in mice exposed for 35, 70 and 105 days and the controls.

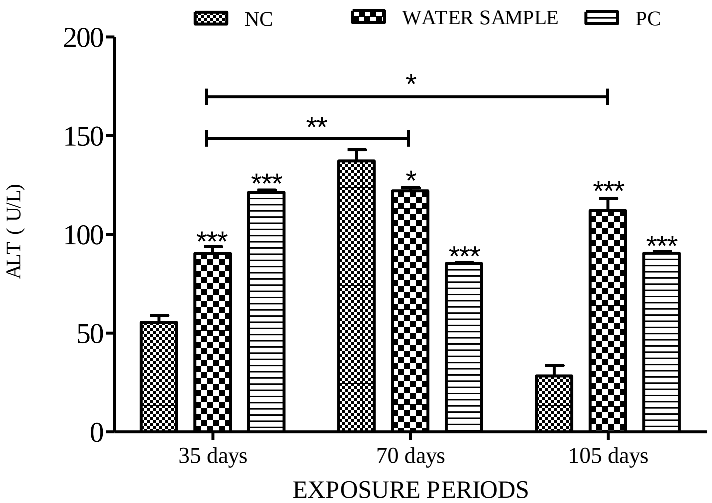

Figure 5. Serum ALT activities induced by ADW sample in mice exposed for 35, 70 and 105 days and the controls.

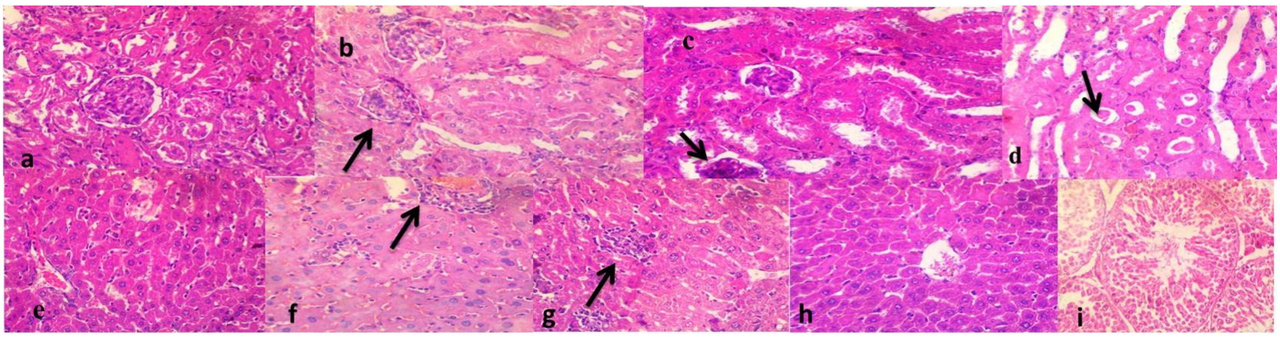

Figure 6. Photomicrograph of kidney, liver and testes of mice exposed to ADW sample for 35, 70 and 105 days. (a) Renal tissue with no lesion (negative control) (b) few foci of cloudy swelling (oedema)/degeneration of tubular epithelial cells (c) mild congestion of interstitial blood vessels (d) tubules containing intraluminal cast (pinkish materials) (e) Hepatic tissue with no lesion (negative control) (f) multiple foci of aggregates of mononuclear inflammatory cells around the portal tracts (g) moderate hepatic necrosis (h) congestion of hepatic sinusoids and veins (i) normal testicular tissue (x1000).

4. DISCUSSION

There are epidemiological evidence of increase risks of nephrotoxicity, cancer and central nervous system disorders resulting from the consumption of contaminated water [31,32]. Also, chemical exposures through drinking contaminated water hold the potential to induce DNA damage and enhance genetic changes in somatic and germ cells; which can result in decrease cell survival or transformation, reproductive abnormalities and cancer formation [33,34]. Awba Dam reservoir, one of the sources of water supply to the University of Ibadan community, receives diverse contaminants from both point and non-point anthropogenic activities in the community. Therefore, there is need for regular monitoring of the water source for toxins and genotoxins to avoid outbreak of diseases and disorder in the community. It is evident from this study that Awba Dam water has elevated levels of some heavy metals (such as Cd, Pb, Ni and Fe); and other unanalyzed organic pollutants recently reported by Adeogun *et al.* [15,35]. We have also showed the potential cytogenotoxic, mutagenic and systemic toxicity of water from the dam in *Allium cepa* and mice (*Mus musculus*).

Significant mitotic inhibition and induction of different chromosomal aberrations in *A. cepa* suggest that Awba Dam water contained cytotoxic, aneugenic and clastogenic agents capable of inhibiting somatic cell growth and inducing DNA damage. The mitotic index is considered to be reliable in identifying the presence of cytotoxin in the environment [36,37]. Decreased mitotic index (MI) in *A. cepa* roots may be due to disturbances in the cell cycle or chromatin dysfunction induced by metal–DNA interactions [38]. It may also be due to pressure on DNA synthesis or the complete halt of metabolic activities preventing the cell from entering mitosis thereby leaving many cells at interphase [39,40].

Aberrant chromosomes such as spindle disturbances, stickiness, distributed metaphase, anaphase bridges and polar deviations were due to chromatin dysfunction or spindle failure. Chromosome bridges result from chromosome and/or chromatid breaks, which indicate the clastogenic effect of the tested water sample [41]. Disturbed spindles and distributed metaphase may have resulted from disturbance of spindle apparatus which makes the chromosomes to spread irregularly all over the cell at different mitotic stages [42]. Sticky chromosomes may be due to increased chromosome contraction and condensation [43] or possibly from the depolymerization of DNA [44] and/or from partial dissolution of nucleoproteins [45]. Sticky chromosomes indicate that the pollutants affected the organization of the chromatin and this could result in cell death [17,46] as well as irreversible changes [47]. The present observations are similar to previous reports wherein underground water samples and man-made lakes [48], urban streams [49] and solid waste contaminated well waters [23,50] caused significant cytotoxic and mitodepressive effects, and also induced different types of chromosome aberrations in *A. cepa*. Also decreased mitotic index and chromosome aberrations had been similarly reported in *A. cepa* exposed to metal polluted water [51].

The mouse sperm morphology assay has potentials in identifying chemicals that are capable of inducing spermatogenic dysfunction and perhaps heritable mutations [19]. The present study showed the spermatotoxic effect of Awba Dam water. The fold increase in abnormal sperm cells observed during the exposure periods were more than double of those observed in the corresponding negative control and thus satisfied the criteria for positive response [19,20]. The induction of the sperm abnormality can either be due to impaired spermatogenesis or damage in the genetic material of spermatogonia and spermatocytes [52]. The increase in abnormalities observed in exposed mice could be as a result of persistent genetic damage which occurred in the spermatogenic cycles. The heavy metals in the water samples individually or interactively might have altered the normal process of gametogenesis and/or the synchronization of the stages in the seminiferous epithelium or cause abnormal chromosome complement or alter the testicular DNA [19,52]. This is because characteristics controlling the sperm head shape are carried on the autosomes and sperm abnormality test identifies agents capable of inducing DNA damage in the sperm head. This observation is in accordance with previous report on the cytogenotoxic effects and reproductive abnormalities induced by solid waste contaminated underground water in mice [53]. Sperm abnormalities have long been associated with male infertility and sterility in most species and their structure play a substantial role in both fertilization and pregnancy outcome [54].

Haematological parameters give important information on *in vivo* toxicological effects, and are used as predictive measures for human risk during assessment. The observed changes in the blood parameters of the exposed mice could be an adaptive response of the bone marrow or peripheral blood cells to physiological and immunological changes due to stress. The concomitant decrease in the RBC and PCV especially with longer exposure period (105 days) may be an indication of anaemia [55,56]. Anaemia occurs as a result of increased destruction of erythrocytes in the blood circulation [57]. This may have occurred from toxic metal interaction with the RBCs. The observed decrease in Hb values in the long-term exposure period (105 days) and reduction in MCV, MCH and MCHC is an indication of stress, macrocytic and hypochromic anaemia [30,58].

Reduction in Hb and MCHC indicates poor haemoglobin carrying capacity of the erythrocytes [59]. This could be as a result of cadmium, lead and nickel altering the properties of haemoglobin by decreasing their affinity towards oxygen and reducing the binding capacity thereby rendering the erythrocytes more fragile and permeable which probably result in cell damage [60].

Quantitative investigations of total white blood cells and differentials (non-specific immune cells) form a basic examination mostly in immunotoxicity studies. The role of the white blood cells is for defense and also a key to diagnose cancer and autoimmune disease. The decrease in WBC count observed throughout the exposure periods is an indication of immunosuppression and this could be as a result of the toxic action of cadmium, lead, nickel and iron, individually or in combination, which can induce leukopenia and thrombocytopenia in cases of severe liver dysfunction [61]. These observations were also made by Lodia and Kansala, [62] and Veena *et al.* [63] in mice treated with lead. It also corroborates the observations of Hounkpatin *et al*. [64] on rats treated with Cd and Hg and their combination. Concomitant increase in neutrophils could be due to the presence of microorganisms in the water sample which are capable of eliciting inflammatory responses of the neutrophils and the lymphocytes. This observation is in accordance with the findings of Mathur *et al.* [65] that neutrophil values increase in rats as a result of inflammatory responses; and pathogenic microorganisms that could elicit such inflammatory response were observed to increase in waste waters [66] that contaminate water bodies.

Liver produce aspartate aminotransferase and alanine aminotransferase which exist mainly in the hepatocytes and rarely occur freely in the circulating blood; and are important biological indicators of liver injury [67]. The presence of ALT and AST in the serum has been associated with hepatic injury [68]. In this study, the increase in AST and ALT levels are indications of liver damage. This may be due to the levels of Cd, Pb, Ni and Fe in the water sample. This is in accordance with the report of Ibrahim *et al.* [69] who demonstrated that Pb^{2+} induced significant increase in serum AST and ALT in mice. Similarly, Alimba *et al.* [30] reported that exposure to solid waste leachate (containing high concentrations of Ni, Pb and Fe) led to the concomitant increase in the activities of AST and ALT.

Histological changes in the liver and kidney tissues of exposed mice suggest toxic effects of the water samples. These were due to the effect of the water constituents especially the heavy metals of which Pb in particular have been implicated in histopathological changes in the liver and kidney of bank voles exposed to lead, cadmium, zinc and iron but with no visible lesion in the testes [70]. Severe morphological changes such as hepatic necrosis, large vacuolisation in Bowman's capsule and degeneration of proximal tubules indicate degeneration in the physiological conditions of the liver and kidney in the exposed mice.
Biochemically, the observed pathological changes, genotoxicity and elevated serum liver injury biomarkers could have been via generation of reactive oxygen species by the toxic metals and other deleterious constituents of the water samples. Sanchez-Chardi *et al*. [71], Bakare *et al*. [23,72] and Alimba *et al*. [30] reported similar observations in rodents exposed to metals, contaminated ground water and solid waste leachates. Our findings suggest unwanted side effects of the water body on resident flora and fauna, and may be transferred to other organisms including man along the food chain. This becomes highly relevant because of the importance of Awba dam water as a source of water (treated by the University water treatment plant) for domestic use in the University community. In addition, different species of fish harvested from the dam are consumed by members of the University community.

5. CONCLUSION

This study implies that there are chemical pollutants in Awba Dam water that induced genomic disruptions in *Allium cepa* and mice, and systemic damage in mice. Heavy metals and other unidentified pollutants are believed to induce the observed anomalies. Awba Dam water if not properly treated could potentially increase the risk of genetic related problems and incidence of liver and kidney dysfunctions among members of the University of Ibadan community over a long time. It can also increase health risk among aquatic biota and other non-human forms that depend directly on the untreated water for sustenance. Further studies are ongoing on *in situ* genetic and systemic damage using resident fish species from Awba dam.

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AUTHOR CONTRIBUTIONS

AAB conceived and designed the study, BOU and AOO did the literature search and executed the field and laboratory works, BOU, AOO, AMG, OIO, OCB and OMF performed the statistical analysis, and wrote the first draft of the manuscript. AAB and CGA oversees the execution of project. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. Ethical approval was obtained from the Animal Care and Use in Research Ethics Committee of the University of Ibadan (UI-ACUREC/17/0078).

REFERENCES

1 United Nations Environment Programme Global Environment Monitoring System/Water Programme (2000). *Water Quality for Ecosystem and Human Health.* National Water Research Institute: Burlington, ON, Canada.

2 West L. (2006). World Water Day: A Billion People Worldwide Lack Safe Drinking Water.

3 Vörösmarty C. J., McIntyre P. B., Gessner M. O., Dudgeon D. and Prusevich A. (2010). Global threats to human water security and river biodiversity. *Nature* 467: 555-561.

4 Dautremepuits C., Paris-Palacios S., Betoulle S. and Vernet G. (2004). Modulation in hepatic and head kidney parameters of Carp (*Cyprinus carpio* L) induced by copper and chitosan. *Comparactive Biochemistry and Physiology C: Toxicology and Pharmacology* 137: 325-333.

5 Adeniyi A. A., Okedeyi O. O. and Yusuf K. A. (2011). Flame ionization gas chromatographic determination of phthalate esters in water, surface sediments and fish species in the Ogun river catchments, Ketu, Lagos, Nigeria. *Environmental Monitoring and Assessment* 172: 561-569.

6 Adeogun A. O., Ibor O. R., Omiwole R. A., Hassan T., Adegbola R. A., Adewuyi G. O., Arukwe A. (2015). Occurrence, species, and organ differences in bioaccumulation patterns of phthalate esters in municipal domestic water Supply lakes in Ibadan, Nigeria. *Journal of Toxicology and Environmental Health Part A* 78: 761-777.

7 Adeyemi D., Ukpo G., Anyakora C. and Uyimadu J. (2009). Polychlorinated biphenyl in fish samples from Lagos Lagoon, Nigeria. *African Journal of Biotechnology* 8: 2811-2815.

8 Asagbra M. C., Adebayo A. S., Anumudu C. I., Ugwumba O. A. and Ugwumba A. A. A. (2015). Polycyclic aromatic hydrocarbons in water, sediment and fish from the Warri River at Ubeji, Niger Delta, Nigeria. African Journal of Aquatic Science 40: 193-199.

9 Anago I. J., Esenowo I. K. and Ugwumba A. A. A. (2013). The Physico-chemistry and Plankton Diversity of Awba Reservoir University of Ibadan, Ibadan Nigeria. *Research Journal of Environmental and Earth Sciences* 5(11): 638-644.

10 Adewuyi G. O. and Adeleye A. O. (2013). Evaluation of polybrominated diphenyl ethers in sediments of Lagos lagoon, Nigeria. *African Journal of Environmental Science and Technology* 7: 686-693.

11 Ugwumba O. A. and Ugwumba A. A. (1993). A study of the physico-chemical hydrology and plankton of Awba Lake in Ibadan, Nigeria. *Fish Academic* 1: 20-39.

12 Fagade S.O., Adebisi A.A., Kolo R.J, Elemi B.F. Adeosun A.A. and Jaafru, A. (1993) Urbanization and degradation of aquatic resources: The Ibadan Experience. In: *Proc. Nat.Conf. Cons. Aq. Res. Fed. Dept. Fish* Abuja.pp 208-219.

13 Adeogun A. O. and Fafioye O. O. (2011). Impact of Effluents on Water Quality and Benthic Macroinvertebrate Fauna of Awba Stream and Reservoir. *Journal Applied Science and Environmental Management* 15.1: 105 - 113.

14 Tyokumbur E. T. and Okorie T. (2013). Studies on the distribution and abundance of plankton in Awba stream and reservoir, University of Ibadan. *Open Journal of Ecology,* 3(4): 273-278.

15 Adeogun A. O., Onibonoje K., Ibor O. R., Omiwole R. A., Chukwuka A. V., Ugwumba A. O., Ugwumba A. A. A. and Arukwe A. (2016a). Endocrine-disruptor molecular responses, occurrence of intersex and gonado-histopathological changes in tilapia species from a tropical freshwater dam (Awba Dam) in Ibadan, Nigeria. *Aquatic Toxicology* 174: 10–21.

16 Fiskesjö G. (1985). The *Allium* test as a standard in environment monitoring. *Hereditas* 102: 99-112.

17 Fiskesjö G. (1997). *Allium* test for screening chemicals; evaluation of cytological parameters. *Plants for Environmental Studies*. Wang, W., Gorsuch J. W. and Hughes, J. S. Eds. New York: Lewis Publishers. 308–333.

18 Rank J. and Nielson M. H. (1994). Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research 312*: 17-24.

19 Wyrobek A.J., Gordon L. A., Burkhart J.G., Francis M.W., Kapp (Jr.) R.W., Letz G., Malling H. G., Topham J. C. and Whorton M. D. (1983). An evaluation of the mouse sperm morphology test and other sperm tests in non-human mammals. A report of the United States Environmental Protection Agency Gene – Tox Programme. *Mutation Research* 115: 1-72.

20 Bakare A. A., Mosuro A. A. and Osibanjo O. (2005). An *in vivo* evaluation of induction of abnormal sperm morphology in mice by landfill leachates. *Mutation Research* 582 (1-2): 28-39.

21 American Public Health Association (APHA) (1998). Standard methods for the examinations of water and waste water, American Public Health Association (APHA, AWWA, WPCF). 20th ed. Washington, DC: APHA.

22 CIOMS (1985). International Guiding Principles for Biomedical Research Involving Animals. The Development of Science-Based Guidelines for Laboratory Animal Care. NCBI Bookshelf. http://cioms.ch/publications/guidelines/1985_texts_of_guidelines.htm. 28pp

23 Bakare A. A., Alabi O. A., Gbadebo A. M., Ogunsuyi O. I. and Alimba C. G. (2013). *In vivo* cytogenotoxicity and oxidative stress induced by electronic waste leachate and contaminated groundwater. *Challenges*.4: 169-187.

24 Oyeyemi I. T. and Bakare A. A. (2013). Genotoxic and anti-genotoxic effect of aqueous extracts of *Spondias mombin* L., *Nymphea lotus* L. and *Luffa cylindrica* L. on *Allium cepa* root tip cells, *Caryologia.* 66(4): 360-367.

25 Grant W. F. (1982). Chromosome aberration assays in *Allium*. A report of U.S. Environmental Protection Agency Gene- Tox Program. *Mutation Research* 99: 273-91.

26 Alabi O. A. and A. A. Bakare. (2011). Genotoxicity and mutagenicity of electronic waste leachates using animal bioassays. *Toxicological and Environmental Chemistry* 93 (5): 1073–1088.

27 Monesi V., Geremia R., D'Agostino A., Boitani C. (1978). Biochemistry of male germ cell differentiation in mammals: RNA synthesis in meiotic and postmeiotic cells. *Current Tropics in Developmental Biology.* 12:11-36.

28 Wyrobek A. J. and Bruce W. R. (1975). Chemical induction of sperm abnormalities in mice. *Proceedings of the National Academy of Sciences* (USA), 72: 4425-4429.

29 Cheesbrough M. (2005). District laboratory practice in tropical countries: Haematology test.

Part 2, 2nd edition. Cambridge Low Price Editions. Pp 263-347.
30 Alimba C. G., Bakare A. A. and Aina O. O. (2012). Liver and kidney dysfunction in wistar rats exposed to municipal landfill leachate. *Resources and Environment*. 2(4): 150-163.
31 Gupta S. K., Gupta R. C. and Gupta A.B. (2001). Recurrent diarrhea in children living in areas with high levels of nitrate in drinking water. *Archives of Environmental Health* **1**, *56*, 369–373.
32 Sia Su G. (2005). Water-borne illness from contaminated drinking water sources in close proximity to a dumpsite in Payatas, The Philippianes. *J. Rural Trop. Public Health*, *4*, 43–48.
33 Shugart L. R., McCarthy J. F. and Halbrook R. S. (1992). Biological markers of environmental and ecological contamination: An overview. *Risk Anal. 12*, 353–360.
34 Russo C., Rocco L., Morescalchi M. A. and Stingo V. (2004). Assessment of Environmental stress by the micronucleus test and comet assay on the genome of teleost populations from two natural environments. *Ecotoxicology and Environmental Safety* 57*:* 168–174.
35 Adeogun A. O., Ibor O. R., Onoja A. B. and Arukwe A. (2016b). Fish condition factor, peroxisome proliferator activated receptors and biotransformation responses in *Sarotherodon melanotheron* from a contaminated freshwater dam (Awba Dam) in Ibadan, Nigeria. *Marine Environmental Research*. 1 – 13.
36 Kaymak F. and Goc-Rasgele P. (2009). Genotoxic effects of raxil on root tips and anthers of *Allium cepa* L*. Caryologia* 62.1: 1–9.
37 Radić S., Stipaničev D., Vujčić V., Rajčić M. M., Širac S. and Pevalek-Kozlina B. (2010). The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test*. Science of the Total Environment* 408: 1228–1233.
38 Glińska S., Bartczak M., Oleksiaka S., Wolska A., Gabara B., Posmyk M. and Janas K. (2007). Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of *Allium cepa* L. roots treated with heavy metals. *Ecotoxicology and Environmental Safety* 68: 343–350.
39 Yuzbasioglu D., Unal F., Sancak C. and Kasap R. (2003). Cytological effects of the herbicide racer, "flurochloridone" on *Allium cepa. Caryologia*. 56: 97–105.
40 Metin M. and Bürün B. (2010). Effects of the high doses of *Urginea maritime* (L.) baker extract on chromosomes. *Caryologia*. 63(4): 367–375.
41 Leme D. M. and Marin-Morales M. A. (2009). *Allium cepa* in environmental monitoring: A review on its application. *Mutation Research* 682: 71–81.
42 Amer S. M. and Ali E. M. (1974). Cytological effects of pesticides V. Effects of some herbicides on *Vicia faba*. *Cytologia* 39: 633-643.
43 Ahmed M. and Grant W. F. (1972). Cytological effects of the pesticides phosdrin and bladex in *Tradescantia* and *Vicia faba*. *Canadian Journal of Genetics and Cytology* 14: 157–165.
44 Darlıngton C. D. (1942). Chromosome chemistry and gene action. *Nature*. 149: 66.
45 Kaufman B.P. (1958). Cytochemical studies of changes induced in cellular materials by ionizing radiations. *Annals of New York Academy of Science*, *59:* 553–559.
46 Metin M. and Bürün B. (2008). Cytogenetic effects of *Urginea maritima* L. aqueous extracts on the choromosomes by using *Allium* test method. *Caryologia*. 61(4): 342–348.
47 Turkoglu S. (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutation Research* 626: 4-14.
48 Olorunfemi D. I., Olorunfemi O. P. and Agbozu I. E. (2014). Genotoxicity Assessment of Contaminated Drinking Water Sources in a Rural Community in Edo State of Nigeria. *Journal of Geoscience and Environment Protection* 2: 52-59
49 Athanásio C. G., Prá D. and Rieger A. (2014). Water Quality of Urban Streams: The *Allium cepa* Seeds/Seedlings Test as a Tool for Surface Water Monitoring. *The Scientific World Journal*, Volume 2014, Article ID 391367, 7 pages.
50 Bakare A.A., Lateef A, Amuda O. S. and Afolabi R. O. (2003). The aquatic toxicity and characterization of Chemical and Microbiological constituents of Water samples from *Oba* River*, Odo–Oba*, Nigeria. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 5(1): 11– 17.
51 Caritá R. and Marin-Morales M. A. (2008). Induction of chromosome aberrations in the Allium cepatest system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*. 72, 722–725.
52 Tasdemir I., Tasdemir M., Tavukcuoglu S., Kahraman S. and Biberoglu K. (1997). Effect of abnormal sperm head morphology on the outcome of intracytoplasmic sperm injection in humans. *Human Reproduction* 12: 1214–1217.
53 Alabi O. A. and Bakare A. A. (2014). Cytogenotoxic effects and reproductive abnormalitiesinduced by e-waste contaminated underground water in mice. *Cytologia* 79(3): 331–340.
54 Saacke R.G. (2001). What is a BSE-SFT standards: the relative importance of sperm morphology: an opinion. *Society for Theriogenology.* 81-87.
55 Dhanapakaim P. and Ramasamy V. K. (2001). Toxic effects of copper and zinc mixture on some haematological and biochemical parameters in common crab, *Cyprnus carpio* (Linn). *Journal of Environmental Biology.* 22:105-111.
56 Horiguchi H. (2007). Anemia induced by cadmium intoxication, Nihon Eiseigaku Zasshi. *Japanese Journal of Hygiene*. 62(3):888-904.
57 Vinodhini R. and Narayanan (2008). Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (common carp). *International Journal Environmental Science and Technology.* 5(2): 179-182.
58 Barger A. M. (2003). The complete blood cell count: a powerful diagnostic tool. *Veterinary Clinical: Small Animal Practice*. 33(6): 1207-1222.
59 Eaton D.L. and Klaassen C.D. (1998). Principals of toxicology. In: Klaassen C.D. (Ed) *Toxicology. The Basic Science of Poison*, 5th edn, pp 335 – 354.
60 Witeska M. amd Kosciuk B. (2003). The changes in common carp blood after short term zinc exposure. *Environmental Science Pollution Research International*. 10:284-286.
61 Lee W.M. (2004). Acetaminophen and the US acute liver failure study group: lowering the risks of hepatic failure. *Hepatology*. 40:6-9.
62 Lodia S. and Kansala L. (2012).Antioxidant activity of *Rubia cordifolia* against lead toxicity. *International Journal of Pharmacology and Science Research* 3(7): 2224-2232.
63 Veena S., Leena K., Arti Shweta L. and Sharma S. H. (2011). Ameliorating effect of *Coriandrum sativum* extracts on haematologial and immunological variables in an animal model of Lead intoxication. *Journal of Pharmacology and Allied Health Science.* 1:16-29.
64 Hounkpatin A. S. Y., Edorh P. A., Guédénon P., Alimba C. G., Ogunkanmi A., Dougnon T. V., Boni G.1, Aissi K. A., Montcho S., Loko F., Ouazzani N., Mandi L., Boko M. and Creppy E. E. (2013). Haematological evaluation of Wistar

rats exposed to chronic doses of cadmium, mercury and combined cadmium and mercury. *African Journal of Biotechnology* Vol. 12(23): 3731-3737.

65 Mathur N., Krishnatrey R., Sharma S., Pathak S. and Sharma K.P. (2003). Certain heamatological responses in Swiss albino mice following exposure to textile dye wastewater. *Journal of Environmental Biology.* 24(2): 161-164.

66 Oshode O. A., Bakare A. A., Adeogun A. O., Efuntoye M. O. and Sowunmi A. A. (2008). Ecotoxicological assessment using *Clarias gariepinus* and microbial characterization of Leachate from municipal solid waste landfill. *International Journal of Environmental Research.* 2(4): 391-400.

67 Hong W. M., Zuo W. Y., Song L. and Hang W. Q. (2013). Study on subchronic toxicity test of jizhikang oral liquid. *Chinese Animal Husbandry & Veterinary Medicine*. 40(9): 140 -143.

68 Harper C. (1979). Wernicke's encephalopathy: A more common disease than realized. A neuropathological study of 51 cases. *Journal of Neurology and Neurosurgery, and Psychiatry*, 42: 226-231.

69 Ibrahim M. N., Esam A. E.., Hossam S. E. and Yasmin E. A. (2012). Effect of lead acetate toxicity on experimental male albino rat. *The Asian Pacific Journal of Tropical Biomedicine.*2(1): 41-46.

70 Wlostowski T., Krasowska A. and Laszkiewicz-Tiszczenko B. (2000). Dietary cadmium induces histopathological changes despite a sufficient metallothionein level in the liver and kidneys of the bank Vole (*Clathrionomys glareolus*). *Comparative Biochemistry and Physiology* 126:21-28.

71 Sanchez-Chardi A., Penarroja-Matutano C., OliveiraRibeiro C. A.and Nadal J. (2007). Bioaccumulation of metals and effects of a landfill in small mammals. Part II. The Wood mouse, *Apodemus sylvaticus*. *Chemosphere* 70:101–109.

72 Bakare A. A., Patel S, Pandey A. k., Bajpayee M, Parmar D. and Dhawan A. (2012). DNA and oxidative damage induced in organs and tissues of mouse by municipal sludge leachate. *Toxicology and Industrial Health.* 28(7):614-623.

73 United State Environmental Protection Agency (USEPA) (2009). Drinking water contaminants. Washington, DC, USA, Available online: http://water.epa.gov/drink/contaminants/ index.cfm#List (accessed on 5 February 2010).

74 National Environmental Standards and Regulation Enforcement Agency (NESREA) (2009). (Federal Republic of Nigeria Official Gazette), National Environmental (Sanitation and Waste Control). Federal Government of Nigeria Printer, Abuja, Nigeria, FGP 112/102009/L000 (OL54). 60.96: 1057–1102.

75 SON (2007). Standard Organization of Nigeria (SON). Nigerian Standard for Drinking Water Quality. Nigerian Industrial Standard, NIS 554.