

Screening of Asa River water in Ilorin, Kwara State, Nigeria for available pollutants and its effects on mitosis and chromosomes morphology in *Allium cepa* cells

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Keywords: Asa River, Nigeria, *Allium cepa*, cytotoxic, genotoxic, pollutants

Posted Date: August 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-50698/v1>

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Abstract

Improper disposal of wastes as an environmental problem is common in African and other developing countries of the world, and it raises concerns due to its potential threats to the life of organisms in both terrestrial and aquatic ecosystems. In this study, Asa River in Ilorin, Nigeria was evaluated for cytogenotoxicity at 25.0 %, 50.0 %, 100.0 % following the *Allium cepa* assay. Water samples were collected from three points tagged A, B, C, and each point was 500 m apart from each other. The water samples were used to grow *A. cepa* for microscopic and macroscopic toxicities screenings. Heavy metals and volatile organic pollutants in the water were elucidated following the Atomic Absorption Spectroscopy and Gas Chromatography-Mass Spectroscopy. The Water samples induced higher mitotic index values, except the sample C which induced smaller mitotic index value than the negative control. Root growth in the exposed *A. cepa* was significantly promoted at 25.0 % of the water samples, while significant reduction was obtained at 50.0 % and 100.0 % of the sample C, and 100.0 % of the sample A. The absolute water sample A induced highest percentage chromosomal aberrations, as the water samples B and C induced higher percentage chromosomal aberration than the negative control. Cadmium was detected at a concentration higher than its permissible limit in drinking water unlike Zinc, Iron, Manganese. Poly aromatic hydrocarbons, Aromatic amines, Acridine dye, Phenolic and Polychlorinated compounds were detected in the water sample. These pollutants may be responsible for the observed proliferative, inhibitory, cytotoxic and genotoxic effects of the water samples on *A. cepa* cells. Our results suggest that Asa River is polluted, having potential to inflict different adverse effects on human, animals and plants utilizing it along its course.

1.0 Introduction

Water is most essential in the life of living organisms perhaps due to the fact that cell (s) that gives life to living organisms contains water to a large extent. The nourishment of cells that form unicellular or multicellular organisms requires water apart from other essentially required nutrients in both macro and micro quantities. The functions of water are best derived from it when it is in a state of purity. However, pollution makes water impure thereby making it to lose its expected functions when used up through chemical reactions. Rivers, lakes and ponds are sources of freshwater providing water for drinking, irrigating farmlands and other human activities globally. Pollution of surface water source occurs through release of industrial wastewater, agricultural and domestic wastes into water body according to Guan et al. [1] Hussein et al. [2] observed that contamination and pollution of aquatic environment has become a serious matter of concern globally due to their adverse effects on the ecological balance including loss of aquatic biodiversity. Although natural contamination of freshwater sources may occur at gradual processes through chemical weathering and soil leaching, but the release from anthropogenic sources is rapid and takes place at alarming rate as reported by Ogunkunle et al. [3]. Therefore, it should be of paramount importance that freshwater sources are regularly screened in order to ensure that drinking water and agricultural products being watered with water from these sources are free of contaminants and pollutants that might find their way into water through series of anthropogenic activities.

Asa river has its source in Oyo state, south west, Nigeria, while in Kwara state, it is located within Asa and Ilorin west local governments. The river is 56 km long, about 100 m wide supplying water to majority of people of Ilorin through building of dams such as Asa, Agba, Unilorin and Sobi lake dams according to Ogunkunle et al. [3] and Balogun and Ganiyu [4]. Asa river is a reservoir to a number of contaminants and pollutants from industrial, municipal and agricultural sources. Poor solid wastes management is a problem common to both rural and urban cities in Nigeria and other developing countries in Africa. Ebikapade and Jim [5] observed that solid wastes are usually dumped on open lands along major highways and riverbanks. Asa river is not exempted in this unhealthy practice as its riverbanks serve as a refuse dumpsite to different kinds of wastes from various sources. These contaminants and pollutants are chemicals compounds and heavy metals capable of inflicting various harmful effects on organisms at their cellular level according to Opasola et al. [6] and Olatunji et al. [7]. Chemical compounds found in water body from different sources have been reported to be cytotoxic, mutagenic and genotoxic as said by Pellacani et al. [8]. Assessment of quality of this water body is necessary in order to ensure that its use for different purposes is not associated with adverse effects in humans, animals and plants. Genetic toxicological assessment of water is an important indispensable quality assurance screening of water before its final consumption. According to Iqbal et al. [9] it reveals possible genetic effects of tested water samples on cell division and chromosomes behavior during mitosis and meiosis in the test model.

Allium cepa is one of the higher plants that is usually employed as an excellent genetic model for assessing cytogenotoxic effects of environmental contaminants and pollutants on eukaryotic cells due to its good chromosomes condition characterized by large metacentric chromosomes [10, 11]. It is easy to carryout and cheap in term cost implication. Interestingly, it produces results that are comparable with results from animal genetic assays. Bhat et al. [12] stated that it can be used to screen cytogenotoxicity of single and complex chemicals in industrial wastes. The *Allium cepa* assay was employed to evaluate cytogenotoxicity of Sungai Dua river water in Pulau Pinang, Malaysia and Guaribas river water in Piauí, Brazil [13, 14]. Other genetic models such as Ames test, SOS/*Umu* test, comet assay and micronucleus test have also been employed in studying potential genotoxicity and mutagenicity of Organochlorine Pesticides (OCPs), their degradation products, and Polycyclic Aromatic Hydrocarbons (PHAs) in river surface waters in Central Chile, Shanghai, China and Adige river basin [1,15, 16, 17]. Previous studies on cytogenotoxicity of heavy metals and their accumulation in the organs of some selected fish species in Asa river water body and Apodu reservoir in Maleté Ilorin, Kwara State, Nigeria employed micronucleus assay and Ames test which can detect only clastogenic effect and point mutations caused by environmental contaminants in this water body as reported by Hussein et al.[2] and Anifowose et al. [18].

This present study was undertaken on Asa river water samples to screen for potential cytotoxic and genotoxic effects of its available chemicals contaminants / pollutants following the *Allium cepa* assay, and their identification using Gas Chromatographic-Mass spectroscopy and Atomic Absorption Spectrophotometric techniques.

2.0 Materials And Methods

2.1 Chemicals reagents

All chemicals; Hydrogen peroxide (CAS 7722-84-1), Hydrochloric acid (CAS7647-01-0), Nitric acid (CAS7697-37-2), n-Hexane (CAS110-54-3), Ethanol (CAS64-17-5), Acetic acid (CAS64-19-7) and Orcein stain used in this study were of high quality analytical grade supplied by the Fisher Scientific, J.T. Baker, USA.

2.2 Study Area

Asa river has its source in Oyo State Southwestern Nigeria and flows through Ilorin, the capital of Kwara State, Nigeria in the South-North according to Balogun and Ganiyu [4]. It is located in Ilorin at the Southwestern part of Kwara state, surrounded by Moro local government to the North, Oyun and Offa local governments to the South and Ilorin local government to the East. Asa river covers an area of 5036.625km² and lies between latitudes 4°12'N and 4°29'N and longitudes 8°7'E and 8°42'E [19, 20]. (Figure 1).

The major occupation which Kwara State's economy depends on is Agricultural practices from which farmers produce cassava, sorghum, yam, cowpea, maize, groundnut and rice as reported by [21]. Olatunji et al. [7] reported that the river receives domestic wastes including sewage and agricultural wastes run off along the bank of the river.

2.3 Collection of water samples

Water samples were collected from three points in Asa river in Ilorin, Kwara state. The collection points were selected and tagged as sampling point A which is close to a refuse dump site at the river bank, point B which is five hundred meters away from point A, point C which is five hundred meters away from point B and this is where farmers who have farms beside the river bank usually pump water to irrigate their farms or gardens along the course of Asa River (Plate 1).

The collected water samples were kept in different three clean 4 liters capacity glass bottles and were immediately transferred for refrigeration at 4°C in the new Biology laboratory complex, LAUTECH, Ogbomoso, Oyo state, Nigeria, until further analyses in line with the study reported by Climents et al. [16].

2.4 *Allium cepa* Assay on water samples

Onions were purchased from Ipata market, Ilorin, Kwara state, Nigeria. They were sun dried for 1 week in order to reduce moisture content to facilitate root growth [23]. Ten onions per concentration were prepared by removing dried outer scales and scraping dried old roots of the primordial root ring for the onions to sprout new roots as previously described by Akinboro et al. [24] and Ahlem et al. [25]. Each of the water samples A, B, and C (absolute at 100%) was diluted with clean borehole water to obtain 25.0% and 50.0% concentrations. Negative and positive controls were borehole water and 0.1% hydrogen peroxide. Ten onions were planted on water sample in 100 ml capacity beakers at each of the selected concentrations and placed in a cupboard for 48 hours and 72 hours in order to evaluate effects of the

water on cell division and chromosomes structures (microscopic evaluation) and on root growth (macroscopic evaluation), respectively [26]. The water samples in the beakers were replaced with fresh one after every 24 hours and the sprouted onions were placed in the fresh water sample, and thereafter put back into the cupboard for root growth to continue. When it was 48 hours of onions root growth in the water sample, root tips from four onions were cut with a scissors and fixed in ethanol acetic acid fixative (3:1). The fixed root tips were rinsed with distilled water and then hydrolyzed in 1N HCL for 10 minutes at 60°C. Slides were prepared and observed under the light microscope (CH-China) using oil immersion objective lens to score dividing cells and chromosomal aberrations as previously described by de Castro e Sousa et al. [14] and Akinboro et al. [27]. Root length from the remaining six onions was measured with a ruler and recorded in centimeter to determine toxicity level of the water samples to *A. cepa* roots as described before by Akinboro and Bakare [28], Feretti et al.[29] and Verma and Srivastava [30].

2.5 Heavy metals analysis of water sample

Fifty milliliters of water sample collected from a point close to the refuse dump site at the River bank was added with 5 ml nitric acid and the mixture was subjected to heat in order to digest the sample completely in line with the method reported by Alope et al. [31]. Complete digestion of the sample was achieved when the sample became light colour which remained unchanged even with continued heating of the mixture of the water sample and nitric acid [32, 33]. The digested solution was filtered through a Whatman® filter paper (No 1, Qualitative Circles 110 mm Ø, Cat No 1001 110) into a 50 ml conical flask, and the filtrate was diluted with deionized water up to the calibrated line of the flask according to Wold et al. [34]. The analysis of the diluted filtrate for Cadmium (Cd^{+2}), Copper (Cu^{+2}), Nickel (Ni^{+2}), Zinc (Zn^{+2}), Lead (Pb^{+2}), Manganase (Mn^{+2}) and Iron (Fe^{+2}) was carried out using each of the standards of the heavy metals to determine their concentration in the water sample using an auto-sampler Atomic Absorption Spectrophotometer (Agilent technology, Series A, USA). The reading was taken in triplicate and the mean concentration of each heavy metal was recorded.

2.6 Gas chromatography- Mass spectroscopy (GC-MS) analysis on water sample

The volatile organic chemical compounds of the water sample were extracted by successive liquid-liquid extraction using analytical grade n-hexane as the solvent. Water sample A was subjected to Gas Chromatography coupled with Mass Spectrometer (Agilent Technologies, Model GC7890A/5975MS, USA) available at the Central Research Laboratory, Federal University of Technology, Akure, Nigeria. The analysis was performed with the equipment interfaced with HP Chemstation data processor fitted with a quartz Agilent J &W DB - 5ms. Ultra inert GC capillary column 30m × 0.53mm I.D., 5.0um film thickness (Hewlett Packard, Palo Alto, USA) was used. The analysis was run setting the column temperature at 40°C for 6min, increased by 2°C/min up to 100°C. The injector temperature was set at 225°C. Detector temperature for the Mass Spectrometer was set at 250°C. Helium was the carrier gas with pressure 5.0328 psi and split ratio 1:10. The sample was diluted in hexane and ethanol and 10µL was injected. The column inlet temperature was 280°C, the flow rate was 1.0mL/min. The ionization energy for recording mass spectra was 70ev. Individual chemical components of the water sample were identified

compared with the NIST Library and the percentage match of the identified chemicals was recorded according to Akinboro et al. [26, 35].

Statistical analysis

Obtained data were summarized as means and standard deviations of the measured parameters. These were transformed into percentage and the mean values from the treatment groups were compared with that of the negative control using Duncan multiple range comparison in one way ANOVA of the SPSS (version 17.0). Significantly different value was considered at $P < 0.05$.

3.0 Results

The water samples from points A, B and C caused varying degrees of effects on the root growth, cell division and chromosomes structure in *Allium cepa*. At the tested concentrations 25.0%, 50.0% and 100.0%, water sample A which was collected at the point close to the refuse dump site at Asa River's bank induced more dividing cells than the negative control (borehole water). The mitotic index (MI) value obtained at 100.0% of the water sample A was 1.70% which was significantly higher ($P < 0.05$) than the MI value of 1.40% induced by the negative control. Water sampled from the point B also induced higher MI value of 1.50% at each of the selected concentrations than the negative control. However, these were not significantly different from that induced by the negative control ($P > 0.05$). The water sample from point C had the same MI value of 1.40% at 25.0%, while at 50.0% and 100.0% concentrations, the MI values were 1.0% and 0.9%, respectively. These were not significantly different from MI induced by the negative control, except at 100% (Table 1).

Table 1: Effects of water samples on mitosis in *Allium cepa* cells

Concentration (%)	(Dividing cell \pm SD)	Mitotic index (%)	(Chromosomal aberration \pm SD)	% chromosomal aberration
Sampling point A				
Negative control	6.80 \pm 3.27 ^{ab}	1.40	0.60 \pm 0.89 ^a	0.6
Positive control	4.80 \pm 3.03 ^b	1.00	1.60 \pm 1.14 ^{ab}	1.6
25.0	7.80 \pm 2.78 ^{ab}	1.60	1.60 \pm 1.14 ^{ab}	1.6
50.0	7.40 \pm 1.82 ^{ab}	1.50	0.80 \pm 0.84 ^{ab}	0.8
100.0	8.60 \pm 2.70 ^a	1.70	2.20 \pm 1.10 ^b	2.2
Sampling point B				
Negative control	6.80 \pm 3.27 ^{ab}	1.40	0.60 \pm 0.89 ^a	0.6
Positive control	4.80 \pm 3.03 ^b	1.00	1.60 \pm 1.14 ^{ab}	1.6
25.0	7.60 \pm 1.52 ^{ab}	1.50	1.40 \pm 1.14 ^{ab}	1.4
50.0	7.40 \pm 1.67 ^{ab}	1.50	1.20 \pm 1.10 ^{ab}	1.2
100.0	7.40 \pm 2.07 ^{ab}	1.50	2.00 \pm 1.00 ^{ab}	2.0
Sampling point C				
Negative control	6.80 \pm 3.27 ^{ab}	1.40	0.60 \pm 0.89 ^a	0.6
Positive control	4.80 \pm 3.03 ^b	1.00	1.60 \pm 1.14 ^{ab}	1.6
25.0	6.80 \pm 3.27 ^{ab}	1.40	1.40 \pm 1.14 ^{ab}	1.4
50.0	5.20 \pm 0.84 ^{ab}	1.00	0.80 \pm 0.84 ^{ab}	0.8
100.0	4.40 \pm 2.07 ^b	0.90	0.80 \pm 0.84 ^{ab}	0.8

Values of the measured parameters in the same column with different superscript letter (s) were significantly different ($P \leq 0.05$).

Water samples from the three points A, B, and C induced normal stages of mitosis and different types of chromosomal aberrations (CA) namely; disturbed spindle, bridge anaphase and sticky metaphase (Plate 2).

Table 1 shows the percentage of CA induced at 100.0% water sample A was 2.2% and the highest. It was significantly different from the CA caused by the negative control ($P < 0.05$). Similarly, other tested concentrations of the water samples A, B, and C induced percentage chromosomal aberrations that were significantly different from CA induced by the negative control ($P < 0.05$)

Effects of the water samples on root growth of *A. cepa* are presented in Table 2. The water sample A at 25.0% and 50.0% concentrations respectively induced 127.60% and 106.60% root lengths which were higher than 100% root length obtained with the negative control. However, there was inhibition of root growth resulted to 90% root length obtained at 100% concentration of the water sample. Water samples at 25.0% concentration of the samples B and C, and 50.0% concentration of the sample B caused higher percentage root lengths than that of the negative control. There was root growth inhibition in the onions exposed to 100% of the water sample B, as well as 50% and 100% concentrations of the water sample C. The root growth inhibitions caused by the water sample C were significantly different from the negative control. The positive control caused the least root length and this was significantly different from that of the negative control ($P < 0.05$).

Table 2: *Allium cepa* roots lengths grown in water samples from Asa River in Ilorin, Kwara state Nigeria

Concentration (%)	Sampling point A		Sampling point B		Sampling point C	
	Root length (cm)	% root growth	Root length (cm)	% root growth	Root length (cm)	% root growth
Negative control	4.10 ± 1.51 ^d	100.00	4.10 ± 1.51 ^d	100.00	4.10 ± 1.51 ^d	100.00
Positive control	0.51 ± 0.15 ^f	12.44	0.51 ± 0.15 ^f	12.44	0.51 ± 0.15 ^f	12.44
25.0	5.23 ± 1.10 ^{ab}	127.60	4.51 ± 0.92 ^c	110.00	5.03 ± 0.76 ^b	122.70
50.0	4.37 ± 0.75 ^{cd}	106.60	5.54 ± 0.73 ^a	135.1	3.49 ± 0.77 ^e	85.10
100.0	3.69 ± 0.78 ^e	90.00	4.05 ± 0.87 ^d	98.8	3.37 ± 0.66 ^e	82.20

Values that have the different superscript alphabet(s) in the same column are significantly different at $P < 0.05$

Assessment of the water sample A from Asa River to detect and quantify heavy metals in it revealed as presented (Table 3). Heavy metals such as Cadmium (Cd^{+2}), Zinc (Zn^{+2}), Nickel (Ni^{+2}), Copper (Cu^{+2}), Manganase (Mn^{+2}), Lead (Pb^{+2}) and Iron (Fe^{+2}) were detected in the water sample in varying concentrations. Cd was detected at a higher concentration of 0.022 mg/L than its WHO and EPA permissible limit of 0.003 mg/L in drinking water. The concentrations of Zn, Mn and Fe detected in the water sample were lower than their permissible limits in drinking water according to the WHO and FAO standards. However, Ni, Cu and Pb were present below zero level.

Table 3: Heavy metals concentrations detected in water sample from Asa river in Ilorin, Kwara State

Heavy metals	Concentration detected (mg/L)	WHO limit (mg/L)	USEPA limit mg/L
Cadmium - Cd	0.022	0.003	0.003
Zinc - Zn	0.014	5.0	5.0-15.0
Nickel - Ni	-0.040	0.02	NA
Copper - Cu	-0.091	1.0	0.05 - 1.5
Manganase - Mn	0.089	0.1	0.5
Lead - Pb	-0.120	0.05	0.02
Iron - Fe	0.028	0.30	NA

WHO = World Health Organization; EPA = Environmental Protection Agency; NA = Not available

The results of analysis of chemical compounds in the water sample following the Gas Chromatography - Mass Spectroscopy technique (GC-MS) are presented in Table 4. Twenty two different chemicals compounds representing 5 groups of aromatic compounds belonging to Polycyclic Aromatic Hydrocarbons (PAH), polychlorinated compounds, aromatic amines, phenolic compound and acridine dye were revealed by the GC-MS technique. The percentage abundance of the detected chemical compounds in the water sample as revealed by the library of the chemicals station of the instrument ranged between 12 – 43%.

Table 4: Chemical compounds detected in Asa river water sample following the GC-MS technique

Peak	Retention time (min)	% Area	Identified chemicals in the library	CAS NO	% Match
1	4.78	0.3594	2-Pyridinamine, 3,5-dibromo-	035486-42	22
2	5.14	0.4813	Phenol, 2,4-dibromo-	000615-58	22
3	5.22	0.3779	Methaqualone	000072-44	30
4	5.38	0.9599	3,5-Di-t-butyl-4-methoxy-1,4-dihydrobenzaldehyde	1000130-2	35
5	5.58	1.0294	Pyrimidine, 5-bromo-2,4-bis(methylthio)-	060186-81	38
6	5.63	0.3446	1,2-Benzenedicarboxylic acid, 4-methyl-5-(1-methylethyl)-, dimethyl ester	055044-59	35
7	6.04	5.1461	4-(4,5-Diphenyl-1H-imidazol-2-ylsufanyl)-3-oxo-2-(phenyl-hydrazono)-butyric acid, ethyl ester	1000318-3	14
8	6.12	4.1711	Propionic acid, 3-bis(diethylphosphonomethyl) amino-	178762-71	22
9	6.27	1.3046	Indan-1,3-dione, 2-(1,3-dimethyl-1H-pyrazol-4-ylmethylene)-	1000316-7	18
10	6.41	2.0764	Adamantane-2,6-dione, bis(ethylene ketal)-	060797-89	25
11	6.49	1.6541	4-Pyridinamine, 3,5-dibromo-	084539-34	18
12	6.57	2.5341	3-(2-Hydroxy-6-methylphenyl)-4(3H)-quinzolinone	052898-72	22
13	6.67	3.2984	Cinnamic acid, p-(trimethylsiloxy)-, methyl ester	027798-69	30
14	7.35	1.2413	Pyridine-3-carbonitrile, 2-amino-4-(4-methoxyphenyl)-5-methyl-6-propyl-	1000264-8	35
15	7.41	2.9268	2-Ethylacridine	055751-83	15
16	7.56	1.8615	(2-Phenyl-1-benzimidazolyl)acetic acid	092437-42	27
17	8.10	2.7038	1H-Indole, 3-(2-methoxyethyl)-2-(2-pyridyl)-	161988-60	43
18	8.89	2.7248	2,6-Pyridinecarboxylic acid, hexyl 3-(2-methoxyethyl)nonyl ester	1000369-2	25
19	10.11	1.109	Benzothiophene-3-carboxamide, 4,5,6,7- tetrahydro-2-amino-6-tert-butyl-	1000272-8	22
20	11.10	0.8412	Phenol, 2,4-dibromo-, acetate	036914-79	12
21	12.18	0.6695	Benzene, 1,2,3,5-tetrachloro-4,6-difluoro-	001198-56	22
22	12.78	0.9187	1,2-Bis(trimethylsilyl)benzene	017151-09	15

4.0 Discussion

The importance of water to living organisms is enormous that it forms the major constituent of the ecosystem. No wonder its availability is always being ensured by man through its various possible

sources such as rain, river, dams, lakes, glaciers and ground water [36]. Water is used for sustainability of aquatic life and for domestic, industrial, agricultural, recreational and commercial purposes [37]. The efficiency of water depends on its good quality, achievable in its state of being free from contamination or / and pollution. Assessment of water quality is necessary and important in order to ensure safe consumption and utilization of water.

Evaluation of Asa river water samples for toxicity to cell division and chromosomes morphology in the root tip cells of *Allium cepa* showed varying degrees of toxicity at different sampling points. Induction of significantly higher mitotic index (MI) value at 100.0% of the water from the sampling point A (a point close to refuse dump site by the River bank), and non-significantly different but higher MI values recorded at 50.0, 25.0% of the water sample A and at all the tested concentrations of the water from the sampling point B compared to the negative control suggests non-cytotoxicity, but rather indicates proliferative potency of the water samples. Similarly, the water sample C was not cytotoxic at 25.0% and 50.0% except at 100.0%. The observed proliferative and cytotoxic effects of the water samples from Asa River indicate that the water body was contaminated and polluted. Our results are different from over 40% decrease in MI value obtained when industrial effluents collected near the Sava River in Croatia were tested for genotoxicity using the *A. cepa* assay by Radić et al. [38]. Mitotic index (MI) as the total number of dividing cells in a cell cycle has been severally employed to evaluate toxic levels of different kinds of agents in *A. cepa* cells. Both significant increase and decrease in MI values observed with a tested substance compared with the MI value of the negative control are important indices in monitoring environmental pollution. This parameter reveals possibilities of the tested water samples to induce uncontrolled proliferation of cells, leading to tumour formation, or / and stunted growth in biological organisms having similar eukaryotic cells and genetic constituents with *A. cepa* cells [10]. Antimitotic activity of chemical compounds in *A. cepa* cells is attributed to prevention of DNA synthesis and G2 from entering M-phase, chemical (s) mitodepressive activity, inhibition of DNA polymerase due to inactivity of specific nuclear proteins, changing of mitotic stage duration and reactive oxygen species, causing disturbance of homeostasis in a cell cycle as reported by Liman et al. [39].

Induction of significantly different chromosomal aberrations (CA) such as disturbed spindle, bridged anaphase and sticky chromosomes at the tested concentrations of the water samples from that recorded with the negative control further corroborates that Asa River water was contaminated or /and polluted. Chromatid breaks develops to anaphase bridge, disturbed spindles must have been caused by the genotoxic action disrupting the tubulin that is cytoskeleton holding the chromosomes together, while sticky chromosomes is an irreversible CA induced by dissolution and depolymerization of histone protein and chromatin fibres binding subunits of the chromosomes together [25, 30, 40]. Induction of these forms of chromosomal aberrations by the tested water samples in this study implies that Asa River water possesses clastogenic, aneugenic and severe genotoxic effects on *A. cepa* cells. Interestingly, the obtainment of highest percentage of CA at 100.0% of the water sample A further confirms its toxic potential in possibly causing tumour and cancer since it significantly promoted uncontrolled proliferation of cells and caused significantly high frequency of CA necessary for the initiation stage of cancer development to be established in a multicellular organisms.

The results of root length measurement in the exposed *A. cepa* to the water samples corroborated the results of microscopic screening. The induction of smaller root lengths by the absolute water samples from points A, B, and C than that recorded for the negative control implied root growth inhibitory effect of the Asa River water body. However, induction of longer root lengths at lower concentrations of the water samples than that induced with the negative control is in support of the proliferative activity of these water samples, and further suggests the ability of the water samples especially the one from points A and B to induce uncontrolled proliferation of cells, while, significant reduction of root length caused by the water sample C further suggests its phytotoxicity.

Our results in this study for the first time using the *A. cepa* plant genetic assay have further confirmed pollution of Asa River water body as previously reported in the study on heavy metals accumulation in some species of fish in this water body, and another one following micronucleus and Ames tests in fish, *Salmonella typhi* tester strain TA100 and SOS chromotests (*Escherichia coli* PQ37) [2, 41]. It is now established that the use of polluted Asa River water to water farmlands along the River bank could cause stunted growth of crops based on the effects of the water sample C on *A. cepa* root growth. Furthermore, the effects of water sample C on *A. cepa* cells and root growth in this study may logically be expected in the crops (along the River bank) that are being watered with water from the sampling point C through bioaccumulation and biomagnification. These effects may be further transferred to consumers of these crops including humans along the food chain as reported by Guan et al.[1]. This extrapolation of the outcomes of this study to predict similar effects in animal cells is based on the fact that there is oxidase enzyme in *A. cepa* cells which makes it suitable to evaluate genotoxicity of promutagens which requires conversion to mutagenic or genotoxic metabolites, although at a lesser rate compared to how it is carried out by P-450 enzyme in the liver cells of higher animals. Furthermore, chemicals that are able to cause chromosome damage (genotoxicity) in plant cells can also pose danger to animal cells since the damaged material is DNA which is common to both plant and animal cells [10].

The observed proliferative, root growth promoting and inhibitory, cytotoxic and genotoxic effects of the water samples along Asa River's course might have been caused by the types and concentration of polycyclic aromatic hydrocarbons (PAH), aromatic amines, acridine dye, polychlorinated and phenolic compounds, and heavy metals detected in the water samples following the Gas Chromatography Mass Spectrophotometry (GC-MS) and Atomic Absorption Spectroscopy (AAS) techniques. Heavy metals viz a viz; Cadmium (Cd^{+2}), Zinc (Zn^{+2}), Manganase (Mn^{+2}) and Iron (Fe^{+2}) were detected in the water sample at various concentrations as previously reported by Hussein et al.[2] and Anifowose et al.[41]. Heavy metals such as Hg, Cd, As, Pb, Sb, Cr, and Sr are very toxic even at low concentrations because they are non-biodegradable leading to their bioaccumulation in the human body to cause damage to nervous system and internal organs according to Ogunkunle et al. [3]. Leme and Marin-Morales [10] reported that generally, at various concentrations they have been associated with mitotic inhibition and chromosomes abnormalities in *A. cepa* cells. They are toxic agents capable of causing DNA damage through interference with the enzymatic processes and DNA repair mechanism in exposed organisms and this may lead to cancer development according to Mouna et al. [42] and Matos et al. [43]. Apart from the

refuses dumped at the river bank of Asa River, fertilizer and pesticide application by the farmers along the river bank may be the source of heavy metals into the water body [21]. Heavy metals are accumulated not only in the soil but also in plants. The concentration of Cd higher than its permissible limit in drinking water according to the WHO and USEPA standards further implies that the water body is polluted and unfit for consumption in any form. High concentration of Cd in water sources could be possibly due to the waste disposal method, natural processes, human /anthropogenic activities, agricultural practices, closeness of water body to the roads with high traffic density, metal melting and electroplating, coal refining and oil fired power stations [31]. The concentration of Cd (0.022 mg/L) in this study was higher than 0.3 – 1.36 µg/L recorded for wastewater treatment plant in the city of Guelma in Algeria [42]. Environmental exposure to Cd at a high concentration is injurious to health as it can cause kidney damage, being an important organ of homeostasis that first displays sign of toxicity to Cd, neurotoxicity and bone damage according to Oluyemi and Olabanji [44] and Chaitali and Jayashree [45]. Rajappa et al. [46] reported that it causes a painful disease called Itai-itai.

Polycyclic aromatic hydrocarbons (PAHs), aromatic amines, acridine dye, phenolic and polychlorinated compounds are well known cytotoxic and genotoxic agents causing mitotic inhibition and various kinds of chromosomal aberrations in *A. cepa* cells and different types of point mutations in the Ames *Salmonella*/microsome assay [47, 48, 49, 50, 51]. In detecting the presence of PAHs as pollutants in any tested samples, *A. cepa* uses its ethoxyresorufin-O- deethylase (EROD), a subunit of dependent mixed function oxidases of cytochrome P450- (CYP-). PAH ligands activate the hydrocarbon receptor (AhR) on the cell membrane and this is translocated into the nucleus and then activates the CYP1A1 genes according to Claudia Ramos deRainho et al. [52]. PAHs are indirect-acting mutagens which need metabolic activation in order to exert their effects [1]. Polycyclic aromatic hydrocarbons among hydrocarbon compounds in the crude oil are the most dangerous environmental contaminants due to their harmful effects such as toxicity, mutagenicity and carcinogenicity on different living organisms. The presence of these aromatic organic compounds and heavy metals in Asa River as we report in this study makes its use unhealthy to water farmlands along the river bank, as sources of underground and surface drinking water through construction of dams and digging wells. These practices portend danger to human, animals and plants coming in contact with this water.

Conclusion

The present study on quality assurance tests on Asa River water using the *A. cepa* genetic assay, GC-MS and AAS techniques has established that the water body was polluted with heavy metals such as Zinc, Manganase, Cadmium and Iron of which the concentration of Cadmium was above the permissible limit according to the WHO and USEPA standards. Furthermore, the water also contained polycyclic aromatic hydrocarbons, aromatic amines, acridine dye, polychlorinated and phenolic compounds which are responsible for induction of higher and reduced number of dividing cells (MI) and root lengths, and various types of chromosomal aberrations (CA) that were significantly different from those recorded for the negative control at different sampling points. These observed effects on *A. cepa* cells suggest that Asa River water body was polluted and its use as source of drinking water to surface or underground

waters, as well as to irrigate farmland along the river bank may be associated with adverse effects in human, animals and plants that utilize it. For the first time, our results have suggested possible effects of this water body on plant cells including its potential to cause different types of chromosomal aberrations using the *A. cepa* assay in addition to the outcomes of the previous studies that have reported the pollution status of Asa River water body.

Declarations

Availability of data and materials – Not applicable

Competing Interest – Not applicable

Funding – Not applicable

Authors' contributions: (1) Akeem Akinboro designed the study and supervised the conduct of the experiments in the laboratory, (2) Nike Peter Aina carried out the experiment in the laboratory, (3) Mohammed Rufai Akinlabi provided the technical supports, (4) Asiata Ibrahim Omotayo provided technical supports.

Acknowledgements – We acknowledge the assistance rendered in supplying some of the equipment used for the study by the department of Pure and Applied Biology, LAUTECH, Ogbomoso, through Mr. T.O. Amusan and Mr. M. A. Ogunwole.

Conflict of Interest:

No conflict of interest is declared in this study.

References

- [1] Guan Y, Wang X, Wong M, Sun G, An T, Guo J, et al. Evaluation of Genotoxic and Mutagenic Activity of Organic Extracts from Drinking Water Sources. *PLoS ONE*; 2017;12(1): e0170454.
- [2] Hussein K, Okoro O, Iyiola A, Simon I, Oladipo SO. Determination of Heavy Metal Genotoxicity and their Accumulation Pattern in Different Fish Organs of Selected Fish Species collected from the Asa River, Ilorin, Kwara State, Nigeria. *J. Appl Sci Environ Mgt*, 2016;20(3):735-745.
- [3] Ogunkunle C O, Kamaldeen M, Stephen O, Paul OF. Assessment of metallic pollution status of surface water and aquatic macrophytes of earthen dams in Ilorin, north-central of Nigeria as indicators of environmental health. *J King Saudi Uni Sci*, 2016;28: 324–331.
- [4] Balogun OS, Ganiyu HO. Study and analysis of asa river hypothetical dam break using Hec-Ras. *Nig J Tech (NIJOTECH)*. 2017;36:315 – 321.
- [5] Ebikapade A, Jim B. Solid Waste Management Trends in Nigeria. *J Mgt Sust*, 2016; 6: 4.

- [6] Opasola OA, Adeolu AT, Iyanda AY, Adewoye SO, Olawale SA. Bioaccumulation of Heavy Metals by *Clarias gariepinus* (African Catfish) in Asa River, Ilorin, Kwara State. *J Health & Pol*, 2019;9:21:1-10.
- [7] Olatunji MK, Kolawole TA, Albert BO, Anthony IO. Assessment of Water Quality in Asa River (Nigeria) and Its Indigenous *Clarias gariepinus* Fish. *Int J Environ Res Public Health*, 2011; 8: 4332-4352.
- [8] Pellacani C, Cassoni F, Bocchi C, Martino A, Pinto G, Fontana F, et al. Cyto- and genotoxic profile of groundwater used as drinking water supply before and after disinfection. *J water health*, 2016; 14(6):901-913.
- [9] Iqbal M, Mazhar A, Jan N, Arif N, Ahmad ZQ. Bioassays based on higher plants as excellent dosimeters for ecotoxicity monitoring: A review, *Chem Int*, 2019;5(1): 1-80.
- [10] Leme DM, Marin-Morales MA. *Allium cepa* test in environmental monitoring: A review on its application. *Mut Res/Rev Mut Res*, 2009; 682:71–81.
- [11] Silveira GL, Lima MGF, dos Reis GB, Jos_e Palmieri M, Andrade-Vieria LF. Toxic effects of environmental pollutants: Comparative investigation using *Allium cepa* L. and *Lactuca sativa* L. *Chemosphere*. 2017, 178 : 359e367.
- [12] Bhat SA, Singh J, Singh K, Vig AP. Genotoxicity monitoring of industrial wastes using plant bioassays and management through vermitechnology: A review, *Agric Nat Resour*, 2017, 51: 325e337.
- [13] Akinboro A, Kamaruzaman M, Selestin R, Vijay RM. Genotoxicity assessment of water samples from the sungai dua river in Pulau Pinang, Malaysia, using the *Allium cepa* test. *Trop Life Sci Res*, 2011c; 22 (2): 23-35.
- [14] de Castro e Sousa JM, Peron AP, da Silva e Sousa L, de Moura Holanda M, de Macedo Vieira Lima A, de Oliveira VA, et al. Cytotoxicity and genotoxicity of Guaribas river water (Piauí, Brazil), influenced by anthropogenic action. *Environ Monit Assess*, 2017; 189:301.
- [15] Kračun-Kolarević M, Kolarević S, Jovanović J, Marković V, Ilić M, Simonović P, et al. Evaluation of genotoxic potential throughout the upper and middle stretches of Adige river basin. *Sci Total Environ*, 2016; 571: 1383–1391.
- [16] Climent MJ, Sánchez-Martín MJ, Rodríguez-Cruz MS, Pedreros P, Urrutia R, Herrero-Hernández E. Determination of pesticides in river surface waters of central Chile using Spe-GC-MS multi-residue method. *J Chil Chem Soc*, 2018; 63:2.
- [17] Chen C, Zou W, Chen S, Zhang K, Ma L. Ecological and health risk assessment of organochlorine pesticides in an urbanized river network of Shanghai, China. *Environ Sci Eur*, 2020; 32:42.
- [18] Anifowoshe AT, Oladipo SO, Owolodun OA, Akinseye MK, Olafimihan TF, Sidiq AG, et al. Ecogenotoxicological assessments of some selected fish species from Apodu Reservoir, Maleté, North

Central, Nigeria. *Manila J Sci*, 2018; 11: 1-14.

[19] Jimoh HI. Effects of River Erosion on Houses: A case study of Asa River catchment in Ilorin, Kwara State, Nigeria. *Soc. Sci*, 2008;3(2):193-195.

[20] Alaaya BA, Adetimirin OI, Alagbe AO. Investigation of Ground Water Reservoir in ASA and Ilorin West Local Government of Kwara State Using Geographic Information System, TS02D - GIS, Mapping Technology and GeoSpatial Implementations – 6694, FIG Working Week 2013 Environment for Sustainability Abuja, Nigeria. 2013; 6 – 10 May 2013.

[21] Aliyu TH, Balogun OS, Namani CS, Olatinwo LK, Aliyu AS. Assessment of the presence of metals and quality of water used for irrigation in Kwara State, Nigeria. *Pol*. 2017,3(3): 461-470.

[22] Ayanshola A, Olofintoye O, Obadofin E. The impact of global warming on precipitation patterns in Ilorin and the hydrological balance of the Awun basin, *Slovak J Civ Eng (SJCE)*, 2018;26(1):40 – 46.

[23] Akinboro A, Kamaruzaman BM, Mohd Zaini A, Othman AS. Mutagenic and antimutagenic potentials of fruit juices of five medicinal plants in *Allium cepa* L.: Possible influence of DPPH free radical scavengers. *Afr J Biotech*, 2011a;10(51):10520-10529.

[24] Akinboro A, Kamaruzaman BM, Mohd Zaini A, Shaida FS, Othman AS. Antioxidants in aqueous extract of *Myristica fragrans* (Houtt.) suppressed mitosis and cyclophosphamide-induced chromosomal aberration in the *Allium cepa* L. cell. *J Zhejiang Univ-SCI B*, 2011b; 12 (11): 915-922.

[25] Ahlem A, Djamel EB, Mouna T, Recep L, Muhsin K, Messaouda K, et al. Mutagenicity and genotoxicity of drinking water in Guelma region, Algeria. *Environ Monit. Assess*, 2015; 187:21.

[26] Akinboro A, Kamaruzaman BM, Mohd Zaini A, Ahmad SO, Tang HY, Siti Marina M. Mutagenic and antimutagenic assessment of methanol leaf extract of *Myristica fragrans* (Houtt.) using *in vitro* and *in vivo* genetic assays. *Drug Chem Toxicol*, 2012;35 (4): 412-422.

[27] Akinboro A, Adedosu O T, Famurewa FO, Olowe T, Oyewole OO. Antioxidant, Proximate and Cytogenotoxic Evaluation of Anti-typhoid Fever Herbal Recipe in *Allium cepa* Cells. *Global J Bio-Sci Biotech (GJBB)* 2017; 6 (1): 24-29.

[28] Akinboro A, Bakare AA. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *J Ethnopharmacol*, 2007; 112:470-475.

[29] Ferrettia D, Zerbinia I, Cerettia E, Villarinib M, Zania C, Morettib M, et al. Evaluation of chlorite and chlorate genotoxicity using plant bioassays and *in vitro* DNA damage tests, *Water Res*, 2008; 42: 4 0 7 5 – 4 0 8 2.

[30] Verma S, Srivastava A. Morphotoxicity and cytogenotoxicity of pendimethalin in the test plant *Allium cepa* L. - A biomarker based study, *Chemosphere*, 2018; 206:248e254.

- [31] Alope C, Uzuegbu IE, Ogbu PN, Ugwuja EI, Orinya OF, Obasi IO. Comparative assessment of heavy metals in drinking water sources from Enyigba Community in Abakaliki Local Government Area, Ebonyi State, Nigeria. *Afr J Environ Sci Tech*, 2019;13(4):149-154.
- [32] Saeed S, Marzieh VD, Akbar H, Toba K. Heavy Metals in Water and Sediment: A Case Study of Tembi River, *J Environ Public Health*, 2014; Article ID 858720, 5.
- [33] Mahugija JAM. Levels of heavy metals in drinking water, cosmetics and fruit juices from selected areas in Dar Es Salaam, Tanzania, *Tanz J Sci*, 2018;44(1): 1-11.
- [34] Wold FG, Ayenew B, Ahmad T. Assessment of heavy metals concentration in Togona River of Goba Town, Oromia Region, Ethiopia. *Int J Chem Sci*, 2016;14(4): 3207-3214.
- [35] Bell AM, Baier R, Kocher B, Reifferscheid G, Buchinger S, Ternes T. Ecotoxicological characterization of emissions from steel coatings in contact with water. *Water Res*, 2020;173: 115525.
- [36] Hefni E. River water quality preliminary rapid assessment using pollution index, *Procedia Environ Sci*, 2016; 33: 562 – 567.
- [37] Taiwo AM, Olujimi OO, Bamgbose O, Arowolo TA. Surface Water Quality Monitoring in Nigeria: Situational Analysis and Future Management Strategy, *Water Quality Monitoring and Assessment*, Dr. Voudouris (Ed.), 2012; ISBN: 978-953-51-0486-5, InTech, Available from: <http://www.intechopen.com/books/waterquality-monitoring-and-assessment/surface-water-quality-monitoring-in-nigeria-situational-analysis-and-futuremanagement-strategy>.
- [38] Radić S, Draženka S, Valerija V, Marija MR, Siniša Š, Branka PK. The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. *Sci Total Environ*, 2010; 408:1228–1233.
- [39] Limana R, Ahmed NK, Ibrahim HC, Mustafa O, Yaser A. Assessment of the cytotoxic and genotoxic potential of pillar[5]arene derivatives by *Allium cepa* roots and *Drosophila melanogaster* haemocytes. *Ecotoxicol Environ Safety*, 2020; 192:110328.
- [40] Datta S, Jaswinder S, Joginder S, Sharanpreet S, Simranjeet S. Assessment of genotoxic effects of pesticide and vermicompost treated soil with *Allium cepa* test. *Sustain Environ Res*, 2018;28:171e178.
- [41] Anifowoshe TA, Oladipo SO, Adebayo OM, Eboh OS, Abdussalam AR, Adegbenro AM, et al. Induction of Micronuclei, Base-pair Substitution Mutation and Excision-repair Deficient by Polluted Water from Asa River in Nigeria. *Annals Sci Tech*, 2019;4 (2):68-77.
- [42] Mouna T, Ahlem A, Djamel EB, Recep L, Muhsin K, Messaouda K, et al. Mutagenic and genotoxic effects of Guelma's urban wastewater, Algeria. *Environ Monit Assess*, 2015;187:26.
- [43] Matos LA, Cunha ACS, Sousa AA, Maranhão JPR, Santos NRS, de MC, et al. The influence of heavy metals on toxicogenetic damage in a Brazilian tropical river. *Chemosphere*, 2017;185:852e859.

- [44] Oluyemi EA, Olabanji IO. Heavy metals determination in some species of frozen fish sold at Ile-Ife main Market, South West Nigeria. *Ife J Sci*, 2011;13(2):355-362.
- [45] Chaitali VM, Jayashree D. Review of heavy metals in drinking water and their effect on human health. *Int J Innov Res Sci Engr Tech*, 2013; 2: issue 7.
- [46] Rajappa B. Monitoring of Heavy Metal Concentration in Groundwater of Hakinaka Taluk, India. *Contemp Eng Sci*, 2010;3(4):183 – 190.
- [47] Cabaravdić M. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of cells to benzo(a) pyrene, *Medicinski arhiv*. 2010, 64(4): 215-218.
- [48] da Costa TC, de Brito KCT, Vaz Rocha JA, Leal KA, Rodrigues MLK, Minella JPG, et al. Runoff of genotoxic compounds in river basin sediment under the influence of contaminated soils. *Ecotoxicol Environ Safety*, 2012;75:63–72.
- [49] Ferettia D, Roberta P, Elisabetta C, Mario D G, Ilaria Z, Gaia Claudia VV, et al. “Risk is in the air”: Polycyclic aromatic hydrocarbons, metals and mutagenicity of atmospheric particulate matter in a town of Northern Italy (Respira study), *Mut Res Gen Toxicol*, 2019; En 842:35–49.
- [50] Zeyad MT, Kumar M, Malik A. Mutagenicity, genotoxicity and oxidative stress induced by pesticide industry wastewater using bacterial and plant bioassays. *Biotech Reports*, 2019;24: e00389.
- [51] Catalin AR, Elena B, Elena R, Liviu AO. Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*. *Int J Environ Res Public Health*, 2019;16:75.
- [52] Claudia R, de R. Sérgio MC, José LM, Claudia AFA, Israel F. Genotoxicity of Polycyclic Aromatic Hydrocarbons and Nitro-Derived in Respirable Airborne Particulate Matter Collected from Urban Areas of Rio de Janeiro (Brazil) *BioMed Res Int*, 2013; 765352.

Figures

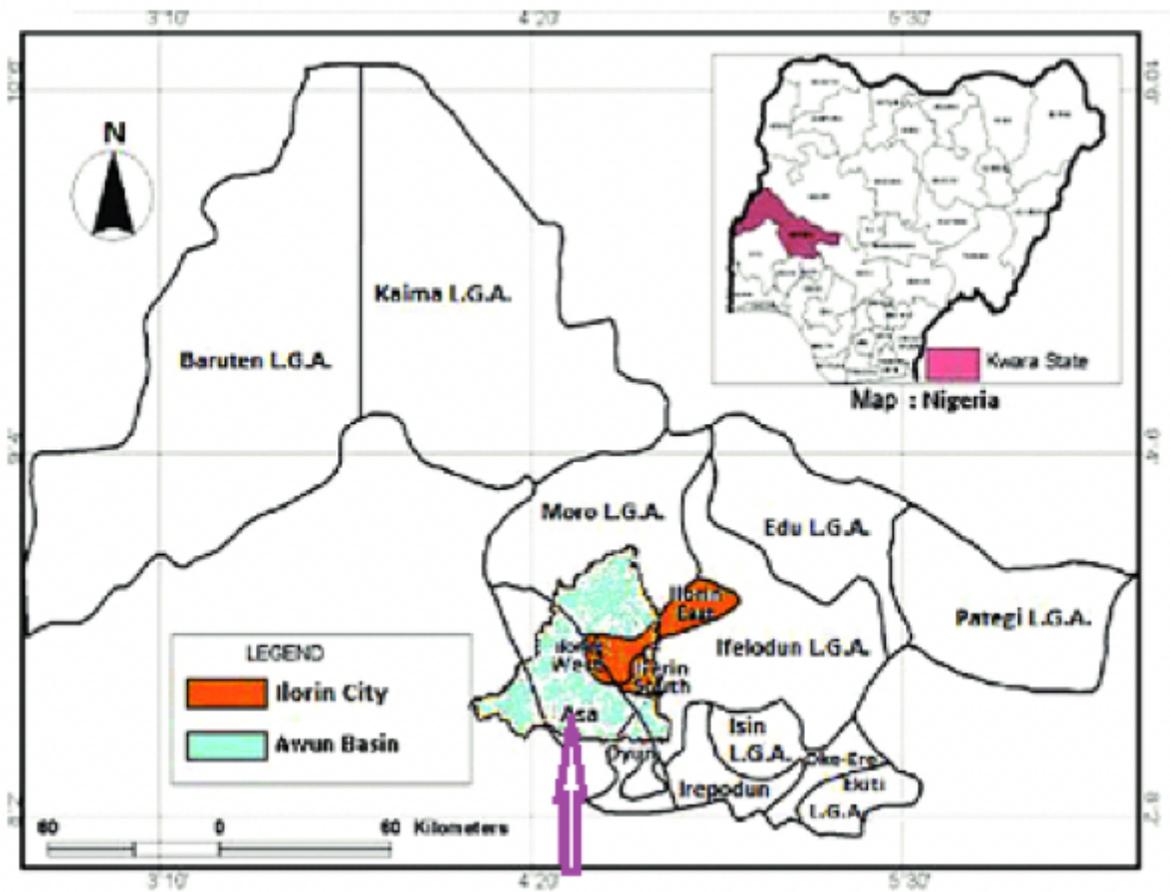


Figure 1

Maps of Nigeria and Kwara State showing Asa local government where Asa river is situated, Ayanshola et al. [22].



Plate 1: Sampling point A



Sampling point B



Plate 1: Sampling point C



irrigated maize farm with water from point C

Figure 2

Plate 1: Water sampling points in Asa River water body

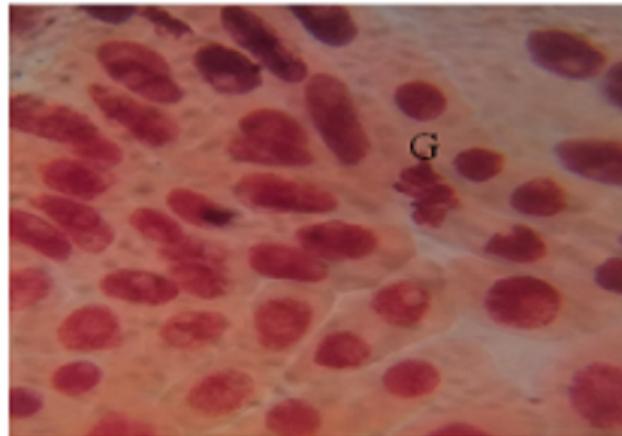
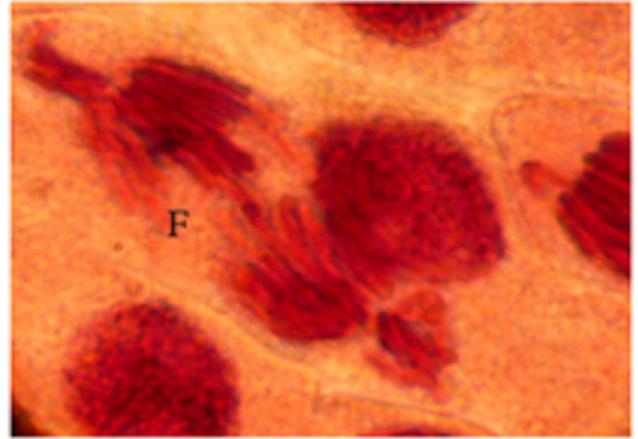
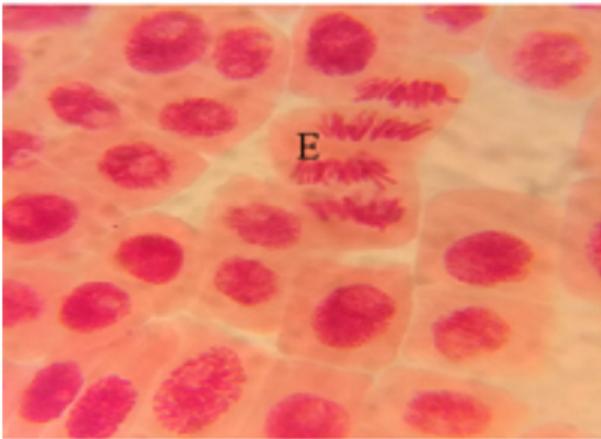
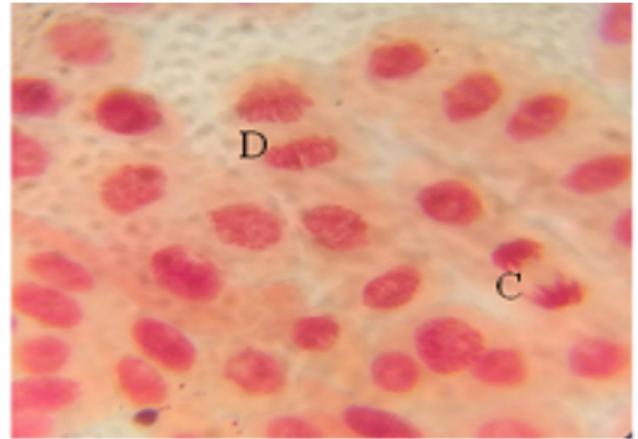
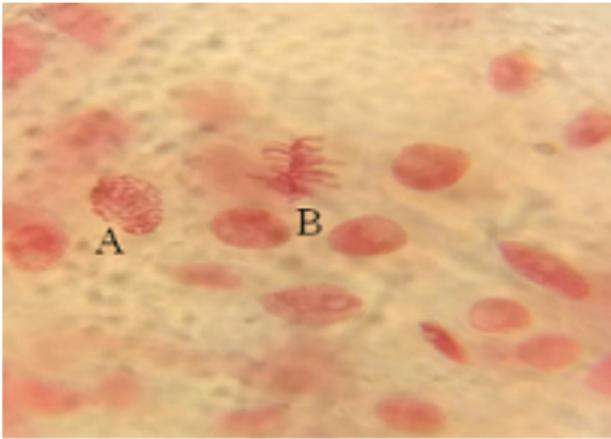


Figure 3

Plate 2: A = prophase; B = metaphase; C = anaphase; D = telophase; E = disturbed spindle; F = Bridged anaphase; G = sticky chromosomes