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Evaluation of Asa River Water in Ilorin, Kwara State, Nigeria for Available Pollutants and their Effects on Mitosis and Chromosomes Morphology in *Allium cepa* Cells

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ABSTRACT: This study evaluated water samples from Asa River in Kwara state, Nigeria, for cytogenotoxicity at 25.0 %, 50.0 %, 100.0 % following the *Allium cepa* assay. Onions were grown in the water samples for microscopic and macroscopic screenings. Heavy metals and volatile organic pollutants in the water were elucidated using AAS and GC-MS techniques. The Water samples except the sample 'C' induced higher mitotic index (MI) than the negative control. Root growth was significantly promoted at 25.0 %, and significantly reduced at 50.0 % and 100.0 % of the sample 'C' and 100.0 % of the sample 'A'. Water sample 'A' (100.0%) induced highest percentage chromosomal aberrations (CA) while the water samples 'B' and 'C' induced higher percentage CA than the negative control. Cadmium was detected at a concentration higher than its permissible limit in drinking water. Poly aromatic hydrocarbons, Aromatic amines, Acridine dye, Phenolic and Polychlorinated compounds were detected in the water sample. The observed proliferative, inhibitory, cytotoxic and genotoxic effects of the water samples on *A. cepa* cells suggest that Asa river was polluted, having potential to adversely affect humans, animals and plants utilizing it along its course.

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Pollution of surface water source occurs through release of industrial, agricultural and domestic wastes into water body (Guan et al., 2017). Contamination and pollution of aquatic environment has become a serious matter of concern globally due to loss of aquatic biodiversity (Hussein et al., 2016). Chemical compounds from different sources into water body were cytotoxic, mutagenic and genotoxic (Pellacani et al., 2016). Therefore, it is important that freshwater sources are regularly screened to ensure that drinking water and agricultural products being irrigated with water from these sources are free of contaminants and pollutants that might find their way into water body through series of anthropogenic activities. Asa river supplies water to majority of people of Ilorin and assessment of quality of this water body is necessary to ensure that its use for different purposes is not associated with adverse effects in humans, animals and plants (Balogun and Ganiyu, 2017). *Allium cepa* is one of the higher plants 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 (Leme and Marin-Morales, 2009). Interestingly, it produces comparable results with that from animal genetic assays. It can be used to screen cytogenotoxicity of single and complex chemicals (Bhat et al., 2017). *Allium cepa* assay was employed to evaluate cytogenotxicity of Sungai Dua river water in Pulau Pinang, Malaysia and Guaribas river water in Piauí, Brazil (Akinboro et al., 2011c; de Castro e Sousa et al., 2017). 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 Malete, Ilorin, Kwara State, Nigeria employed micronucleus assay and Ames test which can detect only clastogenic effect and point mutations caused by the environmental contaminants in this water body (Anifowose et al., 2018). This present study was undertaken on Asa River to screen for potential cytotoxic and genotoxic effects of its available chemicals contaminants / pollutants following the *Allium cepa* assay, and their identification using the AAS and GC-MS techniques.

MATERIALS AND METHODS

Collection of water samples: Water samples were collected strategically from three sampling points; Point 'A' which was close to a refuse dump site at the riverbank, point 'B' which was 500 m away from point

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A, point 'C' which was 500 m away from point B, and this was where farmers who have farms along the riverbank usually pump water to irrigate their farmlands (Plate 1). The collected water samples were kept in 4 liters capacity clean glass bottles stored at 4°C until further analyses.

Plate 1: Water sampling points in Asa River water body

Allium cepa Assay: Onions were commercially purchased, sun dried for 1 week and further treated as described previously (Akinboro *et al.,* 2011a). Absolute water sample 'A', 'B', and 'C' was diluted to 25.0% and 50.0% using borehole water. The negative and positive controls were borehole water and 0.1% hydrogen peroxide, respectively. Ten onions were planted on each water sample in 100 ml capacity beakers and placed in a cupboard for 48 hours and 72 hours for microscopic and macroscopic evaluations, respectively (Akinboro *et al.,* 2011b). Root tips from four onions were cut and fixed in ethanol acetic acid fixative (3:1) after 48 hours. Slides were prepared, observed and scored under light microscope (CH-China) using oil immersion objective lens (Akinboro *et al.,* 2017). Root lengths from the remaining onions were measured to determine toxicity of the water samples to *A. cepa* roots growth (Akinboro and Bakare, 2007; Verma and Srivastava, 2018).

Heavy metals analysis: Fifty milliliters of the water sample was added with 5 ml nitric acid and the mixture was subjected to heat for complete digestion (Mahugija, 2018; Aloke et al., 2019). 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 (Wold *et al.,* 2016). The analysis of the diluted filtrate for Cadmium (Cd^{+2}), Copper (Cu^{+2}), Nickel (Ni^{+2}), Zinc (Zn^{+2}), Lead (Pb^{+2}), Manganese (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.

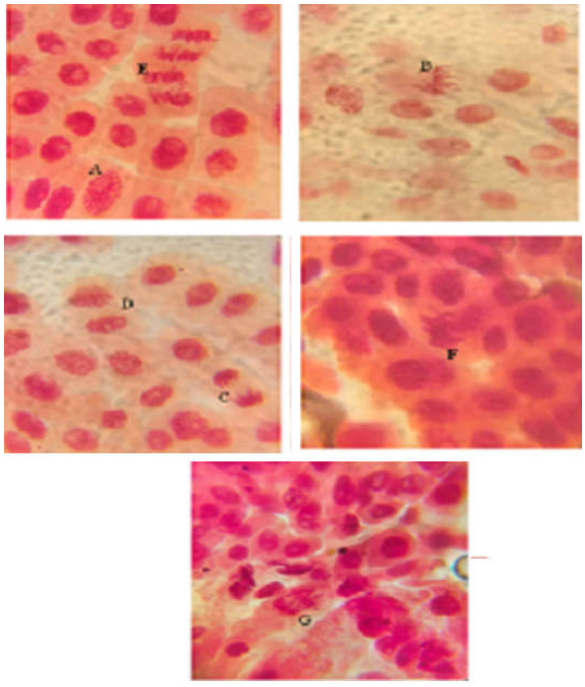

Plate 2: A = prophase; B = metaphase; C = anaphase; D = telophase; E = disturbed spindle F = sticky chromosome; G = anaphase bridge.

Gas chromatography- Mass spectroscopy (GC-MS) analysis: The volatile organic chemical compounds in the water sample were extracted by successive liquid-liquid extraction using analytical grade n-hexane solvent. The water sample was subjected to Gas Chromatography coupled with Mass Spectrometer (Agilent Technologies, Model GC7890A/5975MS, USA). The analysis was performed using ultra inert GC capillary column 30m × 0.53 mm I.D., 5.0 µm film thickness (Hewlett Packard, Palo Alto, USA) following the standardized protocol previously described (Akinboro *et al.,* 2012; Bell *et al.,* 2020). Individual chemical components in the water sample

were identified compared with the NIST Library and the percentage match of the identified chemicals was recorded.

Statistical analysis: The obtained data were summarized and mean values were compared with the negative control using Duncan multiple range comparison in one way ANOVA of SPSS (version 17.0). Significantly different value was considered at $P < 0.05$.

RESULTS AND DISCUSSION

The mitotic index (MI) value 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 sample 'B' also induced higher MI value of 1.50% at the selected concentrations, but not significantly different from that induced by the negative control ($P > 0.05$). The water sample 'C' had MI value of 1.40% at 25.0%, and 1.0% and 0.9%, respectively at 50.0% and 100.0%. These were not significantly different from MI induced by the negative control, except at 100% (Table 1). Water samples '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). Highest and significantly different CA of 2.2% was induced at 100.0% of the water sample 'A' ($P < 0.05$) (Table 1). Effects of the water samples on root growth of *A. cepa* showed that water sample 'A' at 25.0% and 50.0% induced root lengths of 127.60% and 106.60% which were higher than that of the negative control, like was also recorded for the water samples 'B' and 'C' at 25.0 % and 50.0 % 'B'. However, there was inhibition of root growth to 90% by the absolute water sample 'A', at 50% of the sample 'C', and 100% of the samples 'B' and 'C'. The root growth inhibitions caused by the water sample 'C' were significantly different from the negative control (Table 2).Cadmium (Cd^{+2}), Zinc (Zn^{+2}), Nickel (Ni^{+2}), Copper (Cu^{+2}), Manganese (Mn^{+2}), Lead (Pb^{+2}) and Iron (Fe^{+2}) were detected in the water sample in varied concentrations. Only Cd was detected at a higher concentration of 0.022 mg/L than its permissible limit of 0.003 mg/L in drinking water according to the WHO and EPA standards, while Zn, Mn and Fe were lower than their permissible limits (Table 3). Twenty two different chemicals compounds belonging to 5 groups of aromatic compounds were detected. These include Polycyclic Aromatic Hydrocarbons (PAH), polychlorinated compounds, aromatic amines, phenolic compound and acridine dye. The percentage abundance of the detected chemical compounds in the water sample ranged between 12 – 43% (Table 4). Induction of significantly higher mitotic index (MI) value at 100.0% 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 '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 indicate that the water body was contaminated and polluted. Both the significant increase and decrease in MI values compared with 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 the *A. cepa* cells (Leme and Marin-Morales, 2009). Induction of significantly higher chromosomal aberrations (CA) such as disturbed spindle, bridged anaphase and sticky chromosomes than that recorded with the negative control further corroborates that Asa River water was contaminated or /and polluted.

Induction of these forms of chromosomal aberrations implies that the water body 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. The results of root length measurement 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 of the negative control implied root growth inhibitory effect. However, induction of longer root lengths at lower concentrations of the water samples was in support of the proliferative activity of this water body, and further suggests its ability especially those 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* genetic assay have further confirmed pollution of Asa river as previously reported in the studies on heavy metals accumulation in some species of fish and microorganisms (Hussein *et al.,* 2016; Anifowose *et al*., 2019). It is now established that the use of polluted Asa river water to irrigate farmlands along the riverbank could cause stunted growth of crops based on the effects of the water sample 'C' on *A. cepa* root growth.

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

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

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

Table 2: Root lengths of *Allium cepa* 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 $P < 0.05$

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 observed proliferative, root growth promoting and inhibitory, cytotoxic and genotoxic effects of the water samples 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 body.

These 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 (Zeyad et al., 2019). Polycyclic aromatic hydrocarbons 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.

Cadmium (Cd^{+2}), Zinc (Zn^{+2}), Manganase (Mn^{+2}) and Iron (Fe^{+2}) were previously detected in the water sample at various concentrations (Anifowose et al., 2019).

Mercury, 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 (Ogunkunle et al., 2016). Heavy metals 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 (Matos et al., 2017).

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	% Matcl
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(diethylphosphonatomethyl) 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)-quinqzolinone	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-2amino-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

Apart from the refuses dumped at the riverbank of Asa River, fertilizer and pesticide application by farmers along the riverbank may be the source of heavy metals into the water body (Aliyu *et al.,* 2017). 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 was 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 the water body to roads with high traffic density, metal melting and electroplating, coal refining and oil fired power stations (Aloke et al., 2019). Environmental exposure to Cd at a high concentration is injurious to health as it can cause kidney and bone damages, neurotoxicity (Oluyemi and Olabanji, 2011; Chaitali and Jayashree, 2013).

Conclusion: These observed effects of the water samples on *A*. *cepa* cells in this study suggest that Asa river was polluted, and its use as drinking water sources and to irrigate farmlands may be associated with adverse effects in human, animals and plants that utilize it. For the first time, our results have suggested the possible effects of this water body on plant cells including its potential to cause different types of chromosomal aberrations using the *A*. *cepa* assay.

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