CYTOGENOTOXICITY ASSESSMENT OF BOREHOLE WATER SOURCES IN DENSELY POPULATED LOCAL GOVERNMENT AREAS OF LAGOS STATE, NIGERIA USING THE *Allium cepa* TEST

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Abstract: In recent time, increased population in Ikotun, Ikeja and Alimosho local government areas of Lagos State, Nigeria is becoming alarming as this is expected to create pressure on the facilities needed for the basic day-to-day activities of inhabitants to these areas. The cytotoxic and genotoxic potentials of borehole water in these areas were investigated using the *Allium cepa* test. Water samples were collected from three points in each local government. Ikotun (Governor's road, Arida area and Igando Road), Ikeja (Balogun area, Oba Akran area and Opebi road) and Alimosho (Ponle area, Williams layout and Alaguntan area) water. The result showed that test water mitotic index decreased significantly ($p<0.05$) from control.The water samples were characterized by a number of chromosomal aberrations notably bridges, fragments, sticky chromosomes, disoriented chromosomes, and binucleated cells in significant amounts and these were more pronounced in water samples obtained from Ikotun local government (Governor's road, Arida area and Igando Road). The findings in this study are of public health relevance as access to safe water is a fundamental human need and therefore, a basic human right.

Keywords: Allium cepa, borehole water, cytogenotoxicity, public health

Introduction

Lagos state, Nigeria is the most populous city in West Africa, with an estimated population of 7,552,942 in 2006 census report (Ometan *et al.*, 2012). It is the fastest growing city in Africa and the seventh in the world. Lagos is well endowed with water resources, both surface and underground, however it contains over 40% of Nigeria's manufacturing activities with the highest level of emission of 8000 tons of hazardous waste per year (Ekiye *et al.*, 2010). There is need to safeguard it from deterioration arising from anthropogenic activities like agriculture, transportation, fishing, industrial and domestic uses that readily yield pollutants which get into freshwater bodies to contaminate them. One of the primary goals of the World Health Organization (WHO) and its Member States is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water" (Olorunfemi *et al.*, 2014). The most effective way to protect the quality of drinking water is through consistent monitoring of the drinking water supply (Yassi *et al.*, 2001). In this study, we used the *Allium cepa* test to evaluate the cytogenotoxic potentials of borehole water supply used for domestic and commercial purposes in Ikotun, Ikeja and Alimosho local governments of Lagos State.

Materials and Methods

Sample collection

Small sized onion bulbs (sizes between 3.5 and 4.0 cm in diameter) were purchased at Bariga Market, Lagos state, Nigeria. They were sun dried for use. Borehole water samples were collected from Ikotun, Ikeja and Alimosho local government areas of Lagos state. Three water samples were collected from each local government. For Ikotun local government; water sample was collected from Governor's road, Arida area and Igando Road. For Ikeja local government; water sample was collected from Balogun area, Oba Akran area and Opebi road. For Alimosho local government; water sample was collected from Ponle area, Williams layout and Alaguntan area. In total, the samples were collected from 9 areas in 3 local government of Lagos State. 1 litre of each sample was collected into a separate clean plastic bottle and transported to the Cell Biology and Genetics laboratory in University of Lagos for analysis.

Preparation of samples for analysis

For each sample, 200 ml was poured into a glass container. The plastic bottle of each water samples was shaken by hand before dispensing into the glass containers. For control, 200 ml Eva bottled water was used. All samples were prepared in triplicates including the control.

Viability test of Allium cepa

The viability assay was carried out as described by Fiskesjo (1993) and Olorunfemi *et al.* (2011). Outer loose scales of the dried onion bulbs were carefully removed before use, the dry brownish roots at the bottom were scraped without damaging the ring of the root primordial. The onion bulbs were introduced to distilled water for 24 h in optimal condition to determine the viable onions. The onions that sprouted properly were used to carry out the experiment while those with poor or no growth were discarded.

Root length and root length inhibition assay

The viable onion bulbs were transferred into the various glass container of the water sample. The root growth inhibition assay was performed (Samuel *et al.*, 2010). At the end of the exposure period, the root lengths of the onion bulbs for each borehole water were measured after 72 and 96h. The root length was measured using a measuring tape and was expressed in centimeters (cm).

$$\% \text{ Root length inhibition} = \frac{\text{Root length in control} - \text{Root length in test solution}}{\text{Root length in control}} x\,100$$

Cytological/chromosomal aberration studies

The control and treated roots from all water samples were harvested after 72 and 96 h of exposure and were introduced into the fixative immediately (aceto-alcohol 1:3) until slide preparation was carried out in order to arrest mitosis (Fiskesjo, 1987). The prepared slide was placed on the stage of the microscope and viewed under the x40 objective to observe its mitotic stages. The total number of cells, total dividing cells and cells with chromosomal aberrations were taken for each of the different treatments and control. Photomicrographs of normal and aberrant dividing cells were taken.

Mitotic index

This was determined according to Fiskesjo (1993), after 72 h about 896 to 1124 cells were counted and after 96 h 951 to

1110 cells were counted and the cells showing the different phases of mitosis were recorded. The mitotic index was calculated as:

$$\text{Mitotic index} = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cell\ counted} x\,100$$

Scoring for chromosomal aberrations

This was done according to the method described by Fiskesjo (1997) and Rank (2003). The various aberrations were characterized by different parameters such as bridges, fragments, binucleated cells, laggards, and sticky chromosomes. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each water sample (Bakare and Wale-Adeyemo, 2004).

Percentage (%) chromosomal aberration

To determine the percentage chromosomal aberration, the number of total aberration was divided by the total dividing cell and multiplied by 100. It was calculated as:

$$\text{Percentage aberration} = \frac{Number\ of\ total\ chromosomal\ aberration}{Total\ number\ of\ dividing\ cells} x\,100$$

Statistical analysis

Data were presented as mean ± Standard Error (SE) of Mean for each water sample. Statistical significance was estimated at 0.05 level of probability using ANOVA. All analyses were carried out using SPSS 20.0 statistical package.

Results and Discussion

Root growth assay

At 72 h, the lowest mean±SEM root length was 0.56±0.17 cm while the highest was 2.44±0.17 cmfor Igando and Oba Akran, respectively (Table 1). After 96 h of exposure to the different borehole water samples, the least mean root length was 0.86±0.21 cm while the highest mean root length was 3.90±0.17 cm for Igando and Opebi, respectively. The control values were higher that the water samples with mean root length of 3.03±0.04 and 4.06±0.04 cm for 72 and 96 h, respectively. All the borehole water samples showed significant differences lower from control except for Ponle, Opebi and Oba Akran at 72 h and Alaguntan, Balogun and Opebi at 96 h.

Table 1: **Root growth of *A. cepa* in the borehole water samples from different areas**

Areas	Mean Root Length(cm)	
	72 h	96 h
Control (Eva)	3.03±0.04	4.06±0.04
Governor	2.09±0.23*	1.60±0.24***
Arida	1.308±0.27***	1.12±0.16***
Igando	0.56±0.17***	0.86±0.21***
Williams	1.89±0.29**	0.89±0.18***
Ponle	2.39±0.15	2.65±0.34***
Alaguntan	1.86±0.18**	3.35±0.31
Balogun	2.04±0.17*	3.83±0.07
Opebi	2.2±0.17	3.90±0.17
Oba Akran	2.44±0.17	2.10±0.19***

Mean±SEM root growth of *A. cepa;* *p<0.05; **p<0.01; ***<0.001

Table 2: Percentage root length inhibition after 72 and 96 h of exposure to different borehole water samples

Areas	% Inhibition	
	72 h	96 h
Governor	30.17	60.42
Arida	53.86	72.23
Igando	70.17	78.68
Williams	36.75	77.92
Ponle	20.35	34.77
Alaguntan	35.06	17.30
Balogun	31.83	5.51
Opebi	25.86	3.81
Oba Akran	18.62	48.19

Percentage (%) inhibition after 72 and 96 h

Table 3 Showed the mitotic activity of the water samples in *Allium cepa* there were more dividing cells observed at the control after 96 h than after 72 h, at prophase, more cell were observed at 72 h except for Area Balogun at 96 h. At metaphase, more cells were observed after 96 h and the highest number of cell was observed at Area Balogun. There was no difference in the number of metaphase in Area Arida. At anaphase, the frequencies of cells observed were higher at Areas of Balogun and Opebi. At telophase, more cells were seen at Area Balogun at both 72 and 96 h with 20 and 16 numbers of cells, respectively.

Table 4 showed the mitotic index of onion cells after exposure to different borehole water samples. The mitotic index decreased with increase in concentration. The mitotic index of the control; 7.74% and 8.90% at 72 and 96 h, respectively were higher than the sampled water. The closest values were seen at Area Balogun with mitotic indices of 7.36% and 6.76% at 72 and 96 h, respectively. The lowest values of 3.29 % and 2.62% at 72 and 96 h, respectively were seen at Area Arida.At Areas with higher mitotic indices, the numbers of abnormal chromosomes observed were lesser after 72 and 96 h while the reverse was the case for areas with less mitotic indices. The highest numbers of abnormal chromosomes were recorded in Arida and Igando while Opebi recorded the least number of aberrations.

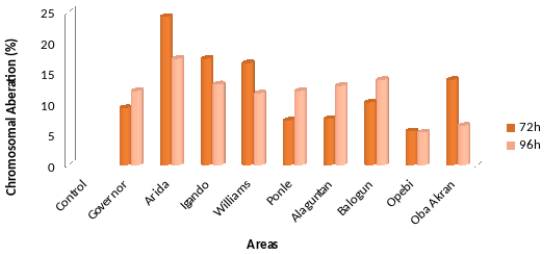

Fig. 1: Percentage chromosomal aberration of onion cells exposed to different borehole water samples

The study showed that all the tested borehole water samples induced a significant inhibition in mean root growth compared to control. However, there were variations in their mean root length at both 72 and 96 h but it was observed that the root growth inhibition became more pronounced at Arida and Igando areas (Ikotun local government) and Williams (Alimosho local government) as well as decrease in mitotic activity and the presence of aberrant chromosomes. Significant number of aberrations were more observed in Governor, Arida and Igando Area.

Table 3: The mitotic activity in the *Allium cepa* root cells exposed to borehole water samples

Area	Number of cells counted		Numbers of dividing cells		Prophase		Metaphase		Anaphase		Telophase	
	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Control	1124	1011	87	90	21	24	24	26	20	20	22	20
Governor	1108	1110	46	32	15	10	11	9	8	6	12	7
Arida	1003	1105	33	29	9	8	9	9	7	5	8	7
Igando	1021	1017	46	38	12	12	11	10	9	6	14	10
Williams	928	1005	36	28	10	6	7	6	8	7	11	9
Ponle	1101	983	54	41	15	10	14	11	10	8	15	12
Alaguntan	1103	951	33	61	7	16	9	15	8	16	9	14
Balogun	896	1005	66	68	16	19	14	18	16	15	20	16
Opebi	1020	1008	59	64	17	15	15	17	14	16	15	15
ObaAkran	899	1022	62	36	19	11	14	9	16	9	13	7

Table 4: Percentage aberration after 72 and 96 h of exposure to different borehole water samples

Area	Number of cells counted		Numbers of dividing cells		Mitotic index (%)		Chromosomal aberration		% Chromosomal aberration	
	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Control	1124	1011	87	90	7.74	8.9	0	0	0	0
Governor	1108	1110	46	32	4.15	2.88	9	10	9.33	12.12
Arida	1003	1105	33	29	3.29	2.62	9	9	24.24	17.39
Igando	1021	1017	46	38	4.5	3.73	10	8	17.39	13.20
Williams	928	1005	36	28	3.87	2.78	6	6	16.66	11.76
Ponle	1101	983	54	41	4.9	4.17	4	5	7.31	12.12
Alaguntan	1103	951	33	61	2.99	6.41	6	8	7.57	12.96
Balogun	896	1005	66	68	7.36	6.76	5	6	10.25	13.95
Opebi	1020	1008	59	64	5.78	6.34	3	4	5.56	5.35
Oba Akran	899	1022	62	36	6.89	3.52	8	5	13.95	6.45

Table 6: Types of chromosomal aberration of onion cells exposed to different borehole water samples

Area	Stickiness		Vagrant		C mitosis		Bridges		Binucleated cells		Total		Mean±
	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	SEM
Control	0	0	0	0	0	0	0	0	0	0	0	0	0
Governor	2	2	1	1	1	1	3	3	2	3	9	10	1.9±0.07**
Arida	1	1	2	2	2	2	3	2	1	2	9	9	1.8±0.20**
Igando	2	1	3	2	2	2	3	1	0	2	10	8	1.8±0.29**
Williams	0	0	2	1	1	1	2	2	1	2	6	6	1.2±0.24*
Ponle	1	1	0	2	1	1	2	1	0	0	4	5	0.9±0.23*
Alaguntan	1	1	0	2	1	1	3	2	1	2	6	8	1.4±0.26*
Balogun	1	0	0	1	2	2	2	2	0	1	5	6	1.1±0.27*
Opebi	0	1	1	1	1	1	1	1	0	0	3	4	0.7±0.15*
Oba-Akran	2	1	1	1	1	0	2	1	2	2	8	5	1.3±0.21*

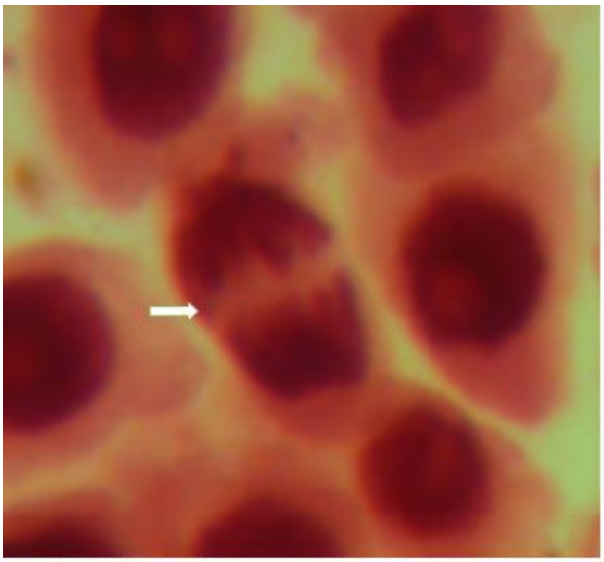

Plate 1. lagging chromosomes at anaphase

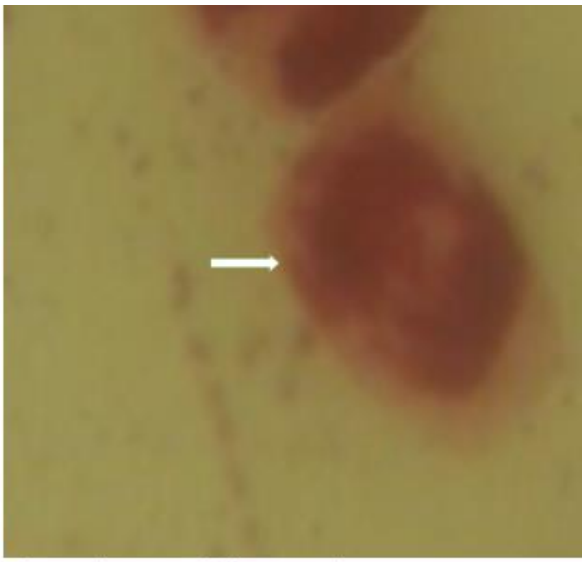

Plate 2. chromosome bridge at anaphase

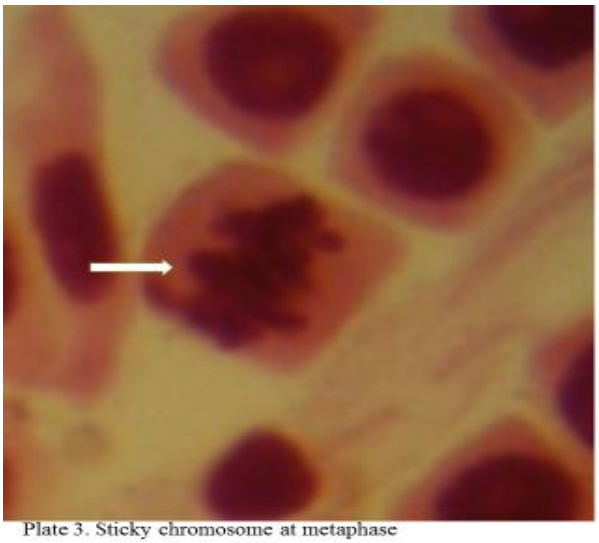

Plate 3. Sticky chromosome at metaphase

Plate 4. Binucleated cell

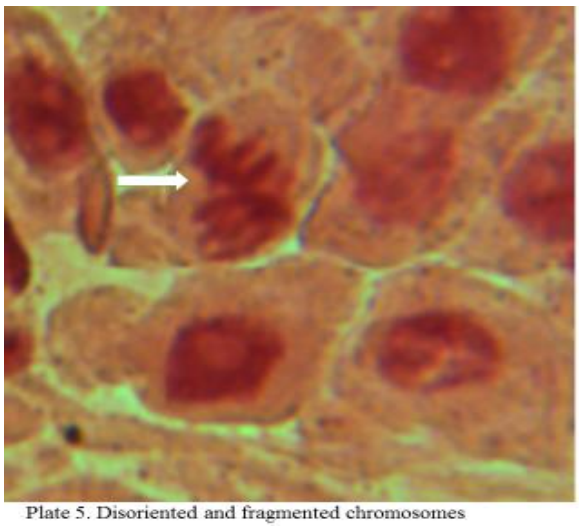

Plate 5. Disoriented and fragmented chromosomes

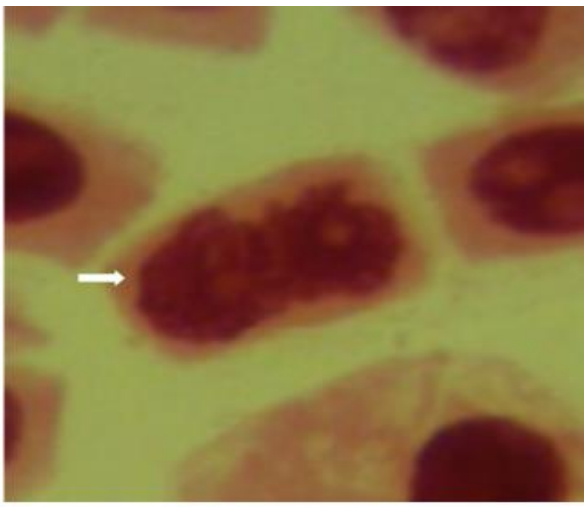

Plate 6. Sticky chromosomes at prophase

Monitoring of underground water in Nigeria and in most developing countries with high pollution levels of the aquifer is mostly restricted to chemical and microbial analysis (Adewoye *et al*., 2011; Rossi *et al*., 2012). These methods of analyses cannot provide information on all the toxic chemicals present in the water samples and the potential synergistic and antagonistic interactions of these chemicals and microbes in living systems. A more comprehensive approach involving *A. cepa* root tip meristems have been widely used and accepted for the evaluation of cytotoxic and genotoxic activities of different physical, chemical and microbial substances in wastewater and underground water (Chandra *et al*., 2005; Akinbola *et al*., 2011; Bakare *et al*., 2013). The *Allium cepa* test is adequately sensitive to detect innumerous substances that cause chromosomal alterations has been used by many researchers mainly as a bioindicator of environmental pollution (Bagatini *et al*., 2009; Leme & Marin-Morales, 2009). Chromosomal aberrations are considered as end result of genotoxic effects of various physical and chemical agents and are also estimates of exposure of various organisms to different physical and chemical agents (Pohren *et al*., 2013). Based on microscopic (cytogenetic effect) and macroscopic (growth inhibition effect) evaluations of the various borehole waters analyzed, abnormalities like sticky chromosomes, binucleated cells, laggards, fragments, disturbed metaphase and bridges were observed. These effects could result in a higher risk of bringing about drinking water contamination. This provides evidence that contaminated borehole water inhibit root growth (causing growth retardation), which juxtapose with the findings of Odeigah *et al*. (1997). The number of aberrant mitotic cells caused by all water samples from the boreholes was apparently different from that of the control. No aberration was observed in the *A. cepa* exposed to the control. It is possible that the observed cytogenotoxicity in the root meristems in *A. cepa* were possibly induced by chemicals and microorganisms likely to be present in the water samples, thus may portends a direct or indirect risk to living organisms. Bridges probably occur by the interruption and joining chromosomes or chromatids (Turkoglu, 2007), or as a result of chromosome stickiness, or it may be ascribed to unequal translocation or inversion of chromosome segments (Gomurgen, 2005). According to Fiskesjö (1997) and Babatunde and Bakare (2006), whenever there is root growth inhibition in *A. cepa*, there is always reduction in the number of dividing cells; the lower the mitotic index, the more toxic the waste water, chemical or pollutant.

The *Allium* test has often been used to determine the cytogenotoxic effects of deleterious chemicals (Akinbola *et al*., 2011; Fiskesjo, 1997) due to its cell cycle duration and its reactions in the presence of known mutagenic agents (Evseeva *et al*., 2003). Most of the observed aberrations in the cytology of the *A. cepa* root meristems (as shown in the photomicrographs) indicate that the possible chemicals and microorganisms in the borehole water samples induced the

observed cytotoxic/genotoxic effects and this may present a direct or indirect risk to living organisms. This alluded to the fact that there is very high population of people due to urbanization in Arida and Igando both in Ikotun local government hence increased anthropogenic activities leading to heavy environmental pollution. Williams area in Alimosho local government also recorded high pollution as reflected in the aberrations observed in the *A. cepa* cells. The presence of laggards, sticky chromosomes and Anaphase Bridge were regarded as mitotic irregularities induced by pollutants (Zhang and Yang, 1994). Sticking of chromosomes probably occurs due to degradation or depolymerization of chromosomes DNA (Grant, 1982) or as a result of DNA condensation and stickiness of inter-chromosome fibres (Schneiderman, 1971). Stickiness reflects high toxicity of substance as well as irreversibility of the change. Bridges probably occur by the interruption and joining chromosomes or chromatids (Turkoglu, 2007), or as a result of chromosome stickiness, or it may be ascribed to unequal translocation or inversion of chromosome segments (Gomurgen, 2005). The most effective way to protect the quality of drinking water may include consistent biomonitoring of the water supply using plant and animal models.

In conclusion, the pollution of borehole waters observed both in Ikotun and Alimosho local government area may be in tandem with the rapid and astronomic increase in population of migrants in these areas and there consequent commercial activities for survival. Different wastes (liquid, solid gaseous) released into the environment be may serve as clastogens that caused reduction in mitotic index and chromosomal aberrations in cells. This study is of public health relevance as DNA damage in eukaryotic systems is associated with diseases. Besides, water is major route to water borne diseases and therefore, proper borehole water management and treatment should be put in place to ensure safety of both human and animals.

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