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ASSESSMENT OF DNA DAMAGE IN ONION ROOT CELLS EXPOSED TO CONTAMINATED RURAL WATER SOURCES USING RAPD MARKERS

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ABSTRACT: Recent advances in molecular biology have led to the development of a number of molecular marker assays for DNA variation analysis in eco-genotoxicology. In this study, the random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in root tip meristems of *Allium cepa* L. cultivated in contaminated drinking water samples obtained from lakes and boreholes in two rural communities in Edo State of Nigeria. Compared to the control (tap water), the drinking water samples in the rural settlements caused greater changes in the RAPD patterns. DNA polymorphism/damage became evident as the presence and/or absence of DNA fragments in the test samples. The number of disappearing bands in profiles increased from 33 of dry season lake water to 45 in the wet season compared to total bands in control. The results showed that it is possible that the detected DNA polymorphism could lead to genotoxic effects in the treated plants due to chemical substances present in drinking water samples. Consequently, the use of water from both the lakes and boreholes for domestic purposes could pose health hazard to humans and the environment.

Keywords: rapd, DNA damage, *Allium cepa*, rural communities, drinking water

INTRODUCTIONS:

In Nigeria, the provision of pipe borne potable water is the primary responsibility of the Federal, State and Local Governments but unfortunately, access to drinking water is grossly inadequate both in quantity and quality in both rural and urban areas of the country (Onokerhoraye, 1995). The problem is more pronounced in the rural areas, leaving the inhabitants no choice but to result to streams, hand-dug wells, rivers, ponds and lakes for their drinking water supply. These water sources are under threat from pollution from either human life style manifested by the low level of hygiene practiced or through non-point source when already polluted water in the area enters into the ground water body by lateral or side movement (Wright *et al.*, 2004). Obazuwa and Ozomu communities are rural settlements located within latitude 5°E, 6°E and longitude 5 °N, 7°N, in Ovia North-East Local Government Area of Edo State. Their main sources of drinking water are boreholes and man-made lakes (one in each community) constructed by the inhabitants through self-help. The communities are characterized by two major tropical climates marked by two seasons (rainy and dry) which run from April to September and October to March respectively.

The complexity of contaminated water makes it almost impossible to carry out a hazard assessment based on physicochemical analysis alone, therefore, a comprehensive approach involving the use of higher plant bioassays alongside chemical and microbial analysis has been advocated for hazard assessment in toxicity screening of contaminated water (Arkhipchuk *et al.*, 2000).

The limitations notwithstanding, higher plants provide a useful genetic system for screening and monitoring environmental pollutants *in situ* and *ex situ* with numerous advantages. Their genotoxicity assays offer attractive alternative to mammalian and non-mammalian animal assays in mutagen screening programmes (Grant, 1994). They are good indicators of cytogenetic and mutagenic effects (Constantin and Owens, 1982).

Among the seven plant bioassays reviewed by the US Environmental Protection Agency EPA Gene-Tox program in 1980, the *Allium* root tip chromosome aberration assay, a higher plant genetic assay was used as an alternative first–tier indicator for safety evaluation of cytogenetic and mutagenic effects, was one of the protocols adopted and standardised by the International Program on Plant Bioassays (IPPB) for monitoring or testing environmental pollutants, which is currently in operation under the auspices of the United Nations Environment Program UNEP (Ma, 1999). The advantage of this test in comparison with others is that it does not require preliminary processing of water samples for establishing toxicity and genotoxicity (Grant, 1994).

Results obtained on the toxicity assessment of water quality of the domestic water sources in Obazuwa and Ozomu communities undertaken by Olorunfemi *et al*. (2014) showed that although the study did not obtain documented evidence on the incidence of major water borne disease outbreak in the communities, the concentrations of physicochemical constituents and microbial load of the water samples were higher than permissible national (Standards Organisation of Nigeria) and international (World Health Organisation) limits (Table 1). In addition, the classical *Allium* chromosomal aberration conventional test used in the study to detect environmental clastogens revealed that, compared with the control,

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there was significant reduction in mitotic index and increased chromosome aberrations in the water samples. The observed frequencies of chromosomal bridge, sticky and laggard chromosomes were indicators of chromosome damage in the water samples.

Table 1.

Physicochemical properties of water samples obtained from the study site (Obazuwa) during dry and wet seasons

Parameters	Lake water (Dry season)	Lake water (Wet season)	Borehole water (Dry season)	Borehole Water (Wet season)	WHO (2006) Limit	SON (2007) Limit
pH	5.15	5.80	6.45	7.59	6.5-9.5	6.5-8.5
Turbidity	13.1	10.5	11.3	10.1	-	-
Conductivity	1350	1540	1420	1040	-	-
Total Hardness	263.33	326.66	236.66	216.66	500	150
Nitrates	100.9	99.3	50.8	29.0	50	50
Phosphates	145.25	123.25	80.05	97.65	-	-
Magnesium	163.9	170.8	59.5	71.3	-	0.20
Chloride	798.75	763.25	745.50	305.75	-	250
Manganese	4.1	5.9	-	0.1	-	0.20
Nickel	0.2	0.3	-	-	0.02	0.02
Cadmium	0.1	0.2	-	-	0.003	0.003
Lead	0.2	0.1	0.1	-	0.01	0.01
Iron	11.1	15.3	-	1.3	-	0.3
Zinc	21.6	30.5	2.2	2.6	0.01	3.0
Chromium	2.4	3.0	-	-	0.05	0.05
Copper	2.8	1.8	1.8	0.1	-	0.1
Silver	0.2	0.1	-	-	-	-
TH Bacteria	5.4	2.6	4.1	3.3	0	0
Coliform Organisms	10	2.0	6.4	4.6	0	0

All values are in mg/L except conductivity (µscm-1),temperature (°C), turbidity (NTU), pH (no unit), total heterophilic bacteria (cfu/g) x105 and coliform organisms (MPN/100ml)

Recent advances in molecular biology have led to development of the number of selective and sensitive assays for DNA analysis in eco-genotoxicology. Among the DNA based techniques, RAPD is used to evaluate the variation at the DNA base pairs level. RAPD is a reliable, sensitive and reproducible assay has the potential to detect a wide range DNA damage, as well as mutation due to heavy metal stress and therefore, it can be applied to study the genotoxicity (Atienzar and Jha, 2004). Random amplified polymorphic DNA (RAPD) technique has been used to detect the differences in DNA fingerprints generated between control and heavy metal (genotoxic agents) treated plant seedlings in the recent past (Liu *et al.*, 2007; Enan, 2006).

Currently, there is no documentation on the genotoxicity evaluation of the drinking water sources in the rural communities using molecular markers, therefore, this study was undertaken to determine whether the RAPD assay could detect DNA damage in onion roots grown in the drinking water sources in these rural settlements.

MATERIALS AND METHODS:

Collection of water samples

Water samples for the study were collected in March and July 2012 from Obazuwa and Ozomu communities are rural settlements located within latitude 5°E, 6°E and longitude 5 °N, 7°N, in Ovia North-East Local Government Area of Edo State, Nigeria (Fig. 1). The lake water samples were collected at different depths from five random points within the lake. Water samples were collected from the boreholes following established protocols (Claaasen, 1982; Barcelona et al., 1985; APHA, 2005). The nozzle of the borehole was swabbed with cotton wool soaked in 70% (v/v) ethanol and flamed for 2-3 min. Samples were collected using washed and sterilized plastic containers after running water to waste for 4-5 min. In both cases, samples were taken in triplicates from each sampling point aseptically into plastic containers and kept in an ice chest and stored in the refrigerator at 4 °C and analyzed within 24 h of collection.

Plant Material and Treatment

The purple variety of average sized onion bulbs, *Allium cepa* L. (about 30 g, 15-22 mm diameter) were purchased from a local market in Benin City, Nigeria (6°15′N, 5°25′E) and sun-dried for two weeks and the dried roots present at the base of the onion bulbs were carefully shaved off with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primordial cells from drying up. The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water. Seven onion bulbs were utilized for each water sample and the control (tap water). The base of each of the bulbs was suspended on the water sample inside 100 ml beakers in the dark for 7 days. Test samples were changed daily. At the end of the exposure period, the roots with the best growth were removed with a forceps and utilized for chromosomal DNA extraction and RAPD analysis.

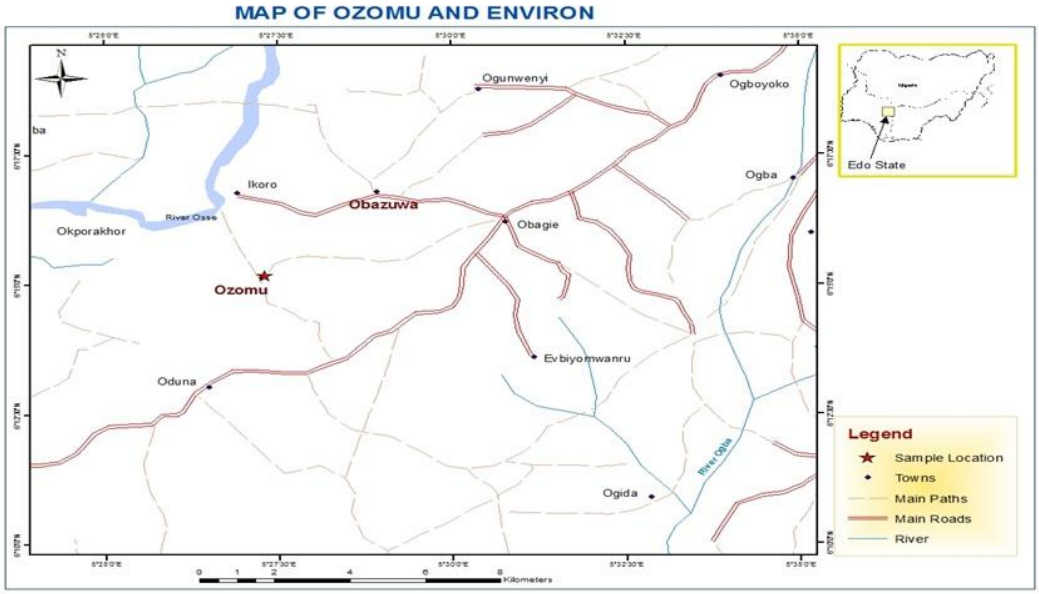

Fig 1: Map showing location of sample collection

DNA Extraction

After one week of growth, approximately 1.5-3.5 cm of the onion roots were collected, ground in liquid nitrogen, and total genomic DNA was isolated by a CTAB method based on that of Padmalatha and Prasad (2006) method with minor modifications (Qari, 2010). Purity of DNA was determined by measuring its optical density in spectrophotometer at 260 nm/280 nm ratios and quality of DNA samples was checked by loading them on 0.8% agarose gel and observing it on UV illuminator.

RAPD Fingerprinting

The conditions of DNA amplification were followed the procedure of Williams *et al.* (1990) with some modifications (Qari, 2010). Random Amplified Polymorphic DNA (RAPD) was performed using five primers OPBO6 (5'-TGCTCTGCCC-3'), OPBO7 (5'-GGTGACGCAG-3'), OPB08 (5'-GTCCACACGG-3'), OPB11 (5'-GTAGACCCGT-3') and OPD03 (5'-GTCGCCGTCA-3') (Operon technologies Inc., Alameda, California, USA) for each amplification. Each reaction (25 µL) consisted of 1 mM of $MgCl_2$, 4 mM each of dATP, dCTP, dGTP, dTTP (Boehringer-Manheim, Germany), 400 nM primer, 1.0 U of Taq DNA polymerase (Appligene-Oncor, France), reaction buffer (1 mM MgCI2, 20 mM Tris HCI pH 8.0, ethylenediaminetetraacetic acid (EDTA) 1 mM, dithiothreitol 1 mM, glycerol 50%). The reaction mixture was overlaid with a drop of mineral oil and incubated in a thermal cycler (thermal cycler 480, Perkin Elmer-Cetus, USA) programmed as follows: 48 cycles of 1 mm denaturation at 94°C, 1 mm annealing at 37°C and 1.5 mm extension at 72°C, followed by final extension at 72°C followed by a cooling at 4°C. Tubes containing all reaction products except template DNA were used as negative control.

PCR reaction products were mixed with one-sixth volume of gel loading buffer (analytical grade water containing 36% glycerol, 0.05% bromophenol, 30 mM EDTA and 0.05% xylene cyanol), and then separated by electrophoresis in a 2.4% agarose gel, using a Tris-borate-EDTA (TBE) system (0.5 × TBE = 45 mM Tris-base, 45 mM boric acid, and 1 mM EDTA). Agarose gel dimensions were 12×6×0.5 cm^3. For comparison, DNA molecular size marker (1 kb) was used for each agarose gel.

Statistical Analysis

The data were expressed as mean±SD. The differences between mean values and the controls were statistically investigated using student t-tests. Genomic template stability (%) was calculated as 100 − (100 **a/n**), where **a** was RAPD polymorphic profiles detected in each sample treated and **n** the total number of bands in the control. Polymorphism observed in RAPD profiles include disappearance of a normal band and appearance of a new band in comparison to control RAPD profiles (Williams *et al.*, 1990; Atienzar *et al.*, 1999) and the average was then calculated for each experimental group exposed to different drinking water sample treatments.

For the Rescaled Distance Cluster Combine, the SPSS version 16.0 Statistical package for Windows was used to construct the UPGMA dendrogram.

RESULTS AND DISCUSSION:

The range of the purity of DNA extracted from root tips of onion bulbs grown in the drinking water samples from Ozomu and Obazuwa lakes and boreholes during the dry and rainy seasons and control was in the range of 1.50 - 1.85.

Result of the RAPD profile of nucleotide sequence of the five primers used is presented in Table 2. The RAPD profile obtained with the five oligonucleotide primers used produced bands between 100 and 1200 bp in length (Plates 1-5). In all, 111 bands were scored, 57 (51%) were polymorphic. The plants treated with dry season water samples yielded a larger number of fragments (11) compared with those treated with wet season water samples which yielded a total of 9 new bands. Similarly, the total number of disappeared bands was found to be 16 in plants treated with dry season water samples, while a total of 9 bands where

found missing in plants treated with wet season water samples. However, Ozomu lake dry season samples (OZLD) gave the highest number of missing bands of 7, followed by Ozomu lake wet season samples (OZLW) and Ozomu borehole dry season samples (OZBD) with 5 missing bands each (Table 3).

Table 2:
Nucleotide sequence of the five primers used showing the total bands and the polymorphism in percentage as calculated from control and treated samples

RAPD Primers	Sequence 5'----3'	G-C Content (%)	Total Bands	Polymorphism (%)
OPB06	TGCTCTGCCC	70	18	48
OPB07	GGTGACGCAG	70	28	58
OPB08	GTCCACACGG	70	25	61
OPB11	GTAGACCCGT	60	11	27
OPD03	GTCGCCGTCA	70	29	54
Total			111	

Table 3:
Changes in RAPD profiles scored compared with control

Source of Water	Wet Season		Dry Season	
	RAPD Band Gain (+)	RAPD Band Loss (-)	RAPD Band Gain (+)	RAPD Band Loss (-)
Ozomu Lake	4	5	4	7
Ozomu Borehole	3	3	1	5
Obazuwa Lake	1	1	4	2
Obazuwa Borehole	1	3	2	2
Total	9	12	11	16

Contaminated drinking water has been implicated in chromosome aberration induction in *Allium cepa* genotoxicity assay in precious studies with borehole water (Olorunfemi *et al.*, 2012; Olorunfemi *et al.*, 2013), hand-dug water (Olorunfemi and Osaretin, 2012) and more recently in drinking water sources in Obazuwa and Ozomu communities in Edo State of Nigeria (Olorunfemi *et al*., 2014). The classical *Allium* chromosomal aberration conventional test used in the studies to detect environmental clastogens revealed reduced mitotic index and chromosome aberrations in the water samples. The observed frequencies of chromosomal bridge, sticky and laggard chromosomes were indicators of chromosome damage in the onion root tip cells.

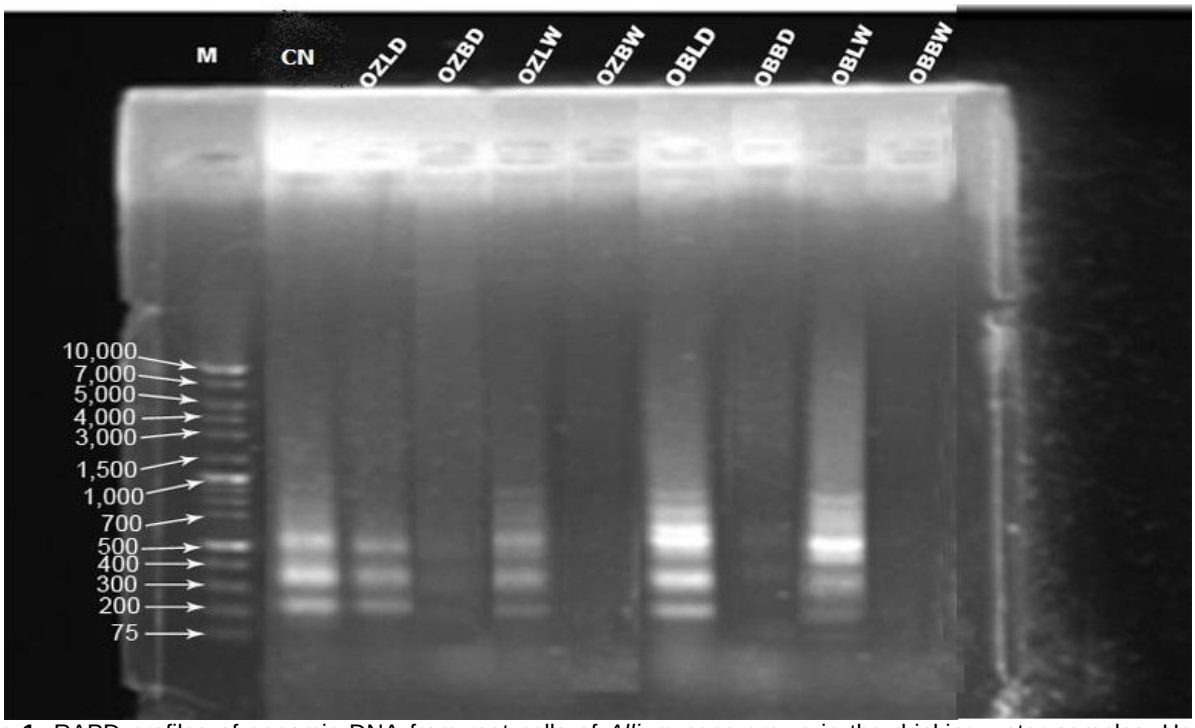

Plate 1: RAPD profiles of genomic DNA from root cells of *Allium cepa* grown in the drinking water samples. Header photo was indicated by M: DNA marker, CN: negative control, OZLD: Ozomu lake water (dry season), OZBD: Ozomu borehole water (dry season), OZLW: Ozomu lake water (wet season), OZBW: Ozomu borehole water (wet season), OBLD: Obazuwa lake water (dry season), OBBD: Obazuwa borehole water (dry season), OBLW: Obazuwa lake water (wet season), OBBW: Obazuwa borehole water (wet season) using primer OPB-08

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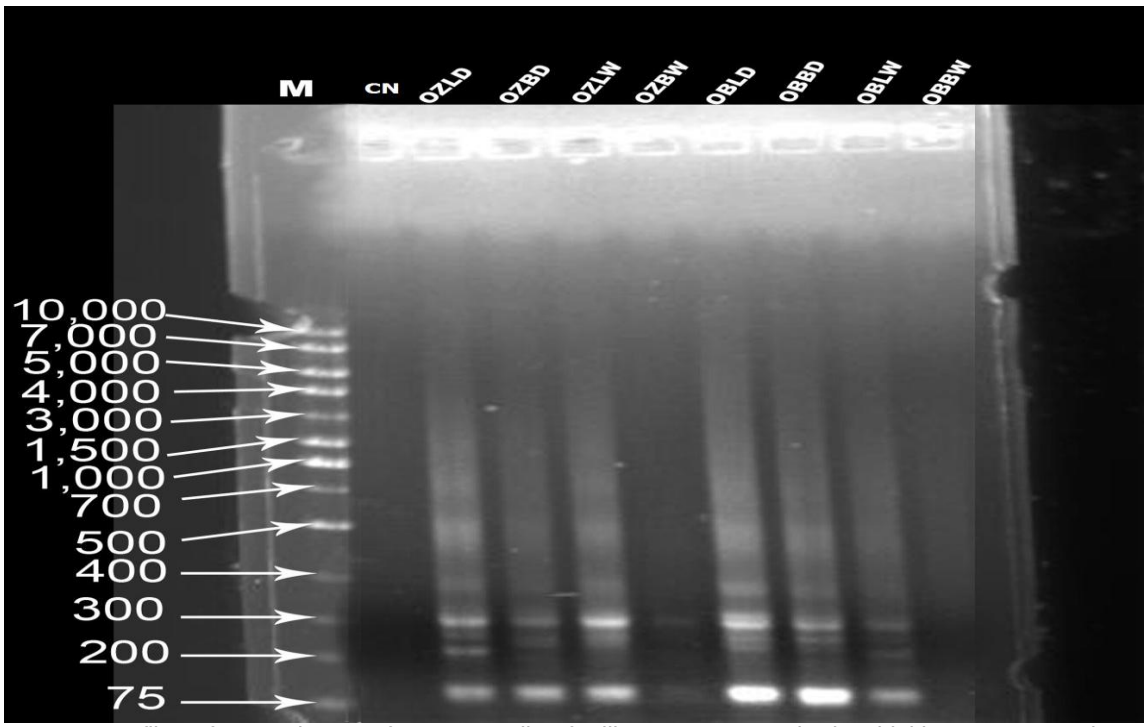

Plate 2: RAPD profiles of genomic DNA from root cells of *Allium cepa* grown in the drinking water samples. Header photo was indicated by M: DNA marker, CN: negative control, OZLD: Ozomu lake water (dry season), OZBD: Ozomu borehole water (dry season), OZLW: Ozomu lake water (wet season), OZBW: Ozomu borehole water (wet season), OBLD: Obazuwa lake water (dry season), OBBD: Obazuwa borehole water (dry season), OBLW: Obazuwa lake water (wet season), OBBW: Obazuwa borehole water (wet season) using primer OPB-07

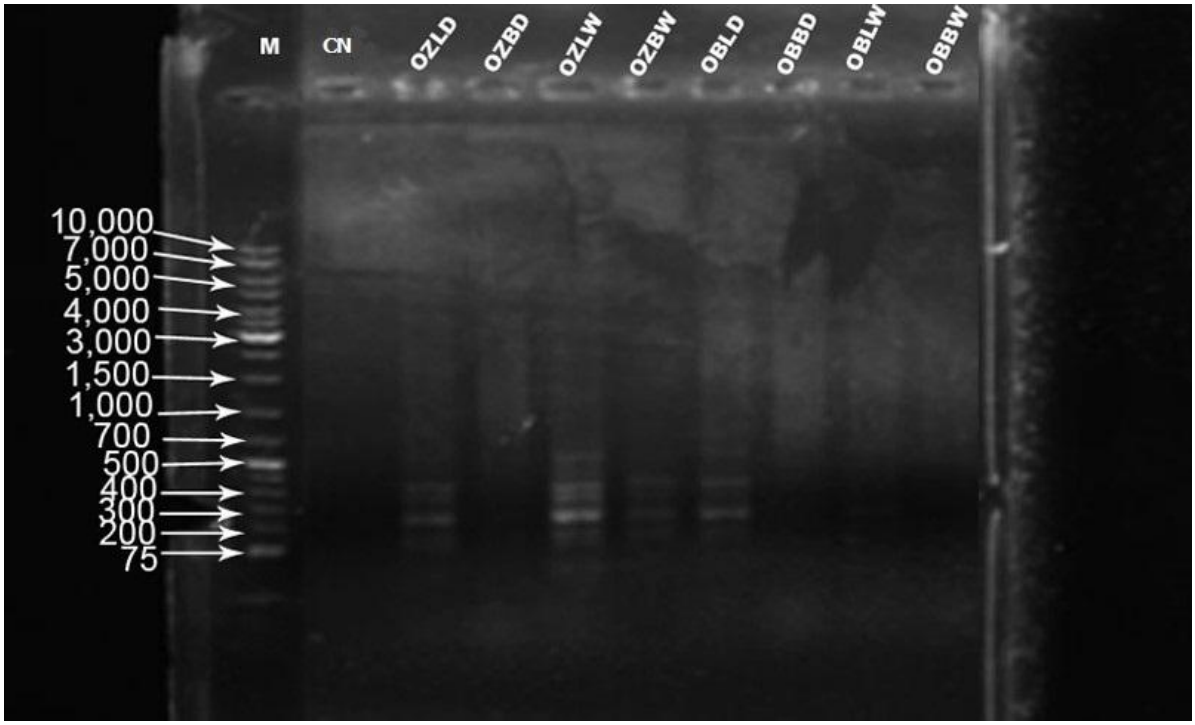

Plate 3: RAPD profiles of genomic DNA from root cells of *Allium cepa* grown in the drinking water samples. Header photo was indicated by M: DNA marker, CN: negative control, OZLD: Ozomu lake water (dry season), OZBD: Ozomu borehole water (dry season), OZLW: Ozomu lake water (wet season), OZBW: Ozomu borehole water (wet season), OBLD: Obazuwa lake water (dry season), OBBD: Obazuwa borehole water (dry season), OBLW: Obazuwa lake water (wet season), OBBW: Obazuwa borehole water (wet season) using primer OPB-06

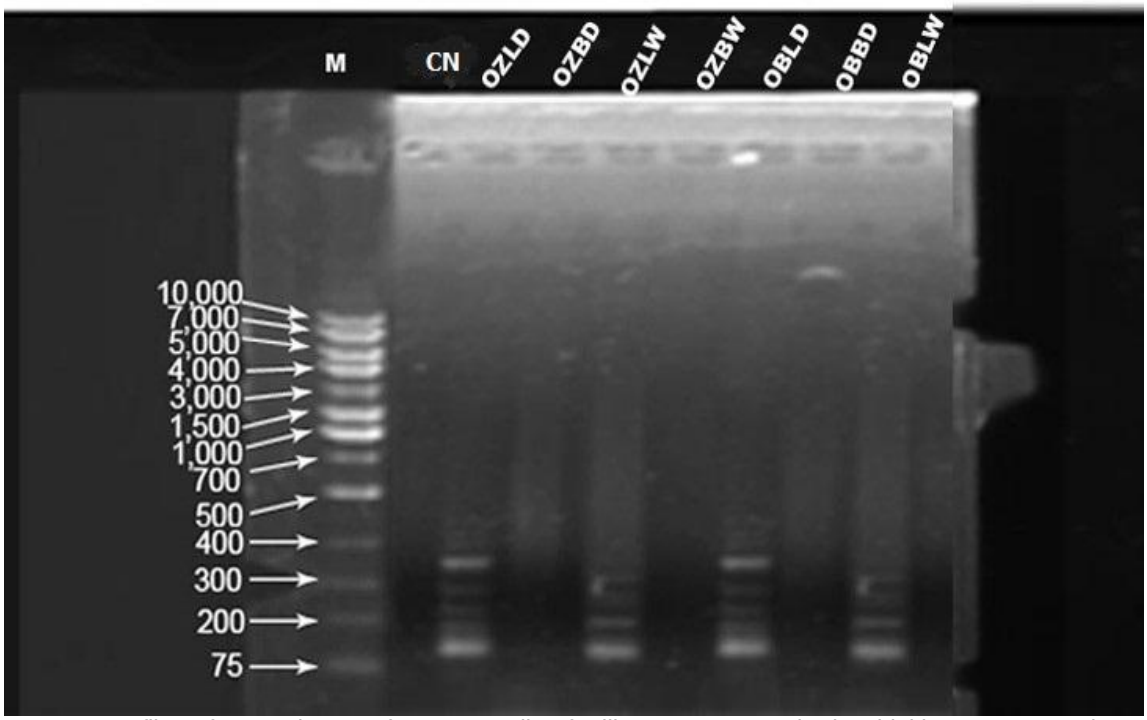

Plate 4: RAPD profiles of genomic DNA from root cells of *Allium cepa* grown in the drinking water samples. Header photo was indicated by M: DNA marker, CN: negative control, OZLD: Ozomu lake water (dry season), OZBD: Ozomu borehole water (dry season), OZLW: Ozomu lake water (wet season), OZBW: Ozomu borehole water (wet season), OBLD: Obazuwa lake water (dry season), OBBD: Obazuwa borehole water (dry season), OBLW: Obazuwa lake water (wet season), OBBW: Obazuwa borehole water (wet season) using primer OPB-11

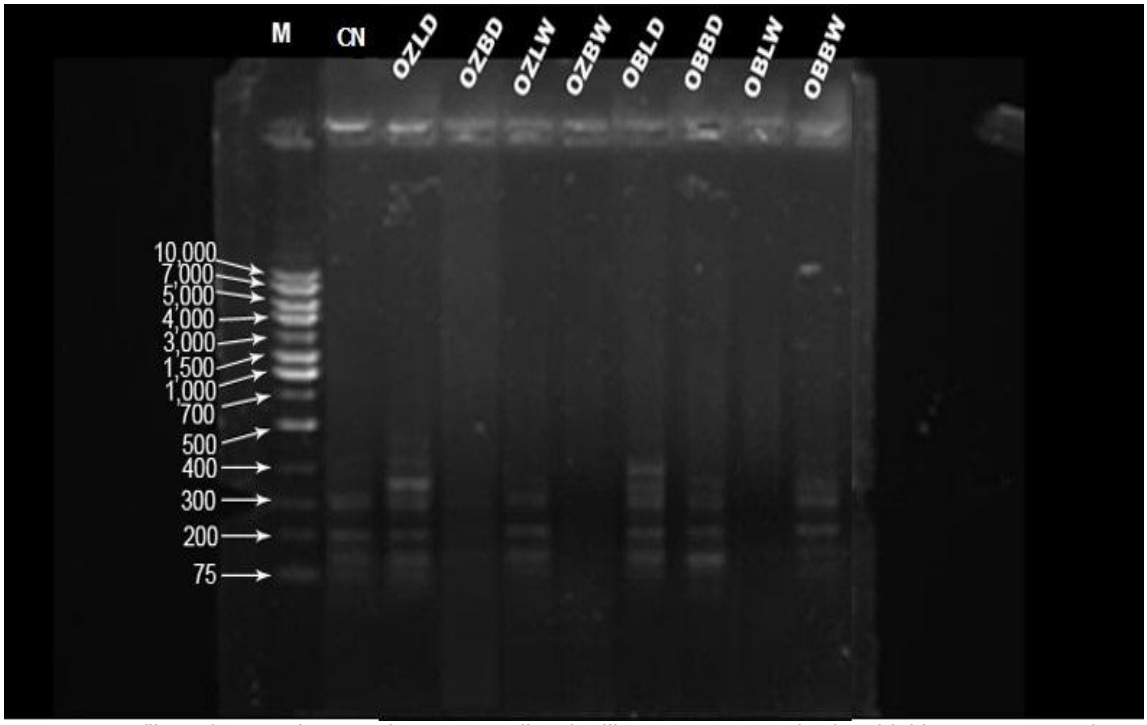

Plate 5: RAPD profiles of genomic DNA from root cells of *Allium cepa* grown in the drinking water samples. Header photo was indicated by M: DNA marker, CN: negative control, OZLD: Ozomu lake water (dry season), OZBD: Ozomu borehole water (dry season), OZLW: Ozomu lake water (wet season), OZBW: Ozomu borehole water (wet season), OBLD: Obazuwa lake water (dry season), OBBD: Obazuwa borehole water (dry season), OBLW: Obazuwa lake water (wet season), OBBW: Obazuwa borehole water (wet season) using primer OPD -03

In order to estimate the level of DNA polymorphism among negative control and treated samples, a dissimilarity values were constructed, using the Squared Euclidean distance method. The Squared Euclidean distance between the control plants and those treated with OZLW, OBLW and OBBW was 5.2, while a Squared Euclidean distance of 10.1 between control and plants treated with OZBW and OBBD, was observed. There was a distance of 20.2 between negative control and those treated with OZBD, OBLD

and OZLD. Dendrogram using Average Linkage (UPGMA) between groups showed that control plants merged with OBBW to form the first cluster with OBLW and OZLW, while OZBD merged with OBLD to affliate with OZLD in a separate cluster (Fig. 2).

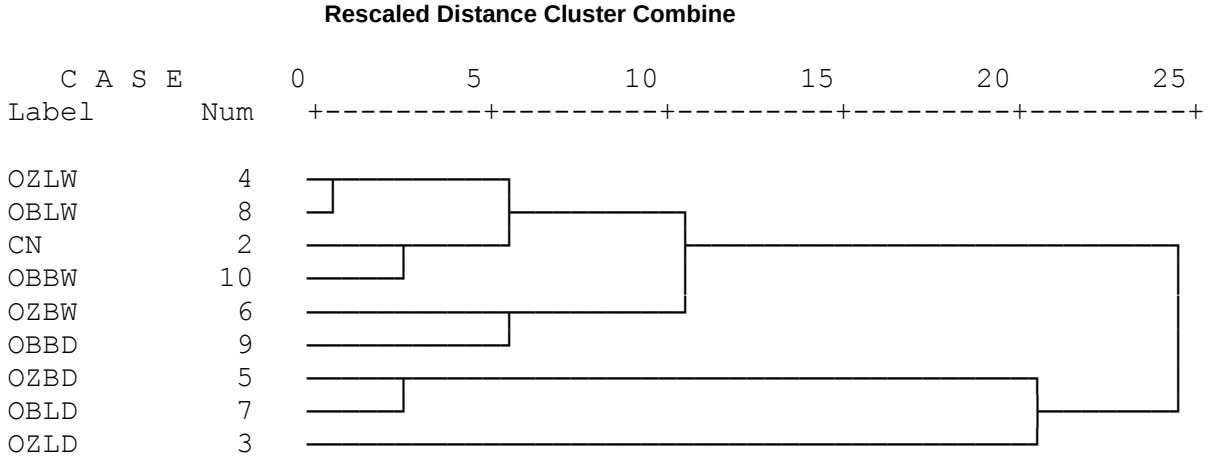

Fig. 2: UPGMA dendrogram showing clustering of control and treated samples of *Allium cepa*. OZLW: Ozomu lake water (wet season), OBLW: Obazuwa lake water (wet season), CN: negative control, OBBW: Obazuwa borehole water (wet season), OZBW: Ozomu borehole water (wet season), OBBD: Obazuwa borehole water (dry season), OZBD: Ozomu borehole water (dry season), OBLD: Obazuwa lake water (dry season), OZLD: Ozomu lake water (dry season).

DNA-RAPD fingerprinting has been used as a biomarker assay to detect DNA damage and mutational events in cells of *Allium cepa* by a number of workers (Qari, 2010; Ozakca and Silah, 2013; Hassan and Yassein, 2014). In this study, DNA damage/polymorphism was evident in RAPD profiles via appearance or disappearance of bands compared with the control. The number of disappearing RAPD bands increased from 12 in plants treated with water samples collected during the wet season to 16 in plants treated with water samples collected during the dry season. Disappearing bands are likely due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Liu *et al.*, 2007).

This method can be applied to a wide range of bioindicator organisms and may become a universal methodology to identify target genes for specific genotoxic agents (Al-Qurainy *et al.*, 2010). The sequence characterized amplified region (SCAR) marker could be developed for detection of genotoxic doses and identification of nucleotides sequence of the affected regions in the plant genome.

CONCLUSION:

Although the study did not obtain documented evidence on the incidence of major water borne disease outbreak in the communities under investigation, notwithstanding, the DNA polymorphism detected by RAPD in this study attests to the suggestion that most of the observed cytotoxic/genotoxic effects in the root meristems in *A. cepa* in earlier studies were possibly induced by the chemicals and microorganisms in the water samples and this may present a direct or indirect risk to living organisms. This study supports the claim that DNA polymorphism detected by RAPD can be considered as a powerful molecular biomarker assay for the detection of the genotoxic effect of contaminants in water for household use or irrigational purpose. Therefore RAPD analysis can be used for evaluation of toxicity on plants caused by environmental pollutants/contaminants.

In conclusion, since the RAPD technique is inconsistent in toxicity evaluation, further studies involving the use of gene sequencing and additional markers including the comet assay and nuclear fragmentation test would be of advantage to ascertain the extent of DNA damage in the root cells of *A. cepa* exposed to contaminated drinking water samples obtained from lakes and boreholes in two rural communities in Edo State of Nigeria.

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