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TOXICITY EVALUATION OF WASTE EFFLUENT FROM CASSAVA-PROCESSING FACTORY IN LAGOS STATE, NIGERIA USING THE ALLIUM CEPA ASSAY

Ogunyemi, A.K[1]; Samuel, T.A[2]; Amund, O.O[1] and Ilori, M.O[1]

[1]Department of Microbiology, University of Lagos, Akoka, Lagos, Nigeria

[2]Department of Biochemistry, University of Lagos, Idi-Araba, Lagos, Nigeria.

[1]Corresponding author email Address: waleogunyemi2002@yahoo.com Tel. no. +2348034864513

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ABSTRACT

Mutagenic and genotoxic effects of cassava wastewater (CWW) were investigated by assay of *Allium cepa* root meristematic cells. The physicochemical parameters of the wastewater samples were also determined. In *Allium* root growth inhibition test, experimental onion bulbs were cultivated in various concentrations of the CWW and distilled water was used as a negative control. After 72 h, the root tips from the treated bulb were processed for cytological studies by orcein squash technique. The mean lengths of root bundles were obtained and effective concentration (EC) values calculated. The cytotoxic effects on the onion root tips showed strong growth retardation at high concentrations of the effluent with EC_{50} value of 5.67%. The mitotic index (MI) rapidly decreased with increasing effluent concentration compared to control. There was significant increase in frequency of chromosome aberrations (stickiness, c-mitosis, vagrant, bridged fragment, binuclei, multipolar anaphase, attached chromosome and laggard chromosome) in root tip meristem cells of *Allium cepa* at all tested concentrations. At lower concentrations (0.005%), binuclei, (0.5%), vagrant chromosome and (1.0%), bridged fragment were the most common aberrations observed while at higher concentrations, (100 %), c-mitosis, vagrant and bridged fragment were the typical aberrations observed. The results indicate that the effluent samples collected were highly mutagenic. The results of physicochemical analysis revealed that the concentrations of some parameters (turbidity, chemical oxygen demand (COD), biological oxygen demand (BOD), conductivity, total dissolved solid (TDS), total suspended solid (TSS), sulphate, nitrate, phosphate and metals-copper, cobalt, chromium, iron, manganese, magnesium, nickel, cadmium, lead, sodium, potassium and calcium) were above the maximum permissible limit set by world health organization (WHO) and could partly be correlated with the toxicity of wastewater. The findings indicate that the substances contained in the cassava effluents may be toxic to living organisms and may pollute the environment if untreated.

Keywords: Mutagenicity, genotoxicity, cassava wastewater, chromosomal aberration, mitotic index, cyanide, *Allium cepa*.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a woody shrub widely cultivated in tropical and subtropical areas of the world for its edible roots (Burrell, 2003). Products derived from cassava are the principal food source of 500 million–1 billion people in tropical countries (Rosling, 1987; Bokanga, 1995). However, it contains the potentially toxic cyanogenic glucosides, primarily linamarin, and a small amount of methyl linamarin (lotaustralin), located inside the plant cells together with a specific hydrolytic enzyme, linamarase (EC 3.2.1.21), located in the cell walls. Cassava is normally processed before consumption as a means of detoxification, preservation and modification (Oyewole, 1991). The extraction of starch from the root requires large amounts of water and the residual water after separation of starch and fibre contains small amounts of starch, proteins and hydrocyanic acid. Wastes generated by cassava processing pose serious environmental pollution threat especially with increased industrial production of cassava flour and starch (Goodley, 2004). Cassava processing is generally considered to contribute significantly to environmental pollution and environmental nuisance (Ubalua, 2007), because the effluents produced during and after processing are usually discharged indiscriminately into the environment, particularly on farmlands (Ogboghodo *et al.*, 2001, 2006). These wastes such as peelings, fibrous by-products and wastewater effluents are indiscriminately disposed into the environment without prior treatment to reduce the volume, toxicity or mobility of the hazardous substances. When the effluent is released directly or indirectly into streams and rivers, it may lead to detrimental effects on fish and other aquatic organisms (Bakare *et al.*, 2003, 2009; Fawole *et al.*, 2008; Kumar, 2008; Oti, 2002; Oboh and Akindahunsi,

2003; Oboh, 2004). Cassava processing-related water pollution problems have been reported as serious in many countries such as Thailand (Kiranwanich, 1977). The continuous indiscriminate discharges of untreated effluents constitute danger to the environment, especially to water sources used for cassava processing. In search of test systems, which combined with the chemical analysis, can be used to provide data as a scientific basis for regulating the discharge of potentially hazardous substances into the environment and suitable for performance of toxicity evaluation, the *Allium cepa* seems to have some advantages. Levan (1938) introduced a method for genotocity evaluation using *Allium cepa* and it has been used on wastewater from other studies (Ravindran, 1978; Shanthamurthy and Rangaswamy, 1979; Smakakinel *et al*., 1996; Mishra, 1993). Fiskesjo (1985) proposed *Allium cepa* test as a standard method in environmental monitoring and toxicity screening of wastewater and river water. Wastewater containing cyanide must therefore be treated before discharge into the environment. Hence, an efficient method for detoxifying cassava is desired. Studies have shown the importance of toxicity evaluation and results obtained provide baseline data that are vital for the formulation of guidelines for pollution control with regard to discharge of cassava wastewaters into the environment. Therefore, this present study was carried out in order to evaluate the genotoxic effect of cassava wastewater collected from a process plant at Odogunyan, Ikorodu, Lagos, Nigeria using the *Allium cepa* chromosome aberration assay.

MATERIALS AND METHODS

Sampling site, Collection of Industrial Effluents and Dilution of the wastewater

A cassava factory at Odogunyan, Ikorodu is a major cassava factory in Ikorodu town, in Lagos State Nigeria. Samples of cassava processing wastes were collected from discharge points in sterile 5-litre bottles and used for physicochemical and genotoxicity analyses. Figure 1 shows the location of the cassava factory, in Ikorodu area of Lagos State. Dilutions of cassava effluents were made using distilled water as diluent to obtain graded concentrations of the cassava wastewater for the genotoxic study.

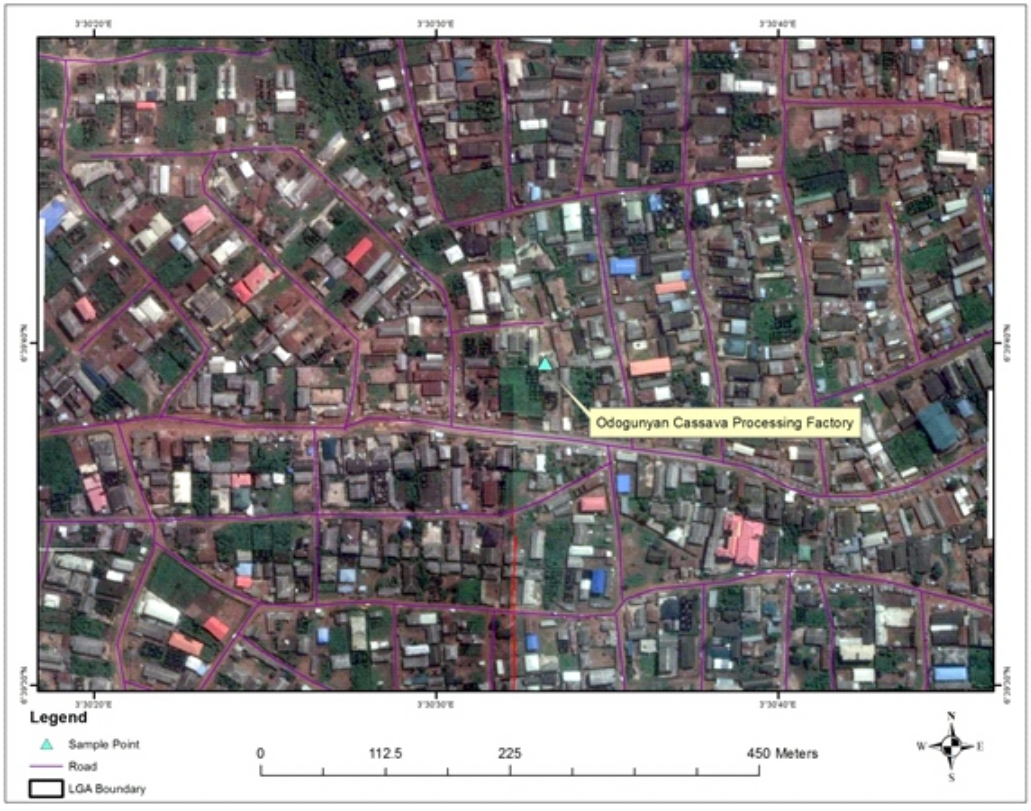

Figure 1: Satellite image showing cassava processing plant at Odogunyan, Ikorodu, Lagos, Nigeria.

Determination of Physicochemical Parameters of Cassava Wastewater

The wastewater samples were analyzed for a number of physicochemical properties including chemical oxygen demand (COD), total alkalinity (TA), total dissolved solids (TDS), biochemical oxygen demand (BOD), sulphate (SO_4), nitrate (NO_3), phosphates (PO_4) and cyanide (CN) were determined according to standard analytical methods (APHA, AWWA and WEF, 2005) while the total solids (TS), hardness and electrical conductivity (EC) were determined by method described by Ademoroti (1996). Fifteen heavy metals (including nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), zinc (Zn), silver (Ag), cobalt (CO), were determined in the wastewater samples according to standard analytical methods (Ademoroti, 1996). Wastewater pH was measured electrometrically with Orion 3 Star bench top pH meter (Thermoscientific, USA) (Ademoroti, 1996).

Source and Treatment of *Allium cepa* bulbs

A. cepa (2n = 16) onion bulbs, of an average size of 15-20 mm in diameter, were purchased locally in Lagos State, Nigeria. They were dried for about six weeks and the dried roots present at the base of the onion bulbs were carefully shaved off with a new razor blade in order to allow fresh meristematic tissues to be well exposed in the CWW. The root length (cm) was measured with a graduated meter rule for three consecutive days and the mean root length of two bulbs for each test sample concentration was determined and recorded.

Allium cepa assay

The *Allium* test for macroscopic as well as microscopic evaluations adopted in this study was as previously described by Fiskesjo (1997) and Bakare and Wale-Adeyemo (2004). The outer scales of the onion bulbs and brownish bottom plate were carefully removed thereby leaving the ring of fresh root primordia intact. The peeled bulbs were transferred into distilled water during the cleaning procedure to prevent the primordia from drying. This followed with the bulbs being exposed directly in 0, 0.05, 0.1, 0.5, 1.0, 5, 10, 25, 50 and 100% concentrations (v/v, effluent/distilled water), of each of the test sample (cassava wastewater). Twenty onion bulbs were set up in each series for each sample, out of which the best eighteen with good root growth were selected for analysis of root growth inhibition. Distilled water was used as negative control. The experiment was set up in the dark at 28 °C for 72 h. Test liquids were changed daily. Photographs of test materials were taken with *Nikon* Digital Camera D80 (Nikon Corp., Japan) and special note was taken of change of colour of root tip and morphology. After 48 h, one root tip was removed from each bulb, fixed in ethanol : glacial acetic acid (3:1, v/v) and hydrolysed with a solution of 1 N HCl at 65 °C for 3 min. After staining the tissue, the specimen on the slide was gently covered with a cover slip, allowing the stain to spread evenly over the square parts of the cover slip to eliminate air bubble. The slide with the specimen was then placed in between two folds of the filter paper and using the blunt end of a pen, gentle tapping and pressure was applied around the square area of the cover slip for even squashing of the specimen. Finally, the square edges of the cover slip of the squashed onion roots was sealed with white transparent nail hardener as suggested by Grant (1982) to prevent drying out of the preparation by the heat of the microscope (Sharma, 1983). Three slides were prepared for each concentration and control. After 96 h, mean length of root bundles were obtained as described by Fiskesjo (1985) and the EC_{50} values and 95% confidence interval were determined from a plot of root length, % of control against the sample concentrations using the Graph pad prism 5.0 version. The slides were viewed under the microscope to observe mitotic stages and chromosomal aberrations to produce photomicrographs. The mitotic index (MI) was calculated as the ratio of number of dividing cells to number of observed cells (Fiskesjo, 1997). The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of each effluent. These are calculated as follows:

$$\text{Mitotic index} = \frac{\textit{Number of dividing cells}}{\textit{Number of cells scored}} \times 100$$

$$\text{Frequency (\%) of aberration} = \frac{\textit{Number of aberant cells}}{\textit{Number of cells scored}} \times 100$$

Statistical Analysis

The results of the root inhibition and chromosome aberrations at each concentration of the effluents were expressed as mean ± standard deviation using the excel software. The differences between the control and different concentrations of the CWW effluents were compared using one-way analysis of variance (ANOVA) (Mason *et al.*, 2003) which assesses whether the means of groups are statistically different from each other. This analysis is appropriate for comparing groups while p-values less than 0.05 were considered significant.

RESULTS

Physicochemical Characteristics of Cassava Wastewater Samples

Table1a shows the results of various physicochemical parameters including pH and the levels of potassium, sodium, calcium, magnesium, aluminum, cadmium, chloride, manganese, iron, zinc, phosphate, sulphate, nitrate, nickel, zinc and cyanide in the wastewater. The concentrations of K, Na, Ca, Mg, Al, Cd, Cl, Mn, Fe, Zn, PO_4, SO_4, NO_3, Ni, Zn and CN ions were not in any definite increasing/decreasing order. However, the concentrations of heavy metals were relatively high. Table1b shows the concentration of heavy metals obtained from the cassava wastewater. Of all the elements and ions measured, the average concentration of these metals at the site were 53.59±5.27 mgL^{-1} for potassium, 29.50±4.2 mgL^{-1} for sodium, 51.50±5.7 mgL^{-1} for calcium, 31.70±4.3 mgL^{-1} for magnesium, 17.97±2.515 mgL^{-1} for aluminum, 0.502+ mgL^{-1} for cadmium, 0.904±0.035 mgL^{-1} for manganese, 4.004±0.373 mgL^{-1} for iron and 1.374±0.087 mgL^{-1} for zinc while average concentrations of the ions were 161.0±9.0 mgL^{-1} for nitrate, 4941±1259 mgL^{-1} for chloride and 118.6±9.3 mgL^{-1} for sulphate and 18.4±0.3.3 mgL^{-1} for phosphate. Interestingly, cyanide anion, of considerable interest was detectable at a concentration of 12.49±1.14 mgL^{-1}

Table 1: Physico-chemical properties of cassava wastewater samples collected from cassava factory site.

Parameters	Value/observation	WHO Standard limit*
Colour	White	Unobjectionable
pH	4.01±0.12	6.5-9.5
Appearance	Cloudy	Unobjectionable
Odour	Objectionable	Unobjectionable
Conductivity (μScm^{-1})	11602±620	1200
TS (mgL^{-1})	14810±286	1500
TDS (mgL^{-1})	9240±472	2000
Alkalinity (mgL^{-1})	BDL	100
Hardness (mgL^{-1})	1300±176	500
BOD (mgL^{-1})	155±21	50
COD (mgL^{-1})	224±12	1000
K^+(mgL^{-1})	53.59±5.27	-
Na^+(mgL^{-1})	29.50±4.2	-
Ca^{2+}(mgL^{-1})	51.50±5.7	-
Mg^{2+}(mgL^{-1})	31.70±4.3	20
NO_3^-(mgL^{-1})	161±9.0	50
PO_4^{2-}(mgL^{-1})	18.4±3.3	-
SO_4^{2-}(mgL^{-1})	118.6±9.3	500
CN^{-1}(mgL^{-1})	12.49±1.14	0.07
Cl^{-1}(mgL^{-1})	4941±1259	250
Al^{3+} (mgL^{-1})	17.971±2.515	0.2
Ag^+ (mgL^{-1})	BDL	-
Cu^{2+} (mgL^{-1})	0.162±0.019	2.0
Cr^{2+} (mgL^{-1})	1.423±0.467	0.05
Cd^+ (mgL^{-1})	0.502±0.114	0.003
Pb^{2+}(mgL^{-1})	BDL	0.01
Mn^{2+}(mgL^{-1})	0.904±0.035	0.4
Fe^{2+}(mgL^{-1})	4.004±0.373	3.0
Zn^{2+}(mgL^{-1})	1.374±0.087	3.0

CWW-Cassava wastewater, TS-Total solid, TDS-Total dissolved solid, BOD-Biochemical oxygen demand, COD-Chemical oxygen demand, BDL-Below detectable level, ±-Standard deviation, WHO-World Health Organization, *-Source (Institute of public Analysts of Nigeria, IPAN, 2005)

Macroscopic effects

The results obtained for the root lengths and morphological properties employed to assess genotoxicity are shown in figure 2 and table 2. It was observed that there was a strong growth retardation in onion roots growing at higher concentrations of the CWW, whereas the effects were less severe at lower concentrations. The growth curve of the onion roots at different concentrations of the wastewater had generally a sigmoid shape, indicating a positive dose-response effect. There was no root growth at all in onion bulbs treated with the undiluted sample, while at 20 and 50 % concentrations of CWW, there were about 22.0 and 66.0% growth retardation in relation to root lengths in the control. The root growth retardation or inhibition is concentration dependent with an EC_{50} value of 5.76%, while a total phytotoxic effect was induced by the undiluted wastewater. The presence of twists, root tips bent upwards resembling hooks ('crochet hooks' and c-tumors (abnormalities appearing as swellings of the root tips) were noted.

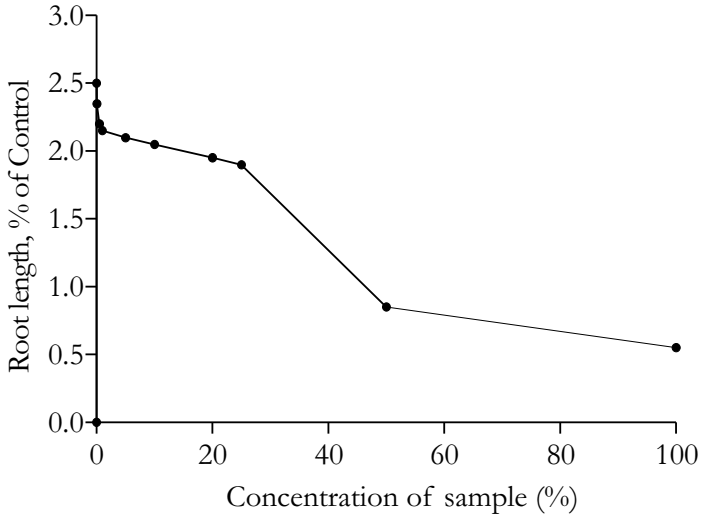

Figure 2: Growth curve of Allium roots (in relation to control) after treatment with cassava wastewater.

Table 2: Results of Allium root growth inhibition test

Treatment (%)	control	0.05	0.1	0.5	1.0	5.0	10	20	25	50	100
Root Length (cm)	4.1+0.1	2.5+0.1	2.35+0.15	2.2+0.1	2.15+0.05	2.1+0.1	2.05+0.05	1.95+0.0	1.9+0.0	0.85+0.0	0.55+0.0
Decrease RL %	0	60.97	57.31	53.65	52.44	51.22	50.00	47.56	46.34	20.73	13.41

±-standard deviation, %-percentage, RL-Root length

Table 3: Cytological effects of treatments at different concentrations of cassava wastewater (CWW)

Concentration (%)	Mitotic index[a]	Number of cells[b]	Number of dividing cells	Stickiness	C-Mitosis	Vagrant	Bridges fragment	Binuclei	Multipolar anaphase	Attached	Laggard	% Aberration (+SD)
Control	10.60	500	53	0	0	0	0	0	0	0	0	0
0.05	10.26	458	47	6	2	5	5	2	0	5	1	26±7.87
0.1	9.25	454	42	6	1	5	6	1	0	5	1	25±7.68
0.5	7.93	429	34	5	0	6	5	1	0	4	0	21±6.59
1.0	7.09	423	30	6	0	5	4	0	0	3	1	19±5.99
5.0	6.69	418	28	4	1	4	3	0	1	3	0	16±4.93
10	6.05	413	25	5	2	4	4	1	0	3	0	19±5.83
20	5.39	408	22	5	1	3	4	0	0	4	0	17±5.33
25	4.96	403	20	4	2	2	3	1	1	1	0	14±4.25
50	3.54	396	14	3	1	2	2	1	0	1	1	11±3.32
100	2.88	382	11	4	1	2	2	0	0	0	0	9±2.96

[a]Mitotic index was calculated as: (number of dividing cells / number of cell) × 100

[b]Chromosome aberrations were scored on 500 cells/slide

P<0.001 (x^2-test), S.D-Standard deviation

Microscopic effects

The effect of the effluents on cell division and chromosome behaviour of *Allium cepa* are shown in table 3. There was no chromosomal aberration in the control which had a mitotic index (MI) value of 10.60. Chromosomal aberrations were induced at all concentrations of the effluents and were statistically significant ($p<0.05$). As the concentration of the effluents increases, there was concentration-dependent decrease in the mitotic index. For example, the MI at 25% effluent concentration was 4.96 compared to the negative control value of 10.60%, at all concentrations. Thus the mitotic index could be another endpoint for general toxicity assessment. Figure 3 represents the micrograph of the observed chromosome aberrations.

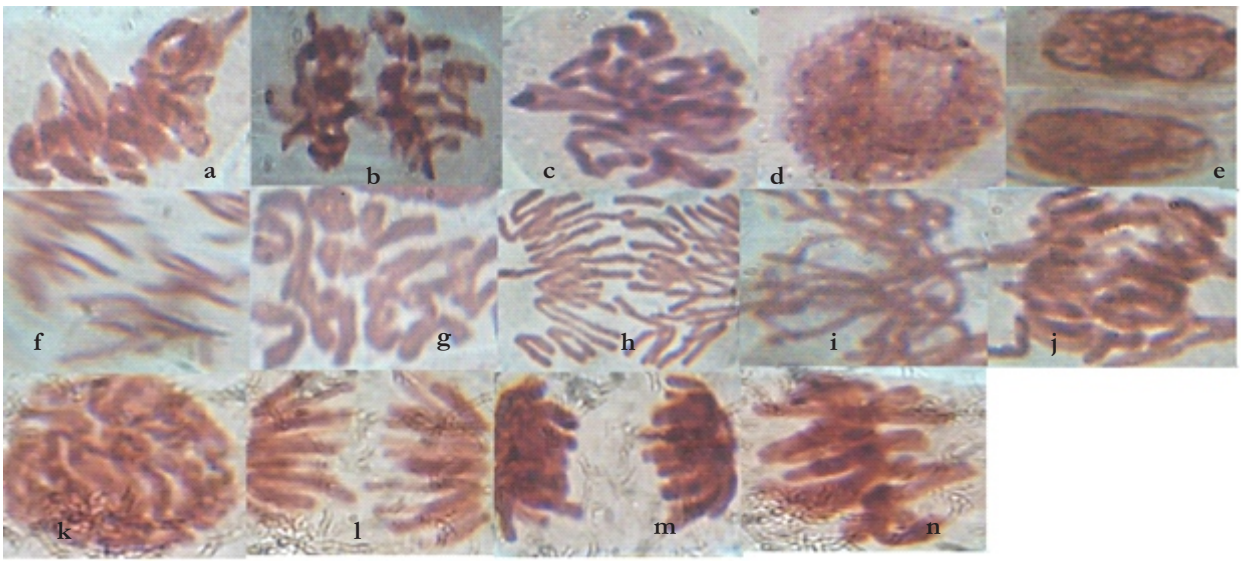

Figure 3: Chromosomal aberrations induced in *Allium cepa* exposed to cassava effluent. (a) attached chromosome; (b) bridged anaphase; (c) vagrant chromosome (d) sticky chromosome (e) binucleated cells; (f)multipolar mitosis (g) c-mitosis; (h) bridged chromosome; (i) laggard chromosome (j) prophase control; (k) bridged chromosome; (l) anaphase control (m) telophase control; (n) metaphase control;

The types of chromosomal aberrations induced by the effluents include stickiness, c-mitosis, vagrant, bridged fragment, binuclei, multipolar anaphase, attached chromosome and laggard chromosome (Figure 3a-n) at various concentrations. At lower concentrations (0.005%), binuclei, (0.5%), vagrant chromosome and (1.0%), bridged fragment were the most common aberrations observed while at higher concentrations, (100%), c-mitosis, vagrant and bridged fragment were the typical aberrations observed.

DISCUSSION

The Allium test has often been used for the determination of cytotoxic and/or genotoxic effects of various substances (Grant, 1982; Smaka-kinel *et al.*, 1996). It is considered to be a standard procedure for quick testing and detection of toxicity and pollution levels in the environment. Results of the Allium test indicate the presence of certain cytotoxic/genotoxic or mutagenic substances in the environment, which represent direct or indirect risks for all living organisms. Fiskesjo (1985) has demonstrated the usefulness of root tips of *Allium cepa* as a test system for monitoring the genotoxic effects from a chemical factory in Sweden. The Allium test was found to be very useful for evaluating and ranking aquatic toxicities for a number of metals including mercury (Fiskesjo, 1988; Dash *et al.*, 1988; Rank and Nielsen, 1998). The cytoxicity level can be determined by the decreased rate of the mitotic index. A mitotic index decrease below 22% of the control causes lethal effects on test organisms (Antonsie-weiz, 1990), while a decrease below 50% usually has sublethal effects (Panda and Sahu, 1985) and is called cytoxic limit value (Sharma, 1983). In this present study, cassava effluent showed a low mitotic index (2.88: control 10.60). These values represent 21.36% of the control attributing to sublethal effects of cassava

wastewater samples from this site. The mitotic index decrease in onion root meristem was found to be a reliable means for quick determination of the presence of cytoxic substances in the environment, for monitoring the cytotoxic pollution level in the natural environments and for evaluation of water pollution levels. This parameter is sensitive enough also to be used for monitoring the pollution levels of slightly polluted water (Smaka-kinel *et al.*, 1996).

Ivanova *et al.* (2002) and Staykova *et al.* (2005) have reported the genotoxic and mutagenic effects of open water contaminated with heavy metals and cyanide, consequently confirming the results of the inhibitory effects of these effluents on seed germination and growth in earlier studies (Olorunfemi *et al.,* 2007). The results from this study suggest that the chromosome aberration induction in the *Allium cepa* root meristem could be as a result of heavy metals-cyanide interaction in the cassava waste waters. Adeyemo (2005), in a similar study conducted to assess the haematological and histopathological effects of cassava effluent on adult female African catfish, *Clarias gariepinus*, reported that the fish was found to show signs of gill and liver damage. Similarly, histopathological examination of the kidney, gill and liver of the fingerlings of the Nile Tilapia, *Oreochromis niloticus* treated with cassava effluent indicated damage (Wade *et al.,* 2002). The genotoxic effects of the cassava effluents established in this study indicates that the effluents contain toxic substances which may constitute a risk to the environment and human health, more especially as the waste generated from cassava processing is not properly treated before their disposal to the environment.

In *Allium cepa* test, there usually seems to be a relative decrease in root growth (cytotoxicity) and chromosomal deviations (genotoxicity). Whenever chromosome aberrations occur, there are almost always definite growth restrictions (Fiskesjo, 1997). Sticky chromosome is an indicator of poisoned chromosomes with sticky surface, which possibly bring about cell death (Fiskesjo, 1985). The abundance of sticky chromosomes at metaphase and anaphase stages in this *Allium cepa* test indicates that these effluents contain toxic substances. This assertion is supported by a previous work on the physicochemical analyses of effluents collected from this same industrial area which showed the presence of some poisonous heavy metals such as nickel, lead, cadmium, copper, zinc and cyanide. The levels of nickel (0.867ppm), cadmium (0.085ppm), lead (ppm), cadmium (ppm), copper (ppm), zinc (ppm) and cyanide (2.21ppm) were beyond the WHO permissible limits of 0.2 ppm, 0.05 ppm, 0.01 ppm, 2 ppm, 5 ppm and 0.200 ppm respectively (Zigham-Hassan *et al.*, 2012; Olaitan *et al.*, 2013). National agency for food and drug administration and control permissible limit for cyanide in wastewater is 0.001 mgL^{-1} (IPAN, 2005). The inductions of bridges at anaphase were frequently observed and such anomaly is also an indication of mutagenic events in the cell (Mishra, 1993). The findings from this study is in agreement with earlier studies by Samuel *et al* (2010) and Olorunfemi and Ehwre (2010) who previously worked on industrial effluents. This identifies cassava effluents to have adverse cytogenetic effects, which when exposed to humans and other living organisms can lead to harmful effects on vital organs of the body and may extend to future offsprings if not well managed. The discharge of cassava effluents without appropriate treatment can result in bioaccumulation of toxic substances in the environment. Hence, it is strongly suggested, as recommended by Samuel *et al.* (2010), root growth should be incorporated in the Whole Effluent Test (WET) programme by giving a particular EC_{50} that must be met by an industrial effluent before being allowed to be discharged into the environment. *Allium cepa* test used in this study has proved to be an effective tool for monitoring the genotoxic effects of industrial effluents before they are discharged into the environment. According to Odeigah *et al.* (1997), the impact of genotoxic wastewater on the environment and the significance to human health are difficult to predict, because wastewater are complex mixtures of chemical substances. A complete interpretation of their effects often requires, in addition a chemical analysis of the constituents that may indicate the components of the wastewater that can persist and accumulate in exposed biota and thus potentially pose a hazard to human health.

CONCLUSION

The *Allium cepa* chromosome aberration assay is useful for many types of environmental samples and can be recommended as a tool for monitoring the genotoxic effects and thereby contributing to environmental risk assessment information.

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