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

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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 showing cyanide content 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 10%. The mitotic index (MI) rapidly decreased with increasing effluent concentration compared to control. There was significant increase in frequency of chromosome aberrations (sticky chromosome, c-mitosis, vagrant chromosome, bridges fragment, binucleated cells, multipolar anaphase, attached chromosome and laggard chromosome) in root tip meristem cells of *Allium cepa* at all tested concentrations. Further analysis using one-way ANOVA revealed that there was a statistically significant difference ($p<0.05$) in concentration-dependent inhibition of onion root growth, mitotic index and induction of chromosomal aberration in the *Allium cepa* test. The results indicate that the effluent samples collected were highly mutagenic. The results of physicochemical analysis revealed that the concentrations of some parameters (conductivity, total suspended solid (TSS), total dissolved solid (TDS), biological oxygen demand (BOD), nitrate, cyanide, chloride and metals-magnesium, aluminum, chromium, cadmium, manganese and iron) 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 addition to chemical analysis, the search for test systems that can be used to provide scientific data to monitor the discharge of potentially hazardous substances into the environment through 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 from cassava processing contains cyanide and ought to be treated before discharge into the environment. Hence, an efficient method for detoxifying cassava wastewaters 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 processing factory in Ikorodu town, in Lagos State, Nigeria. Wastewaters from the processing plant were collected from discharge points in sterile 5-litre bottles and used for physicochemical and genotoxicity analyses. Figure 1 shows the satellite view of the cassava factory and sampling points. Dilutions of cassava effluents were made using distilled water as diluent to obtain graded concentrations of the cassava wastewater used for the genotoxic study.

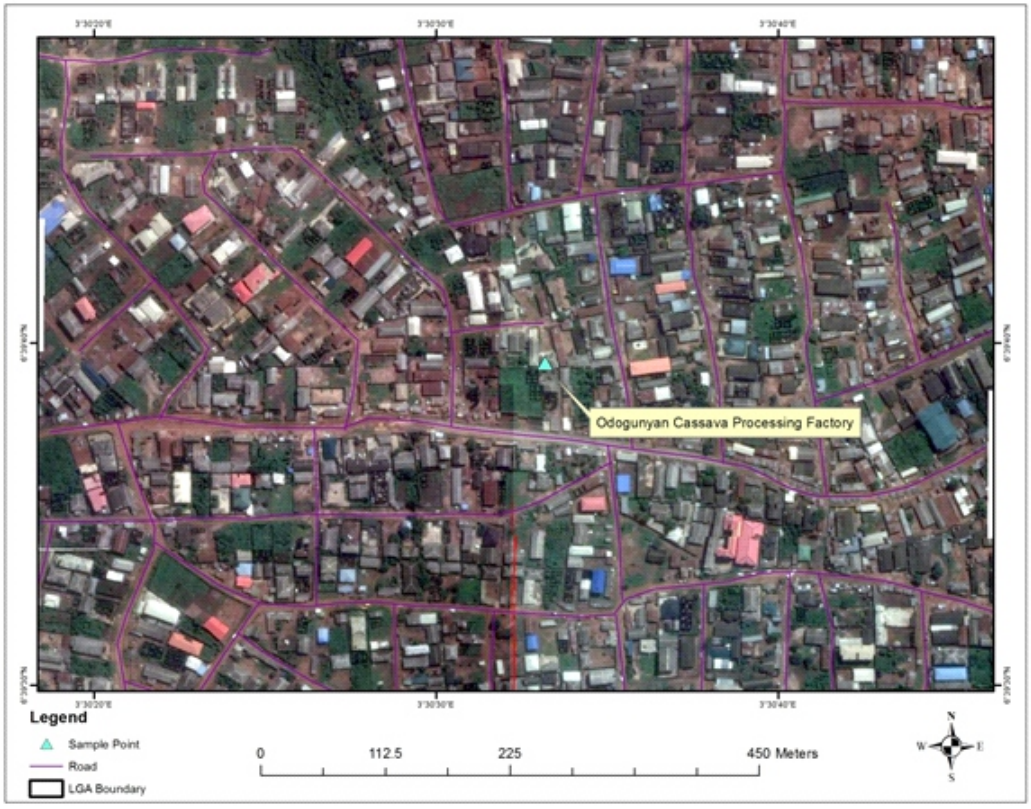

Figure 1: Satellite image showing cassava processing plant at Odogunyan, Ikorodu, Lagos, Nigeria.

Determination of Physicochemical Parameters of Cassava Wastewater

The wastewater samples were analyzed for a number of physicochemical properties including total dissolved solids (TDS), total alkalinity (TA), chemical oxygen demand (COD), biochemical oxygen demand (BOD), nitrate (NO_3), phosphates (PO_4), sulphate (SO_4) and cyanide (CN). They were determined according to standard analytical methods (APHA, AWWA and WEF, 2005) while the electrical conductivity (EC), total solids (TS), hardness and chloride (Cl) were determined by method described by Ademoroti (1996). Fourteen metals (including ten heavy metals) namely aluminum (Al), silver (Ag), copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), manganese (Mn), iron (Fe), zinc (Zn) and nickel (Ni) 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 four 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 was followed with the bulbs being exposed directly to 0, 0.05, 0.1, 0.5, 1.0, 5, 10, 25, 50 and 100% concentrations (v/v, effluent/distilled water). Eight onion bulbs were set up for each concentration of the wastewater, out of which the best four 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 M 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 (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). 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. 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 were calculated as follows:

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

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

(Akinboro and Bakare, 2007)

Statistical Analysis

SPSS 20.0 statistical package was used for all data analysis. The results of the root inhibition at each concentration of the effluents were expressed as mean ± standard deviation. 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). To assess whether the means of groups were statistically different from each other, the least significant difference (LSD) test was adopted. In all cases, a value of $p<0.05$ was considered significant.

RESULTS

Physicochemical Characteristics of Cassava Wastewater Samples

Table1 shows the various physicochemical parameters including pH and the levels of potassium, sodium, calcium, magnesium, nitrate, phosphate, sulphate, cyanide, chloride, aluminum, silver, copper, chromium, cadmium, lead, manganese, iron, zinc and nickel, in the wastewater. The concentrations of the elements and ions were not in any definite increasing/decreasing order. However, the concentrations of some of the heavy metals were relatively high. Of all the elements and ions measured, the average concentration of metals in the sampled wastewaters 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, 0.162±0.019 mgL^{-1} for copper, 1.423±0.467 mgL^{-1} for chromium, 0.502± 0.114 mgL^{-1} for cadmium, 0.904±0.035 mgL^{-1} for manganese, 4.004±0.373 mgL^{-1} for iron, 1.374±0.087 mgL^{-1} for zinc and 0.138±0.032 mgL^{-1} for nickel; while average concentrations of the anions were: 161.0±9.0 mgL^{-1} for nitrate, 18.4±3.3 mgL^{-1} for phosphate, 118.6±9.3 mgL^{-1} for sulphate and 4941±1259 mgL^{-1} for chloride. The concentrations of silver and lead were below detectable level. Interestingly, cyanide anion, was detectable at a concentration of 12.49±1.14 mgL^{-1}

Table 1: Physico-Chemical Properties of Cassava Wastewater Samples Collected from a 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	11602±620	1200
Total solid	14810±286	1500
Total dissolved solid	9240±472	2000
Alkalinity	BDL	100
Hardness	1300±176	500
Biochemical oxygen demand	155±21	50
Chemical oxygen demand	224±12	1000
Potassium (K^+)	53.59±5.27	-
Sodium (Na^+)	29.50±4.2	-
Calcium (Ca^{2+})	51.50±5.7	-
Magnesium (Mg^{2+})	31.70±4.3	20
Nitrate (NO_3^-)	161±9.0	50
Phosphate (PO_4^{2-})	18.4±3.3	-
Sulphate (SO_4^{2-})	118.6±9.3	500
Cyanide (CN^-)	12.49±1.14	0.07
Chloride (Cl^-)	4941±1259	250
Aluminum (Al^{3+})	17.971±2.515	0.2
Silver (Ag^+)	BDL	-
Copper (Cu^{2+})	0.162±0.019	2.0
Chromium (Cr^{2+})	1.423±0.467	0.05
Cadmium (Cd^+)	0.502±0.114	0.003
Lead (Pb^{2+})	BDL	0.01
Manganese (Mn^{2+})	0.904±0.035	0.4
Iron (Fe^{2+})	4.004±0.373	3.0
Zinc (Zn^{2+})	1.374±0.087	3.0
Nickel (Ni^{2+})	0.138±0.032	0.2

All values are means of three replicates and are expressed in mgL^{-1} except colour, odour, conductivity (μScm^{-1}) and pH (no unit). BDL-Below detectable level, 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. There was 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 significant decrease in root growth in onion bulbs treated with the undiluted sample, while at 20 and 50% concentrations of CWW, there were about 47.56 and 20.73% growth retardation in relation to root lengths in the control. The root growth retardation or inhibition was concentration dependent with an EC_{50} value of 10%, while a 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 (Table 3).

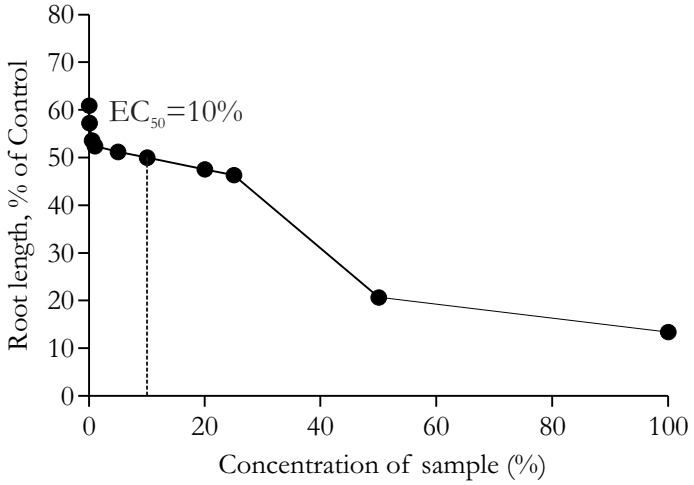

Figure 2: Growth curve of Allium roots (in relation to control) after treatment with cassava wastewater.

Table 2: Results of *Allium* Root Growth Inhibition Test

Treatment (%)	Root length (cm)*	% Root growth of control
Control	4.1±0.05	0
0.05	2.5±0.05	60.97
0.1	2.35±0.05	57.31
0.5	2.2±0.20	53.65
1.0	2.15±0.05	52.44
5.0	2.1±0.05	51.22
10	2.05+0.05	50.00
20	1.95±0.05	47.56
25	1.9±0.05	46.34
50	0.85±0.05	20.73
100	0.55±0.05	13.41

*Values are significantly different from the control at $p < 0.05$ (One-way ANOVA test)

Table 3: Cytological Effects of Treatments at Different Concentrations of Cassava Wastewater (CWW)

Concentration (%)	Number of cells[b]	Number of dividing cells	Mitotic index[a]	Sticky chromosome	C-mitosis	Vagrant chromosome	Bridges fragment	Binucleated cells	Multipolar anaphase	Attached chromosomes	Laggard chromosome	Mean of aberration	% aberrant cells
Control	500	53	10.60±0.12	0	0	0	0	0	0	0	0	0.00±0.00	0.00
0.05	458	47	10.26±0.24*	6	2	5	5	2	0	5	1	3.57±2.23*	5.68
0.1	454	42	9.25±1.19*	6	1	5	6	1	0	5	1	3.43±2.64*	5.51
0.5	429	34	7.93±0.94*	5	0	6	5	1	0	4	0	3.00±2.58*	4.90
1.0	423	30	7.09±0.54*	6	0	5	4	0	0	3	1	2.57±2.57*	4.49
5.0	418	28	6.69±0.41*	4	1	4	3	0	1	3	0	2.29±1.60*	3.83
10	413	25	6.05±0.20*	5	2	4	4	1	0	3	0	2.71±1.80*	4.60
20	408	22	5.39±0.12*	5	1	3	4	0	0	4	0	2.43±2.07*	4.17
25	403	20	4.96±0.62*	4	2	2	3	1	1	1	0	2.00±1.16	3.47
50	396	14	3.54±0.27*	3	1	2	2	1	0	1	1	1.43±0.98	2.78
100	382	11	2.88±0.21*	4	1	2	2	0	0	0	0	1.29±1.50	2.36

[a]Mitotic index was calculated as: (number of dividing cells/number of cell) × 100
[b]Chromosome aberrations were scored on 500 cells/slide
*Values are significantly different from the control at $p < 0.05$ (One-way ANOVA test).

Microscopic effects

Figure 3 represents the micrograph of the observed chromosome aberrations. 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 ($p<0.05$). The effect of the effluents on cell division and chromosome behaviour of *Allium cepa* are shown in table 3. 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.

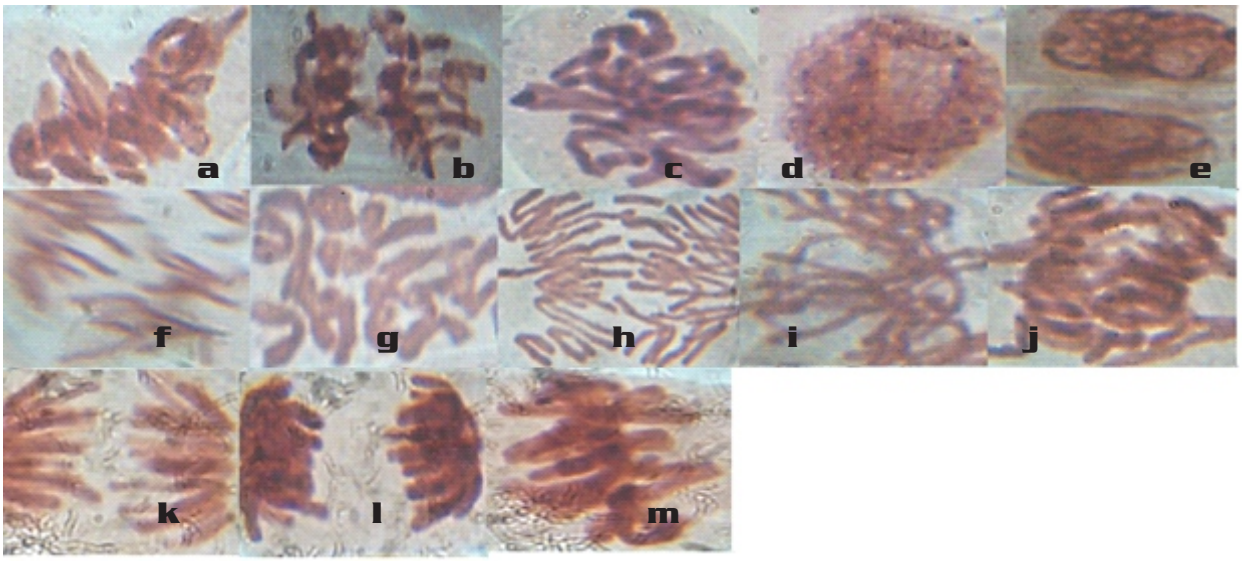

Figure 3: Chromosomal aberrations induced in *Allium cepa* exposed to cassava effluent. (a) attached chromosome (b) anaphase bridge (c) vagrant chromosome (d) sticky chromosome (e) binucleated cells (f) multipolar anaphase (g) c-mitosis (h) bridges fragment (i) laggard chromosome (j) prophase control (k) anaphase control (l) telophase control; (m) metaphase control . (magnification: 1000x).

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 wastewater, 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 cytotoxic limit value (Sharma, 1983). In this present study, cassava effluent showed a low mitotic index (2.88: control 10.60). These values represent 27.17% 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 presence of sticky chromosomes in this *Allium cepa* test indicates that the cassava effluent contains toxic substances. National Agency for Food and Drug Administration and Control (NAFDAC) 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. The discharge of cassava effluents without appropriate treatment can result in bioaccumulation of toxic substances in the environment. Hence, it is strongly suggested that root growth assay 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. This has previously been recommended by Samuel *et al.* (2010), *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.

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