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Genotoxicity of Industrial Paint Effluent on the Root Meristem of Allium Cepa

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Abstract: *The genotoxic effects of the industrial effluent from a paint (100%, 50%, 25%, 10%, 5%, 1%, 0.50% and 0.25%) manufacturing company in Ikotun, Lagos was evaluated using the root meristem cells of Allium cepa. chromosomal aberration assays were used to determine the mitotic index and chromosome aberration rate. There was a significant difference ($p<0.05$) in the average root length of Allium cepa exposed to the various concentrations of the industrial. The phase index for each mitotic phase arrest was evaluated. There was a significant difference ($p<0.0001$) in phase index. There was an increase in the mitotic inhibition as the concentration increased whereas there was a decrease in the mitotic index, indicative of an inverse relationship shared by the two properties. The effluent induced chromosomal aberrations in the meristematic cells of the A. cepa root tip and laggards were the most frequently observed of the aberrations induced. The use of less or non-toxic alternatives, in the manufacturing process, was advocated as well as the suitability of A. cepa chromosomal assay as a tool for biomonitoring of environmental pollution by industrial effluents and wastewater was discussed.*

Keywords*: Chromosomal Aberrations, mitotic inhibition, phase index, biomonitoring*

I. Introduction

The impact of industrial wastewaters on aquatic and terrestrial ecosystems has drawn a lot of attention worldwide because of its overwhelming environmental significance (Olorunfemi*et al.*, 2011). Industrial wastewater originates from the wet nature of most large industries which require large quantities of water for processing and disposal of wastes. Industrial wastewater is not only concentrated but plentiful, so the pollution potential of industrial wastewater is by far greater than that of domestic wastewater (Olorunfemi*et al.*, 2011). Most industrial wastewaters are usually extremely complex mixtures containing numerous inorganic as well as organic compounds (Nielsen and Rank, 1994). The complexity makes it almost impossible to carry out a hazard assessment based on chemical analysis (El-Shahaby*et al*., 2003).

In Lagos, Nigeria, there are many industries that discharge their effluents into the different water bodies around the metropolis (Samuel *et al*., 2010). Of particular interest are paint industries which constitute one of the major industries in Lagos and discharge large amount of effluent among all the industries in the metropolis (Samuel *et al*., 2010). Some industries release effluent such as paint effluent unto vegetation leading to high deposit of contaminants (bioaccumulation) in the plants or in the soil (Oladele*et al* 2013).Most of these effluents contain high concentrations of potentially mutagenic heavy metals and it has been reported that heavy metals are among the most toxic and environmentally dangerous pollutants (Ivanova*et al*. 2005; Abu and Ezeugwu, 2008).

Analysis of chromosomal aberrations serves as a test for genotoxicity and is one of the few direct methods to measure damage to systems exposed to potential mutagens or carcinogens. To enable the evaluation of the effects or damage mutagens can cause, it is necessary that the sample is constantly undergoing mitotic division; identify objective toxic effects and changes that occurred during the cell cycle, *Alliumcepa* test has been widely used to serve this purpose (Silva and Fonesca, 2003).

The use of *Allium cepa*(common onion) was introduced as a biological test system to evaluate the cytogenetic effects of colchicine cells (Levan, 1938 in Rank, 2003). Since then, *A. cepa* L has been a biological material of wide use in laboratory tests, due to the fast growth of its roots and the response of genetic material to the presence of potential cytotoxic and genotoxic substances in test liquids (Vesna*et al*., 1996). The Allium test has been applied to evaluate the quality of underground, surface waters and effluents in a simple way through the study of macroscopic parameters, such as the values for root growth inhibition, cytological parameters such as aberrations cellular metaphase and anaphase and cellular division inhibition (Fiskesjö, 1988, Vesna*et al*., 1996). The chromosomal and the division of meristematic root cells onions are often used to alert population on product consumption (Vicentini*et al*. 2001). The Allium cepa has small chromosome number (2n=16) and can easily be used for cytological work because they are few and can easily be counted. The chromosomes of *A. cepa* also are large enough for chromosomal aberrations to be seen (Okoli and Russom 1986). The small chromosome number of *A. cepa* and the large size of the chromosome of *A. cepa* accounts for its wide use as a biomonitor for genotoxicity assay.The *Allium cepa* assay is low cost, is easy to use and produces similar results to animal tests because of similarity in their genetic compositions, hence same response to mutagens (Akinboro,

et al., 2011). The presence of metacentric chromosomes in *A. cepa*cells allows easier and better microscopic assessment

AlthoughOladele*et al*(2013) reported that paint effluents have hematotoxic effects on Swiss albino mice, little information is available on the genotoxic effects of paint effluents. This work was carried to evaluate the genotoxic effect of paint effluent which is among the effluents that are mostly discharged into the environment. The results of this study will be useful to environmental regulatory agencies in developing *A. cepa* assay as a useful tool in detecting the presence and action of mutagenic agents in industrial effluents discharges. Therefore this will set pace for toxicity identification evaluation (TIE) studies of industrial effluents found to be mutagenic.

II. Materials And Methods

The paint effluent was obtained from Precious Paints Ltd, Ikotun in Alimosho Local Government, in Lagos which was being discharged into a nearby drainage. The waste water was stored at room temperature. The onions used for the test were obtained from a market at Ikotun area of Lagos State.

The *Allium cepa* assay was carried out as described by Fiskesjo (1993) and Olorunfemi*et al*., (2011). The loose scaly part of the onion bulbs carefully removed before use and the dry scaly root at the bottom plates were carefully scraped away without destroying the root primordial. The stock of the wastewater (100%) was diluted into the 50%, 25%, 10%, 5%, 1%, 0.5% and 0.25% (v/v) concentrations respectively. This was done using the volume per volume (v/v effluent/tap water) method in which the concentrations indicated are the respective volumes of the wastewater in the sample mixture for each concentration.

The onion bulbs were introduced to tap water for 48 hours to check for the viable onions. The onions that sprouted (the viable ones) were used for the experiment. The tap water was changed daily. The following day, only the onions with the best growing roots were used to carry out the assay. For each concentration, four onion bulbs were transferred into the wastewater samples at varying concentrations (100%, 50%, 25%, 10%, 5%, 1%, 0.5% and 0.25%) while tap water was used as control.

Four roots from the 10%, 5%, 1%, 0.5%, 0.25% and the control (0%) set-ups were harvested after 48 hours and 96 hours respectively while for the acute toxicity testing, roots from all the concentrations were harvested after 96 hours and were immediately introduced into fixative (aceto-alcohol 1:3) for 24 hours in order to arrest mitosis (Fiskesjo, 1987). After the fixation, the slides were prepared using all the fixed root tips. The tips were treated with 1N-HCl for a period of 5 minutes in order to hydrolyse and soften the tissues of the root tips after which the excess 1N-HCl was blotted out using a filter paper and then the tips were macerated using a dissecting needle. Maceration was done to enhance stain uptake and to ensure the spreading of the cells in a monolayer for easy microscopic examination. After maceration, the root tips were stained using the Lactic-acetic orcein stain which was left to stand for 20 minutes to allow the stain penetrate the cells thoroughly (Fiskesjo, 1993).

A cover slip was applied over the stain to seal it in and the excess stain was carefully removed by applying slight thumb pressure over the cover slip, which was wrapped in between a filter paper (in order to blot out the excess stain and prevent air bubbles) in a uniform manner and then sealed with a nail varnish at the edges for preservation (Fiskesjo, 1997).

The prepared slides were viewed under the X40 objective of the microscope to evaluate the different stages in mitosis as well as the induced aberrations. Micrographs of the aberrations and the other stages of mitosis were taken.The mitotic index, mitotic inhibition as well as the phase index were calculated as described by Fiskesjo (1993).

$$\text{Mitotic inhibition} = \frac{\text{Mitotic Index in Control group} - \text{Mitotic Index in test groups}}{\text{Mitotic Index in control group}} \times 100$$

$$\text{Phase Index} = \frac{\text{Number of cells at each mitotic phase}}{\text{Total Number of cells counted}} \times 100$$

Slide scoring was done in accordance to the method described by Fiskesjo (1997) and Rank (2003). Data analysis was done using 2-way ANOVA at $p<0.05$ level of significance and the Tukey's multiple comparison test using the GraphPad Prism™ 6 software.

III. Results And Discussion

Table 1 shows the cytological effects of the effluents on the meristematic cells of the onion root while table 2 shows the effect of paint effluent on mitotic index and inhibition of onion cells. The mitotic index decreased as concentration of the effluent increased while, the mitotic inhibition increased with concentration with the 100% concentration showing 78% mitotic inhibition whereas there was a major drop in that value at the 0.25% concentration which only inhibited mitosis to 7%. There was an inverse relationship between the mitotic index and the mitotic inhibition such that as the concentration increased, the mitotic index decreased whereas

the mitotic inhibition increased. Mitotic index as was described by Fiskesjo (1993), shows the potential of cell to divide whilemitotic inhibition is a measure of how much mitosis is prevented by the toxicant under study. According to Odeigahet al., (1997), the corresponding decrease in the mitotic index with the increase in concentration of the effluent noticed at the different treatment periods (48hours and 96hours) may be due to prophase arrest or pre-prophase inhibition or an interference of the cell cycle at metaphase or anaphase stage (Oloyedeet al., 2009) or certain components of the paint effluent interfered with DNA synthesis thus causing the aberrations observed. As it is well known, mitotic index shows the proliferation status of a cell. Therefore the decrease of the mitotic index with the increase in the concentration of the effluent indicates that high concentration of paint effluents inhibits cell proliferation. Also, since mitosis can lead to growth, reduction of its activities by paint effluent indicates that paitn effluent inhibits plant growth.

Table 1: Cytological Effects of Paint Effluents on the Root Meristem Cells After 96hours

			CHROMOSOMAL ABERRATIONS								
Conc.	Number of cells	No. of dividing cells	Lag	Brid	Vag	BiN	M. Pol	Sticky	D. Spindle	Total aberrant cells	% Aberration
0%	1000	422	0	0	0	0	0	0	0	0	0
0.25%	1000	391	0	1	1	0	1	1	0	5	10
0.50%	1000	373	2	2	2	2	1	2	0	14	25
1%	1000	352	2	2	3	2	1	2	1	15	28
5%	1000	320	3	4	4	2	1	2	0	21	39
10%	1000	304	3	4	4	3	2	3	2	25	47
25%	1000	206	4	1	1	0	0	1	0	9	14
50%	1000	152	2	0	0	0	0	0	0	2	2
100%	1000	93	6	2	0	0	0	0	0	9	9

Keys: Lag – Laggard; Brid – Bridged; M.POL – Multipolar Cells; Vag – Vagrant Cells; Sticky – Stickiness; C.MET – C-Metaphase; BiN – Binucleated Cell; D.spindle – Disturbed Spindle

Table 2: The mitotic index and mitotic inhibition of *Allium cepa* exposed to paint effluent

Conc.	Number of cells	No. of dividing cells	Mitotic index (%)	Mitotic inhibition (%)
0%	1000	422	42.23	0.0
0.25%	1000	391	39.10	7.4
0.50%	1000	373	37.33	11.6
1%	1000	352	35.17	16.7
5%	1000	320	31.97	24.3
10%	1000	304	30.40	28.0
25%	1000	206	20.57	51.3
50%	1000	152	15.17	64.1
100%	1000	93	9.30	78.0

The phase index of the mitotic cells per concentration after 96 hours is shown in table 3. There was a higher level of mitotic arrest at prophase than at any other phase of mitosis. Anaphase recorded a generally low mitotic arrest and the least mitotic arrest was recorded at 10% concentration of the effluent which showed approximately 7% anaphase arrest during mitosis. There was a significant change in the phase index at $p<0.0001$. This means that there is a 0.01% chance of randomly observing an effect this big or bigger in an experiment of this size. Comparing phase indices at each phase, there was a significant difference (at $p<0.0001$) between the phase index between that of prophase and the other mitotic phases such as metaphase, anaphase and telophase but no significant difference between the phase index of metaphase and anaphase, metaphase and telophase as well as that between anaphase and telophase.

TABLE 3: Phase Index of Mitotic Cells After 96 Hours

	NUMBER OF MITOTIC CELLS	P Ph I (%)	M Ph I (%)	A Ph I (%)	T Ph I (%)
0%	422	64.93	13.27	11.37	10.43
0.25%	391	65.47	12.02	11.76	11.00
0.50%	373	65.42	12.33	11.26	10.99
1%	352	65.34	11.93	11.08	11.65
5%	320	70.31	13.44	7.19	9.06
10%	304	70.39	11.51	6.58	11.51
25%	206	68.93	10.19	10.19	10.68
50%	152	60.53	9.21	15.79	13.82
100%	93	61.29	17.20	11.83	8.60

KEYS: P PhI – Prophase Phase Index, M PhI – Metaphase Phase Index, APhI – Anaphase Phase Index; T PhI – Telophase Phase Index

Most aberrations were recorded at 10% concentration which showed 47% aberration and a total of 25 aberrant cells..Laggards were the most common of the aberrations observed and the highest number of laggards was recorded at the 100% concentration.The different chromosomal aberrations are shown in figure 1. The different chromosomal aberrations in root tip cells of *A. cepa*(vagrant chromosomes, bridges and fragments and laggards chromosomes) suggest the presence of certain cytotoxic/genotoxic substances in the paint effluent used in the study. Vagrant chromosomes have been described as weak C mitotic effects indicating risk of aneuploidy while sticky chromosomes indicate a highly toxic, irreversible effect probably leading to cell death (Fiskesjo, 1988). According to Kong and Ma (1999), there is a hypothesis that stickiness of chromosomes may cause incomplete separation of daughter chromosomes as a result of cross-linkage chromoproteins. Also, according to Konuket al (2007), stickiness an indicator of cell death and may be caused by physical adhesion of proteins to the chromosomes.The presence of these abnormalities in the chromosomes of the test plant used for the study indicates that plants and/or other organisms exposed to paint effluents may suffer from cell death or have risk of non-disjunction of chromosomes. According to the views of Paul *et al* (2013), the occurrence of several types of chromosomal abnormalities, such as stickiness, laggards, c-mitosis, bridges, multipolarity and fragmentation of *Allium cepa*L. root tip cells in this study, clearly shows that the accumulated effect of paint effluent results inactivation of spindle formation, deformation of non-histone chromosomal proteins and mutation of the structural genes

The usual idea that aberrations increase with increasing concentration was not followed as higher concentrations such as 100%, 50% and 25% showed smaller amount of aberrations compared to lower concentrations such as 10%, 5% and 1% showing much higher amount of aberrations. This result contradicts that of Qian (2004) which reported that aberrant rate goes up with the concentrations but in agreement with Odeigah*et al*. (1997). According to Odeigah*et al*. (1997), a possible explanation for this is that, with increasing concentration and consequently, increasing toxicity, there was an inhibitory effect on cell division. This might occur in pre-prophase, where cells are prevented from entering prophase or there may be prophase arrest where cells enter into mitosis but are arrested during prophase resulting in a high frequency of prophase cells. It is suggested that prophase - arrest is the most likely explanation, as it could also explain the decline in chromosome aberrations, without any parallel decline in the mitotic index values (Odeigah*et al*.,1997).

These anaphase and metaphase disorders are indicative of disrupted kinetic of chromosomes and are generated due to qualitative and quantitative changes of chromatin kinetochore (Amin, 2002), therefore may indirectly constitute a risk of aneuploidy (Maluszynska and Juchimiuk, 2005). While Odeigah*et al*., (1997) were of the opinion that bridges are formed as a result of unequal chromatid exchange,Kalcheva*et al*., (2009) suggested that bridges occur as result of chromosome or chromatid breaks. These may imply that paint effluents can cause unequal chromatid exchange or chromosome break as bridges are some the aberration observed in this study. The presence of few binucleated cells usually arises as a consequence of the inhibition of cell plate formation (Grant, 1978), which might be due to the suppression of phragmoplast formation in the early telophase (Borooah, 2011).The lagging of chromosomes was caused by disturbances in the mitotic spindle or the centromere (Ivanova*et al*., 2008). Ivanova*et al*., (2008) suggested that the anaphase and telophase bridges established, as well as the chromosome fragments resulted from different types of chromosome aberrations, associated with a loss of genetic material.

IV. Conclusion

In conclusion, this study has shown that paint effluent has genotoxic effect on *Allium cepa* root. The effects of the paint effluents on the root meristem cells of *Allium cepa* are an indication of the likelihood of a similar if not the same effects on humans. The results shown are indicative of the genotoxic nature of paints effluents which are discharged into the environment.This study advocates the use non-toxic alternatives in place

of the more toxic ones presently in use by paint manufacturing industries for the safety of the environment and its biotic dependents.

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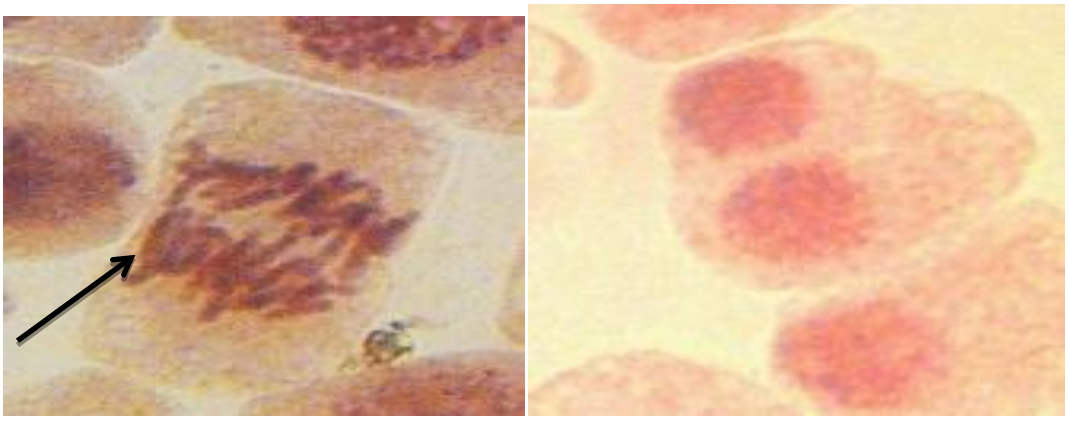
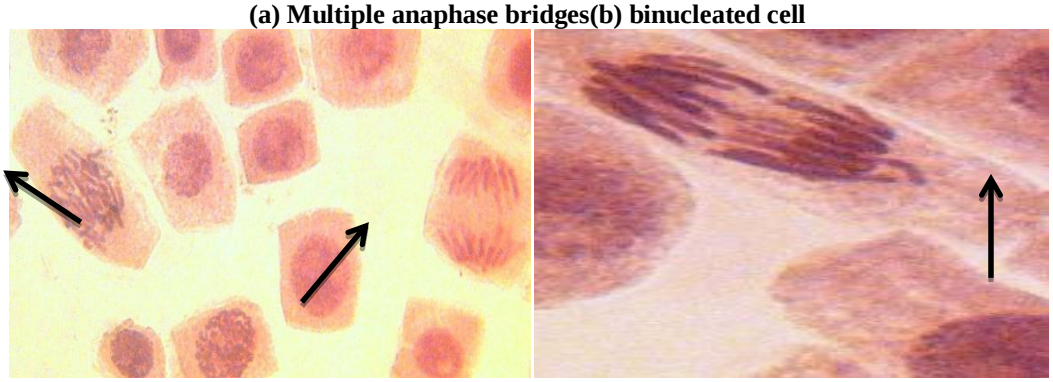
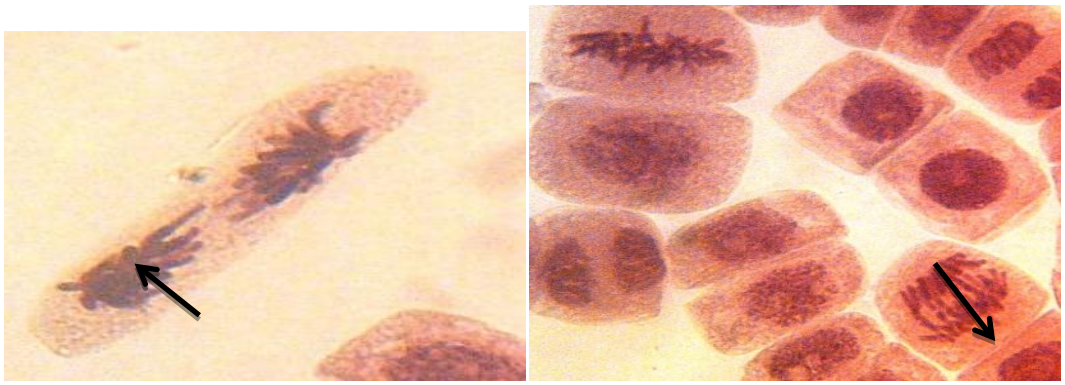


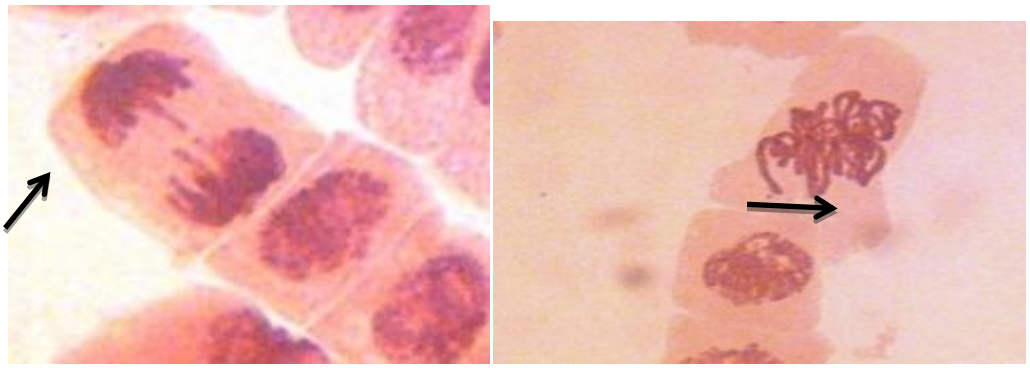
(a) Multiple anaphase bridges(b) binucleated cell



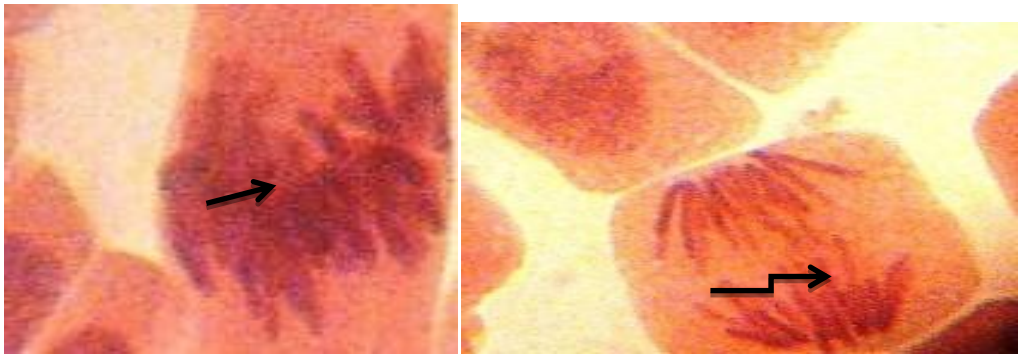
(c)polyploidyand early anaphase **(d) Fragment broken from chromosome**



(e)a multipolar anaphase aberration.(f)vagrant chromosomes in anaphase



(g) Anaphase-Telophase bridge (h)A disturbed spindle



(i)Stickymetaphase (j) Bridged Anaphase

Figure 1: chromosomal aberrations induced by paint effluent on Allium cepa roots