

Full Length Research Paper

## Chemical composition and mutagenic assessment of petrochemical effluents on onion (*Allium cepa*) root tip mitosis

Abu, Ngozi E.<sup>1\*</sup>, Asomba, C. O.<sup>1</sup> and Ubani, C.<sup>2</sup>

<sup>1</sup>Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.

<sup>2</sup>Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.

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The continuous production and release of chemicals into the environment have led to the need to assess their chemical composition and genotoxic effects on cell reproduction. Two petrochemicals, air liquid and polyester resin effluents were assessed. The common onion of the purple variety was used as the test organism. The results of the chemical analysis of the wastewaters showed high concentration of some potentially mutagenic heavy metals. The effects of the wastewaters and their dilutions on root tip mitosis were mitodepressive. The mitotic index (MI%) were 14.0, 5.3 and 4.1 in control, air liquid and polyester resin effluents, respectively. The MI (%) values estimated across concentrations ranged from 15.1 in control to 5.5 in undiluted effluent. Percent abnormal dividing cells increased with increase in wastewater concentration. Abnormal dividing cells observed ranged from C-mitotic effects to precocious chromosomes and anaphase bridges. These findings indicate the cytotoxic and genotoxic effects of these wastewaters on *Allium cepa* chromosomes. Positive results on *Allium* chromosomes could serve as indicator of the deleterious effects of these wastewaters on other organisms at the point of discharge – either on land or water bodies. The need for sound sewerage system that would protect flora and fauna in the ecosystem is advocated.

**Key words:** Chromosome, ecosystem, heavy metal, mitosis, mutation.

### INTRODUCTION

High rate of industrialization means high rate of development. Compounds such as agricultural products, food additives, pharmaceuticals, chemotherapeutic agents and several others produced in different industries are for the well-being of any nation, however, the processes they undergo in these industries to come out

as useful products lead to discharge of effluents into the environment. Most of these toxic composites are released as wastewaters which are discharged into large lakes, rivers, canals or drains and even agricultural land without any further treatment to remove the toxic component (Grover and Kaur, 1999). The indiscriminate

\*Correspondence author. ngozi.abu@unn.edu.ng, abu\_ngo@yahoo.com. Tel: +234 8064664555.

discharge of effluents into the environment by local industries without treatment has been accompanied with pollution (Odeigah et al., 1997). These wastewaters are extremely complex mixtures. The complexity makes it almost impossible to carry out a hazard assessment based on chemical analyses only (El-Shahaby et al., 2005). Moreover, the possibility of bioaccumulation and biotransformation is a risk to environmental and human health. An important task has been to develop test systems which can be used to provide data as scientific basis for regulating the discharge of potentially hazardous substances into the environment. Higher plants have been proposed and also used as test organism for the detection of genotoxic substance in the environment (Rank et al., 2002; Maluszynska and Juclimiuk, 2005). Plant roots are excellent in toxicity assays because the roots are always the first to make contact with the chemicals in both soil and water (Odeigah et al., 1997). *Allium cepa* is one of those plants that have been used in different studies to detect chromosome aberrations induced by chemicals since it was first introduced by Levan (1938) and later was proposed as a standard method for the testing of chemicals, in environmental monitoring and in toxicity screening of wastewater and river water (Fiskesjö, 1985a; 1985b; 1993).

The *Allium* genetic material has been widely exploited for such purpose because of its excellent chromosome conditions. *Allium* test is easy to handle, has low cost and shows good correlation with mammalian test systems (Fiskesjö, 1985a). *A. cepa* root system is particularly sensitive to the harmful effects of such environmental contaminants (Vargas et al., 1993). Gross effects can be quantified by measurement of inhibition of growth of the newly developing root system while the examination of the chromosomes of the individual cells of the root tip could be a pointer to their mutagenic effect.

Environmental degradation along with subsequent agronomic constraints is slowing down the growth in world's food output. The investigations made by various researchers had pointed out that industrial pollution induced reduction in the number of populations and restricted the distribution of species from the fauna of the areas studied (Metchera et al., 1997). Heavy metals (Cd, Zn, Cu, Pb) are among the most toxic and environmentally dangerous pollutants (Luter et al., 2011; Adu et al., 2012). They are potentially mutagenic class of environmental pollutants and some of them are implicated in the induction of tumours in experimental organisms and exposed humans (Minissi and Lombi, 1997). Jiang et al. (2000) had reported that copper could cause rapid decrease of mitotic index. Cd had been reported to cause genome damages (Risso-de Faverney et al., 2001), Pd is capable of causing a wide range of biochemical and enzyme inhibitions, chromosome aberrations, DNA synthesis abnormality and mutation, it forms complexes with many biomolecules thereby influencing

genetic structures (Johnson, 1998).

Dimitrova and Ivanova (2003) reported that the increased heavy metal amount in soil decreased not only the growth of vegetative organs but also the rate of cell division, inducing chromosome aberrations in *Linum usitatissimum*. Abu and Mba (2011) had also reported the induction of diverse chromosomal and cytogenetic abnormalities on *Allium* root mitosis by pharmaceutical effluents. Equally in previous studies chromatin clumping and denaturing effects on DNA by untreated wastewater samples had been reported (Abu and Ogbonna, 2009). Due to the high level of risk associated with the indiscriminate discharge of wastewater without adequate pretreatment and little or no concern by the industrialist and some environmental protection agencies in developing countries, these series of work has been designed to assess the cytotoxic and genotoxic effects of wastewaters. The aim of this study was to assess the chemical, cytotoxic and genotoxic effects of air liquid and polyester resin industrial effluents on *A. cepa* root tip mitosis as a pointer to their possible mutagenic effects on the ecosystem.

## MATERIALS AND METHODS

### Wastewater collection

The test effluents were collected from two industries in the Niger Delta, Southern Nigeria. The two chemical industries produce air liquid and polyester resin, respectively. The wastewaters were collected after primary treatment and stored in opaque plastic gallons in a refrigerator before use. Before the use of the wastewater, the temperature was restored to room temperature in the laboratory.

### Experimental material

Approximately equal sized onion bulbs of the purple variety were purchased from the local market in Nsukka, Enugu State. The outer scale leaves were removed and the compressed stem bases of the bulbs were scrapped to remove dry roots and expose the root primordial before planting.

### Chemical analysis

The chemical analyses of the effluents were determined at the Department of Civil Engineering, University of Nigeria, Nsukka. The wastewaters were analysed for zinc, lead, copper, cadmium, sulphate and nitrate. The pH of the samples were taken and recorded.

### *A. cepa* test

All the already cleaned bulbs for the microscopic assessment were sprouted over tap water before they were transferred to appropriate test solution (Fiskesjö, 1993; Ukaegbu and Odeigah, 2009). The poorly sprouted bulbs in tap water were discarded. The effluent dilutions used for the experiment were 25, 50 and 75% effluent concentrations. The undiluted wastewater was 100% while tap

**Table 1.** Chemical analysis of the effluents.

Effluent	pH	SO <sub>4</sub>	NO <sub>3</sub>	Pb	Cd	Fe	Zn	Cu
Air liquid effl	6.4	485.321	5.92	Nil	Nil	12.168	Nil	11.291
Polyester res. effl	5.8	12813.39	11.84	0.218	170.667	17.73	58.842	36.131
*Nigeria standard values	6-9	500	20	<1	<1	20	<1	<1

\*FEPA (1991).

**Table 2.** Analysis of variance (ANOVA) of the mitotic index values.

Source of variance	df	SS	MS	VR	P
Effluent	2	9.40E+02	4.70E+02	72737.84	< 0.001
Concentration	3	1.98E+02	6.61E+01	10241.49	< 0.001
Duration	1	1.69E+01	1.69E+01	2620.16	< 0.001
Effluent*Concentration	6	1.04E+02	1.73E+01	2671.56	< 0.001
Effluent*Duration	2	9.09E+00	4.55E+00	704.03	< 0.001
Concentration*Duration	3	4.77E+00	1.59E+00	246.05	< 0.001
Effluent*Concentration*Duration	6	3.72E+00	6.20E-01	95.99	< 0.001
Residual	24	1.55E-01	6.46E-03		
Total	47	1.28E+03			

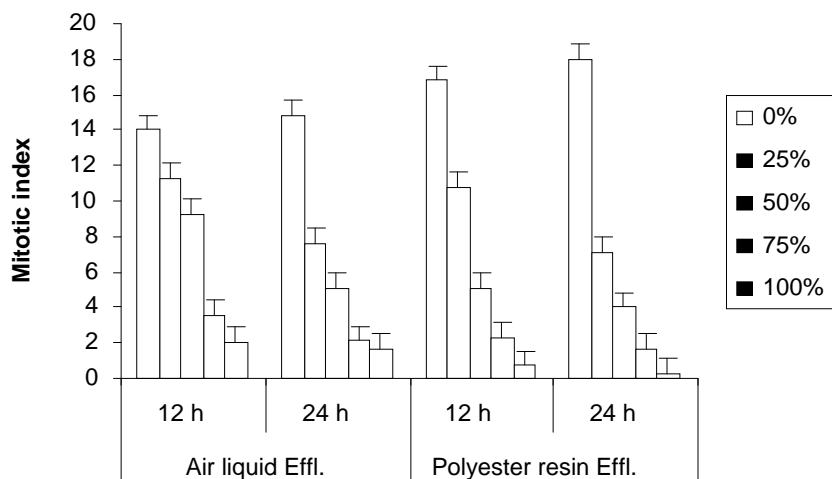
water served as the control (0%). Equal volumes of transparent plastic cups were properly positioned on the laboratory benches. Each sample with its five test solutions - 0, 25, 50, 75 and 100% were replicated three times for two treatment durations - 12 and 24 h, respectively. The bulbs with their roots immersed in the appropriate test solution were monitored for 12 and 24 h before harvesting the roots. The roots were fixed in Carnoy solution at the ratio of 3:1 absolute ethanol to glacial acetic acid and thereafter, transferred to 70% ethyl alcohol and stored at 4°C (Jones and Rickards, 1991). The roots were prepared for microscopic study through the conventional methods and aceto-orcein was used as stain (Sharma and Sharma, 1965; Jones and Rickards, 1991). The stained and macerated milky root tips on glass slides were covered with cover slips and observed in a microscope starting from lower magnifications. Cell counts were made for both dividing and non-dividing cells. Number of dividing cells out of 1000 cells were counted and recorded. Equally, the number of abnormal cells was also counted. The mitotic index was estimated by calculating the number of dividing cells out of 1000 (one thousand) cells expressed in percentage. The abnormal dividing cells were also expressed as a percentage of abnormal dividing cells over number of dividing cells. Different types of aberrant cells were noted and recorded. The mitotic cells were photographed with motic camera fixed on ordinary light microscope.

## RESULTS AND DISCUSSION

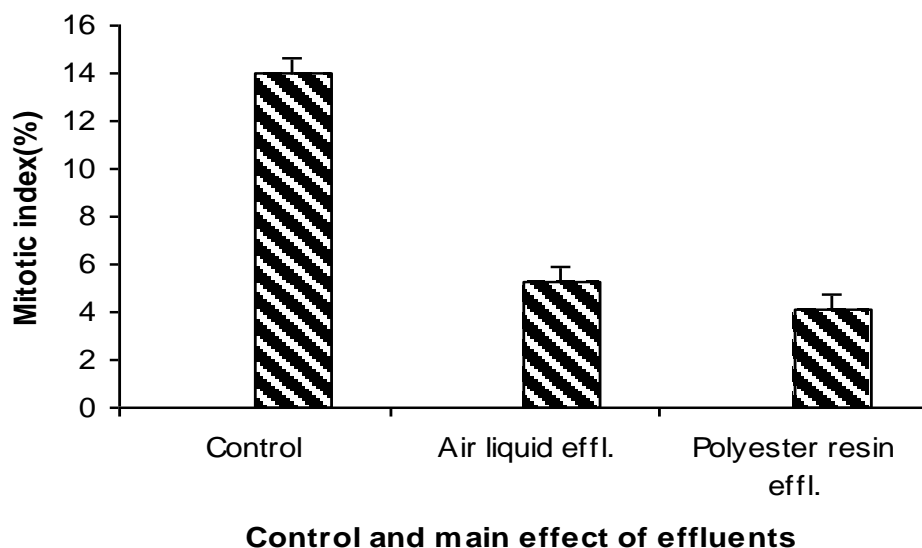
The result of the chemical analysis presented on Table 1 showed that the pH values were 6.4 in air liquid effluent and 5.8 in polyester resin effluents while recommended safe levels for the environment ranged from 6 to 9 (FEPA, 1991). The pH values of the wastewaters were approximately within the range of safe environmental values recommended by the federal environmental

protection agency except that polyester resin wastewater was slightly more acidic. The sulphate value (12813.4 mg<sup>l</sup><sup>-1</sup>) in polyester resin wastewater was very much higher than recommended environmental value of 500 mg<sup>l</sup><sup>-1</sup>. The nitrate, lead and iron contents in the effluent are within environmental safe levels ranging from 5.93 to 11.84, 0 to 0.22 and 12.17 to 17.73 mg<sup>l</sup><sup>-1</sup> in nitrate, lead and iron, respectively (Table 1). The values of cadmium and zinc in polyester resin industrial effluent were 170.67 and 58.84 mg<sup>l</sup><sup>-1</sup>. Copper was high in the two effluents, 11.29 and 36.13 mg<sup>l</sup><sup>-1</sup> for air liquid and polyester resin wastewaters, respectively. The presence of heavy metals in wastewater samples are implicated in diverse cytotoxic and genotoxic effects on organisms. The effects of mutagens on eukaryotic nuclei can be assessed cytologically by observing inhibition of cell growth and division, interruption of metaphase or the induction of numerical and structural chromosomal aberrations and changes among sister and other chromatids (Vieira and Vicentini, 1997).

Beyond the chemical analysis, the effects of the effluents were tested on living system by observing cell reproduction in *A. cepa* root tip cells. The analyses of variance table for mitotic indices estimates showed that the effluent, concentration, time and all the interactions were highly significant at  $P \leq 0.001$  (Table 2). This seems to suggest that the effluent and their dilutions, and the duration/length of time these effluents were allowed to remain in contact with biolife could significantly affect the mitotic indices. The significance of effluent dilutions is a pointer to their effects in natural systems where they may be diluted to varying concentrations by natural sources as rain or down the stream in water bodies. However, rain



**Figure 1.** Effects of effluents, concentrations and treatment durations on mitotic indices.

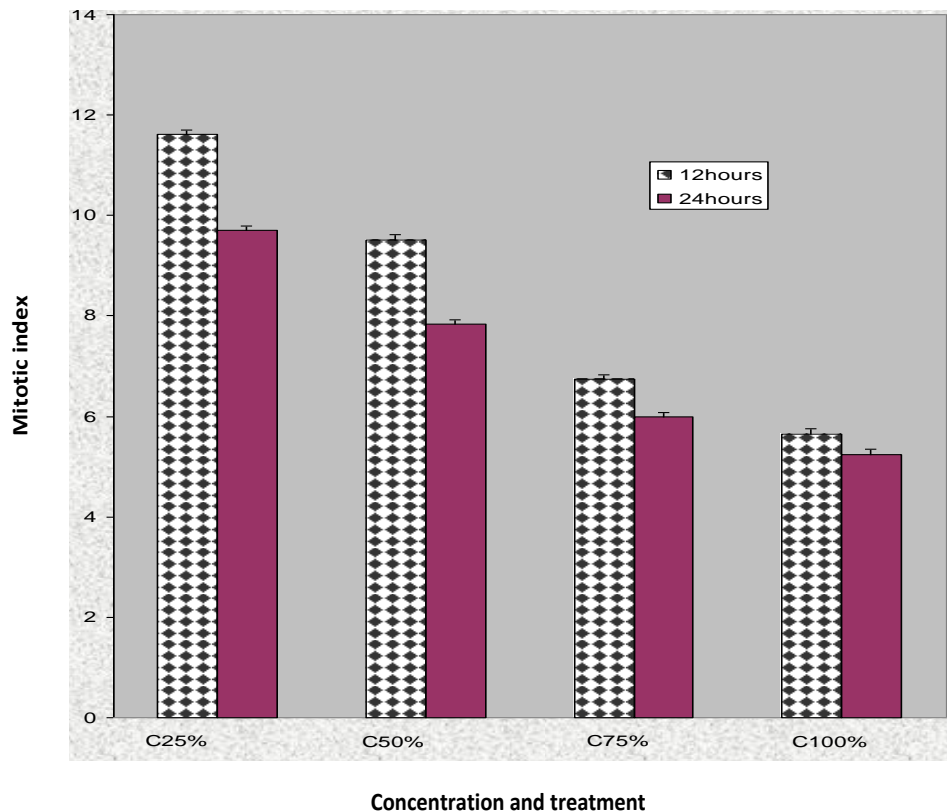


**Figure 2.** The main effects of the wastewaters and the control on the MI (%).

fall may take longer periods depending on seasonal variations, thereby allowing contact between plants and effluents to last for a longer period. The significance of the interactions also indicate that effluent, its concentration and the length of time of contact with biological systems can significantly affect cell reproduction in diverse ways.

The M1 in the effluents, their dilutions and across the treatment durations is shown on Figure 1. Significant differences ( $P = 0.001$ ) were observed on the main effects of effluents on the MI (%). The control had the highest MI in both the wastewater and the treatment durations. The Polyester resin effluent at 100% (undiluted) was more deleterious to cell reproduction at

both 12 and 24 h treatment time Figure 1. The main effects of the industrial wastewater and the control on cell reproduction as estimated by M1 (%) were 14.0, 5.3 and 4.1 in control, air liquid and polyester resin effluents, respectively (Figure 2). This indicates that the main effects of the effluents on MI (%) as it compares with the control value are 37.86 and 29.29% for air liquid and polyester resin, respectively. This places these wastewaters as being capable of causing sublethal effects on contact organisms (Antonsie – wiez, 1990; Panda and Sahu, 1985). Figure 3 shows the effects of concentration and treatment time on mitotic index. The MI (%) reduced significantly as the concentrations increased. The treatment time of 12 and 24 h duration



**Figure 3.** Effect of concentration and treatment time on mitotic index.

also caused significant reduction in cell reproduction as shown by MI (%) values. The 24 h treatment time had lower MI (%) across all the concentrations thereby suggesting that longer period of the wastewater with contact organisms are more deleterious.

The effects of the effluent across the different concentrations showed increase in percent abnormal cells as the concentration of the wastewater increased (Figure 4). The percentage abnormal dividing cells had direct relationship with the concentration of the effluents - increasing with increase in concentrations. The abnormal dividing cells ranged from 0.2% in control to 97.2% in air liquid and 0.4 to 98.79 in polyester resin effluents (Figure 4). At 75% effluent concentrations, abnormal dividing cells were more than 80% of the cells dividing. On close observation all the dividing cells in the undiluted wastewater concentrations in both effluents were abnormal. This implies that all the cells estimated in M1% in Figure 1 were all abnormally dividing cells at 100% effluent concentration. Similarly, treatment time had significant effects in inducing abnormal cells, the longer the *A. cepa* root cells stayed in the wastewater the more the number of abnormal dividing cells (Figure 5). Polyester resin wastewater, which also had higher concentrations of lead, cadmium, zinc and copper, significantly induced higher number of abnormal dividing

cells, even at lower concentrations. This agrees with several reports on the implication of heavy metals in inducing diverse cytotoxic and genotoxic effects in organisms (Bruning and Chronz, 1999; Kovalchuk et al., 2001).

The high reduction in cell reproduction and other observed abnormalities could lead to mutagenic effects of these effluents in the ecosystem. The reduced M1 and high rate of abnormalities could be correlated with the chemical compositions of the effluents. The chemical composition of the air liquid effluent was within the range of safe environmental levels with the exception of the copper which was as high as 11.3%. On the other hand, the polyester effluent had higher levels of sulphate, zinc and copper when compared to environmental standards. The M1 in undiluted polyester effluent was significantly lower than their values in both the control and air liquid effluent. The aberrant cells were also more in the polyester wastewater. The observed reduction in cell reproduction and increased aberrant cell could be linked to the toxic levels of chemicals and heavy metals in the effluent. It has been reported by several authors that heavy metals are deleterious and could cause diverse forms of aberrations (Minissi and Lombi, 1997; Johnson, 1998; Jiang et al., 2000). The very high levels of zinc and copper could be the reason for higher deleterious effects

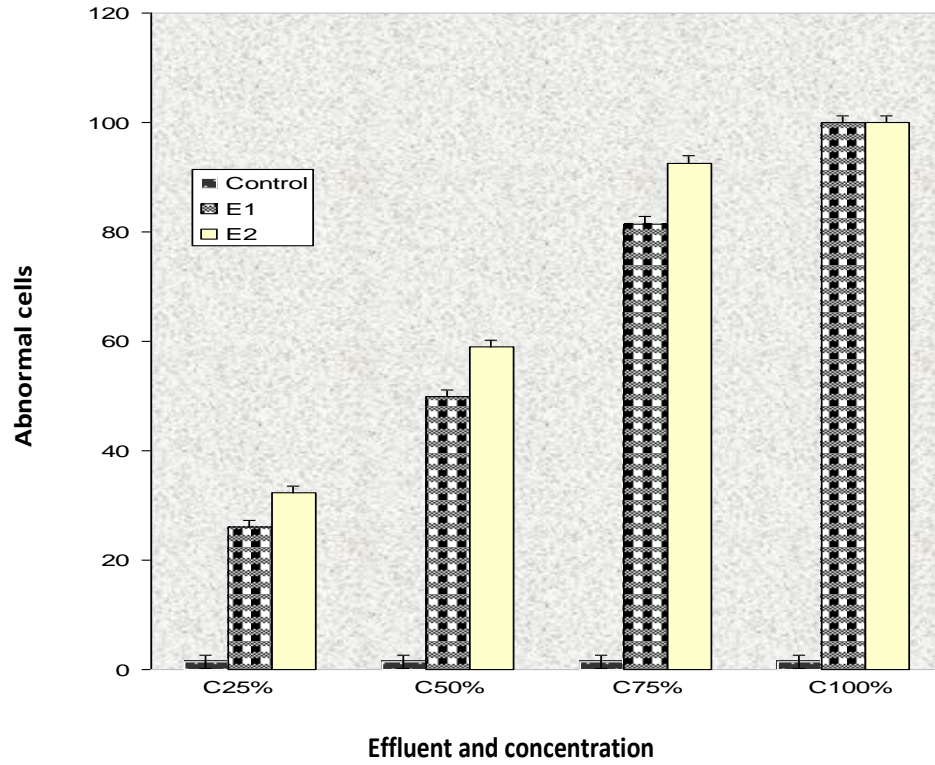


Figure 4. Effects of the effluents and the different concentrations on abnormal cells.

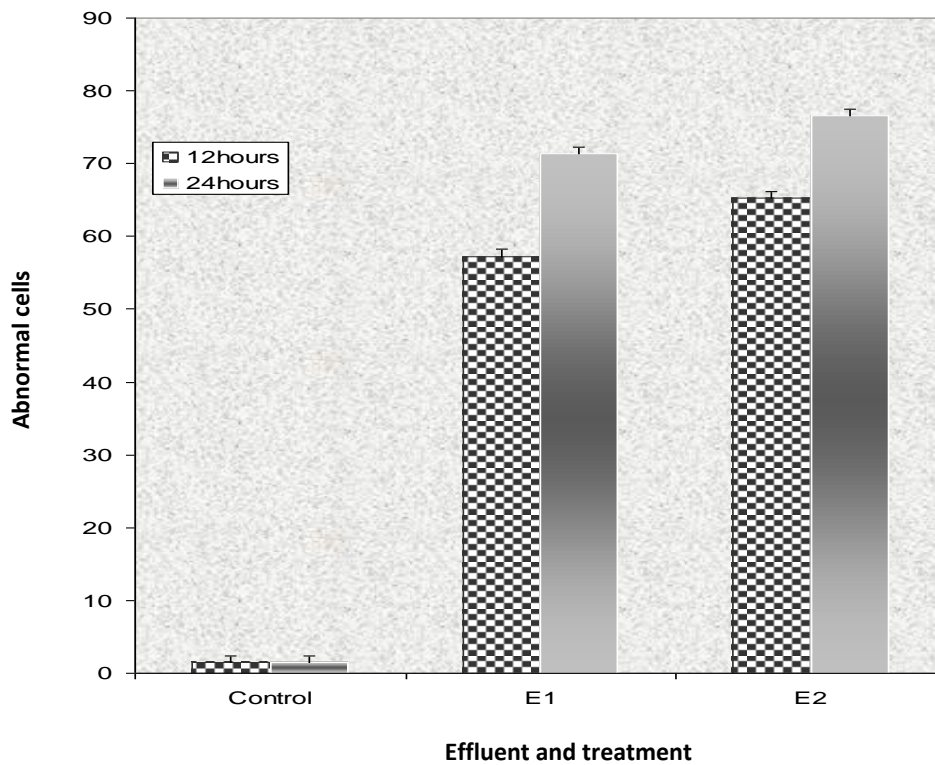
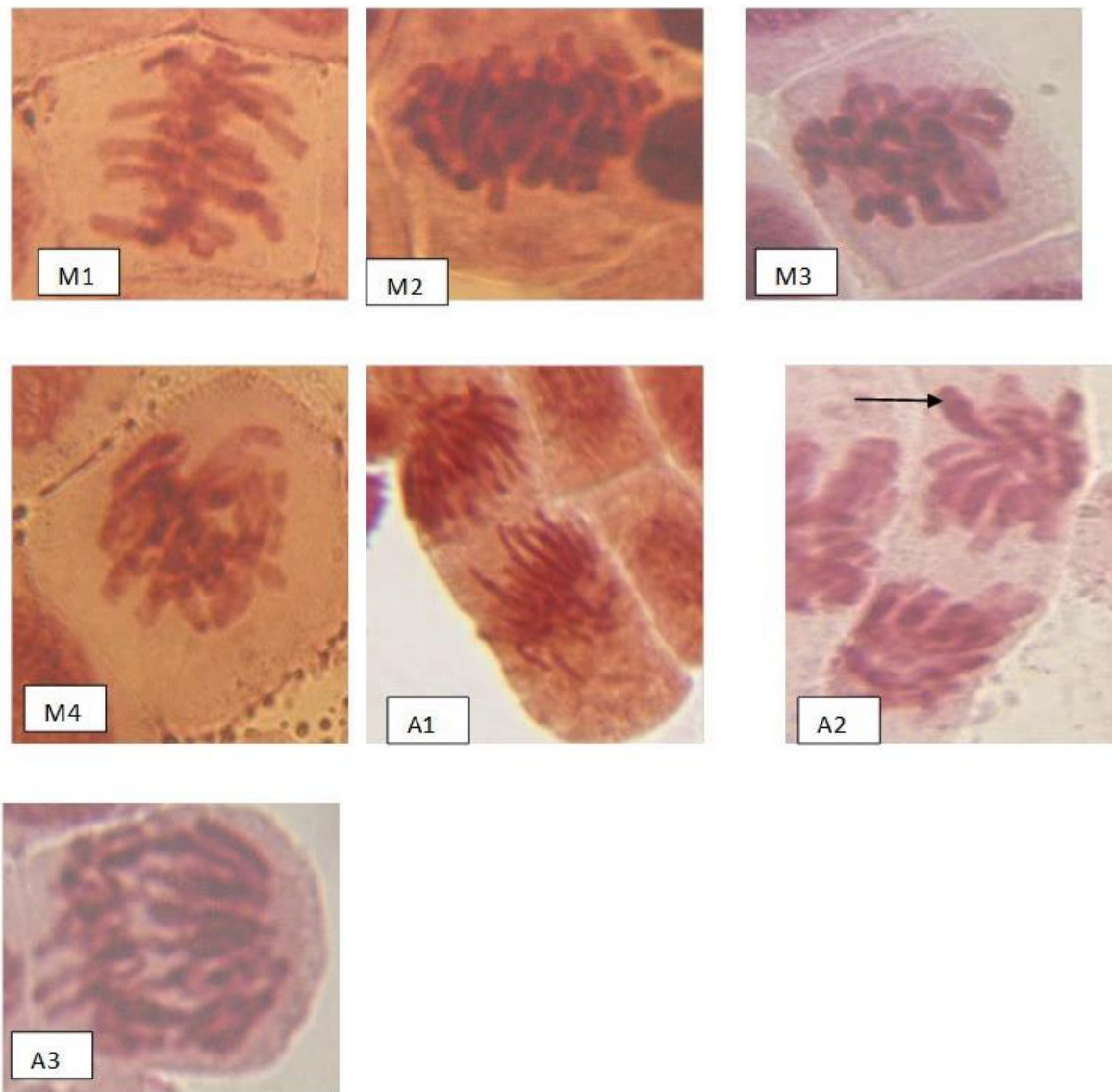


Figure 5. Effect of effluent and treatment time on abnormal dividing cells.



**Plate 1.** M1- normal metaphase, M2 to M4 – abnormal metaphase plates, A1 to A3 – Abnormal anaphase plates, (A1 anaphase with precocious chromatids, A2 anaphase with swollen chromatids- arrowed, A3 anaphase with precocious chromosomes and bridge).

observed in this polyester resin industrial effluent, however, the air liquid which had values within the range of environmental levels were also highly deleterious as can be seen in reduced cell reproduction and high percentage abnormal dividing cells. This is in line with an earlier report that wastewaters are complex mixtures and that chemical analysis cannot be effectively used to assess their toxicity level. Fiskesjö (1985a) and El-Shahaby et al. (2005) reported that wastewaters are extremely complex mixtures containing numerous inorganic and organic compounds, the complexity makes it impossible to carry out a hazard assessment based on chemical analysis only. Therefore, the seemingly low levels of compounds observed in air liquid effluent based

on chemical analysis may not be enough to quantify their risk in the ecosystem. The possibility of bioaccumulation and biotransformation equally increases their risk. Moreover, the action of wastewater in the natural system cannot be attributed to specific compounds in the mixture but to a set of chemical properties and interactions of groups of compounds in the wastewater.

Diverse forms of chromosomal aberrations were observed (Plate 1), these include: C-mitotic effects of diverse kinds signifying disturbance or breakdown effects on the spindle apparatus, precocious chromosomes at anaphase and anaphase bridges which could lead to chromatid breakage and formation of micronuclei or aneuploidy conditions in the parental cells. It has been

reported that cytokinetic failure in addition to diverse forms of chromosomal aberrations could lead to cancerous cells in organisms (Panda and Sahu, 1985; Bruning and Chronz, 1999; Kovalchuk et al., 2001). Another major abnormality observed was prophase accumulation. The effluents at high concentration significantly reduced cell division and equally there seemed to be a delay at prophase as most of the cells dividing at high concentration remained at prophase stage. This phenomenon has been attributed to a delay in the breakdown of the nuclear membrane due to a 'carryover' inhibitory effect from treatments (Wilson, 1965).

The significant reductions in cell reproduction as shown by low MI at high concentrations and diverse forms of chromosomal aberrations are pointers to the possible mutagenic effects of these wastewaters in the ecosystem. Positive results of *Allium* test should be considered as warning and an indication that the samples tested may constitute a risk to environmental health. Therefore, there is need for industries in the third world to maintain a sound sewerage system for proper pretreatment of effluents before discharge into the environment.

### Conflict of interests

The authors did not declare any conflict of interest.

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