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RISK EVALUATION OF INDUSTRIAL WASTEWATER ON PLANTS USING ONION (ALLIUM CEPA L.) CHROMOSOME ABERRATION ASSAY

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ABSTRACT

Rapid strides in industrialization coupled with incomplete sewage treatment system have led to indiscriminate discharge of potentially mutagenic wastewater into agricultural lands and water bodies. The response of Onion plant genetic material to the presence of potential cytotoxic and genotoxic substances in the environment was used to evaluate the toxicity of chemical effluent collected from a chemical industry in Enugu, Nigeria. The Allium test procedure was used. The concentrations of heavy metals (Cu, Co, Cd, Pb) were also determined. The data obtained showed that the mitotic index (MI) decreased as effluent concentration and treatment durations increased. Significant differences in MI at different concentrations and treatment durations were observed. Conversely, percentage of abnormal dividing cells significantly (P<0.05) increased as the concentration and treatment durations increased. Diverse chromosomal aberrations and cytotoxic problems were observed. These ranged from a mild C-mitotic effect at lower concentrations to vacuolated nucleues and even cytokinetic failure at higher concentrations. The adverse effects of this untreated wastewater on the ecosystem of which one of the most vulnerable are plants are discussed.

Keywords: Industrial wastewater; Cytotoxicity; Genotoxicity; Chromosome aberration; Onion.

INTRODUCTION

Rapid growth in industrialization is of great benefits to Nigeria. However, most industries have incomplete sewerage system. The wastewaters from these factories are discharged into land and water bodies. El-Shahaby *et al.* (2003) reported that industrialization movements in several cities have contributed large amounts of pollutants through indiscriminate discharge of effluents into drains. It is well established that pollution lowers the quality of life in various aspects, besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human afflictions like cancer, cardiovascular diseases and premature ageing (Grover and Kaur, 1999). Most industrial wastewater can be characterized as extremely complex mixtures containing numerous inorganic as well as organic compoumds (Nielson and Rank 1994). Most of these effluents contain high concentrations of potentially mutagenic heavy metals. Ivanova *et al.* (2005) reported that heavy metals are among the most toxic and environmentally dangerous pollutants. Dovgaliuk *et al.* (2001) has shown that the heavy metals could induce clastogenic and aneugenic effects including mitosis and cytokinesis disturbances.

The complexity of industrial wastewater makes it impossible to carry out a hazard assessment based on chemical analysis only. In search of test systems which can be combined with chemical analysis 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* test (Fiskesjo, 1985) was proposed. This was proposed as a standard method in environmental monitoring and toxicity screening of wastewater and river water. The author has demonstrated the usefulness of root tips of *Allium cepa* as a test system for monitoring the genotoxic effects of contaminated water from river receiving effluents from a chemical factory in Sweden. Plant root is very useful in this testing because the root tips are often the first to be exposed to chemicals in the soil and water (Odeigah *et al.*, 1997). *Allium* test has also successfully been used in evaluating cytotoxic and genotoxic effects of diverse complex mixtures of herbicides, fungicides, and insecticides as well as water extracts of medicinal plants (Njagi and Gopalan, 1981; Ene-Obong and Amadi, 1987a, b; Ghareeb and George, 1997; Abu and Duru, 2006). The objective of this work is to asses the adverse effects of this effluent on plants via it's cytotoxic and genotoxic effects on *Allium cepa* (Onion) root meristems.

MATERIALS AND METHODS

A chemical factory that produces insecticdes and shoe polish in Enugu, Enugu State, Nigeria was sampled. The wastewater is discharged via a pipe into a piece of land at the back of the site. The effluent was collected at the point of discharge and was analyzed chemically for the presence and concentrations of some potentially mutagenic heavy metals (Cd, Cu, Co, Pb) and other chemicals Cl, SO_4, Na, Ca, Mg as well as chemical oxygen demand (COD), total disolved solids (TDS), and total suspended solids (TSS).

Approximately equal sized Onion bulbs were bought from an open market. The bases of the bulbs were gently scrapped to expose the root primordia. The wastewater sample was diluted with distilled water to produce two dilutions-50% and 75 %. The undiluted wastewater is 100 % while tap water was used as the control. Ten bulbs each for 50%, 75% and 100% concentrations of the wastewater and the control were germinated in tap water and the best eight in root growth were transferred to each test solution. The germinated bulbs were left in the control and effluent concentrations for 6 and 12 hours, respectively (ie four bulbs per concentration per time). There were four replications for each concentration and control at each treatment duration. The roots were harvested after each period of treatment and fixed in aceto-alcohol (1:3). The root tips were hydrolyzed in 1N HCL at 60^0C for 5 minutes. Aceto-orcein squash technique was used in preparing the root tip for cytological examination. Slides were examined and counts were made for dividing cells, non dividing cells, cells at each mitotic phase and aberrant cells were made.

Photomicrographs of some aberrant cells were taken. Mitotic Index was estimated as number of dividing cells over the total number of cells counted expressed in percentage. The phase indices were estimated as number of cells in each mitotic phase over the number of dividing cells expressed in percentage. Similarly, the percentage abnormal cells were calculated as the number of aberrant cells over the number of dividing cells

RESULTS

The results of the chemical analysis showed that the wastewater sample is slightly acidic with pH value of 6.3 (Table 1). The total dissolved solid (TDS) was 520 mg / l. The values for heavy metals Co, Cu, Cd and Pb were 18.859, 1191.117, 43.758 and 2.305, respectively. These values are presented together with their standard environmental levels by the Federal Environmental Protection Agency of Nigeria (FEPA 1991) in Table 1.

The acceptable percentage of heavy metals in the environment are less than 1mg/l for Cu,Cd and Pb(FEPA 1991). The total suspended solids (TSS) in this wastewater is 14,700mg/l as compared to the environmental standard value of 30mg/l. This is equally much higher than the acceptable environmental level for TSS.

The results of the Mitotic index (MI) with the error bar at different effluent concentrations and the control in the two treatment durations are summarized in Fig. 1. Effluent concentrations and treatment durations had significant effect on mitotic index and the number of abnormal dividing cells. The MI. decreased with increasing concentrations of the wastewater and increased duration of the treatment, however, the MI (%) at 50% concentration seemed to have increased more than the control. The MI (%) values in the control were 18.15 and 17.56 (%) at 6 and 12 hours treatment durations respectively. The MI values at 50% and 75% concentrations and at both treatment durations are 20.83 and 19.35; 14.88 and 13.39 respectively. At 100 % effluent concentration, it varied between 9.23 and 7.44 at both treatment durations. Thus the Mitotic Index (%) at 100 % concentration is 50. 85 % and 42.37 % in comparison with the control at 6 hours and 12 hours, respectively. The percentage abnormal dividing cells at different effluent concentrations and treatment durations are shown in Fig. 2.

This was estimated as the number of aberrant dividing cells over the total number of dividing cells expressed in percentage (Abu and Duru, 2006). Low percentages of aberrant cells, 6.56 % and 8.44 were observed in the control. All the other effluent concentrations (50 %, 75 % and 100 %) at both durations of treatment had more than 50 % of the dividing cells as abnormal with the exception of 100% at 6hrs. The phase indices are presented in Table 2. These show both normal and abnormal dividing cells in each mitotic phase. Prophase accumulation was observed in all the effluent concentrations. This probably resulted in a reduction in the number of cells that progressed mitotically to other phases. The percentage of abnormal dividing cells in each mitotic phase increased with increase in concentrations. At 100 % effluent concentration and 12 hours treatment duration, 60 % of the dividing cells were at prophase. Out of these, 32 % were abnormal; at metaphase, there were 24 % out of which 12 % were abnormal; at anaphase there was no normal dividing cells, all were abnormal (Table 2). Fig 3 shows the plates of normal mitotic stages for a quick comparism with abuoamal. The chromosomal aberration micrographs are presented in Fig. 4. The diverse aberrations observed are C – mitotic effect, anaphase with chromatid fragment, bridges and precocious chromosomes, as well as degenerating chromosomes at telophase. Cytokinetic problems like anucleate cell and failure of cell plate formation leading to multinucleate cells were also observed. Vacuolated nucleus was a common feature of cells at 100% effluent concentration.

Table 1: Nigerian Standards and Chemical Analysis of the wastewater.

Standard / Wastewater	pH	TDS	SO_4	Cl	COD	TSS	Na	K	Ca	Mg	Co	Cu	Cd	Pd
Nigerian Standard*	6-9	2,000	500	600	-	30	-	-	3	200	-	< 1	< 1	< 1
Wastewater	6.3	520	71.7	90	512	14,700	275	42.5	1,062	48.6	18.859	1191.117	43.758	2.305

All values except pH are expressed in mg / l.
TDS – Total dissolved solids
TSS – Total suspended solids
COD – Chemical oxygen demand
- **Federal Environmental Protection Agency (FEPA 1991).**

Table2.Normal (Norm) and abnormal (Abn) cells at different mitotic phases

Treatment Conc/duration	No. of dividing cells	Prophase Norm..	Prophase Abn.	Metaphase Norm.	Metaphase Abn.	Anaphase Norm.	Anaphase Abn.	Telophase Norm.	Telophase Abn.
6hrs	61	22.95	1.64	22.95	3.28	19.67	0.00	27.87	1.64
0% 12hrs	59	23.73	1.69	22.03	3.39	18.64	1.69	27.12	1.69
50% 6hrs	70	17.14	20.00	12.86	14.9	11.43	12.86	5.71	5.71
12hrs	65	16.92	21.54	10.77	13.85	9..33	12.31	4.62	10.77
75%. 6hrs	50	16.00	20.00	12.00	14.00	4.00	14.00	4.00	6.00
12hrs	45	13.33	20.00	11.11	15.56	11.11	15.56	4.44	8.89
100%. 6hrs	31	29.03	22.58	16.13	9.68	9.68	6.45	3.23	3.23
12hrs	25	28	32.00	12.00	12.00	0.00	12.00	4.00	0.00

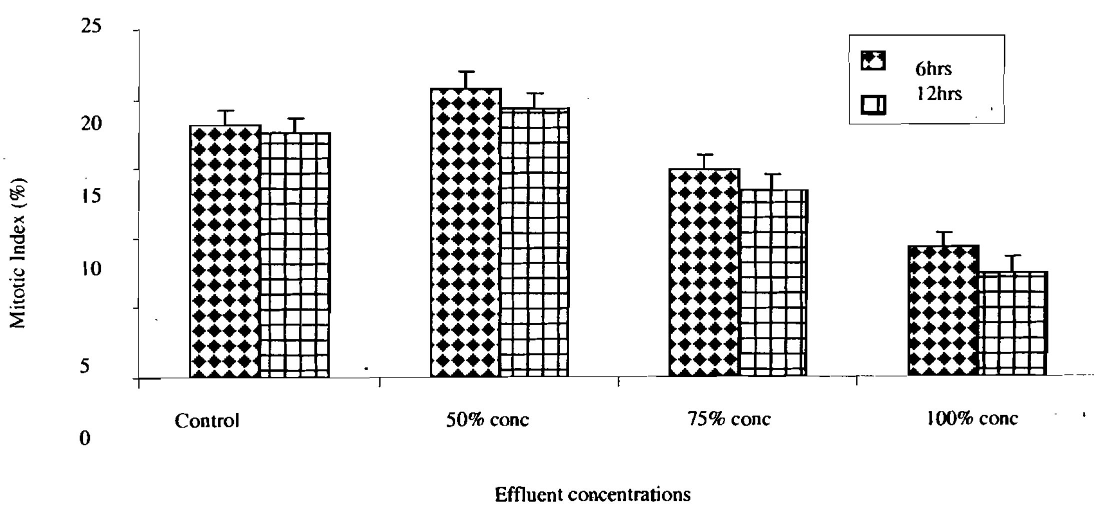

Fig. 1. Mitotic index at different effluent concentrations and treatment duration

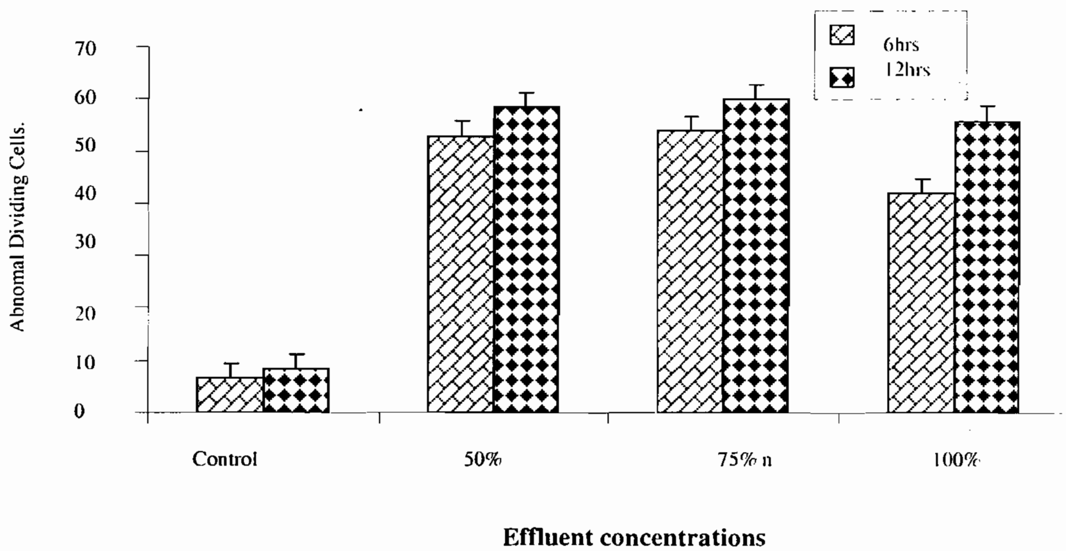

Fig. 2. Effect of effluent concentrations on the number of abnormal dividing cells.

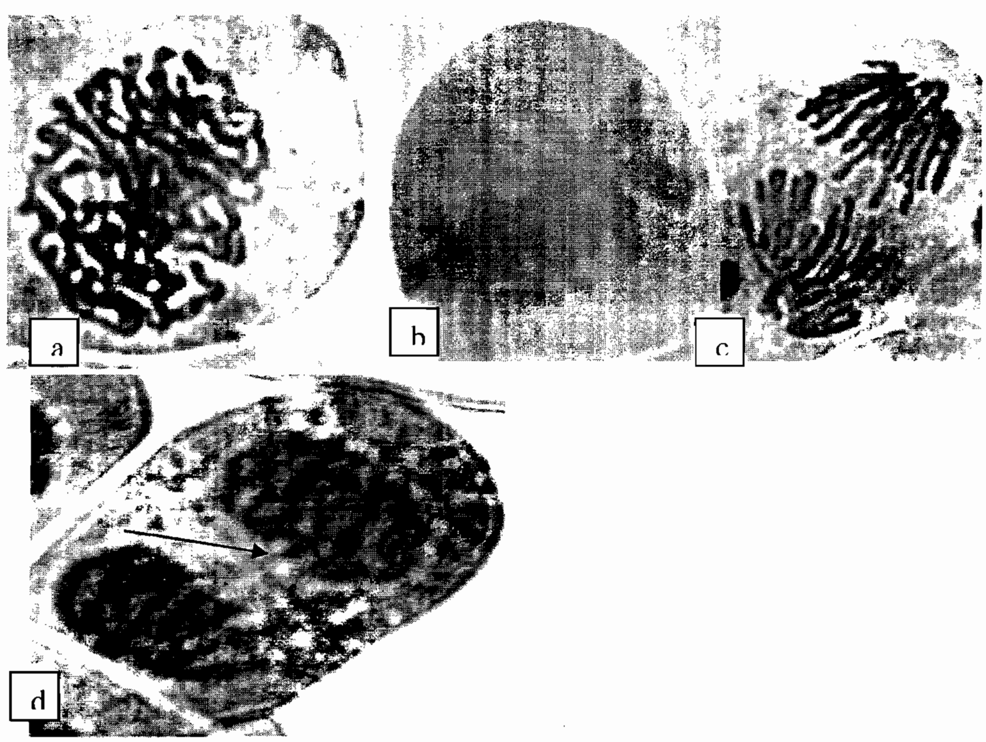

Fig 3. Normal mitotic stages in *Allium cepa* root meristems (a) prophase, (b) metaphase,(c) anaphase and (d) telophase with signs of cell plate formation (arrowed).

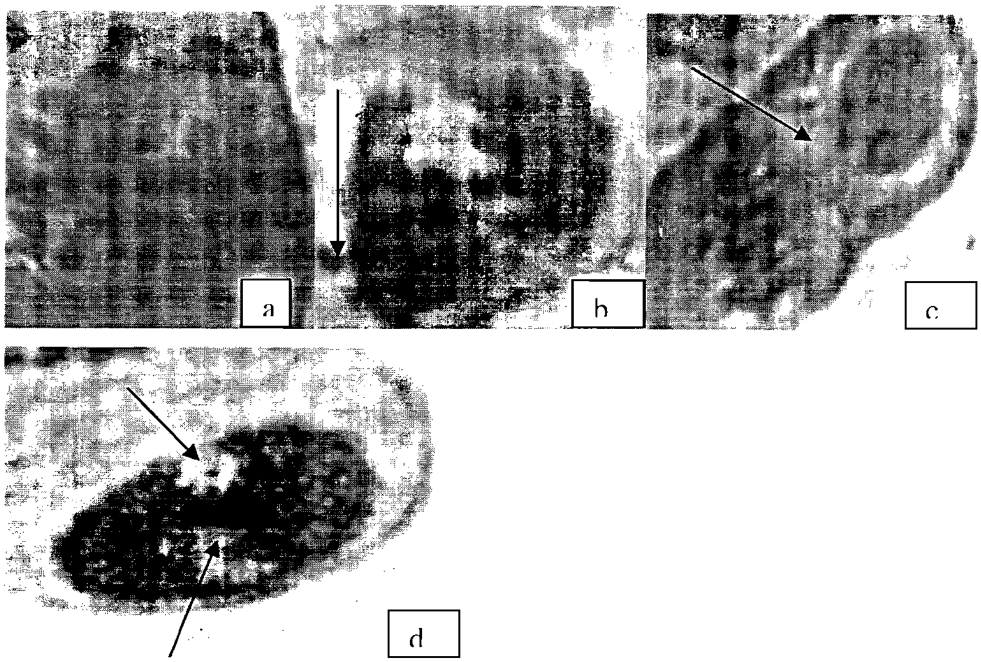

Fig 4. Abnormal dividing cells and an abnormal nucleus (a) c-mitosis, (b) late anaphase with vagrant chromosome 'arrowed', (c) cytokinetic failure ' arrowed', (d) Vacuolated nucleus 'arrowed'

DISCUSSION

Allium test has been proposed as a standard method in environmental monitoring and toxicity screening of wastewater and river water (Fiskesjo, 1993, Rank and Nielson, 1993). The results of the Allium test may indicate the presence of certain cytotoxic / genotoxic or mutagenic substances in the environment, which represent direct or indirect risks for all living organisms (El-Shahaby *et al.*, 2003). The results of the chemical analysis are within the range of standard values by the Federal Environmental Protection Agency of Nigeria (FEPA 1991), with the exception of the concentrations of the heavy metals – Co, Cu, Cd and Pb, which were very high in the chemical effluent. Their values are far above environmental standard levels. Lead and Cadmium are about 230 and 4375% above the environmental standard of less than 1mg/l while Copper and Cobalt in the same trend are about 119111 and 1885% above the standard levels respectively. The high values would tend to imply that the concentrations of these heavy metals could attain a deleterious level in the wastewater. Heavy metals have been reported to be among the most toxic and environmentally dangerous pollutants (Ivanova *et al.*, 2005). Mitotic index is used as a bio-monitor to asses the mutagenicity of effluents (Fiskesjo, 1985). Mitotic index in this study significantly decreased with increased in the concentration of the effluent. This may be due to increase in the concentration of heavy metals as effluent concentrations increased. This implies that the mitodepressive effect of this effluent was dose-dependent. At 75 % and 100 % concentrations the number of cells entering mitotic cell division reduced drastically. The cells seemed to remain or have a delay at interphase nucleus. This phenomenon was also reported by Ene-Obong and Amadi (1987a,b) and Ene-Obong (1995). At 50 % concentration, there seemed to be a higher MI than the control. However, more than half of the dividing cells (52.86% and 58.46%) were abnormal at both treatment durations, respectively. A possible explanation may be that the cells that had contact with the wastewater may have reacted by undergoing rapid cell division with a resultant high level of abnormalities. Panda and Sahu (1985) and Antonsie-Wiez (1990) reported that the cytotoxicity level may be determined by the degree of the MI; a mitotic index decrease below 22 % of the control causes lethal effects on test organisms while that below 50 % has sublethal effects. In the present study, the MI at 100 % concentration and at 12 hour duration was 42.37 % of the control, thereby suggesting that this wastewater has sublethal effect in undiluted form. Moreover the percentage abnormality was found to increase with effluent concentrations, thereby pointing out the toxicity of this wastewater on test organism and the ecosystem in general. Heavy metals are implicated as the cause of this high toxicity. The low percentage of abnormal cells observed in the control may be due to an automutagenic effect. This was also observed and reported by Ivanova *et al* (2005).

Prophase accumulation was a common feature in all the effluent concentrations. At 100 % effluent concentration in both 6 and 12hrs of treatment, more than 50 % of the cells dividing were at prophase stage. Similarly the number of abnormal dividing cells at prophase is more than the normal dividing cells in the same phase.. Prophase accumulation has been reported by other researchers as a common deleterious effect of industrial wastewater and other complex mixtures on the root tip meristem of plant materials. (Raji and Reddy, 1971; Ene-Obong, 1995; Abu and Duru, 2006). Prophase accumulation has been attributed to a delay in the breakdown of the nuclear membrane due to 'carry over' inhibitory effects of treatments from interphase stage (Wilson, 1965). Ene-Obong and Amadi, (1987a, b) and Amor and Fara, (1974) attributed prophase accumulation to a disturbance or breakdown in spindle apparatus. Similarly C-mitotic effect on cells has also been attributed to spindle abnormalities (Grover and Kaur, 1999). Abnormal spindle function and inhibition of cytokinesis in *Allium cepa* L. was equally observed by Panda and Sahu (1985).

Nuclear vacuoles are higher indication of nuclear poison than surface nuclear leisions. Mercykutty and Stephen (1980) and Akaneme and Iyioke (2008) noted that the presence of nuclear lesions and nuclear dissolution offer cytological evidence for the inhibitory action on DNA biosynthesis. Kovalchuk *et al* (2001) stated that pollution with heavy metal salts caused an increase in the frequency of somatic intrachromosomal mutations. Rank and Nielson (1998) ascertained genotoxicity at the lowest industrial load by anaphase – telophase assay and reported that the toxicity could be positively correlated to the industrial load and concentration of the heavy metals (Pb, Ni, Cr, Zn, Cu, Cd). The results of the present work agree with the above report on the mutagenicity of industrial effluents being caused by heavy metals.

Monitoring of hazardous wastes is vital for sustaining the required legal compliance and in real terms prevents irreversible health and ecological damages. There is sufficient evidence from the results obtained that chemical effluent with high concentration of heavy metals could constitute environmental hazards. It is therefore recommended that industries should introduce measures that will remove or reduce mutagenic heavy metals before discharging wastewater into the sewer

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