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Mitotic response of *Allium cepa* L. to toxicity of pharmaceutical effluents

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The need to determine the mutagenic ability of waste waters from pharmaceutical industry is on the rise. With the aid of macro and micro assay of the root tip, this study was carried out to assess the genotoxic effects of varied concentrations of three pharmaceutical effluents (Ciprofloxacin, Arthemetrin and Psuedophedrin) on *Allium cepa* root tip during mitosis. The chemical evaluation of the effluents confirmed the presence of a few heavy metals with mutagenic potentials (Ni, Zn, Pb and Cu). The experiment carried out in the research and teaching laboratory of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The results showed extensive reduction in the length and number of the roots that were grown on the effluents. Noticeable reductions in the reproduction process of the cells were observed in Ciprofloxacin and Psuedophedrin than Arthemetrin effluent. The mitotic index ranged from 18.92 to 22.55% with Ciprofloxacin recording significantly ($P < 0.05$) the highest index. Both effluent concentrations and the time of treatment significantly affected the mitotic index. Conclusively, these untreated effluents are considered to be lethal following the aforementioned results and are environmentally risky. Therefore, a sound sewerage system in the pharmaceutical industry is therefore crucial.

Key words: Cell, mitosis, pharmaceutical effluents, environmental pollution, sewage.

INTRODUCTION

The global amplification of the production of pharmaceuticals had resulted in making the pharmaceutical industry one of the foremost contributor to pollution, mostly in the discharge of effluents and solid wastes into the environment. Pharmaceutical industries by and large create various set of waste in the process of manufacturing and maintenance operation. More so, like other industrial wastewaters, pharmaceutical wastewaters are tremendously a complex mixtures composed of chemicals and micro organic chemicals (salt), surfactants and their metal complexes, toxic organic chemicals, biocides, un metabolized drugs, etc (Sumpter, 2005; Gadipelly et al., 2014; Patel et al., 2019). These chemicals primarily are accountable for the toxicity of

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effluents from the pharmaceutical industries. Effluents are defined as wastewaters (treated or not treated), that is discharged out of a treatment plant, sever or industrial fallout (USEPA, 2006). Pharmaceutical effluents are therefore wastewaters produced by pharmaceutical industry during the process of drug production. A larger number of these industries which are involved in manufacturing of pharmaceutical and personal care product lack inadequate treatment plants because of the presence of recalcitrant substances. Toxic materials which includes a lot of organic materials, acids, alkalis, and non-metallic elements are normally resistant to biological processes except diluted. Metals are frequently capable of precipitating out with varying pH or treatment by means of other chemicals. Pharmaceuticals discharge a lot of xenobiotics acknowledged as lipophilic, which are not biodegradable in nature into the environment (Hernandoa et al., 2006; Lateef et al., 2007). The combining and/or potentially opposing character of organic compounds and metals present in the effluents from industries producing drugs can induce alterations in enzymes biochemistry, bio molecules and cell membrane due to the increased cellular formation of oxidative stress (Bakare et al., 2003). Therefore, their existence in the environment is of grave concern. Once more, most pharmaceutical products in the environment build up in water bodies, soil and biological systems, and with time attains a biologically active level (Hernandoa et al., 2006). A prolong exposure of complex pharmaceutical compounds even in low concentrations on water ecology may possibly cause acute and reproductive damages, changes in behaviour, and inhibits the proliferation of cells (Patneedi and Prasady, 2015). Pharmaceutical effluent has toxic substances that are harmful to health and when released into the surface water makes up biological hazards to human and other organisms living in that environment (Bakare et al., 2003; Olaitan et al., 2014).

Bioassays with numerous higher plant roots have offered alternative methods that are reliable, sensitive, easier, cheaper, useful, and valuable in the evaluation of the deleterious effects of pollutants in the environment as compared to the usual experimental animal assays (Olaitan et al., 2014; Urama et al., 2020). Most of the researches had indicated a strong relationship between abnormalities in the chromosome established in root-tip systems and those observed in mammalian cell systems. Therefore, the observation of plant root tip systems makes up a rapid and sensitive technique for monitoring the environment (Majer et al., 2005). Onions bulbs (*Allium cepa*) have largely been used for culinary purposes, but apart from its significance nutritionally, the common onion is among the majority of outstanding higher plant recommended by USEPA and the ASTM in 1982 and 1994, respectively. *A. cepa* L. has been utilized as a first-level evaluation method for the identification of environmental pollutants that may present genetic dangers from a broad variety of chemicals such as sewage effluents (Ukaegbu and Odeigah, 2009); hence, the research is aimed at studying the cytotoxic effects of pharmaceutical effluents on *A. cepa* root tip during mitosis.

MATERIALS AND METHODS

The three effluents (Ciprofloxacin, Arthemetrin and Psuedophedrin) were collected from a pharmaceutical industry in Enugu, Enugu State, Nigeria. Opague plastic gallons were used to collect the samples at the discharge point of the sewer system and preserved in the refrigerator. The effluents were analyzed for a number of physical and chemical properties, including total dissolved solids (TDS), chemical oxygen demand, biochemical oxygen demand (BOD), turbidity, and total hardiness according to methods described by APHA (2005). The pH of the samples was measured using a digital pH meter (Metrohm, Model: 872 Labo-maga symbol). Selected heavy metals comprising Nickel (Ni), Zinc (Zn), Copper (Cu) and Lead (Pb) were also analyzed following standard analytical methods (USEPA, 2006; APHA, 1998) using an atomic absorption spectrophotometer (AAS) (Analyst 200 Perkin Elimer).

Onion bulbs (*A. cepa*, L, 2n=16) of the purple variety with average size of 15-22 mm diameter were procured from the Ogige market in Nsukka town, Nigeria and used all through the study. The onions were dried under the sun for two weeks and fungi infested ones were discarded while the unaffected ones were utilized for the experiment. The external layers were removed carefully, without interfering with the primordial root ring. Several bulbs of onion were used per treatment to account for a number of bulbs in the population that would be naturally slow or poor growing, as in previous studies (Olorunfemi et al., 2015).

The wastewater samples were diluted with distilled water to produce concentrations of 0, 25, 50, 75 and 100% (v/v, distilled water/effluent).

Macro evaluation of the root

The evaluation of the initiation and growth of the roots was conducted using the modified *Allium* test method adopted by Abu and Mba (2011). The bulbs were planted directly on the different effluent concentrations without any initial rooting over water after scrapping the base. The experiment was laid out in a completely randomized design (CRD) with five replicates.

Micro evaluation of the root tips

The micro assay experiment was conducted using a 3 × 5 × 2 factorial in a completely randomized design (CRD) replicated three times. The factors include 3 effluents (Ciprofloxacin, Arthemetrin and Psuedophedrin), 5 concentrations (0, 25, 50, 75 and 100%) and 2 treatment duration (12 and 24 h). The bulbs were germinated in water before transferring to the different treatment. The root tips were then harvested, fixed with glacial acetic acid: absolute alcohol (1:3 v/v), hydrolyzed in 1 N HCL at 60°C for 5 min and rinsed with distilled water. Two root tips were squashed on each slide and stained with aceto-orcein stain for 10 min. With the aid of a filter paper, the surplus stain was removed and a cover slip was carefully placed to prevent air bubbles being trapped under. The edges of the cover slip were then sealed with a nail polish to prevent preparation from drying out by the heat of the microscope (Olorunfemi et al., 2015; Ibeh and Umeham (2018). Five different slides were prepared per each treatment combination and

Table 1. Chemical analysis of the effluents.

Physicochemical parameter	Effluents			Standards		
	Ciprofloxacin	Arthemetrin	Psuedophedrin	FEPA	NESREA	USEPA
Ni (mg/l)	0.6154	0.3077	0.3077	< 0.386	0.05	0.005
Zn (mg/l)	0.0087	0.0173	0.0173	<1	-	5
Pb (mg/l)	1.00	0.25	0.75	<1	0.05	0.02
Cu (mg/l)	0.00323	0.0647	0.0647	<1	0.5	1.3
pH	4.6	5.2	3.8	6-9	6-9	6.5-8.5
TDS (mg/l)	5730	1193.08	9816.19	2000	500	500
BOD (mg/l)	1152	880	1568	50	50	250
COD (mg/l)	3840	2960	5200	50	NA	NA
Turbidity	45.2	28.6	60.9	NA	NA	NA
Total hardness	154	148.6	141	NA	NA	NA

FEPA-Federal Environmental Protection Agency (2001); NESREA-National Environmental Standards and Regulation Enforcement Agency (2009); USEPA-United States Environmental Protection Agency (2006) Standards for effluent discharge regulation; TDS-Total dissolved solid; BOD- Biochemical oxygen demand; COD-Chemical oxygen demand; NA- Not available (that is, no guideline established).

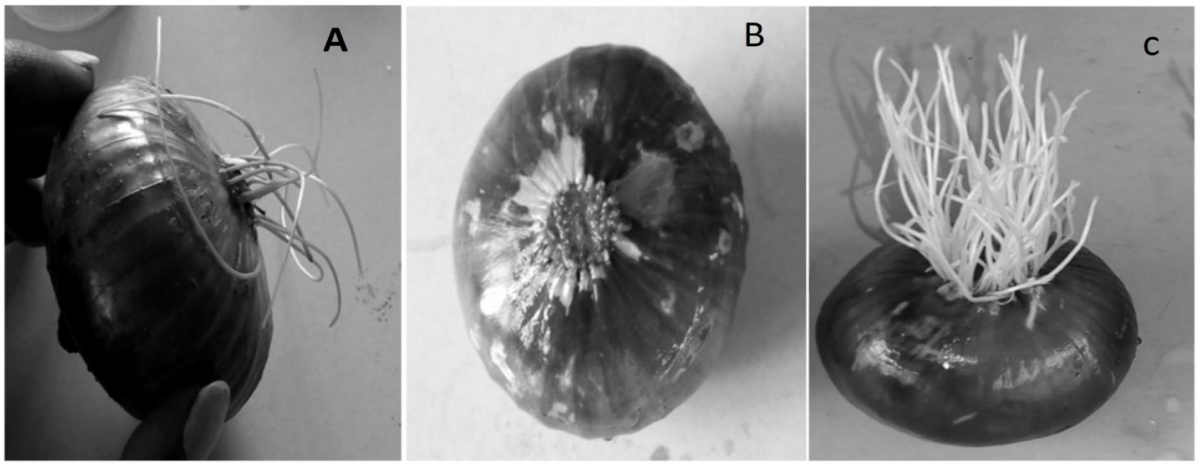

Plate 1. Morphological changes in *A. cepa* root tips induced by pharmaceutical effluents; A - Dry and unhealthy roots, B - C-tumor roots; C- Healthy roots grown in the control.

examined for different mitotic stages and occurrence of aberrant cells at ×1000 magnification. Data were collected on number of dividing cells out of 1000 cells. The mitotic index (MI) was expressed in percentage of the number of dividing cells in 1000 cells and photomicrographs were taken using a motic camera fixed on the ordinary light microscope.

RESULTS

Chemical analysis of the effluents

The heavy metals and physico-chemical properties of the pharmaceutical effluents are presented in Table 1. It was observed that the effluents were slightly acidic and contained varied amounts of heavy metals. The heavy metals (Ni, Zn, Pb, and Cu) analyzed ranged from 0.000323 to 1.00 mg/l. The concentration of Ni and Pb observed in Ciprofloxacin were higher than the other effluent. However, Ni and Pb concentrations in all the effluents were also higher than the recommended standard of NESREA and USEPA while Zn and Cu concentrations were within the three standards limit. TDS ranged from 1193.08 in Arthemetrin effluent to 9816.19 mg/l in Psuedophedrin effluent. More so, TDS, BOD and COD observed in Ciprofloxacin, Arthemetrin and Psuedophedrin were higher than the recommended standards (Table 1).

Effect of effluent on growth and development of roots in *A. cepa*

In a separate experiment, the effect of effluent on growth and development of roots in *A. cepa* as shown in Plate 1 were evaluated and the results are shown in Table 2.

Arthemetrin effluent supported more growth of roots as

Table 2. Effect of effluents on number of roots and root length.

Effluent	Root growth	
	Number of roots	Root length
Ciprofloxacin	0.95 ± 0.53[b]	13.27 ± 8.68
Arthemetrin	2.55 ± 0.70[a]	17.53 ± 8.33
Psuedophedrin	0.43 ± 0.39[b]	7.00 ± 6.38
LSD	0.41	NS

Data are presented with means ± SE. significant means were separated with different superscript alphabets on each culunm using least significant difference (LSD) at P < 0.05.

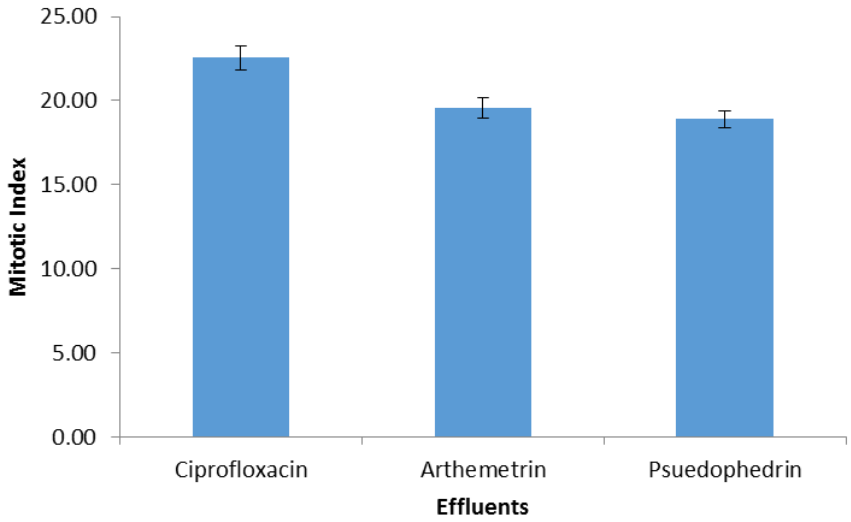

Figure 1. Effect of effluents on mitotic indices.

compared to Ciprofloxacin and Psuedophedrin. *A. cepa* grown in Arthemetrin effluent had 2.55 ± 0.70 roots which was significantly (P < 0.05) higher than 0.95 ± 0.53 and 0.43 ± 0.39 roots observed in *A. cepa* grown in Ciprofloxacin and Psuedophedrin effluent, respectively (Table 2).

Effect of effluents on mitotic indices, number of abnormal and normal dividing cells

The microscopic assessment of the squashed *A. cepa* L. root tip meristem cells showed that mitotic activity, expressed as mitotic index, was significantly affected by the different factors considered in this study. The main effect of the effluents on the mitotic index ranged from 18.92 to 22.55% with Ciprofloxacin recording significantly (P < 0.05) the highest index while Psuedophedrin effluent gave the lowest mitotic index (Figure 1). The main effect of the duration showed that root tips soaked for 12 h recorded higher mitotic index which was significantly different (P < 0.05) from 24 h treatment time (Figure 2). There was a dose dependent effect of the concentration on the mitotic index as shown in Figure 2. The mitotic index ranged from 17.32 to 23.99% with the control recording significantly (P < 0.05) the highest index while 100% concentration recorded significantly (P < 0.05) the lowest index (Figure 3).

The effect of Ciprofloxacin effluent treated for 12 h gave the highest mitotic index of 23.04% while Arthemetrin treated for 24 h recorded the lowest mitotic index of 18.22% (Figure 4). The result on the interactive effect between the concentration and duration shows that control (0%) effluent treated for 24 h recorded higher mitotic index of 24.01% while 100% concentration treated from 24 h recorded the lowest mitotic index of 15.96% (Figure 5).

The result for the interactive effect between the effluents and concentration as presented in Table 2 shows that Ciprofloxacin control recorded significantly the highest mitotic index of 26.11 ± 0.36%. This was followed by 25% Ciprofloxacin (25.04 ± 0.64%) while 75 and 100% Arthemetrin treatment recorded the significantly the lowest mitotic index (Table 3). The mitotic index for the

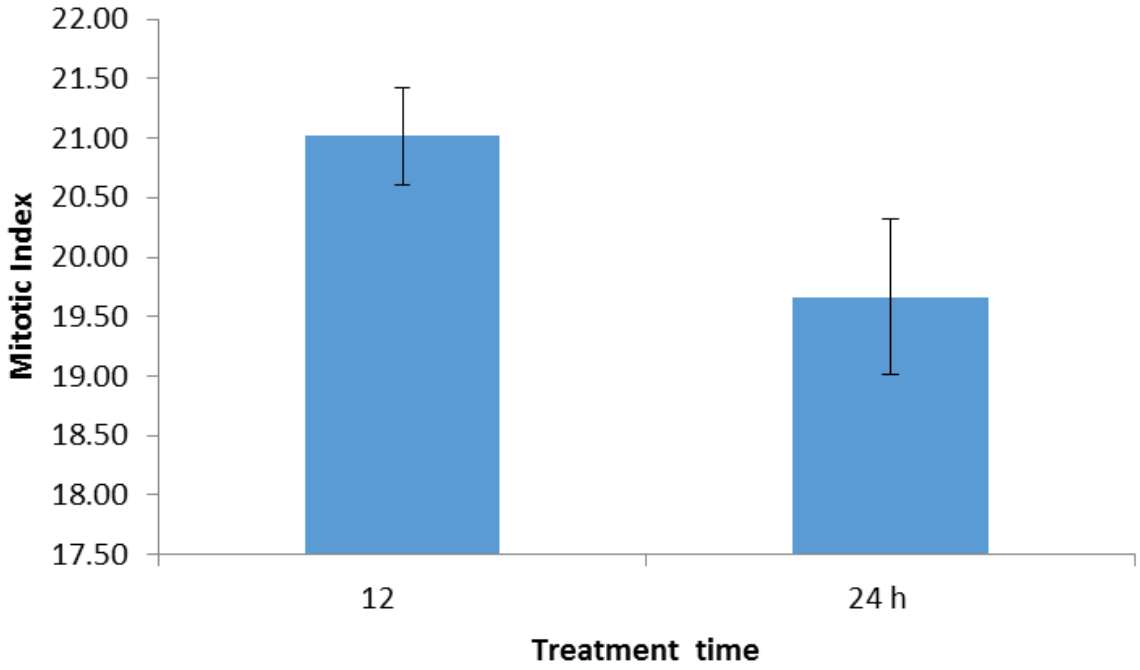

Figure 2. Effect of treatment time on mitotic indices.

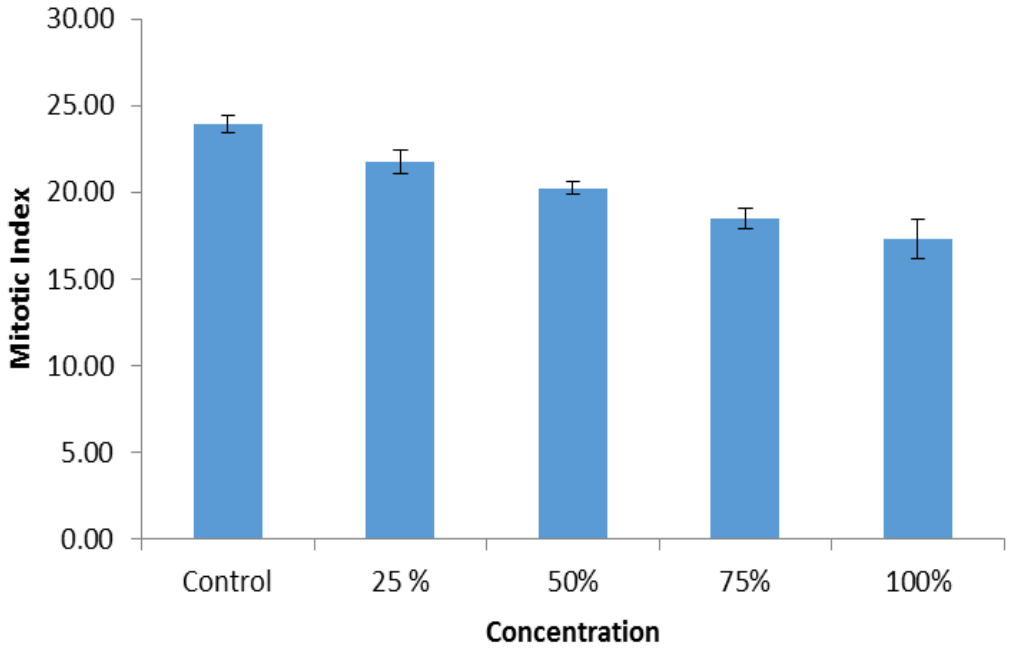

Figure 3. Effect of concentration on mitotic indices.

interactive effect of the three factors (effluent, treatment time and concentration) ranged from 12.89 in 100% Arthemetrin treated for 24 h to 26.84% in the control Ciprofloxacin treated for 12 h. The differences however did not significantly vary (Table 4).

Plate 2 shows the effect of effluents on *A. cepa* cell division process. However, several abnormalities were observed which include spindle disturbance, multiple bridges, diverse forms of C-mitotic effect and vagrant chromosome (Plate 3).

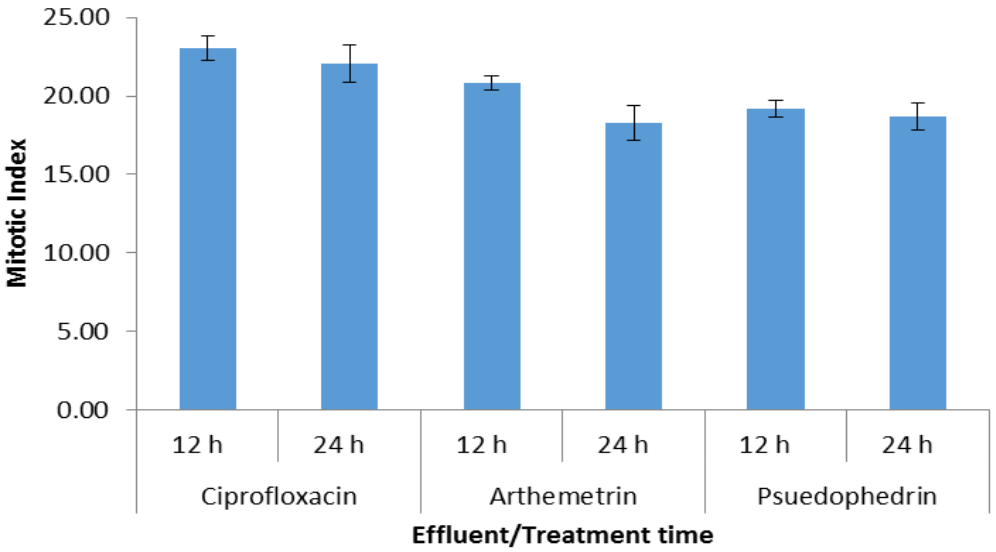

Figure 4. Effect of effluents and treatment time on mitotic indices.

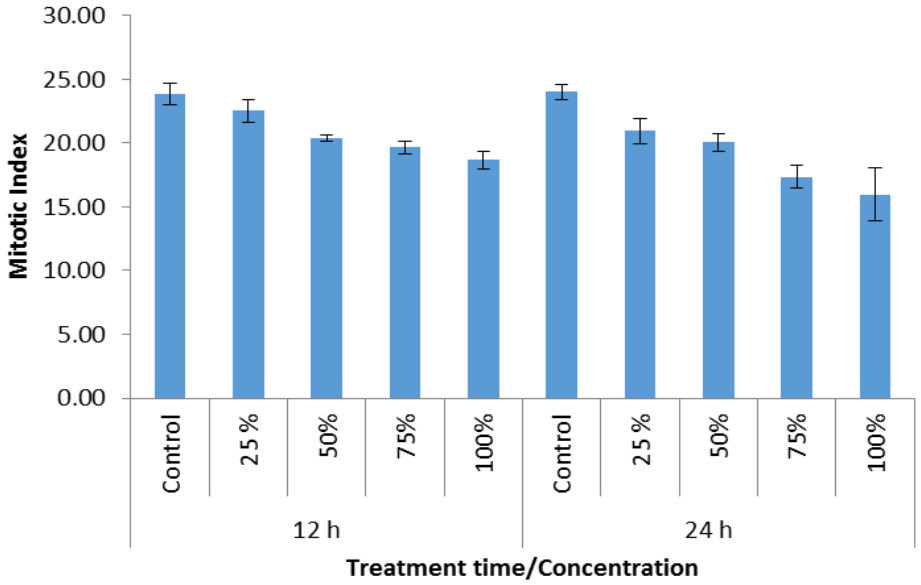

Figure 5. Effect of treatment time and concentration on mitotic indices.

Table 3. Effect of effluent and concentration on mitotic indices.

Concentration	Effluents		
	Ciprofloxacin	Arthemetrin	Psuedophedrin
Control	26.11 ± 0.36^{a}	23.74 ± 0.19^{c}	21.95 ± 0.68^{d}
25%	25.04 ± 0.64^{b}	21.05 ± 0.27^{e}	19.10 ± 0.62^{g}
50%	20.62 ± 0.96^{ef}	19.62 ± 0.45^{g}	20.45 ± 0.20^{f}
75%	20.47 ± 0.61^{f}	16.91 ± 1.05^{i}	18.12 ± 0.78^{h}
100%	20.52 ± 2.56^{ef}	16.45 ± 1.61^{i}	14.99 ± 0.59^{j}

Data are presented with means ± SE. significant means were separated with different superscript alphabets in the table using least significant difference (LSD = 0.55) at $P < 0.05$.

Table 4. Effect of effluent, treatment time and concentration on mitotic Indices.

Parameter	Ciprofloxacin		Arthemetrin		Psuedophedrin	
	12 h	24 h	12 h	24 h	12 h	24 h
Control	26.84 ± 0.32	25.37 ± 0.09	23.42 ± 0.15	24.06 ± 0.24	21.31 ± 0.43	22.59 ± 1.32
25%	25.88 ± 0.96	24.19 ± 0.64	21.34 ± 0.26	20.75 ± 0.46	20.34 ± 0.39	17.85 ± 0.49
50%	20.79 ± 0.33	20.45 ± 2.12	20.13 ± 0.74	19.10 ± 0.43	20.25 ± 0.10	20.64 ± 0.38
75%	21.60 ± 0.29	19.35 ± 0.70	19.24 ± 0.25	14.57 ± 0.19	18.10 ± 0.08	18.15 ± 1.74
100%	20.12 ± 0.33	20.92 ± 5.71	20.00 ± 0.39	12.89 ± 0.43	15.91 ± 0.43	14.06 ± 0.82

Data are presented with means ± SE. significant means were separated with different superscript alphabets in the table using least significant difference (LSD = NS) at $P < 0.05$.

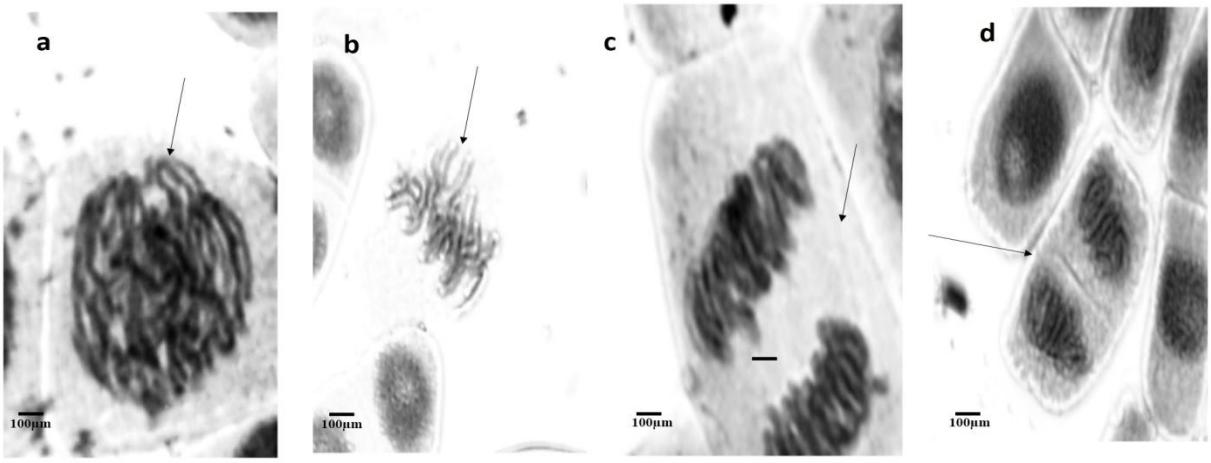

Plate 2. Normal mitotic stage in *Allium cepa*: a) prophase, b) metaphase, c) anaphase and d) telophase.

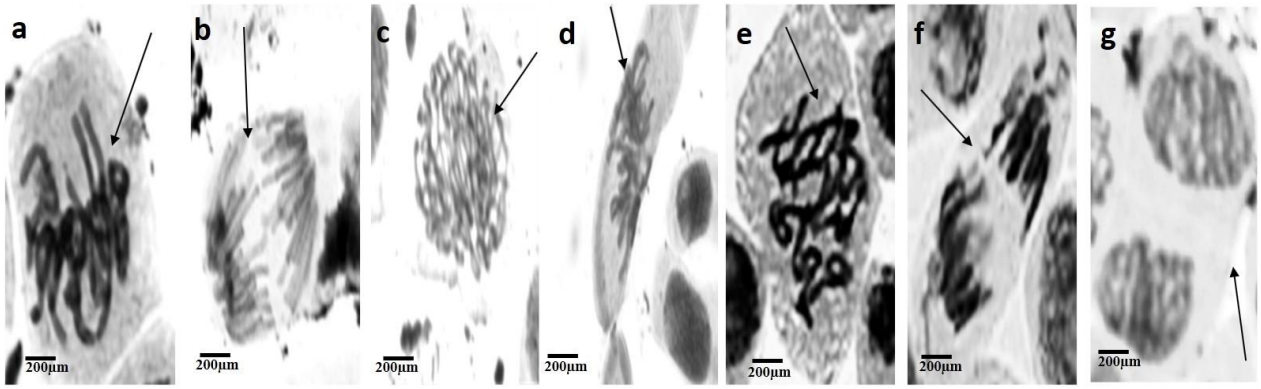

Plate 3. Abnormal dividing cells: (a) spindle disturbance at metaphase; (b) anaphase with multiple bridge, (c-e) diverse forms of C-mitotic effect at prophase and metaphase; (f) anaphase bridge with vagrant chromosomes; (g) cytokinetic failure.

DISCUSSION

This study monitored the toxicity induced by hospital effluents in Enugu. The amounts of heavy metal observed in these three wastewaters are more than the recommended safe levels in the environment. The results for the heavy metals observed in the three effluents except Zn were higher than that observed in pharmaceutical effluents from Sango industrial area by Olaitan et al. (2015). The heavy metals presence in wastewater samples have been acknowledged to be responsible in numerous cytotoxic and genotoxic effects

on organisms (Abu and Mba, 2011; Abu et al., 2015).

The acidic nature of the effluents was in agreement with the report of Abu and Mba (2015) for Jutrim, Flu-J and Ampiclox effluents. The acidic nature of the pharmaceutical industrial effluent is capable of increasing the pH of the water bodies receiving the effluents, thereby distorting the fundamental properties like metal solubility, alkalinity, and the hardness of water (Olaitan et al., 2015). These will in turn affect the metabolic process of aquatic organisms which are reliant on the pH values as well (Wang et al., 2002).

The possible implication of the chemical analysis results is that these wastewaters when discharged into the environment are too toxic without pretreatment. Nevertheless, the complexity of industrial wastewater had been accounted for impossibility of basing hazard assessments on chemical analysis alone (El-Shahaby et al., 2005; Abu et al., 2015). In search for a test system that could be used in combination with the chemical analysis to give information that will serve scientifically as a basis for regulating the release of hazardous substances into the environment and suitable evaluating toxicity, the Allium test was proposed (Fiskesjö, 1985).

The effect of pharmaceutical effluents was further evaluated on the growth and development of *A. cepa* roots. Lower number of roots produced and root length was obtained in undilutated psuedophedrin effluent. The results based on *A. cepa* assay confirms the strong and cumulative cyto- and geno-toxic activities of pharmaceutical industrial effluent, being in agreement with the report of other similar research (Paz et al., 2006; Abu and Mba, 2011; Perrodin et al., 2013; Pedrazzani et al., 2014).

In relation to the growth of roots as a combination of cell division and elongation, the significant reductions of root length and mitotic activity are true indicators of rhizo-toxicity, being a general phenomenon caused by most pollutants (Fiskesjö, 1993; Anila and Ditika, 2015). More so, the induction of malformations in the root of *A. cepa* has been acknowledged as a useful sign of toxicity in earlier studies (Babatunde and Bakare, 2006, Bakare et al., 2009; Olorunfemi et al., 2011; Abu and Mba, 2011). Odeigah et al. (1997) reported that the usefulness of plant roots in the monitoring of effluents since they are frequently the first part to be exposed to chemicals in soil and water. The elevated acidity and amount of heavy metals in these wastewaters had been attributed to have an effect on the initiation and growth of the root through their action on cell reproduction (Abu and Mba, 2011). Fiskesjö (1985) had similarly described that growth retardation and wilting of root are as a result of the suppression of cell division and chromosomal aberrations.

The mutagenic effects on eukaryotic nuclei could also be evaluated cytologically by examining the inhibition of cell division process (Abu et al., 2015; Urama et al., 2020). It was observed that the effluents, concentrations and treatment time were mitodepressive. This is in agreement with Abu and Mba (2011) and Olaitan et al. (2015). The observed effect in this study had been attributed to the presence of heavy metals in complex mixtures like industrial wastewater which causes cytotoxicity and genotoxicity effects in both plant and animal test systems (Grover and Kaur, 1999).

Panda and Sahu (1985), Antonsie-Wiez (1990) and Abu et al. (2015) had reported that a mitotic index decrease below 50 and 22% of the control causes sub-lethal and lethal effects, respectively, on test organisms. Copper has been reported to have the effect of rapidly decreasing the mitotic index (Jiang et al., 1996). In the present study, Arthemetrin and Psuedophedrin had the highest amount of copper and the least MI value thereby agreeing with the findings of Jiang et al. (1996) and Abu et al. (2015). Moreover, all the undiluted effluents (100%) at both 12 and 24 h treatment durations had less than 22% of the control MI value. These are pointers to the fact that these effluents are lethal in undiluted forms. Therefore, when these effluents are released into farm lands for at least 12 h without dilution from the rain or other sources, they can cause lethal effects on contact plants. More so, if discharged into water bodies, the effects would be more at the point of discharge than downstream.

The lethal effects of these effluents could be ascribed to the high levels of heavy metals in them. Several authors have reported a high relationship between the amounts heavy metals in natural and industrial environment, the increased frequency of chromosome mutations and the cancerous processes in organisms (Bruning and Chronz, 1999). C-mitotic effect is a frequent symptom of spindle inhibition. Dash et al. (1998) reported earlier that lead affects the cell in varied ways, some of which are inhibition of enzymes, aberrations in the chromosome and mutation with its clastogenic effects leading to spindle impairment and malfunction.

Cytokinetic failure leading to multinucleate cells is a severe deleterious effect that can lead to cancerous cells in tissues while nuclear disintegration could lead to cell death. The nuclear lesions suggest indication of nuclear poison. Akaneme and Iyioke (2008) reported that the presence of nuclear lesions and nuclear dissolution offer cytological evidence for the inhibitory action on DNA biosynthesis.

Conclusion

This study monitored the toxicity induced by Ciprofloxacin, Arthemetrin and Psuedophedrin effluents and found that these effluents contained heavy metal amounts that are more than the recommended safe levels in the environment. Severe retardation of root growth, lethal effects based on very low MI, and aberrant cells were observed. These are signs of the mutagenicity of these effluents and could cause irreversible health and ecological damages. These untreated effluents are therefore considered to be lethal based on the results

and are environmentally risky. However, a sound sewerage system in industries is therefore crucial.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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