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STUDIES ON THE PHYSICOCHEMICAL PROPERTIES AND GENOTOXICITY OF EFFLUENTS FROM A DAIRY INDUSTRY

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ABSTRACT
In this study, the physicochemical analysis of raw and treated effluents obtained from a dairy industry was carried out and it revealed the presence of zinc, iron, manganese, nitrates and sulphates at levels higher than Standards Organisation of Nigeria (SON) permissible limits. The pH of the effluents was acidic (pH 4.7 and 6.43 respectively). Results obtained from the macroscopic evaluation of Allium cepa after 96 hours of cultivation in both effluents showed a significant ($p<0.05$) concentration-dependent root growth inhibition. Root tip cells of A. cepa processed for cytological studies by the aceto-orcein squash technique after exposure to the effluents for 48 hours at concentrations of 0.5%, 1.0%, 2.5%, 5.0%, and 10% (v/v) showed chromosomal aberrations at all concentrations. Sticky chromosomes with bridges and laggards were the most observed, however, the frequency of these aberrant chromosomes was more in the raw compared with those in the treated effluent. Statistical analysis of microscopic results showed significant ($p<0.05$) reduction of mitotic index in a concentration-dependent relationship. The findings of this study have shown that a combination of physicochemical analysis and genotoxicity assay is effective in assessing the mutagenic components of industrial effluents for environmental monitoring of pollutants. The treated wastewater from the dairy industry, if discharged into water bodies without further treatment, could pollute the receiving water bodies and impair biolife.
Keywords: Dairy effluent, genotoxicity, Allium cepa, physicochemical analysis

INTRODUCTION

Water pollution by industrial effluents has been one of the vital issues of environmental concern in countries around the world. Due to continual disposal of wastewater into the water bodies, water surface quality has deteriorated because of the mixing of various chemicals of the effluent with water (Fayaza *et al.,* 1996). The dairy industry is one of the highest consumers of water and is one of the biggest producers of effluents per unit of production in addition to generating a large volume of sludge in biological treatment (Ramjeawon, 2000). The volume of effluent arising from a dairy plant is dependent on two factors; the type of dairy product being processed and the degree of water management being exercised, and thus the amount of water being conserved. For example, cheese, milk powder and evaporating plants generate larger volumes of effluent than those producing pasteurized milk (Ikhu-Omoregbe *et al.* 2001).

The industry is one of the most polluting, not only in terms of the volume of effluent generated, but also in terms of its characteristics as well. It generates about 0.2–10 litres of effluent per litre of processed milk (Vourch *et al*., 2008) with an average generation of about 2.5 litres of wastewater per litre of the milk processed (Ramasamy *et al*., 2004). The industry generates an almost entire quantity of water as effluent and appreciable quantity of solid wastes (Dhanam, 2009). The wastewaters are primarily generated from cleaning and washing operations in the milk processing plants. It is estimated that about 2% of the total milk processed is wasted into drains (Munavalli and Saler, 2009). Due to the high pollution load of dairy wastewater, the milk-processing industries discharging untreated/partially treated wastewater cause serious environmental problems (Montuelle *et al.,* 1992, Mohana *et al.,* 2011).

Like in most process industries, the dairy industry has effluent disposal problems (Jarawaza, 1997; Ikhu-Omoregbe *et al*., 2001). To enable the dairy industry contribute to water conservation, good pollution prevention practices and stringent control, effluent disposal mechanisms must be put in place (Jarawaza, 1997). Common techniques for treating dairy industry waste waters include grease traps, oil water separators for separation of floatable solids, equalization of flow, and clarifiers to remove suspended solids (SS). Biological treatment comprises of the aerobic and anaerobic processes (Demirel *et al*., 2005) and often times the post-treatment of dairy wastewater is also done using the physicochemical treatment methods (Bickers and Bhamidimari, 1998).

A comprehensive approach for hazard assessment of industrial effluents involving the use of higher plants bioassays in addition to chemical and microbial analyses in environmental monitoring and toxicity screening of contaminated water and other pollutants has been advocated (Arkhipchuk, *et. al*., 2000). The onion bulb (*Allium cepa* L.) root growth inhibition and chromosome aberration assay has been accepted as excellent and alternative first–tier indicator for safety evaluation of cytogenetic and mutagenic effects of drinking water and environmental pollutants (Fiskesjö, 1993, 1997; Rank, 2003). We are not aware of any report on the genotoxicity of effluents discharged from a dairy industry in Southern Nigeria.

In particular, this study was undertaken to determine the nature and extent of toxicity or otherwise of raw and treated effluents discharged from the dairy industry using physicochemical analyses and a higher plant monitoring system; the *Allium cepa* assay. The results obtained from such a study would help to ascertain whether or not adequate treatment methods have been used in the treatment of the dairy wastewaters.

In addition, the study would also ascertain the effectiveness of the *Allium cepa* assay in screening mutagens in complex industrial mixtures.

MATERIALS AND METHODS

Collection of Dairy Effluents

Samples of raw and treated dairy effluents were collected from a dairy industry located in Oyo State situated between latitudes 7°23'47" N and longitudes 3°55'0" E. Raw and treated dairy effluents were collected from discharge points in the factory during work hours and after primary treatment by the aerobic process using sedimentation basins and activated sludge. The effluents were stored in four 4-litre hard plastic screw capped jerry cans. The effluents were diluted to the following concentrations; 0.5%, 1.0%, 2.5%, 5%, 10% and 20%.

Physicochemical Analysis

Raw and treated dairy effluent samples, together with control (tap water of good quality) were analysed for a number of standard physicochemical properties, including total dissolved solids (TDS), total hardness, sulphates, phosphates, nitrates, biochemical oxygen demand (BOD), and dissolved oxygen (DO) according to methods described by APHA (2005). Ten metals namely lead, copper, mercury, cadmium, chromium, iron, zinc, aluminium, nickel, and manganese were analysed in the water samples according to standard analytical methods (USEPA, 1996; APHA, 2005) using an atomic absorption spectrophotometer (AAS) (PerkinElmer A Analyst 100). The metal standards were prepared to known concentrations, labelled, and kept inside plastic bottles that were pre-cleansed with concentrated nitric acid and distilled water. The absorbance of the standards, effluent samples and control was taken in triplicates. Graphs of the concentrations against the absorbance of each of the standards for the metals were plotted. Thereafter, the concentrations of the five metals in the dairy samples were interpolated from their respective graphs.

***Allium cepa* Test**

Procurement and preparation of onions

Allium cepa L. (2n = 16), commonly called onions, were purchased from a local market in Benin City (6°15'N and 5°25'E), and the same batch of bulbs were used throughout. The onions were sun dried for a week, and those attacked by fungi were discarded at the beginning of the experiment. The outer scales were carefully removed, without tampering with the primordial root ring.

Macroscopic evaluation

For evaluation of root growth inhibition, seven onion bulbs were utilized for each dairy effluent sample. The negative control was set up using tap water of good quality only. The tap water was ascertained to be of good quality by having a pH around 7 and has relatively high hardness (Ca + Mg= 50-70 mg/l) and free from any chlorine compounds and toxic ions (Fiskesjö, 1985). The base of each of the bulbs was suspended on the water sample inside 100 ml beakers containing about 75 ml of the test sample in the dark for 96 h. Test samples were changed daily. At the end of the exposure period, the root lengths of at least 20 roots of five onion bulbs with the best growth were removed with a forceps per water sample and measured using a meter rule. From the weighted averages for each water sample and the control, the percentage root growth inhibition in relation to the negative control was calculated.

Microscopic evaluation

For the evaluation of induction of chromosomal aberration, 5 onion bulbs were suspended in the dairy effluent samples and the control for 48 h. At the end of 48 h, root tips from these bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v) inside universal bottles and kept at 4°C for 24 h before use. The already fixed root tips were hydrolysed in 1N HCl at 60°C for 5 minutes. The hydrolysed root tips were washed several times with distilled water. Three root tips were squashed on each slide and stained with aceto-orcein for 10 minutes. Excess stains were removed, and the cover slip's edges were sealed as suggested by Grant (1982). A total of 5000 cells from 5 slides per sample were observed (at 1000 x magnification) for different mitotic stages and chromosomal aberrations using a Nikon Eclipse (E400) light microscope.

The mitotic index (MI), mitotic inhibition and the frequency of chromosomal aberrations (CA) were calculated (Fiskesjö 1997; Bakare *et al.* 2000).

$$Mitotic\ index\ (MI) = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells\ counted} \times 100$$

$$Mitotic\ inhibition = \frac{Mitotic\ index\ of\ control - Mitotic\ index\ of\ treated}{Mitotic\ index\ of\ control} \times 100$$

$$Frequency\ of\ CA = \frac{Number\ of\ Aberrent\ cells}{Total\ number\ of\ cells\ counted} \times 100$$

Statistical Analysis

Quantitative data were summarised as means ± standard errors and percentages, which were then subjected to Duncan multiple comparison and Dunetts tests in a one-way ANOVA, using SPSS version 15.0 for Windows 2007. The effects of the dairy effluent samples and controls on root growth, cell division and chromosome aberrations of *A. cepa* were compared. Significant differences were set at $p \leq 0.05$.

RESULTS

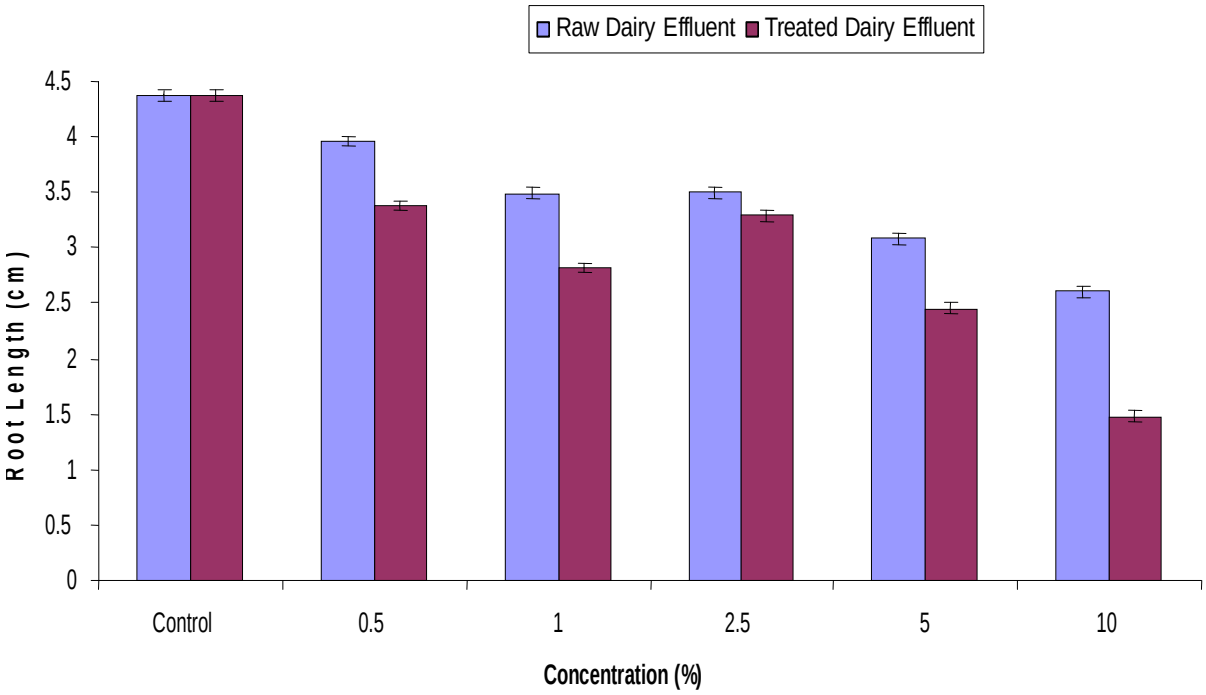
The results of the physical and chemical analysis of the raw and treated dairy effluent samples are presented in Table 1. Both effluents had stringent and highly unpleasant odour with acidic pH values of 4.78 and 6.43 respectively. The effluents were slightly hard with total hardness of 367.67 mg/l and 290 mg/l respectively. The amount of zinc was high in both effluents with the raw and treated dairy effluents having a concentration of 7.1 mg/l and 4.1 mg/l respectively. Although copper, chromium, and lead were not detected in the treated effluent, they were present in the raw sample (0.1, 0.1 and 0.2 mg/l respectively). Manganese, nitrates and phosphates were present in both effluents in high concentrations while aluminium, nickel, cadmium and mercury were not detected in both effluents.

Table 1: Physicochemical properties of raw and treated dairy effluents

Parameter	Raw Effluent	Treated Effluent	FEPA (1991) Limit	USEPA (1999) Limit	SON (2007) Limit
pH	4.78	6.43	6 -9	6.5 -8.5	6.5 -8.5
Total hardness	367.67	290.00	NS	0 -75	150
BOD	7.2	7.1	50	NS	NS
DO	10.5	7.95	NS	NS	NS
TDS	0.14	0.02	2000	500	500
Alkalinity	1.83	2.58	NS	NS	NS
Nitrates	85.0	54.5	20	10	50
Sulphates	96.0	65.0	500	250	100
Phosphates	98.5	60.0	5	NS	NS
Aluminium	ND	ND	NS	NS	0.2
Manganese	0.2	0.3	5	0.05	0.20
Lead	0.1	ND	<1	0.003	0.01
Iron	5.9	0.3	20	0.3	0.3
Zinc	7.1	4.1	<1	0.12	3.0
Chromium	0.1	ND	NS	NS	0.05
Copper	0.2	ND	<1	0.009	1.0
Nickel	ND	ND	<1	0.005	0.02
Cadmium	ND	ND	<1	0.002	0.003
Mercury	ND	ND	0.05	NS	0.001

All values are means of 3 replicates and are expressed in mg/l except pH with no unit.
BOD: Biochemical oxygen demand, DO: Dissolved oxygen, TDS: Total Dissolved Solids, NS: Not specified, ND: Not detected.

Results of the root growth inhibition evaluation of onion bulbs grown in raw and treated dairy effluents showed that compared to control, root growth inhibition was concentration dependent at all concentrations used in this study (Fig. 1). For example, the mean root length of *A. cepa* grown in 0.5% raw effluent was 3.42±0.50 cm while at 10% the mean root length was 1.41±0.54 cm. Conversely, the root lengths of the onion bulbs grown in treated effluent were 4.10±0.49 cm and 2.71±0.48 cm at the same concentrations respectively. Unlike the roots of treated effluents, those grown in 5% and 10% of raw effluents were characterized by twists and crotchet roots (roots bent upward resembling hooks) (Plates 1 & 2). The onion bulbs exposed to raw effluents showed greater root inhibition than those exposed to treated effluent (Figure 1).



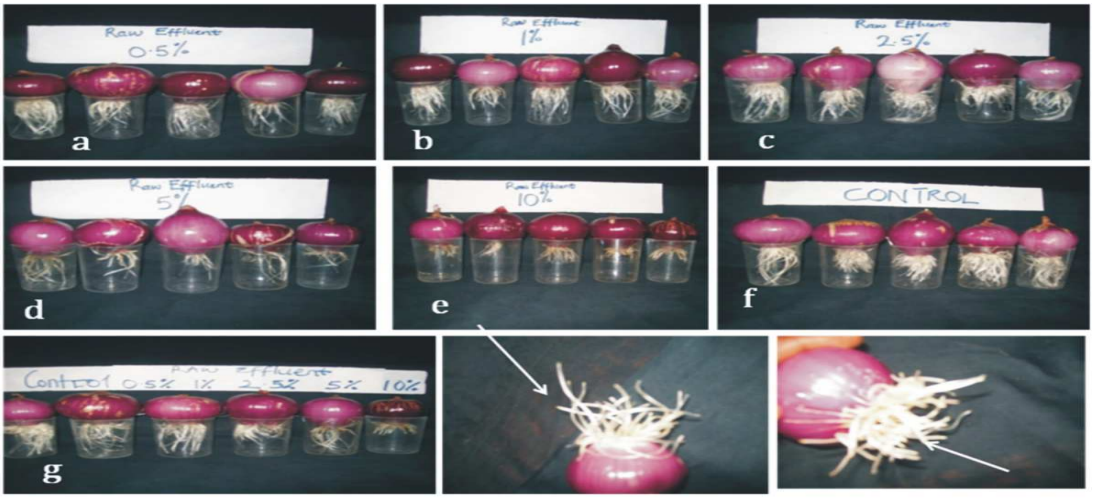


Plate 1: Growth response of *Allium* roots exposed to raw dairy effluents. Root malformations (twists and crotchets) are arrowed

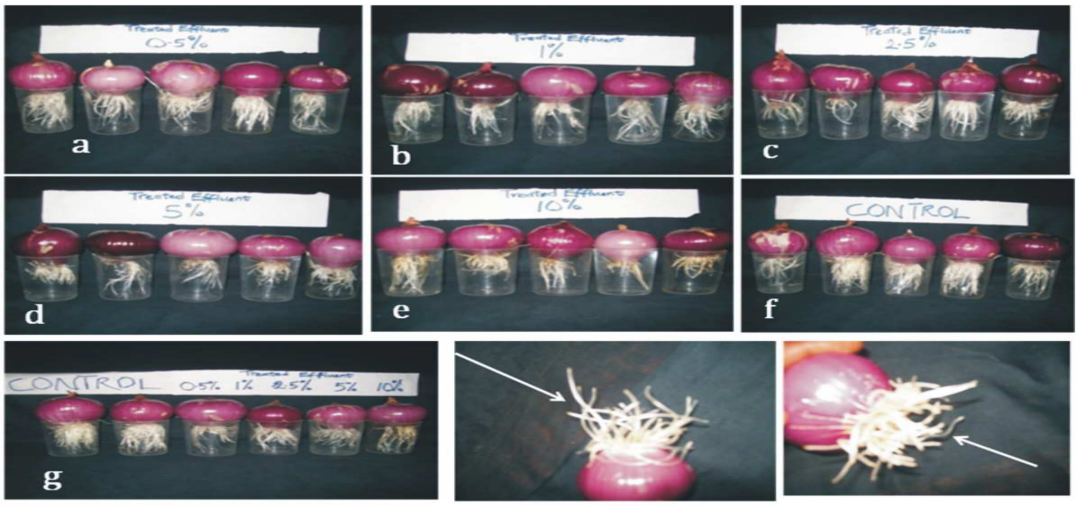


Plate 2: Growth response of *Allium* roots exposed to treated dairy effluents. Root malformations (twists and crotchets) are arrowed

Results of the microscopic evaluation are presented in Table 2. Root tips of onion bulbs exposed to both raw and treated dairy effluents were replete with aberrant cells at all the tested concentrations. As concentration of the effluents increased, the number of dividing cells decreased. In the same manner, the number of aberrant cells increased as the mitotic indices decreased. There were no dividing cells at the 20% concentrations of the dairy effluents.

Table 2: Cytological effects of dairy effluents on cells of *Allium cepa*

Conc. (%)	Raw dairy effluent				Treated dairy effluent			
	No. of dividing cells	Mitotic index (Mean ± SE)	Mitotic inhibition (Mean ± SE)	% of aberrant cells	No. of dividing cells	Mitotic index (Mean ± SE)	Mitotic inhibition (Mean ± SE)	% of aberrant cells
0	409	81.8±0.72	-	-	409	81.8±0.72	-	-
0.5	379	75.8±0.89*	12.1±0.46	5.6±0.52	376	75.2±0.91*	8.1±0.37	4.2±0.47
1	367	73.4±0.83*	14.9±0.53	6.8±0.55	352	70.4±0.81*	13.9±0.51	4.9±0.44
2.5	345	69.0±0.75*	20.0±0.49	8.6±0.45	336	67.2±0.74*	17.9±0.47	6.4±0.48
5	318	63.6±0.91*	27.0±0.51	10.7±0.53	315	63.0±0.90*	22.3±0.48	7.6±0.51
10	312	62.4±0.75*	28.0±0.55	12.9±0.51	305	61.0±0.73*	25.4±0.53	9.0±0.55
20	-				-			

5000 cells (5 slides) per concentration of each effluent and the control
*Significant difference from control at $p<0.05$

A comparison between the onion bulbs exposed to raw effluents and those exposed to the treated effluents showed lower mitotic indices in the former than those exposed to treated effluent with increasing concentration (Figure 2). All the tested effluents induced chromosomal aberrations in all the concentrations and they were statistically significant ($p<0.05$). The most frequent aberrations were sticky chromosomes, laggards, and vagrants (Plate 3).

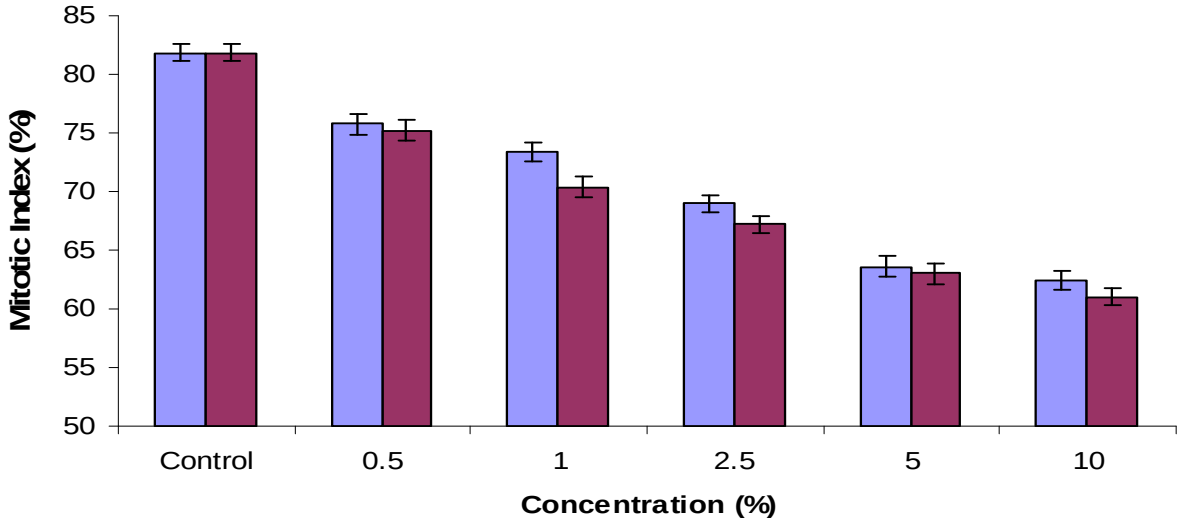


Figure 2: Mitotic index *A. cepa* grown in various concentrations of raw and treated dairy effluents.

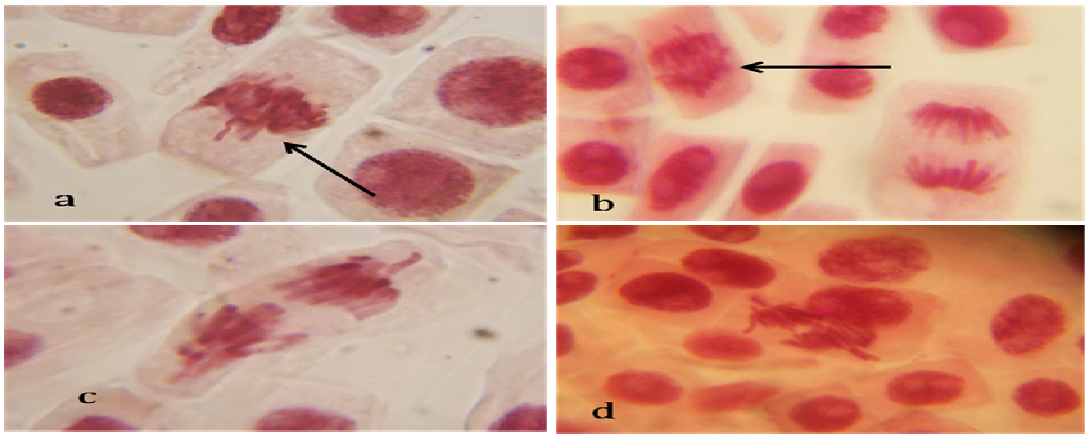


Plate 3: Some chromosomal aberrations induced in *Allium cepa* grown in raw and treated dairy effluents (a) Sticky chromosomes (b) bridges (c) vagrants (d) multiple bridges Magnification 1000*

DISCUSSION

One of the aims of screening wastewater for toxicity is to identify sources of pollution in the environment and suggest measures to be taken to reduce the levels of toxicity of the wastes. In this study, the physicochemical parameters of raw and treated dairy effluents were analysed. Compared to the standard limits (FEPA, 1996; USEPA, 1999; SON, 2007), most of the analysed parameters were present at manageable concentrations; however, lead, copper and zinc were present in appreciably high amounts. Magnesium, phosphates, and nitrates were also present in concentrations above the permissible limits. Heavy metal contamination may have devastating effects on the ecological balance of the environment and a diversity of aquatic organisms (Ashraj, 2005, Vosyliene and Jankaite, 2006, Farombi, *et al*., 2007). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.,* 2004; Clarkson, 1998; Dickman and Leung, 1998).

A positive correlation between genotoxicity assay and physicochemical analysis was observed. Cytological analysis indicated that the dairy effluents in this study were genotoxic as significant differences ($p<0.5$) in chromosomal aberrations were induced at all concentrations of the effluents. The induction of root malformations (e.g. twists and crotchet roots) in *Allium cepa* by the effluents has been shown to be useful signs of toxicity in previous studies (Babatunde and Bakare, 2006, Bakare *et al*., 2009, Olorunfemi *et al*., 2011a).

In the *A. cepa* test, there is usually a relationship between root growth retardation, mitotic indices and chromosomal damage (genotoxicity). Whenever chromosome aberrations occurred, there was almost always certain growth restriction and reduction in the number of dividing cells (i.e. mitotic indices). The mitotic index is considered to reliably identify the presence of cytotoxic pollutants in the environment (Smaka-Kincl *et al*., 1996; Grover and Kaur, 1999; Chandra and Kulshreshtha, 2004). The increase in the number of aberrant cells with increase in the concentration of the raw and treated effluents in this study is an indication of toxicity. This is in agreement with observations in earlier studies (El-Shahaby *et al*., 2003, Olorunfemi *et al*., 2011b).

Trace elements and other pollutants were considered responsible for the diminished mitotic index of the *A. cepa* roots exposed to industrial effluents (Awode *et al*., 2008). Decreased mitotic index in *A. cepa* roots grown in the dairy effluents is probably due to either disturbances in the cell cycle or chromatin dysfunction induced by metal-DNA interactions (Glinska *et al*., 2007). Lead has been known to cause reduction in root growth and frequency of mitotic cells in the meristematic zone of onions. It also induced chromosome damage and disturbance of mitotic processes in onions (Lerda, 1992). The raw dairy effluents showed the strongest genotoxic effects on the root meristem cells.

The most frequent abnormalities were stickiness and anaphase bridges. Chromosome bridges result from chromosome and/or chromatid breaks indicating the clastogenic effect of raw and treated dairy effluents. Bridges and fragments are clastogenic effects, both resulting from chromosomal and chromatid breaks (Kovalchuk *et al*. 1998) while vagrants arise as a result of irregular separation and dislocation of chromosomes; thereby constituting a risk of aneuploidy (Maluszynska and Juchimiuk, 2005). Stickiness is considered a common sign of toxic effect of pollutants on chromosomes probably leading to cell death (Fiskesjo, 1997). Sticky chromosomes have been reported in *Allium* roots after treatment with various heavy metals such as mercury, nickel and copper (Fiskesjo, 1993, 1997).

CONCLUSION AND RECOMMENDATION

Although there was no significant difference ($p>0.05$) between the genotoxic parameters of raw dairy effluents on one hand and treated dairy effluents on the other, there was however, ample evidence of toxicity of both effluents compared to the control. The most likely reason for this observation is the complex assortment of toxic metals present in raw dairy effluents and the incomplete or partial treatment of treated dairy effluents. However, the strong toxic effect demonstrated by treated dairy effluents, for example, can hardly be explained by the relative low chemical levels measured in physicochemical analysis. It goes to show that the effects of chemical interactions and the influence of complex matrices on toxicity cannot be determined from chemical tests alone. Even with the partial treatment of dairy effluents, low concentrations of treated dairy effluents caused chromosomal aberrations. This proves that new/improved treatment methods for treating industrial effluents should be developed and adopted and there should be regular monitoring of discharge of waste waters into water bodies and agricultural lands by regulating bodies.

The positive and consistent results of the *A. cepa* genotoxicity test underscore the importance of the bioassay as a first-tier procedure for environmental monitoring. This study also shows the usefulness of combining physicochemical analysis with cytogenetic methods to bring about better understanding of the toxicity of industrial effluent pollutants and their influence on human health and plant life.

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