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Comparative Physicochemical and Genotoxicity Assessments of Textile Mill Company Effluent and Local Tie-And-Dye Textile Wastewater

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ABSTRACT: The textile industry has become indispensable in view of its basic and social importance to human life, but its environmental impact has continued to be a subject of concern. The objectives of the present study were to assess and compare the physico-chemical and genotoxicity properties of a corporate textile mill effluent and the wastewater generated by a local Tie-and-dye (Adire) business in Western Nigeria. While the *Allium cepa* assay was used to assess genotoxicity, standard APHA and other procedures were used to analyse the physico-chemical properties. Results showed that many physicochemical qualities of the two effluent types were higher than the recommended limits. The root growth and mitotic inhibition potential of the two effluent types increased with rising effluent concentrations and period of exposure. The chromosome aberrations induced by the two effluent types were binucleated, attached, vagrant, sticky, and bridged chromosomes. Independent samples t-test analyses revealed that the differences between the two effluent types were only marginal and not significant ($P > 0.05$). This implies that the textile mill company whose effluent was used for this study did not subject its effluent to treatment before discharging, and if it did, the treatment was grossly inadequate. Governments at all levels in Nigeria, and other countries in which similar results may be obtained, are called upon to reinvigorate their regulatory agencies to enable them to discharge their oversight functions effectively.
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Key words: Chromosome, contaminants, environment, effluents, Nigeria, textile.

The textile and clothing industry is a major player in the economy of many countries. It is a global industry that provides employment for different categories of people including the skilled, semi-skilled and the unskilled. Textile and clothing production process consists of spinning, printing, weaving, dyeing and many other finishing processes that result in garment production. During the production process, various chemicals and large volumes of water are used, and correspondingly large quantities of wastewater containing vast amounts of dyes and metal-bearing chemicals are released as effluent (Bello *et al*., 2013; Ghaly *et al*., 2014; Ado *et al*., 2015).

'Adire' is literally translated as 'Tie-and-Dye'. Adire is the indigo-dyed cloth made by the Yoruba people of mainly Southwest Nigeria (Carr, 2001; Oloyede *et al*., 2014). A popular variant of Adire is called Kampala. Just as the textile industry is a major global provider of income and employment, the Adire business is a major source of employment and income to many people, especially women, of Southwest Nigeria. Originally, Adire making involved the use of fermented Indigo plant leaves to create patterns on locally spun and woven fabrics. These days, synthetic indigo, dyes and chemicals have almost completely replaced the natural indigo.

Adire dyeing is usually done first by vatting the dyes and the chemicals into two separate containers where one contains the dyes dissolved with warm water, and the other contains sodium hydroxide (NaOH) and sodium thiosulphate ($Na_2S_2O_3$) dissolved with warm water. In spite of the large volumes of dyes, chemicals, and water that are used, the wastewater generated from the dyeing process is discharged into the environment without adequate treatment, if any (Oloyede *et al*., 2014).

Though, the once vibrant textile industry in Nigeria has witness a steep decline (Bello *et al*., 2013), it is on record that prior to 2003, a substantial portion of the world's textile chemicals was used by the Nigeria's textile industry (Akintayo, 2013). It therefore implies that Nigeria's environment has been exposed to high volumes of dyes and chemical-bearing wastewaters from both corporate textile mills and local Tie-and-dye businesses over the years. Many studies have confirmed the genotoxicity of corporate textile effluents (Samuel *et al*., 2010; Giorgetti *et al*., 2011; Alimba *et al*., 2013; Ghaly *et al*., 2014), however, the present study assessed and compared the physico-chemical and genotoxic properties of a corporate textile mill effluent and the wastewater generated by a local Tie-and-dye (Adire) business in Western Nigeria.

MATERIALS AND METHODS

Collection of test samples: The textile mill effluent used for this study was collected from the effluent channel leading away from a textile mill company in Ibeshe, Ikorodu, Lagos State, Nigeria (Latitude $6^0$55'N and longitude $3^0$47'E). The 'Tie-and-Dye' (Adire) effluent was obtained from Itoku, Abeokuta, Ogun State, Nigeria (Latitude $7^0$15'N and longitude $3^0$25'E). In each case, the effluent was collected in a 10 litre plastic container, brought to the laboratory, and refrigerated at 4^0C pending further actions.

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The average sized onion bulbs (*Allium cepa*, L.) used for the study were procured from Bariga market in Lagos, Nigeria (Latitude $6^0$54'N and Longitude $3^0$39'E). The onions were sun-dried for one week after which the dry ones were selected for the test. The chemicals, beakers, test tubes and other glass wares used for the study were collected from the laboratory store of the Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Nigeria. All chemicals used were of analytical grade.

Physicochemical analyses of textile mill effluent and Adire (Tie-and-dye) wastewater: The effluents obtained from corporate textile company and local Tie-and-dye (Adire) business were analysed for physico-chemical properties (including pH, DO, BOD_5, COD, total nitrogen, metals) using the methods prescribed by the American Public Health Association, APHA, (1998) and Ademoroti (2006). The metals, namely: lead (Pb), Zinc (Zn), Cadmium (Cd), Copper (Cu), Chromium (Cr) were quantified using Atomic Absorption Spectrophotometer (Perkin Elmer model 460). The results of the physico-chemical analyses were compared with the limits set by the National Environmental Standards and Regulations Enforcement Agency of Nigeria (NESREA, 2009). Some of the procedures used are briefly described.

Determination of pH: The pH of test effluents was determined with a digital pH meter. The meter was switched on, rinsed with deionized water, and calibrated with buffer solutions 10, 7 and 4. The probe of the pH meter was inserted into the test solution and readings were recorded.

*Determination of dissolved oxygen***:** Dissolve oxygen meter was rinsed, calibrated and inserted into the samples. Appropriate readings were then taken.

Determination of BOD_5: To determine the 5-day biological oxygen demand, DO was taken at day 1 and day 5. BOD_5 was computed from the difference in the DO value of day 1 and day 5, multiplied by the dilution factor.

Determination of COD: Chemical oxygen demand was determined by titrimetric/dichromate oxidation (Ademoroti, 2006).

*Quantification of metals in effluent samples***:** To determine the concentrations of metals in the effluent samples, 20.0 ml of the test sample was measure into a conical flask. Dilute HNO_3 (HNO_3 : deionized water = 1 : 3) was added to the sample in a conical flask and was heated in a Bunsen burner until all the reddish yellow fumes were expelled. The solution was brought down, allowed to cool, and transferred into a 10 ml standard flask. The standard flask was made up to mark with distilled water. After the digestion, analysis of metal content was carried out using Atomic Absorption Spectrometer, AAS, (Perkin Elmer model 460).

*Genotoxicity assessment of textile mill and Adire (Tie-and-dye) effluents***:** The genotoxicity assessment of textile mill and Adire (Tie-and-dye) effluents was carried out using the *Allium* assay test. *Allium* assay test was adapted from Fiskesjo (1993), Bakare *et al*. (2009) and Olorunfemi *et al*. (2011). The outer scales and brownish bottom plate of sun dried onion bulbs were carefully removed, leaving the ring of primordial root intact. The peeled bulbs were placed in dechlorinated tap water during the cleaning procedure to prevent the primordial from drying up. Onion bulbs were grown in tap water at room temperature (25-30^{o}C) for 24 hours. When the roots were 2-5 cm long, the bulbs were transferred to the prepared effluent concentrations of 5 %, 10 %, 20 % and 100 % (v/v, effluent/water). The test substrates were changed daily. Six onion bulbs were set up for each concentration, out of which the best five were selected for evaluation. The root lengths of the onion were measured from 24 hours to 72 hours using a metre rule and were expressed in centimetres (cm) as described by Fiskesjo (1993).

$$\text{MRL (cm)} = \frac{\text{Summation of RL}}{\text{Total number of RL counted}}$$

$$\text{PRL} = \frac{\text{RL in test solution}}{\text{RL in control}} \times 100$$

$$\text{PRLI} = \frac{\text{RL in control} - \text{RL in test solution}}{\text{RL in control (water)}} \times 100$$

Where RL root length, MRL – mean root length, PRL = percent root length, PRLI – percent root length inhibition

The squash technique for onion root as described by Adegbite and Olorode (2002) was used for the chromosomal investigation. Slides were observed under the light microscope (Leica 2000 phase contrast microscope). Data on total cells, total dividing cells, and cells carrying chromosomal aberrations were taken from the slides prepared for each of the different concentrations and the control.

Mitotic index was calculated by expressing the number of dividing cells as a percentage of total cells counted for each of the treatments and the control.

$$\text{MI} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{PMI} = \frac{\text{MI in control} - \text{MI in test solution}}{\text{MI in control}} \times 100$$

The frequency of chromosomal aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment. Scoring of chromosomal aberrations was taken from 5 microscopic fields for each of the different test solutions (Fiskesjo, 1985).

$$PA = \frac{\text{Number of total CA}}{\text{Total number of dividing cells}} \times 100$$

MI = mitotic index, PMI = percent mitotic inhibition, PA – percentage aberration, CA = chromosal aberration

Data Analyses: Independent samples t-test analyses were conducted to examine the differences between textile mill effluent and Adire (Tie-and-dye) wastewater in terms of physicochemical parameters of the effluents, root growth inhibition, mitotic inhibition, and chromosomal aberrations induced by the two wastewater types. All analyses were done at 5 % level of significance. The statistical analyses were carried out using the SPSS (version 20).

RESULTS AND DISCUSSION

Physico-chemical characteristics of textile mill and Tie-and-dye (Adire) effluents: Many of the physicochemical properties of both corporate textile mill and local Adire (Tie-and-dye) effluents including oil and grease, lead (Pb), copper (Cu), cadmium (Cd), biological oxygen demand, chemical oxygen demand were higher than the recommended limits set by the National Environmental Standards and Regulations Enforcement Agency of Nigeria (Table 1). Moreover, many parameters of the textile mill effluent such as phosphate, potassium, oil and grease, chloride and trace/toxic metal levels were marginally higher than those of Adire (Tie-and-dye) wastewater. However, the differences in the physicochemical properties of the two effluent-types were not statistically significant (p > 0.05). Many past studies have reported similar findings in textile mill effluent in Nigeria (Samuel *et al*., 2010; Odjegba and Bambgose, 2012; Alimba *et al*., 2013) and other places (Giorgetti *et al*., 2011; Zhang *et al*., 2012; Mehari *et al*., 2015).

Many of these physicochemical properties have been implicated in the incidences of adverse environmental and health conditions. Metals like Pb and Cd are known to be toxic and carcinogenic, and may result in synergistic chemical combinations that are more harmful than individual constituents (Bakare *et al*., 2009). Though the DO of the two effluent types were found to be within regulatory range, the high BOD_5 and COD are nevertheless indications of high organic matter in the effluents. Textile dye stuff contains a large amount of organic substances which are difficult to breakdown, and are resistant to aerobic degradation. High BOD_5 and COD are also traceable to the mordant and reducing agents like sulphides, acetic acid and soap that are used during the process of dying fabrics (Ghaly *et al*., 2014).

Percentage growth inhibition, mitotic inhibition and chromosomal aberrations induced by Textile mill and Tie-and-dye (Adire) effluents in A. cepa roots: Textile mill effluent and Adire (Tie-and-dye) wastewater both induced root growth inhibition in *A. cepa* (Figure 1). In both cases, root growth inhibition increased with rising effluent concentration and period of exposure. However, Adire (Tie-and-dye) wastewater had higher root growth inhibiting effect relative to the textile mill throughout the sampling period. At 24 hours of exposure, the percentage root inhibition induced by 5 % Adire (Tie-and-dye) effluent was 34.37 % while the inhibition induced by 5 % textile effluent during the same period was 26.25 %. The Independent T-test analysis conducted to compare the % root growth inhibition induced by textile and Adire (Tie-and-dye) effluents indicated that the mean % inhibition exerted by Adire (Tie-and-dye) wastewater (m = 51.26%) was not significantly higher (p > 0.05) than the inhibition exerted by textile effluent (m = 48.80 %).

Mitotic inhibition by both textile mill and Tie-and-dye effluents was concentration dependent, with the least mitotic inhibition induced by 5 % effluent concentration, and the highest by 100 % effluent concentration in both cases. An exception was at 24 hours of the experiment when textile effluent induced the highest mitotic inhibition of 49.17 % at 5 % concentration and the least inhibition of 5.17 % at 100 % concentration. Independent-samples t-test analysis showed that Tie-and-dye effluent induced a significantly higher (p < 0.05) mean inhibition (m = 50.80 %) relative to the mean inhibition (m = 35.13 %) induced by textile mill effluent (Table 2).

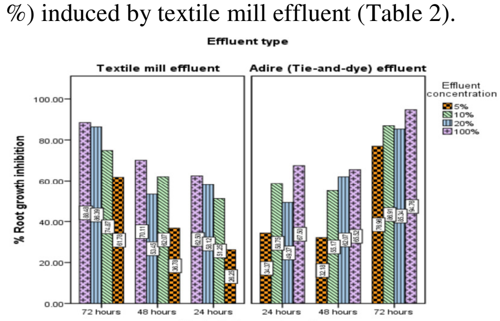

Fig 1: Percentage growth inhibition induced by Textile mill and Adire(Tie-and-dye) effluents in *A. cepa* roots

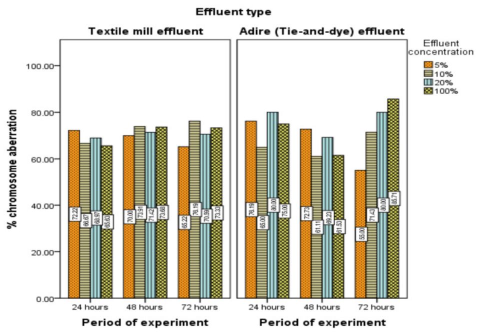

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Fig 2: Percentage chromosomal aberrations induced by textile mill and Adire (Tie- and-dye) effluents

The chromosomal aberrations induced by the two effluent types were binucleated, attached, vagrant, sticky, and bridged chromosomes (Table 3). In absolute term, textile effluent generated a significantly higher ($p < 0.01$) total aberrations (184) relative to the total aberrations (129) generated by Adire (Tie-and-dye) wastewater. The highest aberrations induced by the two effluent types were sticky chromosomes, while the lowest were binucleated chromosomes. Results also showed that at 72 hours, 10 % textile mill and 100 % Adire (Tie-and-dye) effluent concentrations induced the highest percentage chromosome aberrations of 76.19 % and 85.71% respectively (Figure 2). The difference between the percentage chromosome aberrations generated by textile mill effluent and Adire (Tie-and-dye) wastewater was not significant ($p > 0.05$). Some representative normal and aberrant chromosomes observed in the study are presented in Plates 1- 9.

Table 1: Physicochemical Properties of Textile mill and Adire (Tie-and dye) effluents

Effluent Parameters	Effluent concentrations		
	Textile mill	**Adire (Tie-and-dye)**	**NESREA limit**
PO4 (mg/L)	2.291	2.372	ns
K (mg/L)	2.801	3.709	ns
TN (mg/L)	6.723	6.992	10
MC (mg/L)	94.83	96.21	ns
O & G (mg/L)	699.00	672.00	10.00
Pb (mg/L)	0.290	0.233	0.10
Cu (mg/L)	4.109	4.942	1.00
Cr (mg/L)	1.782	2.860	0.01
Zn (mg/L)	1.098	4.210	5.00
Cd (mg/L)	0.396	0.341	0.10
pH	5.2	5.4	6.0-9.0
DO (mg/L)	3.60	3.20	ns
BOD_5 (mg/L)	200.00	198.00	20.0
COD (mg/L)	2,046.0	1,981.00	40.0
Turbidity (NTU)	600.00	526.00	ns
Chloride (mg/L)	596.00	600.00	100.0
Alkalinity (mg/L)	300.40	298.60	ns
Acidity (mg/L)	442.60	439.40	ns
TDS (mg/L)	1,691.0	1,546.00	ns
Conductivity (uS/cm)	3,382.0	3,892.00	ns

NESREA = National (Nigeria) Environmental Standards and Regulations Enforcement Agency (2009); TSS = Total suspended solids; TDS = Total dissolved solids; DO = Dissolved oxygen; COD = Chemical oxygen demand; BOD_5 = Biological oxygen demand.

Table 2: Mitotic index and mitotic inhibition induced in *A. cepa*roots exposed to textile and Adire (Tie-and-dye) effluents

EFFL CONC	TEXTILE MILL EFFLUENT				ADIRE (TIE-AND-DYE) EFFLUENT			
	CC	DC	MI	MIH	CC	DC	MI	MIH
				24 hours				
Control	500	54	10.80	0.00	500	54	10.80	0.00
5%	328	18	5.49	49.17	307	21	6.84	36.67
10%	323	24	7.43	31.20	302	20	6.62	38.70
20%	318	29	9.12	15.56	297	15	5.05	53.24
100%	313	32	10.22	5.17	292	12	4.11	61.94
				48 hours				
Control	500	54	10.80	0.00	500	54	10.8	0.00
5%	315	20	6.35	41.20	287	40	6.97	35.46
10%	310	23	7.42	31.30	279	14	5.02	53.52
20%	305	21	6.89	36.20	273	10	3.66	66.11
100%	301	19	6.31	41.59	267	7	2.62	75.74
				72 hours				
Control	500	54	10.80	0.00	500	54	10.80	0.00
5%	313	28	7.34	32.04	295	22	7.46	30.93
10%	308	21	6.82	36.85	291	18	6.19	42.69
20%	303	17	5.61	48.06	285	13	4.56	57.58
100%	297	15	5.05	53.24	283	13	4.59	57.50

EFFL CONC = Effluent concentration; CC = total cell count; DC = number of dividing cells; MI = mitotic index; MIH = mitotic inhibition.

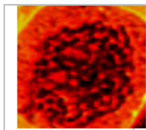
Plate 1: Normal prophase

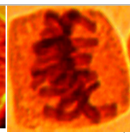
Plate 2: Normal metaphase

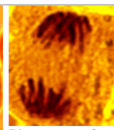
Plate 3: Normal anaphase

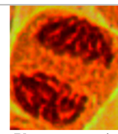
Plate 4: Normal telophase

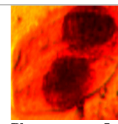
Plate 5: Binucleated cell

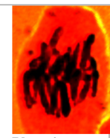
Plate 6: metaphase chromosomes

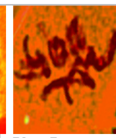
Plate 7: Vagrant chromosomes

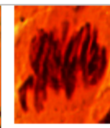
Plate 8: Stickymetaphase chromosomes

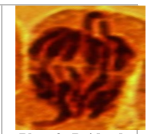
Plate 9: Bridged anaphase chromosomes

Table 3: Chromosome aberrations induced by textile mill and Adire (Tie-and-dye) effluents

Conc	Chromosome aberration type											
	Binucleated		Attached		Vagrant		Sticky		Bridged		Total	
	Tex	Adir	Tex	Adir	Tex	Adir	Tex	Adir	Tex	Adir	Tex	Adir
24 hours												
5%	0	0	2	2	4	3	5	7	2	4	13	16
10%	0	0	1	2	4	3	7	6	4	2	16	13
20%	0	1	3	0	4	4	7	5	6	2	20	12
100%	0	2	3	0	5	2	8	4	5	1	21	9
48 hours												
5%	1	0	1	2	3	4	5	6	4	4	14	16
10%	0	0	2	1	4	2	6	5	5	3	17	11
20%	0	0	2	0	4	2	5	5	4	2	15	9
100%	0	0	2	2	4	1	5	4	3	1	14	8
72 hours												
5%	0	0	3	3	3	2	6	4	3	2	15	11
10%	0	0	3	1	4	2	6	5	3	2	16	10
20%	0	0	0	0	4	3	5	3	3	2	12	8
100%	0	0	2	0	3	1	4	5	2	0	11	6
Total	1	3	24	13	46	29	69	59	44	25	**184**	**129**

Conc = Effluent concentration; Tex = Textile mill effluent; Adir = Adire (Tie-and-dye) wastewater.

Inhibition of mitotic index is an indicator of cytotoxicity (Samuel *et al*., 2010). The occurrence of chromosome aberrations will always lead to growth restrictions (Fiskesjo, 1997). The mutagenic (mutation-inducing) potentials of the effluents in this study could be partly related to their physicochemical qualities and constituents including metals and dyestuffs. For instance, some metals like Cd, Pb, Cu, and Zn, have been found to be capable of inducing clastogenic (chromosome fragmenting) and aneugenic (chromosome loss) effects including mitosis and cytokinensis disturbances (Minissi and Lombi, 1997; Dovgaliuk *et al*., 2001; El-Shahaby *et al*., 2003). Cadmium is particularly associated with bridges, stickiness, micronuclei, and chromosome breaks (Zhang and Yang, 1994). Lead has been implicated in enzyme inhibition, mutation, and chromosomal irregularities (Johnson, 1998). Studies have pointed out that there is a good correlation between chromosome abnormalities and mutagenic activity found in root-tip systems and those found in mammalian cell systems (Majer *et al*., 2005; Olorunfemi *et al*., 2011). Therefore, the effluents emanating from the local Adire (Tie-and-dye) textile business and the corporate textile mill company are potentially genotoxic to plant, animals and humans. The water bodies into which such effluents are discharged are not fit for farm irrigation or as sources of potable and domestic water supply.

Unlike corporate textile mills, Adire (Tie-and-dye) textile businesses in Nigeria are not regulated, and as a result, discharge untreated wastewater into the surrounding soil and water bodies. The fact that no significant differences were found between the physicochemical qualities, mitotic inhibiting and chromosomal inhibiting actions of the two effluent types therefore suggests that the textile mill company whose effluent was used for this study did not subject its effluent to treatment before discharging, and if it did, the treatment was grossly inadequate. This finding casts doubt on the effectiveness of regulatory authorities in Nigeria. Moreover, the findings may be a reflection of what obtains in many other developing countries.

Conclusion: In order to save the environment and the health of citizens, governments at all levels in Nigeria, and other countries in which similar results may be obtained, are called upon to reinvigorate their regulatory agencies to enable them to discharge their oversight functions effectively. The activities of the local tie-and-dye (Adire) business in Nigeria should be regulated to arrest the spate of discharge of untreated, contaminant-laden wastewater into the environment.

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