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HEAVY METALS ANALYSIS AND TOXICITY EVALUATION OF SOME TEXTILE AND DYEING EFFLUENTS IN KANO, NIGERIA USING *Allium cepa* BIOASSAY

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ABSTRACT
Insufficiencies in dyeing and textile processing coupled with incomplete sewage treatment system lead to indiscriminate discharge of large amount of dyestuff into water bodies mostly used for irrigation and fishing. These wastes are potentially mutagenic and some are even carcinogenic. In this research, cytotoxicity of three industrial effluents viz; African Textile Manufacturer (ATM), Kofar Naisa and Kofar Mata dyeing centres were evaluated using Allium cepa bioassay. The concentrations of some heavy metals (Cr, Cd, Zn, Fe, Cu, Co, Pb, Mn and Ni) were also determined. Three sets of small onion bulbs were cultivated in 25%, 50% 75% and 100% effluent concentrations (v/v) and a control for each site. Root tips from each bulb were harvested and processed for cytological studies using squashing technique. After 48hrs of exposure, cytotoxic effects of the effluents on root tips (compared to control) showed root growth retardation which was more apparent at higher concentrations. This indicated that, the root growth inhibition was concentration dependent. Number of dividing cells observed and the Mitotic Index (MI) were also concentration dependent. There were decrease in number of dividing cells and MI with increase in concentration of the treatment. The effective concentrations that caused 50% effect (EC_{50}) was 95% for ATM and K/Naisa dyeing points while it was 100% in K/mata dyeing point. Diverse structural aberrations and abnormalities were observed ranging from chromosomal bridge, which was apparent even at low concentrations of the treatment to cytokinesis failure, Micronucleus (MN) and nuclear buds or vacuolated nuclei. Analysis of Variance (ANOVA) showed no significant difference ($P>0.05$) in mean concentrations of Cr, Fe, Cu and Mn for K/Naisa and K/Mata dyeing centres, but there was statistical difference ($P<0.05$) for these parameters in ATM. Likewise, there was significant difference ($P<0.05$) in the root growth of A. cepa exposed to different concentrations of the effluent in ATM and K/Naisa sites but no statistical difference existed in root growth of A. cepa exposed to K/Mata effluents.
Keywords: Allium cepa, Bioassay, Effluents, Cytotoxicity, Mitotic Index

INTRODUCTION

Environmental pollution is the second main constrain dooming the progress of developing countries apart from poverty and exponential growth in population (Grover and Kaur, 1999; Olusegun *et al.,* 2010). According to Chan *et al.* (2003); Lah *et al.* (2004) and Smolder *et al.* (2004), industrial effluents are the main sources of direct and often continuous input of pollutants and toxicants into aquatic ecosystem causing long term implications onto the ecosystem functioning. Among these are textiles and dyeing industries. Effluents from textiles/dyeing industry act as the most important cause of environmental pollution, because the products are diverse in chemical composition (Ali *et al.,* 2008). Insufficiencies in dyeing during textile processing always results in large amount of dyestuff being directly lost to the wastewater, which unavoidably find its ways into water bodies mostly used for irrigation and fishing (McMullan *et al.,* 2007). These dyes undergo chemical changes under certain environmental conditions and the transformed products may even be more toxic and carcinogenic than the parent molecules (Weber and Adams, 1995; Ratna and Padhi, 2012). The effluents from textile/dyeing industries thus carry a large number of dyes and other additives which are difficult to remove in conventional water treatment procedures and can therefore be transported easily to the water bodies especially because they are designed to have high water solubility and can undergo degradation to form products that are highly toxic and carcinogenic (Rindle and Troll, 1975). The complexity of industrial effluents makes it impossible to carry out hazard assessment study based on physical and chemical analysis only.

Thus, in search of a test systems which can be combined with chemical analysis to provide a scientific data that will be useful as the basis for regulating the discharge of potentially hazardous substances into the environment and as well serve as a suitable method for toxicity evaluation, Devis and Ford (1992), Matcalf and Eddy (2003) proposed the use of biotoxicity test or bioassay, which involves the use of living organisms. Bioassay is typically conducted to measure the effect of substances (effluents in this case) on living organisms (*in-vivo* bioassay) or their tissues (*in-vitro* bioassay) in monitoring environmental pollution. True and Hayward (1990) reported that living organisms (plants or animals) serve as the most sensitive indicators of toxicity in an environment provided that they are judiciously monitored. Fiskesjo (1985; 1993) and Rank and Nielsen (1993; 1998) further considered the use of *Allium cepa* test as a standard method in environmental monitoring and toxicity screening of any wastewater. Odeigah *et al.* (1997) moreover, showed that the plant's root is the most useful tool in this test because the root tips are often the first predisposed to chemical contaminants in water and soil. The study therefore aimed at assessing the heavy metal concentrations as well as evaluating the toxicity effect of textile and dyeing effluent samples collected using *Allium cepa* bioassay.

MATERIALS AND METHODS

Study Area

Kano, located at 11°59′18′′N and 08°32′06′′E about 418 metres above the sea level, occupies the central position in Northern Nigeria. It is one of the most populous and developed cities in Nigeria with dyeing and textile being among the dominating activities in the city (Akan *et al.,* 2007). Within Kano metropolis, Textile Manufacturers (ATM) (11°53′15′′N; 08°28′96′′E), Kofar Naisa dyeing point (11°58′95′′N; 08°30′86′′E) and Kofar Mata dyeing point (11°39′95′′N; 08°31′41′′E) were selected for the study.

Sample Collection

The samples were collected in the early morning hours using 5litre capacity jerry-can from each location as described by Maiti (2004). The samples were used for toxicity evaluation using *Allium cepa* chromosomal aberration bioassay and the heavy metals (Cr, Cd, Zn, Fe, Cu, Hg, Pb, Mn and Ni) using Atomic Absorption Spectrophotometry (AAS).

Determination of Heavy Metals

Heavy metals were determined by aspirating the samples using Atomic Absorption Spectrophotometer (AAS) as described by Maiti, (2004) and APHA (2005). This was done after pre-digestion of the sample using single acid digestion method (Maiti, 2004 ; APHA, 2005).

Allium Chromosomal Bioassay of the Effluents

The design of the experiment was four groups of three onions. The groups are the different concentrations made (i.e. 25%, 50%, 75% and 100%) while the 3 onions per each concentration are the replicates making a total of 36 onion bulbs.

The experiment was set-up allowing the rootlets to grow as described by Fikesjo (1985; 1993) and Rank and Nielson (1993). The onion bulbs of the size 7-12cm in diameter were selected. The bases of the bulbs were gently scrapped to remove the older roots and expose the root-primodia. The bulbs were placed in plastic cups containing distilled water. The set-up was then allowed to stay for 3 days for new roots to emerge. The root length (before and after the treatment) were determined using centimeter rule.

The onion bulbs were transferred to another plastic cups containing these different concentrations of effluent (as treatment) for 48 hours. The set-up was kept in the dark where by the concentration of the effluents was changed at 24 hour interval. The rootlets were harvested after 48 hours and immediately fixed in ethanol : glacial acetic acid (3:1, v/v) in a clean petri dish (Rank and Nielson, 1993; Abu and Ezeugwu, 2008; Olurunfemi *et al.,* 2011). These were then hydrolyzed in 1N HCl at 60°C (using thermostat water bath) for 5 minutes after which the preparation was washed with distilled water. Two tips were squashed on each slide and stained with safranin stain for 2 minutes, the cover slip was carefully lowered on the preparation excluding the air bubbles. Several slides were prepared for each concentration and a control. The slides were then analyzed at ×10, ×40 and ×100 magnification using Olympus electric microscope (XSZ-107BN- model).

The induced chromosomal aberrations were determined and Mitotic Index (MI) calculated as the number of dividing cells per 300 observed cells by 100 (Fiskesjo, 1985, 1997) as showm below:

$$MI = \frac{No\ of\ dividing\ cells}{Observed\ Cells\ (300)} \times 100$$

Percentage aberration was also calculated based on the total number of aberrant cells at each concentration of the effluent per total cells scored or examined (Bakare *et al.,* 2000; Olorunfemi *et al.,* 2011).

$$\% \, aberration = \frac{Total\ No\ of\ aberrant\ cells}{Number\ of\ cells\ scored} \times 100$$

And EC_{50} (effective concentration that causes 50% effect on the experimental materials) was determined from the plot of root length as percentage of control against various sample concentrations otherwise called dose response curve (using Microsoft Excel computer program).

RESULTS AND DISCUSSION

The results of mean heavy metals for the three different sites were presented in Table 1, and these were compared with the Federal Environmental Protection Agency Act (FEPA, 2002) and General Environmental Standard for Discharge of Effluents (Textiles/Dyestuffs and Dye Intermediate). Meanwhile, Table 2 shows the mean root length of *Allium cepa* before and after exposure to different concentrations of the effluents. Table 3 showed the numerical aberration (that includes number of dividing cells and mitotic index) which are among the effects observed in *A. cepa* on exposure to different concentrations of textile and dyeing effluents in different sites. and Table 4 shows the structural aberration observed in *A. cepa* root exposed to different concentrations of the effluent samples.

Table 1.: Mean Values for Heavy Metals of the Effluent Sample Collected and Analyzed Between September, 2016 and July, 2017

Parameters(mg/L)	E1	E2	E3	FEPA/GES
Cr	1.24±0.20[a]	1.66±0.25[b]	1.82±0.19[b]	2.0
Cd	1.46±0.38	1.47±0.30	1.53±0.23	1.0
Zn	2.43±1.23	2.53±1.33	3.19±1.31	1.5
Fe	2.52±0.57[a]	4.79±0.62[b]	4.41±0.49[b]	3.0
Cu	1.22±0.21[a]	1.65±0.29[b]	1.71±0.55[b]	3.0
Co	0.69±0.10[a]	0.85±0.21[b]	0.72±0.20[a]	-
Pb	0.51±0.10[a]	0.61±0.15[a]	0.78±0.14[b]	1.0
Mn	1.17±0.19[a]	1.43±0.21[b]	1.38±0.32[b]	2.0
Ni	1.10±0.43	1.24±0.58	1.25±0.69	3.0

[ab] Means within the same rows with different superscript are significantly different ($p<0.05$)

FEPA (2002): Federal Environmental Protection Agency Act/ General Environmental Standard for Discharge of Effluents (Textiles/Dyestuffs and Dye Intermediate)

Key: E1= African Textile Manufacturer (ATM), E2= K/Naisa Dyeing Point, E3= K/Mata Dyeing Point

Table 2: Mean Root Length of *A. cepa* Exposed to Different Concentrations of Effluent (in cm)

Conc. (%)	ATM		K/Naisa		K/Mata	
	MRLBT±S.D	MRLAT±S.D	MRLBT±S.D	MRLAT±S.D	MRLBT±S.D	MRLAT±S.D
25	6.9±0.45	5.6±0.37	6.9±0.35	5.4±0.17	6.0±1.70	5.5±0.90
50	5.8±0.76	5.1±0.53	5.6±1.50	4.7±0.76	6.4±1.80	6.0±1.91
75	5.9±0.67	4.7±0.89	6.9±0.99	3.9±0.82	6.2±0.57	5.8±0.90
100	5.2±0.25	4.2±0.49	5.6±0.72	2.6±0.31	4.2±1.60	4.1±1.50
Control	(5.7)	**(7.2)**				

N.B: The Values are Mean± S.D of 3 replicates at each concentration

Key: MRLBT = Mean Root Length Before Treatment

MRLAT = Mean Root Length After Treatment

S.D = Standard Deviation

Table 3: Numerical Aberrations Observed in *A. cepa* Roots Exposed to Different Concentrations of Textile and Dyeing Effluents for 48hours

Samples	Concentration (%)	No. of Dividing Cells	Mitotic Index (%)
E1	25	68	22.67
	50	53	17.67
	75	40	13.33
	100	21	7.00
E2	25	70	23.33
	50	61	20.30
	75	38	12.67
	100	15	5.00
E3	25	59	19.67
	50	46	15.33
	75	33	11.00
	100	18	6.00
	Control	**115**	**38.33**

N.B: 100 cells per onion were examined

Table 4: Structural Aberrations Observed in *A. cepa* Roots Exposed to Different Concentrations of Textile and Dyeing Effluents for 48hours

Samples	Conc.(%)	Bridge	Incomp. Cytok.	MN	Chr. Adher.	Nuclear Buds	Multi polarity	Total	% Aberr.
E1	25	2	1	0	0	1	1	05	7.35
	50	3	1	1	1	1	1	08	15.09
	75	2	3	1	0	1	2	09	22.50
	100	0	1	0	3	0	0	04	19.05
E2	25	1	1	0	0	1	0	03	4.29
	50	2	1	0	0	1	1	05	8.20
	75	2	4	1	1	1	1	10	26.32
	100	0	0	0	2	0	0	02	13.33
E3	25	2	3	0	0	1	1	07	11.86
	50	1	1	1	0	2	0	05	10.87
	75	1	1	0	0	1	0	03	9.10
	100	0	0	0	1	0	-	01	5.56
	Control	-	1	-	2	-	-	03	**2.61**

Key: MN= Micro Nucleus, Chr. Adher.= Chromosome Adherence

Table5: EC_{50} and Regression Coefficient of Effluents at Different Sites Extracted from Dose-Response Curve

Sites	EC_{50} (%)	R^2	R
E1	95	0.8881	0.94
E2	95	0.9776	0.99
E3	100	0.4361	0.66

Key: EC_{50} = Effective Concentration causing effect on 50% test material R= Regression Coefficient

DISCUSSION

Analytical results for heavy metals revealed that, the mean concentration of chromium (Cr) in textile and dyeing effluents were 1.24, 1.66 and 1.82 mg/l for ATM (E1), K/Naisa (E2) and K/Mata (E3) textiles and dyeing points respectively. These mean values are lower than 2.38 mg/l of chromium metals recorded by Ugoji and Aboaba (2004) for textile industry effluent in Lagos metropolis, Nigeria. Chromium causes allergic reactions to the skin of exposed organism especially when discharged in wastewater into water-ways, streams and canals which humans use for their daily activities (ATSDR, 2005).

The concentrations of Cadmium (Cd) and Zinc (Zn) 1.46, 1.47, 1.53 and 2.43, 2.53 and 3.19 mg/l for sites E1, E2 and E3 respectively, exceeded the recommended limit by FEPA (2002) (Table 1) for discharged effluents into the environment. These mean values for Cadmium are higher than 0.20 mg/l observed by Dubey *et al*. (2003) in local dye house effluent in New Delhi India.

Exposure to small concentration of cadmium for long time in food and water according to ATSDR (2005) leads to a build-up of cadmium in kidneys and results to possible kidney disease. Other potential long-term effects are lungs damage and fragile bones (ATSDR, 2005).

Likewise, concentration of iron (Fe) in E2 (4.79 mg/l) and E3 (4.41 mg/l) also exceeded 3.0 mg/l recommended limit for the discharge of effluent set by FEPA (2002). These values are greater than 2.14 mg/l the concentration of iron observed by Yusuff and Sonibare (2004). The mean concentrations of Copper however, were 1.22, 1.65 and 1.71 mg/l for the three sites under study; these values were lower than 5.14 mg/l and 4.0 mg/l of copper reported by Aslam *et al.* (2004) and Yusuff and Sonibare (2004) in Kaduna, Nigeria. Copper is an essential element in mammalian nutrition as a component of metallo-enzymes in which it acts as an electron donor or acceptor (Deepali and Gangwar, 2010), but exposure to high level of copper can result in a number of adverse health effects such as diarrhea, stomach cramps, and nausea. (Bremner, 1998); chronic exposure may lead to liver damage and kidney disease. Moreover, the results revealed the concentration of Lead (Pb) for the three sites as 0.51, 0.61 and 0.78 mg/l which are all below 1.0 mg/l standard limit for discharge of textile effluent by FEPA (2002), but research by Dubey *et al*. (2003) showed that long term exposure to lower concentration of lead may result in the accumulation which can cause serious effect to central nervous system and immune system particularly in children. Moreover, Dash *et al.* (1988) reported the range value of 0.29-0.43 mg/l of Pb and found the effect as clastogenic due to impairment of spindle function in *A. cepa*.

The present study also revealed that, the average concentrations for manganese were 1.17 mg/l for ATM, 1.43 mg/l for K/Naisa and 1.38 mg/l for K/Mata dyeing points. These values correspond with the average range of 1.15-1.65 mg/l for manganese observed by Yusuff and Sonibare (2004) in the effluent of textile industries in Nigeria. Although manganese is among the least toxic trace elements, but excessive discharge of effluents containing this amount may gradually accumulate and could provide source of continuing exposure that may lead to harmful effect in long term.

Statistically, no significant difference was observed between different sites (E1, E2 and E3) for the mean concentration of Cd, Zn Mn and Ni ($P>0.05$), but a significant difference ($P< 0.05$) was observed for the mean concentration of Fe, Cu, Co and Pb between different sites (Table 1).

The *Allium cepa* test have been used as a standard and quick method of detecting cytotoxic effect of chemicals and pollution levels in an environment (Grant, 1982; Smaka-kinel *et al.,* 1996). The results of *A. cepa* test indicated the presence of cytotoxic and mutagenic substances in the environment which represent direct or indirect risks to all living organisms by inhibiting the mitotic activities and interfering with growth and development of the test organisms.

The mean root length of *A. cepa* root before and after exposure to different effluent concentrations are presented in Table 2. The result revealed that, there is growth retardation in roots after been exposed to different effluent concentrations, and that, the root growth retardation is concentration dependent, thus, growth retardation was more apparent at higher concentration. That is, high growth rate was recorded in onion bulb exposed to lower concentration and *vice versa*. This agreed with the finding of Olorunfemi *et al.* (2011) where similar results were obtained in *A. cepa* root exposed to different concentrations of effluents from textile and rubber processing industries. Root growth retardation according to Odeigah *et al*. (1997) are indication of irreversible toxicity effect leading to possible cell death during prolong exposure, hence, roots decay were observed in higher (75% and 100%) concentrations during the experiment. On comparing with control, it was observed that, there was no root-growth retardation in control, because, the root length increased from 5.7cm to 7.2cm after the period of the experiment.

Aberrations are among the noticeable effects of the effluent action on the root of *A. cepa.* The number of dividing cells and Mitotic Index (MI) in *A. cepa* exposed to different concentrations of textile and dyeing effluents were shown in Table 3. The results revealed that, there is dependency of cell division in *A. cepa* on concentrations of the effluents used. Thus, increase in concentrations of the treatments (the effluents) caused decrease in number of dividing cells, or in other words, less dividing cells were observed at higher concentration of the treatment than at lower concentrations, and more dividing cells were observed in control than in the treated *A. cepa* roots (Table 3).

Mitotic Index (MI) was also concentration dependent. There was decrease in MI with increase in concentration of the treatment on the test materials (the *A. cepa* roots) across both sites (Table 4).

Generally, MI lower than the control according to Fernandes *et al.* (2007) is an indication of alteration or interference derived from chemical action in growth and development of exposed organism, where as MI higher than control is the consequence of increased or uncontrolled cell division that may lead to disordered cell-proliferation which results to cancerous cells or tumour tissues in an exposed organism (Hoshina, 2002).

The results of this study showed lower MI than the control at each concentration of the treatment. In Site 1 (E1), there was 22.67%, 17.67%, 13.33% and 7.00% MI for 25% 50%, 75%, and 100% concentrations respectively. These values are all lower than 38.38% MI of the control (Table 3). The highest MI was 23.33% in 25% concentration of Site 2 (E2), while the lowest MI was 5.00% in 100% concentration of the same site. These values were also lower than 38.33% MI of the control, and hence this according to Fernandes *et al.* (2007) indicates the interference of the action of effluent concentration on the ability of the cell to divide which directly affects or interferes with growth and development of *A. cepa* roots under study.

Moreover, different structural aberrations were observed in *A. cepa* root exposed to different concentrations of textile and dyeing effluents at various sites (Table 4). Chromosomal bridge and incomplete cytokinesis are the most common aberrations observed in all sites. It was observed that percentage aberrations are inversely proportional to the concentration, thus less number of aberrant cells were observed at highest concentration (100%) at both sites. This may be as a result of root decay observed at high concentrations of the effluents, and may be due to the toxicity effect of the effluent at high concentration which leads to reduced mitotic activities at higher concentrations.

The effective concentrations that caused 50% effect on *A. cepa* roots (EC_{50}) for E1and E2 was 95%, while it was 100% in E3. This showed that 95% was the concentration capable of causing effect to 50% experimental material (*A. cepa)* but 100% in E3. According to Odeigah *et al.* (1997), the higher the $EC_{50,}$ the less toxic are the effluent samples. On the other hand, the regression coefficient (R^2) was 0.8881, 0.9776 and 0.4361 for E1, E2 and E3 respectively, where R equals to 0.94, 0.99 and 0.66 implying that 94%, 99% and 66% of the total effects observed on *A. cepa* was as a result or associated with the treatment (the effluents) for the three sites respectively.

Statistical analysis (ANOVA) showed that, there is significant difference ($P<0.05$) in the root growth of *A. cepa* exposed to different concentrations of effluents in ATM (E1) and K/Naisa (E2) sites. Tukey post-hoc analysis revealed that, the difference exists between the pair mean of 25-50%, 25-75% and 25-100% concentrations in E1, while the difference exists between the pair mean of 25-75% and 50-100% concentrations in E2, but no significant difference exists in root growth of *A. cepa* exposed to K/mata (E3) effluents.

CONCLUSION

Although, textile and dyeing activities are promoting phenomenon for economic growth in Kano metropolis, their effluents should be effectively managed before discharge. This is because, the results of some heavy metals analysis as well as toxicity test showed that there was anomaly in the effective management of the effluents discharged and that poses negative effects on aquatic biota.

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