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Genotoxicity of Hairdressing Salon Effluent Using *Allium cepa*

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***Abstract*— Genotoxicity of hairdressing salon effluent using Allium cepa was carried out using different concentrations of 12.5%, 25%, 50% and 100% of hairdressing salon effluent obtain from different outlets. The presence and concentrations of some heavy metals such as lead, Mercury, Zinc, Cadmium and Arsenic in the effluent were determined using Atomic Absorption Spectrophotometer (AAS). Chromosomal aberration assays were used to determine the mitotic index and chromosome aberration rate. There was an increase in the mitotic inhibition as the concentration increased whereas there was a decrease in the mitotic index, indicative of an inverse relationship shared by the two properties. The effluent induced chromosomal aberrations in the meristematic cells of the A. cepa root tip, and laggards were the most frequently recorded aberrations induced. Morphological abnormalities were also recorded in the roots of the Allium cepa. The genotoxic effects of hairdressing salon effluent on Allium cepa indicates that it contains toxic substances which may constitute environmental risks particularly on soils and plant crops planted near hairdressing salon facilities.**

***Index Terms*— Chromosomal Aberrations, Hairdressing Salon, Effluent, Genotoxicity, Mitotic inhibition.**

I. Introduction

The United States Environmental Protection Agency (USEPA, 2006) defined effluent as wastewater (treated or untreated) that flows out of a treatment plant, sewer or industrial outfall. Industrial effluents or wastewater carry various types of contaminants such as heavy metals, organic and inorganic matters, Polycyclic Aromatic Hydrocarbons (PAHs), microorganisms, etc. into the environment, especially the aquatic systems (HO *et al*., 2012). Among these industries is the cosmetic industry which has given rise to the establishment of hairdressing salons.

Hairdressing salons offer a wide range of services from skin treatment and hair styling to manicure, make-ups and tanning application. These services generate wastes in the form of relaxers, dyes, alkalis, acids and other chemical which can greatly influence the physicochemical properties of the receiving environment (Bowers *et al*., 2002). Hairdressers and their customers are frequently exposed to high concentration of several compounds that are components of the various chemical products used in the hairdressing salon facilities (Wenniger *et al*., 1993), and some of these cosmetic products have been implicated in some health problems (Okereke *et al*., 2015). Many of these products are unregulated and may contain carcinogens and volatile organic compounds (VOCs) such as lithium hydroxide, calcium hydroxide, guamidine carbonate and ammonium thioglycolate; when released into the environment (Leino *et al*., 1999).

ECVAM (2013), defined genotoxicity as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxicity assessment is an essential component of the safety assessment of types of substances, ranging from pharmaceuticals, industrial chemicals, pesticides, biocides, food additives, cosmetics ingredients, and veterinary drugs, which are relevant in the context of international legislations.

This study seeks to evaluate the genotoxic impacts associated with the indiscriminate disposal of hairdressing salon effluents into the environment; using *Allium cepa* (Onion bulb) as a bio-indicator to monitor such effects so as to maintain a healthy, sanitary and supportive environment to man, and for ecosystem, sustainability.

II. Materials and Methods

In line with APHA (1998), the following physicochemical parameters were evaluated in the study, the pH and temperature were determined using HANNA pH meter (HI 8424) after being calibrated with buffer 4.0, 7.0 and 10.0. The Total Dissolved Solids (TDS) was determined using TDS model (H1 98301), the Dissolved Oxygen (DO), alkalinity, Iron HR (high range); turbidity, nitrate, Phosphate, Sulphate, Chloride, electrical conductivity, Cadmium, Zinc, Chromium HR, Manganese, Aluminum, Nickel, Chemical Oxygen Demand MR (COD medium range) and the Biological Oxygen Demand for 5 days (BODs) were all determined using HANNA Multiparameter bench photometer (HI 83099) where each parameter has its reagent kit for its estimation (Hanna 83200 Instruction Manual),

For the genotoxicity analysis, equal sized onion bulb (*Allium cepa*) for the study were air-dried for 14 days before being used for evaluation of the root inhibition and *in vivo* induction of chromosomal aberration (genotoxicity index) according to the methods described by Fiskesjo (1993).

The outer, dried, brown scales of the bulb (dead roots) of

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the onions were carefully removed leaving the rings of primordial root intact. Micrographs of the aberrations including other stages of mitosis were taken. The mitotic index, mitotic inhibition as well as the phase index were calculated as described by Fiskesjo (1993). However, slide scoring was done in accordance to the method described by (Fiskesjo, 1997; Rank, 2003).

III. Result

A. Physicochemical Composition of the Hairdressing Salon Effluent

The Physicochemical composition of the hairdressing salon effluent analyzed showed a higher concentration for some of the micro-element test; these include lead, mercury, cadmium, nickel, selenium, zinc and arsenic.

B. Macroscopic and Microscopic Effect of the Hairdressing Salon Effluent

It was observed that the hairdressing salon effluent suppressed root growth when compared with the control. Strong growth retardation was recorded in onion roots growing at high concentrations of 50% and 100%; the effects were less severe at low concentrations of 12.5% and 25%. The root malformations recorded at high concentrations were twists, crotchet hooks (root tips bent upwards resembling hooks) and C-tumors (abnormalities appearing as swellings of the root tips (Figures 1a, b; 2a -2f).

Table 3.1: Physicochemical Composition of the hairdressing salon effluents

Parameters	Values in Hairdressing Salon effluents	Effluents limitation guide for industries in Nigeria
pH	7.01 ± 0.01	6.00 -9.00
Temperature (^{0}C)	29.00 ± 0.05	Less than 40.00
Conductivity (us/cm)	104.70 ± 0.08	200.00
Turbidity (NTU)	434.10 ± 1.03	-
Acidity (mg/l)	2.54 ± 0.04	-
Total Solid (mg/l)	1.56 ± 0.02	-
Total Dissolved Solids (mg/l)	2.26 ± 0.05	2000.00
Total Suspended Solids (mg/l)	0.76	30.00
Phosphate (mg/l)	0.86	5.00 – 10.00
Nitrate (mg/l)	19.78	20.00
Hardness (mg/l)	160.00 ± 1.12	-
Chloride (mg/l)	300.00	600.00
BOD_5 (mg/l)	7.00 ± 0.00	30.00 - 50.00
Sulphate (mg/l)	206.98 ± 0.82	500.00 – 1000.00

Potassium (ppm)	0.22 ± 0.00	-
Sodium (ppm)	0.34 ± 0.00	-
Calcium (ppm)	1.11 ± 0.00	200.00
Molybdenium (ppm)	0.22 ± 0.00	-
Aluminum (ppm)	0.01 ± 0.00	-
Selenium (ppm)	1.44 ± 0.00	Less than 0.1
Silicone (ppm)	0.89 ± 0.00	-
Lead (ppm)	2.92 ± 0.00	Less than 0.1
Mercury (ppm)	0.35 ± 0.00	0.05
Cadmium (ppm)	0.09 ± 0.00	Less than 0.1
Cobalt (ppm)	0.04 ± 0.00	-
Nickel (ppm)	0.39 ± 0.00	Less than 0.1
Zinc (ppm)	1.76 ± 0.00	Less than 0.1
Chromium (ppm)	0.00 ± 0.00	Less than 0.1
Arsenic (ppm)	1.40 ± 0.00	0.1

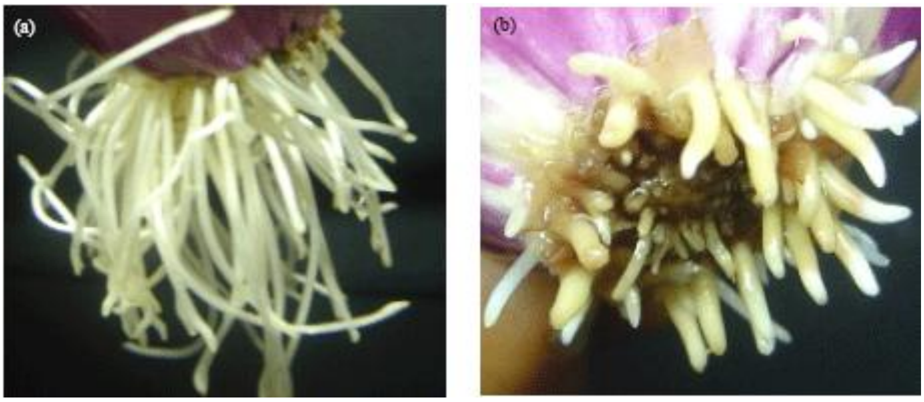
Table3.2: Genotoxic effects of hairdressing salon effluents on *Allium cepa*

CHROMOSOMAL ABERRATION							
Conc. (%)	**Number of dividing cells**	**Lag**	**Brid**	**Vag**	**Sticky**	**Bin**	**Total Aberration cells**
Control	126	0	0	0	0	0	0
12.5	78	3	4	4	3	3	17
25	45	4	2	1	2	1	10
50	36	2	0	0	0	0	2
100	0	0	0	0	0	0	0

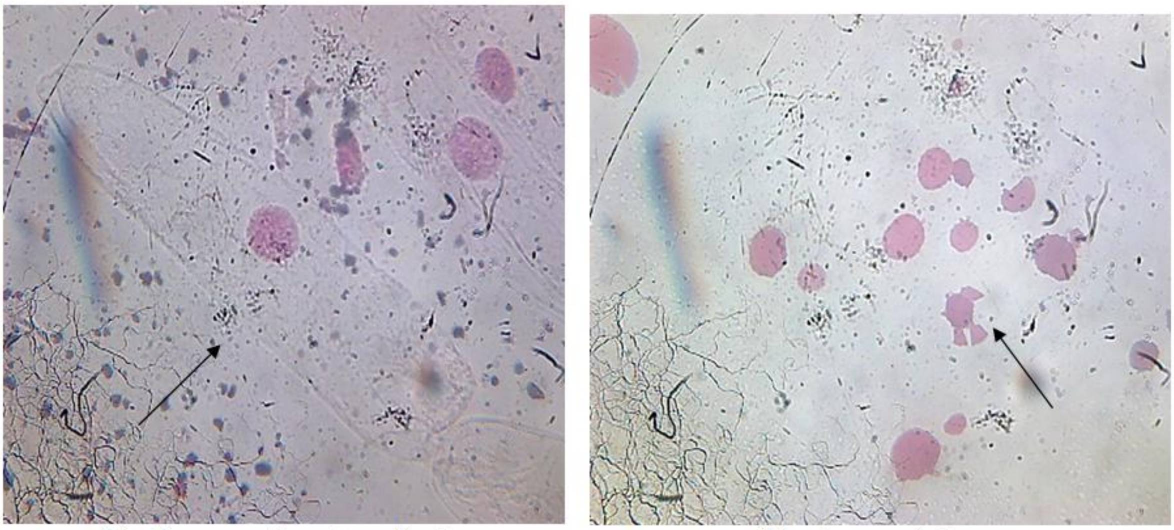
Keys: Lag-Laggard; Brid – Bridged; Vag – Vagrant cells; Sticky – Stickiness; Bin- Binucleated cell 1000 cells per slide.

Table 3.3: The Mitotic index and mitotic inhibition of *Allium cepa* exposed to hairdressing salon effluent

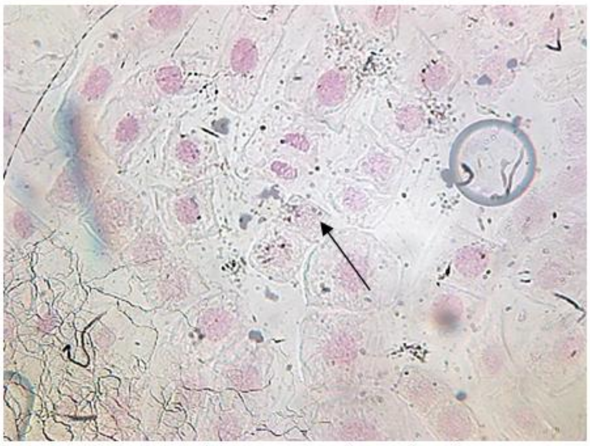
Concentration	Number of dividing cells	Mitotic index (%)	Mitotic inhibition (%)
Control	126	12.6	0.0
12.5%	78	7.8	38.1
25%	45	4.5	64.3
50%	36	3.6	71.4
100%	0	0	100



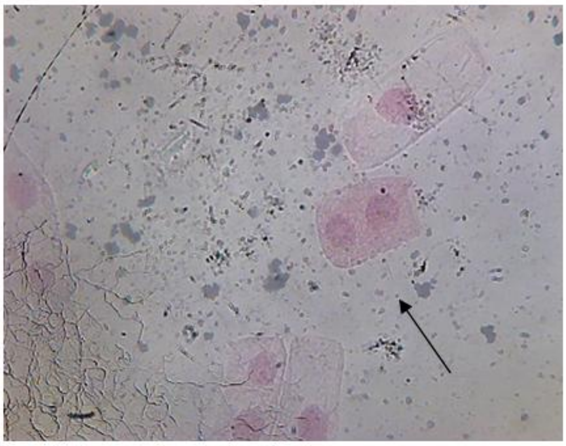
Figures3.1a, b: Macroscopic effects of Hair salon effluents on *Allium cepa* roots (a) Crochet roots at 25% concentration (b) C-tumor roots at 100% concentration



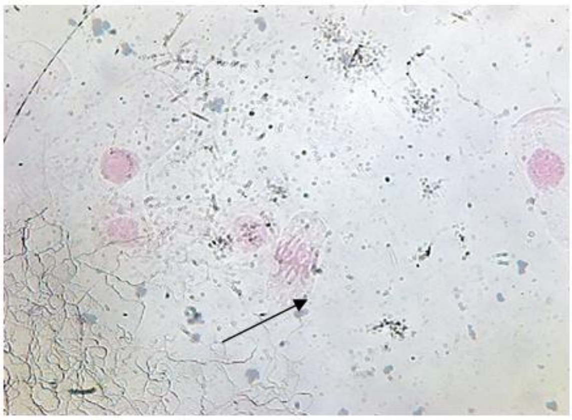
(a) disproportionate cell size (b) micronuclei



(c) Anaphase-Telophase bridge



(d) Binucleated cell



(e) Stickymetaphase

(f) Bridged anaphase

Figure 3. 2(a-f): Chromosomal aberrations induced by hair salon effluent on *Allium cepa* roots

The effect of the hairdressing salon effluent on cell division and chromosomal aberration of *Allium cepa* and the effects of the hairdressing salon effluent on mitotic index and inhibition of onion cells show that mitotic index decreased as concentration of the hairdressing salon effluent increased while, the mitotic inhibition increased with concentration of the 100% concentration. This is an indication of a 100% mitotic inhibition. Thus, mitosis was completely prevented by the toxicant (i.e. the hairdressing salon effluent) under study.

More than one kind of aberration was observed per cell. The highest was recorded in 12.5% concentration. Laggard chromosomes were the most frequent kind of aberration induced by the hairdressing salon effluent. Abnormal chromosome orientation and movement such as multi-polar anaphase and bi-nucleus were observed in 12.5%, 25% and 50% concentrations. Number of dividing cells was highest, and decreased significantly ($p < 0$) as concentration increased (Figures 2a – f).

IV. DISCUSSION

Some of the physicochemical parameters of the hairdressing salon effluent exceeded the effluent limitation guideline for industries in Nigeria (Lead, Mercury, Cadmium, Selenium, Nickel, Zinc and Arsenic). This is synonymous with the findings of Wenniger *et al*. (1993). Okereke *et al*. (2015) documented related findings, too. WHO (2018) noted that lead is among the 10 chemicals of major public health concern, needing action by member States to protect the health of workers, children and women of reproductive age. Young children are particularly vulnerable to the toxic effects of lead and can suffer profound and permanent adverse health effects, especially in the development of the brain and nervous system. Lead also causes long-term harm in adults, including increased risk of high blood pressure and kidney damage.

The level of mercury observed in this study may have contributed to the genotoxic effect on *Allium cepa*. Generally, World Health Organization has documented toxic effects ofexposure to mercuryon the nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes. Mercury is harmful to the developmental stage of a child and early life stages (WHO, 2018). Such exposure mainly occurs when people consume fish and shellfish that are contaminated with methylmercury and through workers inhalation of elemental mercury vapours during industrial processes. Cadmium mainly affects the kidneys, livers and bones. It is also a carcinogen by inhalation. In the environment, cadmium is toxic to plants, animals and microorganisms (Adedapo &

Adeoye, 2014). Due to lack of regulations, cadmium now enters the water through disposal of effluent from households or industries. This could be the possible reason for the distortion of the plant genes. Selenium exposure takes place either by uptake through the food chain or water, or when humans come in contact with soil or air that contains high concentrations of selenium (Lenntech, 2018). Selenium from hazardous waste-sites and from farmland will end up in groundwater or surface water through irrigation (Lenntech, 2018). Over exposure of selenium fumes may produce accumulation of fluid in the lungs, bronchitis, pneumonitis, fever, headache, shortness of breath, conjunctivitis, vomiting, diarrhoea, enlarged liver, etc. Nickel accumulates in soils and sediments and may possibly have an adverse effect on water quality (Gary & Stephen, 2008). However, at vegetative stages, high concentration of Nickel retard shoot and root growth, affect branching development, deform various plant parts, produce abnormal flower shape, decrease biomass production, induce leaf spotting and disturb mitotic root tips (Nagajyoti *et al*., 2010). This could be the possible reason mitosis was completely inhibited by the hairdressing salon effluent.

High concentrations of zinc can be toxic to vegetables and other food crops by inhibiting the developmental processes. If these plants are eaten by man, it can cause stomach upset and interfere with the absorption of other minerals. Zinc toxicity includes bloody enteritis, diarrhoea, paralysis of extremities, lowered leukocyte count and depression of the Central Nervous System (Kathleen, 2017).

Arsenic compounds cause short-term and long-term effects in individual plants and animals and in populations and communities of organisms. These effects are evident in aquatic species of concentrations ranging from a few micrograms to milligrams/per litre. The effects include death, inhibition of growth (inhibition of onion cells by the hairdressing salon effluent) photosynthesis and reproduction, and behavioural effects (GreenFacts, 2018).

Amin (2002) and Nielsen &Rank(1994) noted that root shape and growth, frequencies of mitosis and abnormal cell division can be used to estimate the cytotoxicity and genotoxicity of environmental pollutant. Since, mitotic index shows the proliferation status of a cell, thus, the decrease of the mitotic index with the increase in the concentration of the hairdressing salon effluent indicates that high concentration of hairdressing salon effluents inhibits cell proliferation. And, since mitosis can lead to growth, reduction of its activities by the hairdressing salon effluent indicates that it inhibits plant growth.

Furthermore, it was observed that the hairdressing salon effluent induced chromosomal aberration through interactions with DNA and proteins, leading to chromosome stickiness, mitotic disturbances and cell damage which is in agreement with the findings of Paul *et al*. (2013).The occurrence of several types of chromosomal abnormalities, such as stickiness, laggards, bridges, multipolarity and fragmentation of *Allium cepa* root tip cells, clearly shows that the accumulative effect of hairdressing salon effluent resulted to inactivation of spindle formation, deformation of non-histone chromosomal proteins, and mutation of the structural genes. In this case, the usual idea that aberrations increase with increasing concentration was not followed as higher concentrations such as 100% and 50% showed smaller amount of aberrations compared to lower concentrations showing much higher amount of aberrations. These findings contradicts that of Qian (2004) which reported that aberrant rate is directly proportional to increase in concentrations, but agrees with Odeigah *et al*. (1997), which revealed that with increased concentration and consequent increase in toxicity, there was an inhibitory effect on cell division. This might occur in pre-prophase, where cells are prevented from entering prophase or there may be prophase arrest where cells enter into mitosis but are arrested during prophase, resulting in a high frequency of prophase cells. It is suggested that prophase-arrest is the most likely explanation, as it could also explain the decline in chromosome aberrations, without any parallel decline in the mitotic index values (Odeigah *et al*., 1997).

V. Conclusion

The genotoxic effects of the hairdressing salon effluent reported indicates that the effluent contain toxic substances which may constitute risks to the environment and human health, more especially since it is unregulated. This was evident on the results obtained, as *Allium cepa* root growth was retarded when compared with the control. The root malformations recorded at high concentrations were twists, crotchet hooks (root tips bent upwards resembling hooks) and C-tumors (abnormalities appearing as swellings of the root tips. Adequate measures such as the use of less toxic materials in the manufacturing of cosmetic products used in hairdressing salons, is hereby advocated. Furthermore, wastewater from hairdressing salons should be given some treatment before discharge so as to reduce the its adverse environmental impacts.

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