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Cytogenotoxicity Assessment of Asphalt Plant Discharge Water using Plant Assay

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Abstract

The Allium cepa assay test was used to evaluate cytogenotoxic effects of asphalt plant discharge water at the concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% (v/v) asphalt plant discharge water/distilled water. The distilled water served as the negative control. The analysis of the physicochemical properties and heavy metals concentrations showed that most parameters were higher than the established standards of WHO and FMENV. The concentrations of polycyclic aromatic hydrocarbon detectd in the discharge water were also high. The onion bulbs were grown in the asphalt plant discharge water and distilled water for 72 hours and the roots were properly cut, processed for cytotoxic and genotoxic analysis using the aceto-orcein squashed technique. The results showed that the effects of asphalt plant discharge water on the Allium cepa root tip cells were concentrations dependent. An analysis of variance (ANOVA) revealed that there were significant reduction (P<0.05) in the mitotic index and chromosome aberrations of Allium cepa root cells exposed to the discharge water than the control. Asphalt plant discharge water induced chromosome aberrations such as sticky chromosome, chromosome lag, binucleated cells, bridge chromosome and disproportionate nucleus. Sticky chromomes being the most common aberration detected is an indication that the asphalt plant discharge water contain toxic compounds that can cause pollution in the environment and may directly or indirectly harm living organisms. The results in this study calls for proper treatment of asphalt plant discharge water before emission into the environment.

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INTRODUCTION

Industrial waste discharges have become one of the greatest challenges facing mankind in developing and developed countries [1]. Currently, there is a rising concern on the resultant effects of pollution on plants, animals, environment and humans [2]. Industrializations have contributed majorly to these menace that have bewildered human race [3].

Although there had been many researches focusing on indiscriminate discharges of untreated waste substances and the toxic effects on the environment [4]. However, little information exist on the toxicity effects of asphalt plant discharges [5, 6]. Asphalt plant industries make use of bitumen as one of the most important components of the asphalt mixture obtained from a fractional distillation of crude oil [7]. The heating of bitumen at high temperature during asphalt production releases inorganic gases, volatile organic compounds, polycyclic aromatic hydrocarbons, heavy metals, nitrogen oxide, sulfur, carbon monoxide, particulate matters into the environment [8, 9]. These pollutants from asphalt plant have direct or indirect effects on the environment and human health [2]. In 1987, International Agency for Research on Cancer (IARC) listed bitumen as a potential source of carcinogen [10]. More than hundred polycyclic aromatic hydrocarbons have been identify in nature and sixteen of them have been classified as likely sources of contaminants, carcinogenic and mutagenic according to US Environment Protection Agency [11].

Genetic mutation and the occurrence of chromosome aberration is a significant test in evaluating genotoxicity of pollutants. The resultant effects of genotoxicity include growth reduction and decrease in the number of dividing cells [12]. There are various ways of testing for toxicity to detect pollutants in the environment. One of the common methods is the Allium cepa Assay Test [13].

The Allium cepa Assay Test enables the evaluation of potential mutagenic or carcinogenic environmental pollutants that would be difficult from direct chemical measurements of pollutants in the field [1, 14]. This test is adequately sensitive to detect chemical compounds that can cause chromosome damage even at low concentration [1, 15]. The report of [12] documented that among the seven plant bioassays reviewed in 1980 by Environmental Protection Agency EPA Gene-Tox Programme, the Allium cepa root chromosome aberration assay become one of the protocols adopted by the International Programme on Plant Bioassay for monitory environmental pollutants, which is also in operation under the United Nation Environmental Programme [16, 12]. This test had been used by many researchers as bioindicator of environmental pollutions [17, 18]. Bioindicators of contaminants make it possible to detect low concentration of pollutants that are difficult to measure in the field because plants are good bioindicators [14, 19]. The usage of plant samples as bioindicators for genetic toxicity studies of environmental pollutants has been documented by several studies [14].

The aim of this study is to assess cytogenotoxicity effects of asphalt plant discharge using Allium cepa Assay Test.

MATERIALS AND METHODS

Collection of Asphalt Plant Discharge Water

Asphalt plant discharge waters (treatments) were collected in different asphalt plant locations from T_2 (Imo State Locations), T_3 (Abia Station Location) and T_4 (Rivers State Location) using the downward displacement method. The materials used in the collection of the asphalt plant discharge waters consist of hollow iron pipe. The top of the hollow pipe is connected to a funnel which was suspended in the chimney of the asphalt plant to collect discharges during emission while the bottom of the hollow pipe is suspended in 18 litres of water. The high speed through which the discharges are emitted passes through the hollow pipes and dissolved in the water which was collected and refrigerated for further use.

Laboratory Analysis

The Physicochemical analysis of the asphalt plant discharge water was carried out on a number of standard physicochemical parameters, namely: pH, conductivity, total dissolve solid, turbidity, hardness, sulphate, nitrate, phosphate, alkalinity, chemical oxygen demand, biochemical oxygen demand and heavy metals: chromium, lead, copper, zinc, iron, cadmium, nickel, mercury and arsenic using standardized analytical procedures [20, 21]. The asphalt plant discharge waters were also analyzed for the presence of sixteen (16) Polycyclic Aromatic Hydrocarbons (PAHs) commonly designated by Environmental Protection Agency as environmental pollutants using Gas Chromatography-Mass Spectrometry (TQ8080). The extraction of PAHs from asphalt plant discharge water was accomplished using liquid-liquid extraction method as described by [22, 23] before the commencement of the laboratory work.

Procurement of onions for Allium cepa Assay

Onion bulbs were purchased from Ekeonunwa Market Douglas Road, Owerri Imo State. They were sun- dried for two weeks so that those infected by fungi or showing signs of rottenness can be removed at the beginning of the experiment [24]. The outer dried layers were carefully remove without damaging the primordial of the root tissue. The concentrations of the test samples used were 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 % (v/v) asphalt plant discharge water/distilled water while 00: distilled water was used as the negative control. Each of the treatment and the negative control were replicated three times with the base of the onions suspended in the asphalt plant discharge water and distilled water in 100 ml beaker with 80 ml of the test samples at 26 ±0.5^{c}, which were kept in the dark for 72 hours.

For the assessment of chromosome aberration, root tips of onion bulbs were cut and fixed in ethanol:glacial acetic acid (3:1. v/v) inside a universal bottle and it was stored at 4^{o}C for 24 h before the observation of chromosome aberration [13]. The fixed roots were then placed in the petri- dish and hydrolyze with 1N HCL and heated in an oven at 60^{oC} for 5 minutes in order to soften the cell wall [25]. They were washed with distilled water three times and stored. The root tips were transferred to a glass slide and a small portion of each root tip (1–2 mm) were cut using surgical blade and dipped in a drop of 2 % aceto-orcein for 2 minutes. The root tip were squashed on a slide by adding another drop of aceto-orcein for at least 2 minutes and the cover carefully placed over the slide in order to avoid the entry of air bubbles. Also, the slides containing the root tips was pressed with the thumb and excess stain was removed from the edges of the cover slips with blotting paper. The edges of the cover slips cover was sealed with clear nail varnish as suggested by [26], to prevent drying out of the preparation by the heat of the microscope. Four slides were prepared for each sample and they were examined at 1000 X magnification using Nikon Eclipse (E400) light microscope. Photomicrographs was taken from the attached digital camera. Each slide was examine for chromosome aberrations.

The mitotic index was calculated according to the standard method describe by [27].

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

RESULTS

The results of analysis of the physicochemical properties of asphalt plant discharge water is presented in Table 1.

Physicochemical Properties

From the results Table 1, T_3 treatment had the highest pH value of 5.94 and T_4 treatment had the lowest mean value of 5.94. The pH indicates that the various treatments (T_2, T_3 and T_4) discharge waters were acidic and above WHO and FMENV permissibe limit. Meanwhile, the conductivity of the asphalt plant discharge waters were in the range of 260 – 278 µsm/cm. They were within the acceptable limit of 400 µsm/cm stipulated by FMENV. The mean concentrations of total dissolve solids of T_2 (260.0 ± 42.12 mg/L), T_3 (262.0 ± 44.17 mg/L) and T_4 (248.0 ± 37.23 mg/L) were within the limit of FMENV of 500 m/L but higher than the WHO standard of 250 mg/L. The mean concentrations of turbidity, sulphate, phosphate, biological oxygen demand and chemical oxygen demand detected in the asphalt plant discharge water were above the acceptable limits of WHO and FMENV standards. Also, the mean value of hardness and alkalinity were high. The amount of cadmium, mercury, nickel in T_2, T_4 and lead in T_4 of the asphalt plant discharge waters were higher than the acceptable limits of WHO and FMENV. Meanwhile, zinc, iron, arsenic, copper and chromium did not exceed the recommended limit of WHO and FMENV.

Polycyclic Aromatic Hydrocarbon Concentrations Detected in the Asphalt Plant Discharge Water

The results of the present study as shown in Table 2 also revealed the concentrations of polycyclic aromatic hydrocarbons in the asphalt plant discharge waters.

From the results, T_4 treatment had the highest PAHs content of 3608.25 mg/L followed by T_3 treatment With a total value of 1,722.mg/L while T_2 treatment was the least with a mean concentration of 971.13 mg/L. It was observed that Naphthalene was not detected in T_3 and T_4 treatments. Similarly, Acenaphthene was not detected in any of the treatment. Benzo(b)fluoranthene, Benzo(k) fluoranthene, Benzo(a)pyrene and Dibenzo(a,h) anthracene were absent in T_2 treatment. Ben(g,h, i) perylene and Indeno 1,2,3-c,d pyrene were not detected in T_2 and T_3 treatments. The results also showed that the asphalt plant discharge waters were dominated with High Molecular Weight Polycyclic Aromatic Hydrocarbons with a total concentrations of 5099.88 mg/L while Low Molecular Weight Polycyclic Aromatic Hydrocarbons had a total mean value of 1203.503 mg/L. High Molecular Weight Polycyclic Aromatic Hydrocarbons are very toxic and can be very persistent in the environment.

The Effects of Asphalt Plant Discharge Water on Mitotic Index and Chromosome Aberration of Allium cepa

The results of the effects of asphalt plant discharge water on cell division and chromosome aberration of Allium cepa roots exposed to the discharge water at different concentrations are summarize in Table 3 and 4.

The number of dividing cells decreased as the concentration increases indicating that the asphalt plant discharge water had effect on the onion root cells. The mean reduction in the number of dividing cells is concentrations dependent. The total number of dividing cells in the negative control were significantly difference (P<0.05) than those grown in the discharge water at different concentrations. There were growths between 00–60 % (v/v) concentrations. However, there was no growth (barren cells) observed in the Allium cepa root cells exposed to asphalt plant discharge water with a

Table1. Physicochemical parameters of asphalt plant discharge water collected from different locations.

Parameters	Irete (imo state) T_2	Umuahia (abia state) T_3	Elele (rivers state) T_4	FMENV	WHO
pH	5.78 ± 0.37^b	5.94 ± 0.39^b	5.49 ± 0.28^b	6–9	6.5–8.5
Conductivity (µsm/cm)	179.26 ± 31.42^a	189.45 ± 39.11^a	170.49 ± 30.23^a	400	250
Total dissolve solid (mg/L)	260.0 ± 42.12^a	262.0 ± 44.17^a	278.0 ± 52.22^a	500	250
Total solids(mg/L)	229.0 ± 31.32^c	267.0 ± 42.43^a	248.0 ± 37.23^b	-	-
Turbidity (NTU)	$171.5 \pm 37 .44^c$	169.22 ± 49.59^b	181.5 ± 41.88^a	5	5
Hardness (mg/L)	90.0 ± 25.11^b	70.0 ± 17.16^b	132.0 ± 32.71^a	-	-
Sulphate (mg/L)	93.103 ± 28.37^a	95.402 ± 29.21^a	71.647 ± 24.80^b	50	-
Nitrate (mg/L)	9.157 ± 1.12^a	7.972 ± 1.09^a	7.409 ± 1.02^a	-	50
Phosphate (mg/L)	10.996 ± 0.55^a	10.829 ± 0.41^a	9.73 ± 0.29^a	5	-
Alkalinity (mg/L)	92.5 ± 22.31^b	50.0 ± 18.33^c	150.0 ± 48.71^a	-	-
COD (mg/L)	149.33 ± 31.63^c	101.33 ± 25.32^c	128.0 ± 37.39^c	50	40
BOD (mg/L)	121 ± 22.01^b	132 ± 29.43^a	126 ± 24.51^b	10	10
Chromium (mg/L)	0.020 ± 0.04^b	0.0425 ± 0.08^a	0.016 ± 0.02^b	0.05	0.05
Lead (mg/L)	0.0192 ± 0.06^b	0.0114 ± 0.03^c	0.0520 ± 0.09^a	0.050	0.001
Copper (mg/L)	0.2427 ± 0.09^a	0.0458 ± 0.02^a	0.2443 ± 0.07^a	1.00	2.0
Zinc (mg/L)	1.988 ± 0.42^b	1.478 ± 0.28^c	1.378 ± 0.23^d	5.00	1.0
Iron (mg/L)	0.2758 ± 0.08^b	0.0488 ± 0.01^c	0.00 ± 0.00^d	0.300	-
Cadmium (mg/L)	0.087 ± 0.05^a	0.076 ± 0.04^b	0.099 ± 0.06^a	0.005	0.005
Nickel (mg/L)	0.1323 ± 0.07^b	0.0371 ± 0.05^c	0.1885 ± 0.09^a	0.070	-
Mercury (mg/L)	0.092 ± 0.09^a	0.087 ± 0.08^a	0.089 ± 0.07^a	0.001	-
Arsenic (mg/L)	0.026 ± 0.04^a	0.016 ± 0.02^b	0.028 ± 0.06^a	0.050	-

**Mean ± standard error along the row having different superscript of alphabets differ significantly at (P <0.05) level, mg/L = Milligram per litre; µsm/cm = Microsiemen per centimeter; NTU = Nephelometric turbidity unit; COD = Chemical oxygen* *demand; BOD = Biological oxygen demand; FMENV = Federal Ministry of Environment; WHO = World Health Organisation*.

Table 2. Concentration of polycyclic aromatic hydrocarbons (PAHs) in asphalt plant discharges water from different locations.

Parameters(mg/l)	Number of rings	PAHs	Irete (imo state) T_2	Umuahia (Abia State) T_3	Elele (rivers state) T_4	Total
Naphthalene	2	Nap	$1.493±0.5^b$	ND	ND	1.493
Acenaphthene	3	Ace	ND	ND	ND	ND
Fluorene	3	Flr	$99.89±53.79^b$	ND	$261.25±87.09^a$	361.14
Phenanthrene	3	Phe	$107.55±0.03^b$	$40.72±0.05^a$	$173.93±0.05^c$	322.2
Anthracene	3	Ant	$128.21±0.5^b$	$10.43±0.2^a$	$136.68±0.6^c$	275.32
Acenaphthelene	3	Acy	$120.86±1.63^b$	$39.74±39.74^a$	$82.75±41.37^a$	243.35
Fluoranthene	4	Flr	$442.73±0.20^b$	23.91±0.15a	$35.10±0.49^c$	501.74
Pyrene	4	Pyr	$2.57±0.2^a$	$10.87±0.3^b$	$13.65±0.5^c$	27.09
Benzo(a)anthracene	4	B(a)A	$34.66±0.5^b$	$23.39±0.5^a$	$86.55±0.25^c$	144.6
Chrysene	4	Chy	$34.66±0.10^b$	$11.71±0.4^a$	$11.57±0.03^a$	57.94
Benzo(b)fluoranthene	5	B(b) Flt	ND	$39.27±0.3^a$	$99.10±.0.11^b$	138.37
Benzo(k) fluoranthene	5	B(k) Flt	ND	$89.45±0.06^a$	$210.35±0.15^b$	299.8
Benzo(a)pyrene	5	B (a) pyr	ND	$1407.75±0.53^b$	$837.25±0.33^a$	2245
Dibenzo(a,h) anthracene	5	Db (a,h) A	ND	$25.27±0.11^a$	$807.92±0.58^b$	833.19
Ben(g,h, i) perylene	6	B (g,h,i) p	ND	ND	$622.35±0.15^a$	622.35
Indeno 1,2,3-c,d pyrene	6	Indo 1,2,3	ND	ND	$229.80±0.13^a$	229.8
Total LMWPAHs			456.51	90.89	654.61	1203.503
Total HMWPAHs			514.62	1631.62	2953.64	5099.88

**Mean ± standard error along the row having different superscript of alphabets differ significantly at (P <0.05) level., LMWPAHs = Low Molecular Weight Polycyclic Aromatic Hydrocarbons; HMWPAHs = High Molecular Weight Polycyclic Aromatic Hydrocarbons; ND = Not detected*

Table 3. Mitotic index test.

Conc. v/v (%)	Mean number in the field	Mean number of dividing cells						MI = DC/NC
		P	PM	M	A	T	TOTAL DC	%
00	16±2.34	0.001	0.00	0.001	1.00	0.001	1.00±0.24	6.25
10	240±0.014	0.00	0.00	3	5	2	10 ±6.02	4.10
20	104±6.08	0.00	0.00	2	2	0.0	4.00±1.04	3.8
30	185±2.06	0.00	0.00	2	3	0.00	5.00 ±6.84	2.7
40	190±1.26	0.00	0.00	1	3	1	5.00±2.68	2.6
50	200±0.28	0.00	0.00	1	3	1	5.00±0.62	2.5
60	205±6.04	0.00	0.00	2	2	1	5.00±0.12	2.4
70	180±1.18	00	00	00	00	00	00	00
80	150±0.22	00	00	00	00	00	00	00
90	52±1.04	00	00	00	00	00	00	00
100	00	00	00	00	00	00	00	00

**P = Prophase., M = Metaphase., A = Anaphase., T = Telophase., MI = Mitotic Index., DC = Dividing cells., NC = Number of cells*

concentrations between 70–100% (v/v) Table 4. Also, the different concentrations of the asphalt plant discharge water induced various chromosome aberrations in the *Allium cepa* root cells as shown in the various plates Table 4. The effects of the asphalt plant discharge water had cause damage to the cells such as lag chromosome, chromosome bridge, sticky chromosome, disproportionate nucleus and binucleate cells. These aberrations were absent in the control. These damages can be attributed to the presence of genotoxic chemicals in the asphalt plant discharge water.

Table 4. Mitotic aberration test.

Conc. v/v (%)	Number of cell (NC)	Laggard	Bridge	Sticky	Binucleate	Disproportionate nucleus	Plate
00.00	1000	00	00	00	00	00	DI
10.00	1000	-	-	+	-	-	A9
20.00	1000	-	-	+	-	-	A4
30.00	1000	-	-	-	+	-	A7
40.00	1000	-	-	-	+	-	A5
50.00	1000	-	+	-	+	+	B3 B2 B4
60.00	1000	+	-	+	-	-	C2 C7
70.00	1000	Barren cells					B5
80.00	1000						B5
90.00	1000						B5
100.00	1000						B5

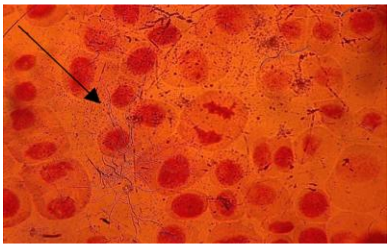
Plate D1: Normal cell (control)

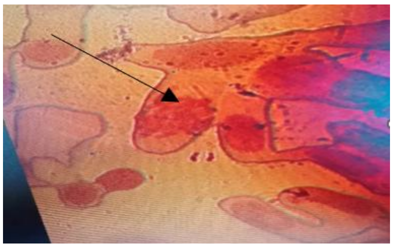
Plate A9: (10 %) Sticky Chromosome

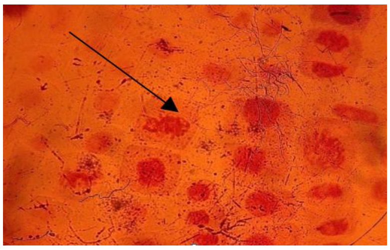
Plate A4: (20 %) Sticky Chromosome

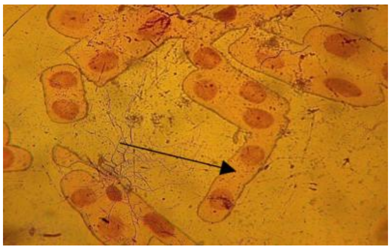
Plate A7: (30 %) Binucleate cells

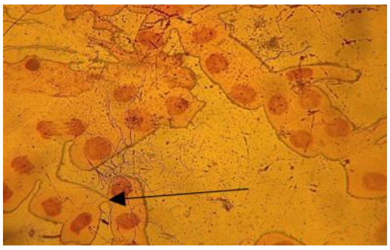
Plate A5: (40 %) Binucleate cells

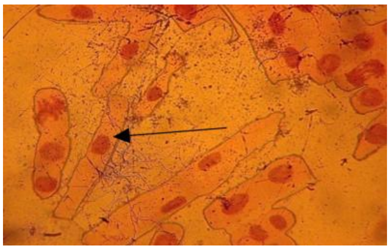
Plate B2: (50 %) Binucleate cells

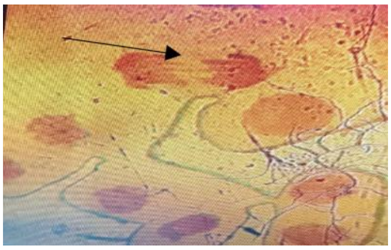
Plate B3: (50 %) Chromosome bridge

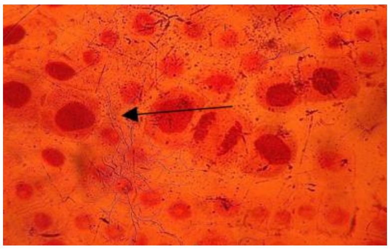
Plate A4: (50%) Disproportionate nucleus

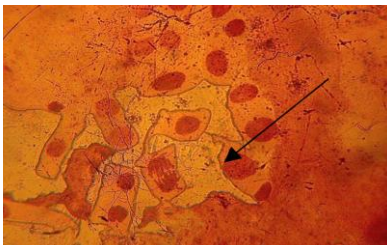
Plate C2: (60 %) Lagard Chromosome

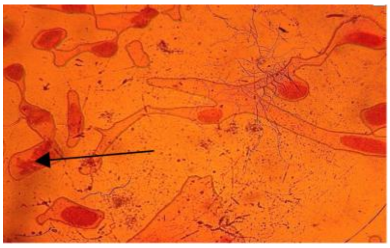
Plate C7: (60 %) Sticky chromosome

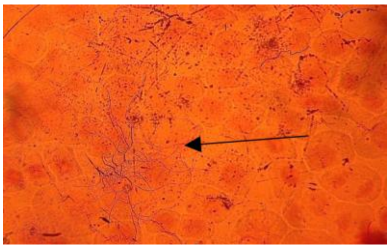
Plate B5: (70 – 100 %) Barren cells

**Plates: Chromosomes aberrations observed in onion root cells exposed to different concentrations of asphalt plant discharge waters.*

Figure 1. Chromosomes aberrations observed in onion root cells exposed to different concentrations of asphalt plant discharge waters.

DISCUSSION

The results obtained in this investigation revealed that the asphalt plant discharge waters contain harmfull chemicals at different concentrations. The Allium cepa test was used as a biomarker for cytogenetics screening of the discharge water for its environmental pollutants. This method had been accepted by USEPA and WHO to collate data related to environmental pollutants that are mutagenic and carcinogenic [28].

The Physicochemical Parameters of the Asphalt Plant Discharge Water

The results of the physicochemical properties are presented in Table 1. The asphalt plant discharge waters were acidic and most other parameters were above the acceptable limit of WHO and FMENV [29, 30]. These discharge waters may not be very suitable for emmision into the environment without proper treatment as plant roots can absorbed toxins from the soil that be may be translocated to the aerial parts of the plants where they may cause cytogenotoxicity [31]. These alterations of the physicochemical parameters of discharge waters may have contributed to the mean reduction of mitotic index and chromosome aberration observed in this study.

The obtained results showed that heavy metals such as Lead, Cadmium, Mercury and Nickel were above the recommended standards of WHO and FMENV permissible limit. These trace elements are known to be very toxic at low concentration [14]. Heavy metals such as Lead, Chromium, Copper and Zinc had been observed to decrease mitotic index and caused chromosome aberration [32]. A study conducted by [47] on borewater supply in tertiary institution in Benin City attributed the presence of heavy metals such as Maganese, Zinc, Chromium, Iron and other inorganic pollutants to cause decrease in mitotic index, chromosome abnormalities and root growth inhibition. The primary cause of decrease in the mitotic index is the action of heavy metals preventing the cells of *Allium cepa* to enter cell division. The presence of heavy metals in the discharge water can disturb the processes controlling the arrangement of microtubule and tubulin. Heavy metals can also induce inhibition of DNA repair mechanisms through competition with some ions that are importants for DNA polymerase. These toxic effects lead to reduction in the mitotic index and chromosome aberrations. Heavy metals can induce different kinds of chromosome aberrations such as stickiness, C-mitosis, anaphase bridge, vagrant chromosome as observed in this study [32–34].

Effects of Polycyclic Aromatic Hydrocarbon (PAHs) Concentrations Detected in the Asphalt Plant Discharge Water

The results showed that the asphalt plant discharge water consist of different compounds of PAHs in varous concentrations. The concentrations of the High Molecular Weight PAHs are higher than low Molecular Weight PAHs. Generally, polycyclic aromatic hydrocarbon are toxics especially the High Molecular Weight PAHs. The presence of these PAHs may have acted singly or in combinations to interfere with normal mitotic processes. The reason for the reduction in mitotic index may be as a result of blockage of G2 phases of the cell cycle, interference with DNA synthesis and blockage of glucose metabolism [35, 36]. Reduction in mitotic index can also result from failed segregations of the chromosomes which suggest cytotoxic and genotoxic effects of the discharge water [37]. PAHs have the ability to induce chromosome aberrations which were also observed in the *allium cepa* test in this study. The laggard chromosome, chromosome bridge, binucleate cells, sticky chromosome and disproportionate nucleus are reflection of pollutants in the asphalt plant discharge water [36, 38].

Effects of Asphalt Plant Discharge Water on Mitotic Index and Chromosome Aberration of Allium Cepa Root Cells

The results revealed that majority of the physicochemical parameters and the heavy metal concentrations detected in the asphalt plant discharge water were above the limit of WHO and FMENV recommendations as given in Table 1. The value of PAHs determined in the asphalt plant discharge water were also high as shown in Table 2, indicating that the asphalt plant discharge water may not have been treated before discharge into the environment. Industrial waste waters are often sources of pollutants which usually interferes with the normal functioning of mitotic cell division. This observation agree with the earlier report of [31, 12, 38].

The cytogenotoxicity effects of the asphalt plant discharge water are shown in Table 3 and 4. It was observed that the asphalt plant discharge water resulted to decrease in mitotic index of the root cells of the Allium cepa except the negative control. Inhibition of mitotic index is used for predicting the presence of cytotoxic substances [38]. The effects of the treatments were concentrations dependent formulated in various concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% (v/v) and 00% is negative control. The total number of dividing cells in the control was significantly higher at ($P<0.05$) than those grown in the asphalt plant discharge water at different concentrations. Meanwhile, there was no evidence of cell division in the concentrations between 70 – 100% (v/v). The number of dividing cells decreased as the concentration increases indicating that the asphalt plant discharge water had effect on the root cells. These observations agree with the results of [31, 39, 12]. However, the results were at variance with [37] who observed a higher number of dividing cells. This may be due to the difference in concentrations of the effluent discharge. These damage can be attributed to the presence of genotoxic chemicals in the asphalt plant discharge water [10].

The asphalt plant discharge water induced chromosomal aberration in the root cells of Allium cepa except the negative control. The aberrations observed were laggard,bridge, sticky chromosomes, disproportionate nucleus and binucleate cells (See Plates). The observation corroborates with that of [40] who exposed Allium cepa roots to tobacco industrial discharge. These chromosome aberrations may have effects on the growth and wellbeing of the organisms [41]. Generally, asphalt plant discharge water induced more sticky chromosomes than other forms of chromosome aberrations. Bridge and laggard chromosome had the lowest frequency. These findings are similar to the report of [42]. The effects of Sticky chromosomes is irreversible and a reflection that the asphalt plant discharge water contain high toxic compounds which may have poisoned the chromosome and may cause the death of the cells. There was no growth observed between 70–100 % concentrations. The results are in agreement with previous studies of [43, 44, 37]. Binucleation is a nuclear division without cytoplasmic division which can lead to doubling haploid genome to diploid cells. Binucleate chromosome observed in this study may be due to malfunction of the cytokinensis which implied that asphalt plant discharge water can interfere with the process of cytokinensis [45]. The observed chromosome bridges in the treatments may be as a result of breakage and fusion of the chromosome, chromosome stickiness and unsuccessful separation of chromosome at anaphase cell division. According to the observation of [46], lagging chromosomes can be associated with the failure of chromosome movement. The observation of chromosome bridges and laggards in this study is an indication that the asphalt plant discharge water has clastogenic effects on the gene constituents of Allium cepa roots [17]

CONCLUSION

The use of Allium cepa test provide fast and convenient method in understanding potential effects of environmental pollutants and predicting the likely effects on humans. The reduction in mitotic index and observed chromosome aberration can be attributed to environmental pollutants present in the asphalt plant discharge water. Hence, it is necessary for industries to treat their industrial waste waters before discharge into the environment.

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