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Investigation of Cytotoxicity and Mutagenicity of Cement Dust Using *Allium cepa* Test

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ABSTRACT

In this study, the mutagenesis of cement dust on plants and animals was investigated. The cytotoxicity and mutagenicity of cement dust was monitored using *Allium cepa* test model. *Allium cepa* grouped into 4 of 10 *A. cepa* per group, after taking their baselines, were exposed to cement dust over three different periods of time at about 100 m from a cement factory. The control group (group 1) was kept in an environment free of cement dust pollution, about 6 km from the cement factory. The test groups (groups 2-4) were exposed to the dust for 2 weeks, 4 weeks and 6 weeks, respectively. The elemental analysis of the *A. cepa* in the test groups revealed significant ($p < 0.05$) levels of calcium, silicon, aluminum, chromium and lead compared to the control group. Also, significant differences ($p < 0.05$) exist among the levels of the elements detected in the *A. cepa* in various test groups. Furthermore, the mean root length growths and the relative growth rates of the test groups were higher than the control, but there was no statistical difference ($p > 0.05$) among them. However, there was a direct linear relationship between the concentrations of calcium and root length growths of the *A. cepa* across the groups. Chromosomal aberrations observed in the test groups are stickiness, c-mitosis, chromosomal bridge, chromosome fragmentation, vagrant chromosomes, bi-nucleus chromosomes and multi-polar anaphase. No chromosomal aberration was observed in the control group. The total number of chromosomal aberrations increases significantly ($p < 0.05$) with the length of exposure. The findings of the research highlight the toxicity of cement dust and the need for pollution control measures to safeguard plants and animals in the environment.

Key words: Element, chromosome, root length, *A. cepa*, aberration, vagrant, stickiness

INTRODUCTION

Air pollution is the release of chemicals, particulate matter, or biological materials into the atmosphere through human activities, causing harm to his health, other living organisms and the environment (Seyyednjad *et al.*, 2011). Pollution stress can alter plant and animal growth as well as quality and the effects are often extensive (Krupa *et al.*, 1982). Furthermore, air pollution can have both short-term and long-term effects and physical injuries to the leaves of plants and skin of animals are the immediate effects of air pollution (Colls, 2002). Of all the main poisonous gases in polluted air, sulfur dioxide appears to be the most toxic to plants and animals and has been implicated in some diseases. Other pollutants that may adversely affect plants and animals are nitrogen dioxide, ammonia, carbon monoxide and troposphere ozone (TRF, 2008). The

calcinations and burning processes of cement production produce these poisonous gases that cause injuries to plants and animals (Abimbola *et al.*, 2007; Gbadebo and Bankole, 2007).

The cement industry is involved in the development of structures in this advanced and modern world because it is the basic ingredient of concrete use in constructing modern edifices and structures. In fact, life without cement in this 21st century is inconceivable. Cement, however, generates dust during its production (Meo, 2004). Cement is a fine, gray or white powder which is largely made up of Cement Kiln Dust (CKD), a by-product of the final cement product, usually stored as wastes in open-pits and landfills (Hansen, 1998) Although, the basic constituents of cement dust are calcium (CaCO_3), silicon (SiO_2), aluminum (Al_2O_3), ferric and manganese oxides (Akpan *et al.*, 2011) its production produces known toxic, carcinogenic and mutagenic substances, such as particulate matters, sulfur dioxide, nitrogen dioxide, volatile compounds, long lived dioxins and heavy metals (Davidovits, 1994). Exposure to cement dust for a short period may not cause serious problem, however prolonged exposure can cause serious irreversible damage to plant and animal (Heather, 2003). Cement dust of sufficient quantities have been reported to dissolve leaf tissues and cause injury to both plants and animals (TRF, 2008). Other reported effects of cement dust on plants and animals include reduced plant and animal growths, reduced chlorophyll of plants, clogged stomata of plants leaves, cell metabolism disruption in plants and animals, respiratory diseases in animals, hematological disease, cancers, eye defects and genetic problems (Iqbal and Shafug, 2001; Meo, 2004; Mohammed and Sambo, 2008; Ogunbileje and Akinosun, 2011).

Several methods are being used to monitor mutagenesis of pollutants or chemical agents, but *Allium cepa* test has proved to be more effective. *Allium* test has been extremely useful in biological monitoring and determination of toxicity of chemical agents and pollution. Application of *Allium* test as a model to detect mutagens dates back to the 1940s and has been used to this day to assess a great number of chemical agents due to its effectiveness. It is characterized as a low cost test, easy to handle and give accurate and reliable results (Sehgal *et al.*, 2006).

Although, much work has been published on the health risks posed by cement dust on the survival of plants and animals, the cytotoxicity and mutagenicity of cement dust are still not clear. While many researchers confirm the cytotoxicity and mutagenicity of cement dust, cement manufacturers deny the claims. They argue that the individuals affected in several studies could have developed the diseases from previous environments they have lived in (Tajudeen *et al.*, 2011). The cement manufacturers argued further that most of the studies were based on spirometry, radiology or questionnaire and the studied organism, man, is mobile. Therefore, one of the objectives of this study was to clear the controversy surrounding the cytotoxicity and mutagenicity of cement dust using *A. cepa* test model. In the face of the advantages that *A. cepa* test model offers, there is no doubt that the results of the study will clear the controversy surrounding the cytotoxicity and mutagenicity of cement dust.

MATERIALS AND METHODS

Description of study site: The West African Portland Cement Company, Ewekoro is the oldest cement company in Nigeria. The company is along the ever-busy Lagos-Abeokuta motorway in South-West Nigeria, about 43 km from Lagos and 37 km from Abeokuta. There are human settlements around the company made up of mostly artisans, farmers, traders, children and factory workers.

Allium cepa Bulbs: Healthy purple variety of *Allium cepa* bulbs (25-32 g) were purchased from Sango-Ota market, Ogun state, Nigeria, in November, 2010. Eighty of the *Allium cepa* bulbs were grown in the dark for 48 h in beakers containing 100 mL of tap water at ambient temperature until the roots have grown to about 2-3 cm. The 40 viable bulbs were selected and used for the research.

Experimentation: The research commenced in mid November, 2010. The 40 viable *Allium* bulbs selected were divided into 4 groups of 10 *Allium* bulbs per group. The control group (group 1) was kept in a cement dust-free environment in the same climatic zone, about 6 km from the company. The test groups (groups 2-4) were exposed to cement dust at about 100 m from the cement factory for 2 weeks, 4 weeks and 6 weeks, respectively. At the end of the exposures, the *Allium cepa* across the groups were taken to the Environmental Biology Laboratory, University of Lagos. Elemental analysis of the *Allium* bulbs was done by Atomic Absorption Spectroscopy using UNICAM model 969 Spectrophotometer and cytotoxicity and mutagenicity of the elements in the *Allium* bulbs were determined using *Allium* test as described by Fiskesjo (1988).

Examination of chromosomal aberrations: The ten viable onions in each group were grown over tap water in beakers under ambient temperature and humidity. The root tip growths of the onions were monitored for seven days after which they were cut and fixed immediately in aceto-alcohol in ratio 1:3. About 4-5 cm root tips from each bulb were macerated in drops of 1 N HCl at 60°C for about 3 min followed by staining in Carbol Fuchsin stain (Koa, 1975). The root tips were then squashed in a 2% aceto-orcein in 45% acetic-acid. Permanent slides were made and mounted on Canadian balsam where chromosomal aberrations were examined and photographed. Mitotic index and chromosomal aberrations were determined by examination of 500 cells per slide and calculated as mitotic cells per 100 cells. Chromosomal aberrations were characterized and classified as bridges, c-mitoses, vagrant, fragment, stickiness, bi-nucleus and multi-polar.

Statistical analysis: A database file was created in a personal computer and all statistical analysis was carried out with the Statistical Package for Social Sciences (SPSS) version 17 for windows and Microsoft Office Excel 2007. Comparison of data among exposed and control groups were calculated using Student's t-test. The $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 showed that the concentrations of the elements detected in the test groups were significantly higher ($p < 0.05$) than the concentrations of the elements detected in the control group. Furthermore, significant difference ($p < 0.05$) exist among the concentrations of the elements detected in various test groups and the amount increased with the length of exposure. For example, the final mean concentration of calcium in the control (group 1) is 1.25 mg kg^{-1} , while the final mean concentrations of calcium in groups 2, 3 and 4 are 2.69, 3.16 and 6.14 mg kg^{-1} , respectively. Furthermore, the final mean concentration of silicon in the control group is 0.033 mg kg^{-1} , while the final mean concentrations of silicon in groups 2, 3 and 4 are 0.12, 0.14 and 0.17 mg kg^{-1} , respectively. Also, the final mean concentration of aluminum in the control group is 0.033 mg kg^{-1} , while the final mean concentrations of aluminum in groups 2, 3 and 4 are 0.063, 0.103 and 0.293 mg kg^{-1} , respectively. Moreover, the final mean concentration of chromium in the control group is 0.003 mg kg^{-1} , while the final mean concentrations of chromium in groups 2, 3 and 4 are 0.008, 0.012 and 0.021 mg kg^{-1} , respectively. Finally, the final mean concentration of lead in the

control group is 0.0004 mg kg⁻¹, while the final mean concentrations of chromium in groups 2, 3 and 4 are 0.008, 0.013 and 0.020 mg kg⁻¹, respectively.

Period of exposure: Group 1 (Un-exposed): Group 2 (2 weeks): Group 3 (4 weeks): Group 4 (6 weeks).

Table 2 showed that the test groups gained more root length than the control group. However, there was no statistical difference (p>0.05) between the root length growth and relative growth rate of the test and control groups. For instance, the minimum and maximum root lengths of the control group are 1.2 and 5.5 cm, respectively, while the minimum and maximum root lengths of groups 2, 3 and 4 are 0.8, 1.4, 0.8 cm and 6.2, 5.8, groups 2, 3 and 4 are 0.008, 0.013 and 0.020 mg kg⁻¹, respectively 5.7 cm, respectively. Furthermore, the relative growth rate of the control group is 44.3% while the relative growth rate of groups 2, 3 and 4 are 67.1, 58.6 and 58.6%, respectively.

Period of exposure of the chromosomal analysis of the *Allium cepa* across the groups:

Table 3 showed the results of the chromosomal analysis of the *Allium cepa* across the groups. The mitotic index of the test groups were higher than the control group but there was no statistical difference (p>0.05) between them. Furthermore, the control group showed no chromosomal aberration (Fig. 1a) while groups 2, 3 and 4 showed 14 (2.8%), 20 (4.0%) and 25 (5.0%) chromosomal aberrations, respectively. Chromosomal fragmentation, bridges and stickiness were found in groups 2, 3 and 4 (Fig. 1b, c). Moreover, vagrant, scattered and multi-polar chromosomes were found in groups 3 and 4 (Fig. 1d, e).

Table 1: Concentrations of the elements (mg kg⁻¹) in the *Allium cepa*

A. cepa group	Calcium			Silicon			Aluminum		
	Initial Conc.	Final Conc.	p-value	Initial Conc.	Final Conc.	p-value	Initial Conc.	Final Conc.	p-value
1	1.25±0.015 ^a	1.25±0.01 ^a	0.7678**	0.027±0.006 ^a	0.033±0.006 ^a	0.2314**	0.03±0.01 ^a	0.033±0.06 ^a	0.6433**
2	1.13±0.06 ^a	2.69±0.01 ^b	<0.0001*	0.020±0.01 ^a	0.12±0.01 ^b	0.0002*	0.03±0.006 ^a	0.063±0.015 ^b	0.033*
3	1.20±0.01 ^a	3.16±0.01 ^b	<0.0001*	0.013±0.006 ^a	0.14±0.01 ^b	<0.0001*	0.02±0.01 ^a	0.103±0.015 ^b	0.0018*
4	1.11±0.01 ^a	6.14±0.98 ^b	<0.0001*	0.013±0.006 ^a	0.17±0.01 ^b	<0.0001*	0.02±0.01 ^a	0.293±0.015 ^b	<0.0001*

A. cepa group	Chromium			Lead		
	Initial Conc.	Final Conc.	p-value	Initial Conc.	Final Conc.	p-value
1	0.003±0.001 ^a	0.003±0.0006 ^a	1.000**	0.0003±0.0001 ^a	0.0004±0.0001 ^a	0.4917**
2	0.0013±0.00006 ^a	0.008±0.0006 ^b	<0.0001*	0.0002±0.0001 ^a	0.008±0.001 ^b	0.0002*
3	0.00013±0.00006 ^a	0.012±0.0001 ^b	0.0075*	0.0002±0.0001 ^a	0.013±0.001 ^b	0.0029*
4	0.0002±0.0001 ^a	0.021±0.0015 ^b	<0.0001*	0.0002±0.0001 ^a	0.020±0.003 ^b	<0.0001*

Data are expressed as Mean±SD: When *p<0.05 = Significant from control and when **p>0.05 = Not significant from control: Mean values in the same row with different superscripts are significantly different at p<0.05

Table 2: Root length growth [CM] of the exposed *A. cepa*

A. cepa groups	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Min. length	Max length	RGR	p-value
1 [control]	1.60±0.4 ^a	2.43±0.4 ^a	3.60±1.1 ^a	4.27±1.0 ^a	4.43±1.0 ^a	4.57±0.9 ^a	4.70±0.8 ^a	1.2	5.5	44.3	
2	1.23±0.4 ^a	2.43±0.4 ^b	3.71±0.9 ^b	4.50±0.5 ^b	5.32±0.3 ^b	5.63±0.3 ^b	5.92±0.3 ^b	0.8	6.2	67.1	0.0720**
3	1.62±0.3 ^a	2.63±0.6 ^b	4.22±1.1 ^b	5.28±0.3 ^b	5.44±0.3 ^b	5.53±0.2 ^b	5.67±0.2 ^b	1.4	5.8	58.6	0.1105**
4	1.21±0.4 ^a	2.23±1.2 ^b	4.42±0.6 ^a	4.64±0.6 ^a	4.83±0.6 ^a	5.02±0.6 ^a	5.20±0.6 ^a	0.8	5.7	58.6	0.4123**

Data are expressed as Mean±SD: When *p<0.05 = Significant from control and when p>0.05 = Not significant from control: Mean values in the same row with different superscripts are significantly different at **p<0.05

Table 3: Chromosomal Analysis of the Exposed *Allium cepa*

<i>A. cepa</i> groups	No. of <i>A. cepa</i>	Total No. of cells screened	Total No. of dividing cells	Mitotic index	Chromosomal aberrations						Total No. of Multi-polar chromosomal aberrations
					Stickiness	C-Mitoses	Vagrant	Bridges fragment	Bi-nucleus	Anaphase	
Baseline	10	500	33 (P ₉ M ₇ A ₆ T ₁₁)	0.066	0	0	0	0	0	0	0 (0%)
Control	110	500	32 (P ₁₁ M ₇ A ₆ T ₉)	0.064	0	0	0	0	0	0	0 (0%)
2	10	500	45 (P ₆ M ₉ A ₁₂ T ₁₈)	0.09	3	1	2	3	3	2	14 (2.8%)
3	10	500	40 (P ₉ M ₁₃ A ₇ T ₁₁)	0.08	5	3	4	2	3	3	20 (4.0%)
4	10	500	36 (P ₁₀ M ₁₃ A ₄ T ₉)	0.072	7	5	1	3	4	5	25 (5.0%)

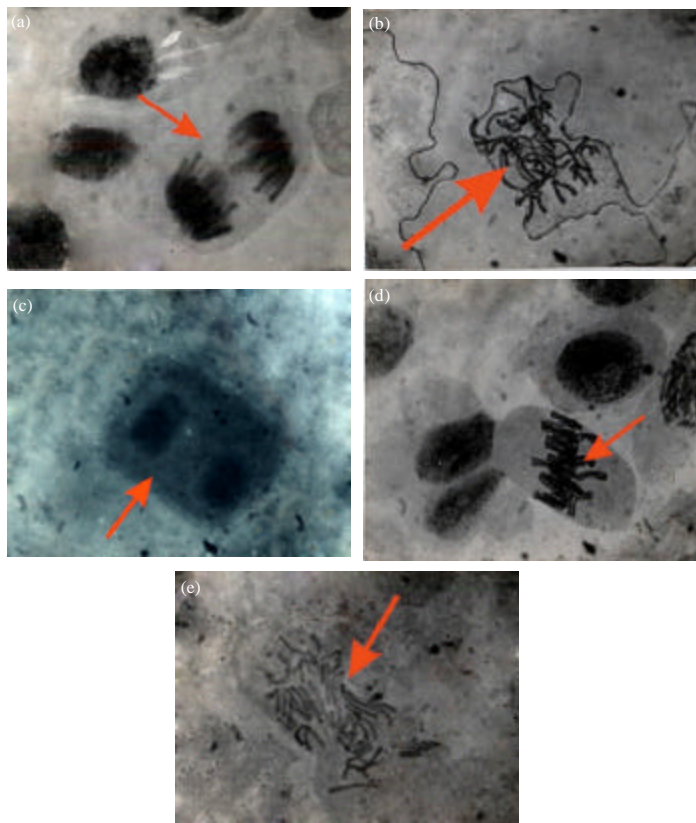


Fig. 1(a-e): (a) Control [anaphase], (b) Bridges and fragmentation observed in groups 2, 3 and 4, (c) chromosome stickiness observed in group 2, 3 and 4, (d) Vagrant chromosome in observed in groups 3 and 4, (e) Multi-polar and scattered chromosome observed in groups 3 and 4

DISCUSSION

The results of the elemental analysis of the exposed *Allium cepa* support the findings of Abimbola *et al.* (2007), Davidovits (1994), Gbadebo and Bankole (2007) and Ade-Ademilua and Obalola (2008). They reported that apart from the basic constituents of cement dust, the calcination

and burning process of cement making produce poisonous substances such as particulate matters, dioxin, heavy metals, sulfur dioxide, nitrogen dioxide and volatile compounds. But this research revealed high concentrations of calcium, silicon, aluminum, chromium and lead. The results further confirm the submission of Bilen (2010) that cement factories are one of the great polluters of the environment with the release of poisonous dust and gases. Considering the short period of exposure and the high levels of the elements detected in the exposed *A. cepa*, it showed that the cement company is badly polluting the environment. The high level of pollution from the cement company might be due to several factors. Firstly, the machines and technologies in place in the company might be old with non-efficient dust collectors and dust-filters. This assertion is more probable because the factory is the oldest in the country and no major turn-around maintenance has been done since its establishment in 1978. Secondly, the company might be burning hazardous waste substances as alternative fuels. This assertion has been corroborated by IPC (1996), who found that the levels of heavy metals and dioxins are higher in cement kilns burning hazardous wastes as fuels than those burning coal or gas alone. Thirdly, the high level of pollution might be due to accumulation of excavated limestone and leftover cement kiln dust which were not packed promptly.

Although many researchers, including Abdullah and Iqbal (1991), Akinola *et al.* (2008), Gupta and Mishra (1994), Iqbal and Shafug (2001), Nigragau and Davidson (1986) and Krupa *et al.* (1982) reported reduction in plant and animal growth from cement dust pollution, this research did not find such results. Contrary, the results of the research revealed that cement dust promotes plant and animal growth. Calcium, the main component of cement dust, is involved in the metabolism of plant and animal and serves as regulator of plant and animal growth and development (Hepler, 2005; Tajudeen and Okpuzor, 2011; Tajudeen *et al.*, 2011). Hence, the increase in the root length growth of the exposed *A. cepa* is not surprising. But aluminum, chromium and sulfur dioxide emitted by cement plants may also affect plant and animal growth negatively. Root apex seems to be the major target of aluminum toxicity where it inhibits cell division and cell extension (Mossor-Pietraszeweska, 2001), while sulfur dioxide and chromium disrupts metabolic activities (Shanker *et al.*, 2005; Zou *et al.*, 2006). So, these toxic metals and calcium work in antagonistic order. While calcium is building plant and animal cells and growth, aluminum, chromium and sulfur dioxide are slowing down the rate at which it does it. This explains variations that exist among the growth rates of the test groups. As the levels of these toxic elements increase with length of exposure, the growth rate decreases. However, the net root lengths gained by the test groups were higher than the control groups probably because of the preponderance of calcium against the toxic elements in the cement dust.

The chromosomal aberrations observed in the study confirms the findings of Calistus Jude *et al.* (2002) who reported that exposure to cement dust may increase the frequency of sister chromatid exchanges, decreased cell kinetics and significantly increased the frequency of chromosomal aberrations in men environmentally and occupationally exposed to cement dust. However, our results revealed high frequency of chromosome stickiness, c-mitosis, chromosomal bridges and fragmentation, multi-polar anaphase, bi-nucleus chromosome and vagrant chromosome. Chromium VI has been implicated to cause chromosomal bridge, chromosome stickiness, decrease in mitotic index, c-mitoses, aneuploid and sister chromatid exchange in plant and animals (IARC, 1990; Zou *et al.*, 2006). Aluminum has also been reported to cause chromosome stickiness, laggards, sticky bridge, occurrence of micronuclei, bi-nucleated and multi-nucleated cells (Balasubramanyam *et al.*, 2009; Mohanty *et al.*, 2004). Furthermore, silica has been fingered in

gene mutation, DNA strands break and bi-nucleus chromosome in exposed animals (NTP, 2009). Finally, lead has been reported to cause bi-nucleus chromosome and cytokinetic effects in exposed earthworms (Muangpha and Gooneratne, 2011).

CONCLUSION AND RECOMMENDATION

The results of this research had clearly shown that the environment surrounding the cement factory is highly polluted with poisonous gases and elements. The effects of these pollutants had shown in chromosomal aberrations in the exposed *Allium cepa*. Definitely, all other organisms including man in the cement polluted environment will be experiencing similar problems. Apart from the fact that we need to protect ourselves, the integrity and population of plants and animals around cement factories must also be protected. This is because man depends on plant and animal for survival and the chromosomal aberrations may be transferred to them. Therefore, environmental pollution from cement factories must be checked by using efficient dust collectors and dust-filters. Cement companies must put in place new machines and technologies and must ensure prompt packaging and transportation of both finished product and left-over cement kiln dust. The use of hazardous waste substances as fuels should be discouraged and there must be a policy on minimum distance from cement companies in which settlements and farming activities will be allowed. Finally, the use of medicinal plants as detoxifiers should be introduced to people living around industrial areas. These will go a long way in preserving the populations of plants and animals as well as health of humans in polluted environments.

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