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Evaluation of Genotoxic Effect of Photographic Effluent Using *Allium cepa* Assay

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Abstract

The increased need to keep graphic records of events has led to the use of photography in investigation and surveys and hence increased photographic activities. Toxicological survey of photographic effluents has received little attention in Nigeria, though the constituents have been shown to be very toxic. This study investigated the genotoxic effects of photographic effluents from selected locations in Lagos, Nigeria using *Allium cepa* assay, viability test, root length measurements and cytological studies. There was inhibition of root length development in addition to several chromosomal aberrations observed in the root of *Allium cepa* exposed to the effluents. Furthermore, there were statistical differences among the aberrations produced by the different concentrations of the effluent (p<0.05; p<0.01; p<0.001). It was inferred that photographic effluents have genotoxic and cytotoxic effects. Thus, there is need for biological detoxification of photographic effluents before disposal into the environment to remove or reduce the pollutant load in them.

Introduction

Industrial effluents have become one of the biggest problems in many developing and developed countries. It is known that these effluents, when not treated properly, can cause mutagenic or toxic effects directly or indirectly on humans, resulting in diseases such as cancer, congenital malformations, and cardiovascular diseases (Grover and Kaur, 1999). Although much work has been done on the toxicity of some effluents, little information exists on the toxicity of photographic effluents.

Printing, photographic film and associated industries' processing and activities generate photographic wastes such as spent fixers and bleaches (KODAK, 1996). Many photographic processes have been found to be hazardous and produce toxic gases which may be released slowly into the environment. In addition, these gases are usually generated at faster rates if the photo chemicals are heated or if certain chemicals are mixed with acid. The film-processing effluents are classified as hazardous waste since they may cause soil and water pollution, if not properly disposed of or treated (KODAK, 1996).

The typical constituents of the photo-processing wastewater streams are organic chemicals, chromium compounds, ferricyanide, silver, thiocyanate, ammonium compounds, sulfur compounds, phosphate and heavy metals (Bober et al., 2004). A wide variety of chemicals are used in black-and-white photographic processing. These include developers, stop baths and fixer, intensifiers and reducers, toners, hardeners, and hypo eliminators. For coloured processing, the chemicals include hydroquinone, ammonia, n-Butanol, Boron, 1,1,1-trichloroethane, methanol, 1,1,2,2-tetrachloroethane, 1,2-dichloropropane, silver and silver salts.

Silver is one of the most toxic metals regulated by regulatory bodies. The different health effects of silver 1,1,1-trichloroethane, an ozone-depleting chemical whose production has been highly regulated was

outlined by Drake and Hazelwood (2005). Hydroquinone is a very toxic chemical with long lasting carcinogenic effects on animals and humans (Enguita and Leitao, 2013). Dissolved borates in high concentration show some toxicity on plants. High vapour concentrations of n-butanol produce narcosis and death in animals (Stellman, 1998).

Some of the most predominantly used and controversial chemicals in photographic developers are metol, hydroquinone, EDTA, DTPA, and NTA. Metol has been known for quite some time to cause a dermatitis condition of the skin known as metol poisoning. Metol is also known to cause eye and respiratory tract irritation with unknown long term effects. In addition to the human health concerns, metol is extremely harmful to aquatic organisms (Andreozzi et al., 2000). There are different forms of toxicity testing to detect the hazards posed by toxicants in the environment. One such toxicity testing methods is the *Allium cepa* test which is an excellent model *in vivo*, where the roots grow in direct contact with the substance of interest (i.e. effluent or complex medicinal mix being tested) enabling possible damage to the DNA of eukaryotes to be predicted (Nefic et al., 2013).

The *Allium cepa* test is one of the few direct methods for measuring damage in systems that are exposed to mutagens or potential carcinogens, and enables the evaluation of the effects of these damages through the observation of chromosomal alterations. An extensive review on the *Allium cepa* test and its use in environmental contamination was carried out by Leme and Marin-Morales (2009), who reported that vascular plants are recognized as excellent genetic models for detecting environmental mutagens and are frequently used in monitoring studies.

Data from *Allium cepa* test can be extrapolated for all animal and plant biodiversity. The Allium cepa test has been used by many researchers mainly as a bioindicator of environmental pollution by Leme and Marin-Morales (2009); Bagatini et al. (2009), testing crude extracts of cyanobacteria, as well as to evaluate the genotoxic potential of medicinal plants (Camparoto et al., 2002; Lubini et al., 2008; Rashed et al., 2015; Tedesco et al., 2012) because it uses a model that is adequately sensitive to detect innumerous substances that cause chromosomal alterations. Evaluation of the ability of a compound to cause chromosomal damage is essential in the assessment of the potential genotoxicity of any compound (Repetto et al., 2001).

The aim of this work was to investigate the cytotoxicity and genotoxicity of photographic laboratory effluent using *Allium cepa* root tip cells.

To the best of our knowledge no information on the genotoxicity assay of the photographic effluents exists. This is worrisome as much importance is attached to photography as means of preserving information.

Materials and Methods

Sources and preparation of materials

The Onion bulbs (*Allium cepa*) of the purple variety of average size (15-22 mm diameter) were purchased at Ile-Epo Market, Lagos. The photographic effluents were collected from three locations (i.e Agbado-Oke, Ifako-Ijaiye, and Yaba areas) in Lagos State, Nigeria. Each effluent was diluted into three concentrations including 50%, 25%, and 10%. Tap water was used as the control (0%). The experimental setup was done in duplicates of each concentration of the photographic effluent (Rank and Nielsen, 1998).

Viability Test

The onion bulbs were sun-dried for two weeks and the dried roots present at the base of the onion bulbs were carefully shaved off, with a sharp razor blade to expose the fresh meristematic tissues. The rings of the root primordial were left intact. The clean and healthy onion bulbs were selected and suspended in distilled water to determine viability as described by Fiskesjö (1997). After 24 hr, the onion bulbs with freshly emerged roots were selected. As soon as the roots were about 1 cm long they were suspended in different concentrations of photographic effluent (50, 25 and 10% respectively).

Root Length Measurement

The onion bulbs were removed from the distilled water and placed on a blotting paper to remove excess water. At the end of the exposure period in effluent and in tap water (control), the roots of the onion bulbs with the best growth at each concentration were removed with forceps and their lengths measured (in cm) with a calibrated ruler. Growth inhibition was estimated as EC50 (the effective concentration of a chemical producing 50% of the total effect). The mean root length of each onion in each concentration was calculated by dividing the total root length for each concentration by the number of roots measured. The root length of the control was also calculated and the result plotted on a graph.

Cytological Study

The emerged root tips of the onion bulbs in the different concentrations of the photographic effluents were fixed in aceto-alcohol (1:3) after the exposure periods. The conventional feulgen-squash method (Sharma and DPhil, 1980) was used to prepare permanent slides of root meristems. The root tips were

put in 1-2 normal hydrochloric acid (HCl) for five minutes to soften the tissue. The tips were then macerated and stained with aceto-orcein (a mixture of acetic acid and orcein) stain for 20 minutes. Four root tip squashes were prepared for each treatment and a minimum of 500 cells were examined for each concentration.

The slides were examined and counts were observed for dividing cells, non-dividing cells, and cells at each mitotic phase and aberrant cells were made (aberration test, bridges, fragments, laggards, and multipolar anaphases were scored) using the method described by Adeyemo and Farinmade (2013). The mitotic index (MI) was determined by dividing the number of dividing cell with the total number of cells counted as described by Adeyemo and Farinmade (2013).

Characterization of mitosis and chromosomal aberrations were scored in 100 cells per slide. Photomicrographs of some aberrant cells were taken in comparison with the normal cells. Mitotic Index was estimated as number of dividing cells over the total number of cells counted expressed in percentage. Similarly, the percentage abnormal cells were calculated as the number of aberrant cells divided by the number of dividing cells and multiplying the quotient with 100 (Bhatta and Sakya, 2008)

Statistical analyses

The data obtained were statistically analysed using the Graphpad Prsim 5.0 version software. The mean values were compared using two way analysis of variance followed with Bonferroni Posthoc test at $P<0.5$, $P<0.1$ and $P<0.01$ respectively.

Results and Discussion

Root length growth and inhibition

The effects of the effluents on the root lengths of *A. cepa* are shown in Table 1. The effluents inhibited the root length development in a dose dependent manner. The root length inhibition for each effluent had a direct relationship with the concentration. Generally, the effluent collected from Ifako-Ijaye exhibited more inhibitory effects than others. Also, effluent collected from Agbado-Oke had the least inhibitory effect compared to the others. The median effective concentrations (EC50) for samples from Agbado-Oke and Ifako-Ijaye areas is 16% while that of sample from Yaba is 17%.

Table 1: Effect of photographic effluent on root growth of *A. Cepa*

	AGBADO-OKE		IFAKO-IJAYE		YABA	
	Mean Root Length	% Root Inhibition	Mean Root Length	% Root Inhibition	Mean Root Length	% Root Inhibition
Control	6.00	0.00	6.58	0.00	6.63	0.00
10%	2.43	59.58	2.33	64.59	2.48	62.59
25%	1.65	72.50	1.81	72.49	1.88	71.72
50%	1.53	74.50	1.50	77.20	1.59	76.02
Median effective Concentration	10%		16%		17%	

The effect of photolab effluent on the cell division and mitotic index of A. Cepa

The number of dividing cells decreased with the increase in the concentration of photographic effluent used (Table 2). Generally, *A. cepa* root exposed to effluents from Yaba had more dividing cells and mitotic index for each concentration compared to the effluents from Ifako-Ijaye and Agbado –Oke. For effluents from Ifako-Ijaiye and Agbado-Oke areas, no dividing cell was observed in 50% treatments. Also the mitotic index of the *A. cepa* exposed to effluents from those areas was 0. In addition, the mitotic indices generally decreased as the concentration of the effluents increased. The total number of dividing cells from the control treatment was significantly higher than those from the roots treated with various concentrations of the effluents ($P<0.05$; $P<0.01$). Significant differences in the number of dividing cells were observed between cells of the roots treated with various concentrations of the effluents.

Table 2: Effect of photolab effluent on the cell division and mitotic index of *A. cepa*

Sampling Site	Concentration (%)	Number of cells	Number of dividing cells	Mitotic index
Agbado-Oke	Control (0%)	1983± 0.00	341± 0.00	17.20± 0.00
	10%	1416±174. 00	122± 3.00	8.77± 1.29
	25%	1699.5± 131.50	41± 31.00	4.455± 0.14
	50%	100± 100.00	00	00
Ifako-Ijaye	Control (0%)	1945±0.00	313± 0.00	16.09± 0.00
	10%	1319± 85.00	94.5± 25.5	7.07±1.48
	25%	1398± 59.00	59.5± 4.50	4.28± 0.50
	50%	0	0	0.00
Yaba	Control (0%)	1889±0.00	289± 0.00	15.30± 0.00
	10%	1625±165.00	202± 0.00	11.075± 0.22
	25%	1563±6.00	151.5± 2.50	9.695±0.20
	50%	1389± 166.00	23.5± 0.50	1.40±0.56

Chromosomal aberrations in A. cepa due to exposure to photolab effluents

The chromosomal aberrations due to exposure of *A. cepa* roots to photographic effluents are shown in Table 3. Generally all the effluents at different concentrations produced more sticky chromosomes than other forms of aberrations. However, the photographic effluent from Yaba produced more chromosomes with multipolar anaphase (at 50% concentration) than the other forms of aberration observed. Although, no aberration was recorded generally for the roots treated with 50% concentration of the effluents from Agbado-Oke and Ifako-ijaye areas due to none dividing cells, the 50% concentration of effluent from Yaba produced the highest percentages aberration of 61.70%.

The lowest percentage aberration (9.5%) was produced in roots treated with 25% concentration of effluents from Yaba. The total number of aberrations in cell of roots treated with 10% effluents from Agbado-Oke and Yaba and 25% effluent from Ifako-Ijaye was significantly higher than the vagrant cell at P<0.001 and for the 10% effluent from Ifako-ijaye (P<0.05). Also, the total chromosomal aberrations were significantly higher than the bridged, and the binucleated cells for cells treated with 10% effluents from Agbado-Oke and Yaba and 25% effluent from Ifako-Ijaye (P<0.001). In the case of roots treated with 10% effluent from Ifako-Ijaye, the bridged chromosomes and the binuleated cells are significantly lower than the total aberration at P<0.05. Similar difference was noticed between the total aberrations and the multipolar cells and cells with chromosome breaks.

The cytotoxicity level of environmental pollutants can be determined by a decreased rate of mitotic index (MI); this is an acceptable measure

for all living organisms (Smaka-Kinel et al., 1996). Several chemicals and food preservatives have been reported to inhibit mitosis (Rencuzogullarý et al., 2001; Bushra et al., 2002) in *Allium cepa* and animal cells. Inhibition of DNA synthesis or a blocking in the G2 –phase of the cell cycle, preventing the cell from entering mitosis, are reasonable explanations for the reduction in the mitotic activity (Sudha et al., 2010). Reduction in the mitotic activity is accompanied by decreased amounts of DNA, which could be due to inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis (Türkoðlu, 2008; Türkoðlu, 2009). Decline of mitotic index below 22% in comparison to negative control can cause lethal effects on the organism (Antonise-Wiez, 1990).

The decrease in mitotic index caused by the photographic laboratory effluent treatments was associated with failed chromosome segregation in anaphase and the appearance of micronuclei as suggested by the results of the mitotic indices. This also, suggest that the effluent has cytotoxic effect which is not lethal at the concentrations used in the study. The decrease in the mitotic index (MI) with increasing concentrations of photographic effluents as well as the formation of chromosomal aberrations such as vagrant, sticky chromosomes, binucleus and severe reduction in root length can be attributed to the presence of some cytotoxic and genotoxic compounds in the effluent. Root length is an important parameter which reflects the toxicity of substances. The elongation zone of the onions may serve as a sensitive external signal of ongoing internal cellular events (Adeyemo and Farinmade, 2013). Plant roots interact with each of the physical factors in their environment and this interaction can lead to modification of the roots. In the *A. cepa* assay, inhibition of root growth has been shown to indicate retardation of growth and cytotoxicty (Odeigah, et al., 1997; Grant, 1982). Also, growth

inhibition can be caused by reduction in mitotic activities and occurrence of various chromosomal aberrations (Adeyemo and Farinmade, 2013). According to Rank (2003), vagrant chromosomes are also indicators of spindle poisoning and the induction of vagrant chromosomes is a consequence of spindle disturbances. Thus the presence of vagrant chromosomes noticed in this work, suggests that photographic effluents have the ability to poison and disturb the mitotic spindles. Such can lead to arrest of mitosis and cell death (Matson and Stukenberg, 2011).

The different forms of aberration observed in this study are similar to the findings of Akinsemolu, et al. (2015) who reported various chromosomal aberrations when *Allium cepa* roots were exposed to tobacco industrial effluents. Previous study by Bhatta and Sakya (2008) also reported chromosomal aberrations in a study of the mitotic activity and chromosomal behavior in root meristem of *A. cepa* treated with magnesium sulphate. Such aberrations have some significance to the development and wellbeing of organisms.

Our results also corroborates that of Dragoeva et al.(2009) who evaluated genotoxic potential of agricultural soil and reported various chromosomal abnormalities like vagrant chromosomes, chromosomal fragments at anaphase and telophase and multipolar anaphases. Binucleate cells are due to a defect in cytokinesis. Similar inhibitions and formation of binucleated cells were reported by (Jensen et al., 1996; Yasui et al., 2015). These cells can arise in a culture as a result of an incomplete process of cell division, i.e. karyokinesis with incomplete cytokinesis or as a result of the mitotic division of a pre-existent binucleate cell. The mitotic division of a binucleate cell can give rise to different types of daughter cells (Rodilla, 1993). The binucleation is the result of nuclear division not followed by cytoplasmic division (Uetake and Sluder, 2004). This could imply that photographic effluents inhibit or interfere with cytokinesis. Binucleate formation therefore may be a way of doubling up the haploid genome, to produce diploid cells of androgenetic origin as seen in placental mesenchymal dysplasia, a rare condition associated with intrauterine growth restriction, prematurity and intrauterine death.

All multipolar anaphases contain extra centrosome. Multipolar anaphase might be a result of partial or incomplete C-mitosis (Grant, 1982). Thus, our data demonstrate that multipolar mitoses can activate the spindle assembly checkpoint (SAC) in S2 cells. Multiple centrosomes in tumor cells create the potential for multipolar divisions that can lead to aneuploidy and cell death (Kwon et al., 2008). Multipolar spindle, aneuploidy, disruption of cell polarity and failure of asymmetric cell division can occur due to failure to properly control centrosome number and function (Nigg, 2002).

The bridges noticed in the cells are probably formed by breakage and fusion of chromosomes and chromatids. Such chromosome bridges were also reported to be induced by other chemicals (Donbak et al., 2002). Chromosome bridges may also be due to chromosomal stickiness and/or unsuccessful separation of chromosomes at anaphase, otherwise, it may be attributed to unequal translocation or inversion of chromosome segments (Gomurgen, 2000).

According to Onyemaobi et al. (2012), bridges arise from joined ends of broken sister chromatids while lagging results from failure of chromosome movement or acentric fragments. Bridges and fragments are clastogenic effects, both resulting from chromosomal and chromatid breaks Kovalchuk et al., 1998). Bridges probably occur by the interruption and joining chromosomes or chromatids (Turkoglu, 2007), as a result of chromosome stickiness, or due to unequal translocation or inversion of chromosome segments (Gömürgen, 2005). This may suggest that the effluents can cause chromatid break. Chromosome stickiness is classified as a physiological effect exerted by the wastewater in *A. cepa*, which alter the proteins of the chromosomes. Stickiness is irreversible and reflects high toxicity of substances, while acentric fragments in anaphase are the result of chromosome or chromatids interruptions, indicating interference with DNA (Olorunfemi et al., 2012).

Sticky chromosomes represent poisoned chromosomes with sticky surface and probably lead to cell death (Fiskesjö, 1997). Chromosome stickiness can also lead to apoptosis due to toxic effects of the wastewater. These results are in line with other studies on the effects of different chemicals on plants and human cells (Rencuzogullarý et al., 2001; Gomurgen, 2000; Shahin and El-Amoodi,1991; Nwangburuka and Oyelana, 2011). Sticky bridges might be as a result of incomplete replication of chromosomes by defective or less active replication enzymes (Sinha, 1979). Sticking of chromosomes probably occurs due to degradation or depolymerization of chromosome DNA (Grant, 1982) or as a result of DNA condensation and stickiness of inter-chromosome fibers (Schneiderman et al., 1971).

This may imply that the sticky chromosomes observed in this study could have been caused by either depolymerization of the chromosome DNA or due to DNA condensation. Stickiness is a highly toxic and irreversible effect, generally leading to cell death (Fiskesjö, 1997) and the folding of chromosome fibres into single chromatids (Klásterská et al., 1976). The

statistically higher number of stickiness compared to other aberrations may suggest that the photographic effluents are highly toxic and that most cells may die if exposed to photographic effluents.

Table 3: Chromosomal Aberration in *A. cepa* due to exposure to photolab effluent

Location	Concentration (%)	Stickiness	Vagrant	Bridges fragment	Binucleated cells	Multipolar anaphase	Chromosomal break	Total aberration
Agbagdo-Oke	Control (0)	35±0.00	00±	00±	00±	0±0	00±	0± 0.00
	10%	19.5±8.50	13.5±4.50	11.5±5.50	6.5±3.50	3.0± 3.00	2.5± 0.50	57±26.00 (46.72%)
	25%	8.0± 1.0	3.5± 0.5	4.5±0.5	0.5±0.5	0.5±0.5	2.5±2.5	19.5± 0.5 (47.56)
	50%	0±	0	0	0	0	0	0
Ifako-Ijaye	Control (00	30±	00	00	00	00	00	30
	10%	9.5±2.50	2.0±2.0	3.0±	3.0±0.00	0.5±0.5	0.5±0.5	18.5± (19.58)
	25%	23.5±3.50	14.5±3.5	12.5±3.5	5.5±2.50	2.0±1.00	6.0± 1.00	59±10.00
	50%	0	0	0	0	0	0	0
Yaba	Control (0)	32±0.00	00	00± 0.00	00± 0.0	00	00	0
	10%	12.5±0.50	8.5±1.5	7.0±1.0	1.5± 0.50	2.5±	2.0±1.00	34 ± (16.83)
	25%	5.0±1.00	2.0±0.00	0.5±0.5	0±0.00	1.0±	1.0±0.00	9.5± (6.27)
	50%	5.0±3.00	1.5±0.5	0±0.00	2.5±2.5	5.5±	0	14.5 (61.70)

Conclusion

This study revealed that photographic effluent has genotoxic effects on *Allium cepa* and can pose a potential health risk to plants, animals and humans. The results suggest that photographic effluent is of ecotoxicological importance and has the ability to interfere with the DNA biosynthesis. A. cepa may be a sensitive biosensor for screening the genotoxicity of photographic laboratory effluents.

References

Adeyemo, O. A. and Farinmade, A. E. 2013: Genotoxic and Cytotoxic Effects of Food Flavor Enhancer, Monosodium Glutamate (MSG) Using *Allium cepa* Assay. Afr. J. Biotechnol. 12(13): 1459 – 1466.

Akinsemolu, A. A. Nwangburuka, C. C. and Ogunwenmo, K. O. 2015: Evaluation of Tobacco Industrial Wastewater for Genotoxic Characteristics on *Allium cepa* L. Root Cell Mitosis. J. Adv. Biol. Biotechnol, 2(3): 165-173.

Andreozzi, R., LoCasale, M. S., Mantta, R. and Pollio, A. 2000: N-methyl-P- aminophenol (metol) ozonation in aqueous solution: kinetics, mechanism and toxicological characterization of ozonized samples. Water Res., 34(18):4419-4429.

Antonise-Wiez, D. 1990: Analysis of the Cell Cycle in Root Meristem of *Allium cepa* under the Influence of Ledakrin Folia. Histochem. Cytobiol. 26: 79-96.

Bagatini, M.D., Vasconcelos, T.G., Laughinghouse IV, H.D. Martins, A.F. and Tedesco, S.B. 2009: Biomonitoring Hospital Effluents By *Allium cepa* L. Test. Bull Environ. Toxicol. and Cont. 82: 590-592.

Bhatta, P. and Sakya, S. R. 2008: Study of Mitotic Activity and Chromosomal Behaviour in Root Meristem of *Allium cepa* L. Treated with Magnesium sulphate, Ecoprint 15: 83-88.

Bober, T.W., Vacco, D., Dagon, T.J. and Fowler, H.E. 2004: Treatment of Photographic Processing Wastes. In: *Handbook of Industrial and Hazardous Wastes Treatments* (2nd edn) Wang, L.K., Hung, Y., Lo, H.H. and Yapijakis, C. (Eds), Marcel Dekker, Inc. New York pp 275 -317

Bushra A, Farah M. A, Ali M. N, and Ahmad W. 2002: Clastogenicity of Pentachloro-phenol, 2, 4-d and Butachlor Evaluated by *Allium* Root Tip Test. Mutation Research/Genetic Toxicol. Environ. Mutagen. 514:105–113

Camparoto, M. L., Teixeira, R.O., Mantovani, M. S. and Vicentini, V.E.P. 2002: Effects of *Maytenusilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells. Genet. Mol. Biol. 25: 85-89.

Donbak L, Rencuzogullarý E, and Topaktas M. 2002: The cytogenetic Effects of the Food Additive Boric Acid in *Allium cepa* L., Cytologia, 67: 153–157.

Dragoeva A., Kalchevai,V. and Slanev S. 2009: Genotoxicity of Agricultural Soils after One Year of Conversion Period and under Conventional Agriculture. J. Appl. Sci. Environ. Manage. 13: 81-83.

Drake, P.L. and Hazelwood, K.L. 2005: Exposure-

Related Health Effects of Silver and Silver Compounds: A Review. The Ann. Occup. Hygiene, 49 (7): 575- 585.

Enguita, F. J. and Leitao, A. L. (2013). Hydroquinone: Environmental Pollution, Toxicity and Microbial Answers. BioMed Res. Int. 2013: 1-14

Fiskesjö, G. 1997: *Allium* Test for Screening Chemicals; Evaluation of Cytologic Parameters, in: W. Wang, J. Gorsuch, J. Hughes (Eds.), Plant for Environmental Studies, CRC Lewis Publishers, New York, pp. 308–333.

Gomurgen A.N. 2000: Cytological Effect of the Herbicide 2, 4-disooctylester 48% on root Mitosis of *Allium cepa*. Cytologia, 65:383–388.

Gömürgen, A. N. 2005: Cytological Effect of the Potassium Metabisulphite and Potassium Nitrate Food Preservative on Root Tip of *Allium cepa* L. Cytologia,, 70: 119–128.

Grant, W.F. 1982: Chromosome Aberration Assays in *Allium*. A Report of the United States Environmental Protection Agency's GENE TOX Program. Mutation Res. 99: 273–291.

Grover, I. S. and Kaur, S. 1999: Genotoxicity of Wastewater Samples from Sewage and
Industrial Effluent Detected by The *Allium* Root Anaphase Aberration and Micronucleus Assays. Mutation Res. 426: 183–188.

Jensen, C.G, Jensen L.C., Rieder, C.L., Cole, R.W., and Ault J. G. 1996: Long Crocidolite Asbestos Fibers Cause Polyploidy by Sterically Blocking Cytokinesis. Carcinog.17: 2013–2021.

Klásterská, I., Natarajan, A.T., and Ramel, C. (1976). An Interpretation of the Origin of Sub-Chromatid Aberrations and Chromosome Stickiness as a Category of Chromatid Aberrations. Hereditas, 83 153–162.

KODAK, (1996). *The Regulation of Silver in Photographic Processing Facilities*. Publication no: J-214 Eastman Kodak Company. Available at: http://www.kodak.com.

Leme, D.M. and Marin-Morales, M.A. 2009:.*Allium cepa* Test in Environmental Monitoring: A Review on its Application. Mutation Res. 682: 71–81.

Kovalchuk, O, Kovalchuk, I, Arkhipov, A, Telyuk, P,

Hohn, B and Kovalchuk, L. 1998): The *Allium cepa* Chromosome Aberration Test Reliably Measures Genotoxicity of Soils of Inhabited Areas in the Ukraine Contaminated by the Chernobyl Accident. Mutation Res. 415: 47–57.

Kwon, M., Godinho, S. A., Chandhok, N. S., Ganem, N. J., Azioune, A., Thery, M. and Pellman, D. 2008: Mechanisms to Suppress Multipolar Divisions in Cancer Cells with Extra Centrosomes. Genes Dev. 22: 2189-2203.

Lubini, G., Fachinetto, J.M, Laughinghouse IV, H.D., Paranhos, J.T., Silva, A.C.F. and Tedesco, S.B. 2008: Extracts Affecting Mitotic Division in Root-tip Meristematic Cells, Biologia, 63: 647-651.

Matson, D. R. and Stukenberg, P. T. 2011: Spindle Poisons and Cell Fate: A Tale of Two Pathways. Mol. Interv. 11(2): 141–150.

Nefic, H., Musanovis, J., Metovic, A. and Kurteshi, K. (2013). Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by Alprazolam. Med. Arch. 67(6): 388-392.

Nigg E.A. 2002: Centrosome Aberrations: Cause or Consequence of Cancer Progression? Nat. Rev. Cancer. 2:815–825.

Nwangburuka C. C, and Oyelana O. A. 2011): Cytological Effect of Chloroquin on Root Mitosis of *Allium cepa* (L.).actaSATECH J. 4(1):25-30.

Odeigah, P. G. C.; Nurudeen, O. and Amund, O. O. 1997: Genotoxicity of Oil Field Waste Water in Nigeria. Hereditas, 126:161-167.

Olorunfemi, D. Duru, E. and Okeimen, F. 2012: Induction of Chromosome Aberrations in *Allium cepa* L. Root Tips on Exposure to Ballast Water. Int. J. Cytol. Cytosystem. Cytogenet. 65(2): 147-151.

Onyemaobi, O. I, Williams, G. O. and Adekoya, K. O. 2012: Cytogenetic Effects of Two Food Preservatives, Sodium Metabisulphite and Sodium Benzoate on the Root Tips of Linn. Ife J. Sci. 14(1): 155-165.

Rank, J. and M.H. Nielsen, 1998: Genotoxicity Testing of Waste Water Sludge Using *Allium cepa* Anaphase-Telophase Chromosome Aberration Assay. Mutation Res. 418: 113-199.

Rank J. 2003: The Method of *Allium* Anaphase-Telophase Chromosome Aberration Assay. Ekologija (Vilnius), 1:38-42.

Rashed, K., Anthonissen, R., Cappoen, D and Verschaeve. L. 2015: Phytochemical Composition and Potential Genotoxic effects of Important Egyptian Medicinal Plants. Pharmacog. Comm. 5(3): 207-216.

Rencuzogullarý E, Ila, H.B, Kayraldýz A, and Topaktas M. 2001: Chromosome Aberrations and Sister Chromatid Exchanges in Cultured Human Lymphocytes Treated with Sodium Metabisulfit a Food Preservative. Mutation Res. 490:107–112.

Repetto G, Jos A, Hazen M. J, Molero M. L, del Peso A, Salguero M, del Castillo P, Rodriguez-Vicente M. C and Repetto, M. 2001: A test Battery for The Ecotoxicological Evaluation of Pentachlorophenol. Toxicol. *in Vitro*, 15:503-509.

Rodilla, V. 1993: Origin and Evolution of Binucleated Cells and Binucleated Cells with Micronuclei in Cisplatin-treated CHO Cultures. Mutation Res. Genet. Toxicol. 300 (3–4): 281-291.

Schneiderman, M. H., Dewey, W.C. and Highfield, D. P. 1971: Inhibition of DNA Synthesis in Synchronized Chinise Hamster Cells Treated in G1 with Cyclohexamid. Exp. Cell Res. 67:147–155.

Shahin, S. A, and El-Amoodi, K.H.H. 1991: Induction of Numerical Chromosomal Aberrations During DNA Synthesis Using the Fungicides Nimrod and Rubigan-4 in Root Tips of *Vicia faba* L., Mutation Res. 26(1):169–176.

Sharma, A. K. and DPhil, A. S. 1980: Chromosome Techniques. Fakenham Press Ltd., Fakenham, Norfolk, pp: 95-105.

Sinha, U. 1979: Cytomorphological and Macromolecular Changes Induced by P-flurophenylalanine in Allium Cepa and Triticale. J. Cytologia. Genet. 14:198

Smaka-Kinel, V, Stegnar, P, Lovka, M. and Toman, M.J. 1996: The Evaluation of Waste, Surface and Ground Water Quality Using the *Allium* Test Procedure. Mutation Res. 368: 171–179.

Stellman, J.M. (ed) 1998: Encyclopedia of Occupational Health and Safety, 4th edn. International Labour Office, Geneva, pp104.32 – 104.34

Sudha S, Shibily P, Shyn J, Kripa S, and Vellingiri B. 2010: Genotoxic Effects of Textile Printing Dye Exposed Workers in India Detected by Micronucleus Assay. Asian Pac. J. 10:56-90.

Tedesco, S B. and Laughinghouse, H. D. IV 2012: Bioindicator of Genotoxicity: The *Allium cepa* Test, Environmental Contamination, Dr. Jatin Srivastava (Ed.), ISBN: 978-953-51-0120-8, InTech, Available from : http://www.intechopen.com/books/environmental-contamination/bioindicator-of-genotoxicity-the-allium-cepa-test

Turkoglu, S. 2007: Genotoxicity of Five Food Preservatives Tested on Root Tips of *Allium cepa* L. Mutation Res. 626: 4-14.

Türkoðlu, Þ. 2008: Evaluation of Genotoxic Effects of Sodium Propionate, Calcium Propionate and Potassium Propionate on the Root Meristem Cells of *Allium cepa*. Food Chem. Toxicol. J. 46: 2035–2041.

Türkoðlu, Þ. 2009: Genotoxic Effects of Mono-, di-, and Trisodium Phosphate on Mitotic Activity, DNA Content, and Nuclear Volume in *Allium cepa* L. Caryologia, 62(3): 171–179.

Uetake, Y. and Sluder, G. 2004: Cell Cycle Progression after Cleavage Failure Mammalian Somatic Cells Do Not Possess a Tetraploidy Checkpoint. J. Cell Biol. 165(5): 609–61.

Yasui, M., Kamoshita, N, Nishimura, T and Honma, M. 2015: Mechanism of Induction of Binucleated Cells by Multiwalled Carbon Nanotubes as Revealed by Live-Cell Imaging Analysis. Genes Environ. 37: 6-11