Toxicological
Communication

Biosci. Biotech. Res. Comm. 11(3): 486-495 (2018)

BBRC
Bioscience Biotechnology
Research Communications

Evaluation of industrial effluent and domestic sewage genotoxicity using *Allium cepa* bioassay

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ABSTRACT

Living organisms including both plants and animals being exposed to polluted water bodies can be impacted. In current investigation the genotoxic effect of industrial effluent and domestic sewage of Barak valley region, Assam, India was investigated using both morphological and root chromosome assay on *Allium cepa*. The mean root lengths of onions exposed to different concentrations of the effluent and sewage (10%, 25% and 50%) were measured for 3 consecutive days for 24, 48 and 72 hrs and the results were compared. The mean root length was statistically evaluated by the analysis of variance. There was both significant increase and decrease in root length among the exposed onion bulbs. Total aberrations increased significantly as concentration increased ($p<0.05$). Both effluent and sewage samples were recorded to cause harmful damages in the exposed onion test samples. These results demonstrated that the Allium test is a useful screening test for the evaluation of toxicity caused by sewage and effluent samples not only at the morphological level but also at the cytogenetic level; and hence pollution in waster bodies in a major cause of concern. Thus, sincere measures should be undertaken regarding the direct disposable of industrial effluents and domestic sewage and protection of water bodies including its flora and fauna.

KEY WORDS: PAPER MILL EFFLUENTS, DOMESTIC SEWAGE, *ALLIUM CEPA* TEST

ARTICLE INFORMATION:

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Received 19th July, 2018
Accepted after revision 17th Sep, 2018
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007 CODEN: USA BBRCBA
Thomson Reuters ISI ESC / Clarivate Analytics USA and Crossref Indexed Journal
NAAS Journal Score 2018: 4.31 SJIF 2017: 4.196

Online Contents Available at: http//www.bbrc.in/
DOI: 10.21786/bbrc/11.3/19

INTRODUCTION

Over two third of Earth's surface is covered by water; less than a third is taken up by land. As Earth's population continues to grow, people are putting ever-increasing pressure on the planet's water resources .In a sense, our oceans, rivers and other inland waters are being squeezed by human activities, not so that they take up less room, but so their quality is reduced. Poorer water quality leads to pollution in water bodies. Water pollution nowadays is a major global problem. It requires ongoing evaluation and revision of water resource policy at all levels. It has been recorded that water pollution is the leading worldwide cause of death and diseases and it accounts for the death of more than 14,000 people daily. India and China are the two countries with high levels of water pollution: An estimated 580 people in India die of water pollution related illness including water borne diseases. Water pollution results in contamination of water bodies like rivers, lakes, aquifer, etc. due to human activities, (Bennet, 1997, Prasad and Rao, 2010. Bakare et al, 2017, Anacleto et al, 2017).

Polllution in water bodies occurs when contaminants gets introduced into the natural environment. For example, releasing inadequately treated waste water into natural water bodies leading to degradation of aquatic ecosystem. This in turn can cause public health problems for people living in downstream as people are using this polluted water for regular domestic activities like bathing, washing, drinking, irrigation, etc. Researches have revealed that the sources of water pollution may be point sources and non-point sources. Point sources have an identifiable cause as storm drain, waste water treatment plant or streams. Non-point sources are more diffuse like agricultural runoff, (Zaiad, 2010). With the increasing development of industrial resources the risk of water pollution has also increased. Not only the industrial resources but also the inadequate system of dumping of municipal sewage has resulted in pollution of water bodies and its aquatic ecosystem including both aquatic flora and fauna.

Researches till date have revealed that dissolved contaminants in both effluents and sewages when exposed to water bodies not only harms plant growth but also forces plants to absorb dangerous chemicals and pollutants which gets passed to animals and human through consumption and other modes (Sik *et al,* 2009). In a work done on the variable actions of *Alium sepa* and its usage as a bio indicator of cadmium toxicity, plants were exposed to increasing concentrations of cadmium, where cadmium was observed to cause inhibition of root and leaves growth and elongation which serve as a tool for characterizing the bio indication of cadmium exposure in waste and effluent condition, (Bakare et al, 2017).

The direct application of industrial sludges were recorded to harm the local biota in an investigation where the genotoxicity of industrial sludges was assessed using various plant including *A.cepa* where *A.cepa* test was found to be effective in detection of damages (Anacleto et al, 2017).

Studies about phytotoxic effects of waste waters and effluents started in 1970s where researches were conducted taking sugar cane, eucalyptus, *Triticum aestivum*, *Brassica campastris*, Sorghum, rice, and many more. *Allium cepa* is the largest genus of petaloid monocotyledons, containing hundreds of species naturally distributed in temperate climates of the northern hemisphere (Koçyi ğit & Özhatay, 2010). This test has important advantages (Zegura, 2009) and has been used from many years in investigating physical and chemical mutagenesis and cytogenetic effects in mitotic cell division. *Allium cepa* is important since it is an excellent model in-vivo, where the roots grow in direct contact with the substance of interest enabling possible damage to the DNA of eukaryotes to be predicted. It is advantageous to use the *Allium cepa* test system since its main component is a vascular plant, making it an evaluating environmental pollutant , detecting mutagens (Gupta et al, 2009).

The present investigation was designed to examine the level of morphological and genotoxic damages caused by industrial effluents as well as domestic sewages on *Allium cepa* so that proper safety measures can be taken not only for the protection of water quality but also preventive measures can be taken against the damages caused to aquatic ecosystem prior to exposure of effluents and sewage.

MATERIALS AND METHODS

For present investigation raw paper mill effluent sample was collected from the outlet pipes in the local river Barak of the valley. The domestic sewage which was selected for comparative analysis was collected from Silchar, Municipal drainage system at Tarapur area, where all the debris and discharges of the whole locality have been found to be discharged. Both effluent and sewage samples were collected in plastic gallons, pH was measured and stored at -20°C to prevent further microbial growth.

The common purple onion, *Allium cepa* (2n=16) bulbs (1.5–2.0 cm diameter) used for this study was procured from organic farmers of almost equal weight age. The dried out scales were carefully removed leaving the ring of the root primordial intact (Fiskesjo, 2011). Then they were kept in moist condition to let root grow for three days, this help select onion with synchronous growth. For each test, 10 *A. cepa* bulbs purchased from organic farmers were set up to produce roots in filtered and dechlorinized tap water for three days and then

transferred to the test solutions. Tap water was previously filtered in a bio-activated coal filter to remove chlorine and its by-products commonly used for disinfecting drinking water. Three litres of water were aerated over a period of 24 h before filling the test tubes. For positive control mitomycin C was selected . Mitomycins are a family of azinidines containing natural products insulated from *Streptomyces lavendulae*. Mitomycin C is a potent DNA crosslinkers. A single cross link per genome has shown to be effective in killing bacteria. This is accomplished by reductive activities followed by 2 N –Alkylation. Both alkylation are sequence specific for a guanine nucleotide sequence.

Three different concentrations of both the effluents and sewage were selected as 10%, 25% and 50% for exposure through prior standardization. During the Allium cepa assay, all selected onions were exposed to the selected concentrations of effluent and sewage for 24hrs, 48hrs and 72 hrs, respectively. The growth in roots were recorded till the third day of exposure in water and after that the variation in root growth were recorded after every 24 hrs for next three consecutive days till 72hrs and the data were recorded and compared. For mitotic studies, growth inhibition tests were carried out for each sample, to find its toxicity level. After every 24hrs of exposure, 3 to 4 healthy root tips from each bulb were prepared for the microscopic slides.

The emerged root tips of the onion bulbs in the different concentration of sewage and effluent were fixed and macerated in a solution of 45% acetic acid (9 parts) and 1 N HCl (1 part) at 50 °C for 10 min, followed by squashing in 2% Acetocarmine stain for 15 minutes. The modified conventional Feulgen-squash method (Sharma and Dphil, 2012) was used to prepare permanent slides of root meristems. The root tips were put in 1 normal hydrochloric acid for five minutes to soften the tissue. The macerated and stained root tips were covered with cover slip and squashed. Minimum 3-4 Slides were prepared per bulb for microscopic observation. Approximately three thousand cells were examined per onion to remove the errors and classified according to the chromosomal aberrations presented including bridges, fragments and chromosome lagging.

Results were presented as Mean±SE where mean value was calculated from three individual readings of a particular set. ANOVA was performed to determine the level of significance from the set of onion bulbs. ANOVA was done using graph pad PRISM (Graph pad Inc., san Diego, CA, USA).

RESULTS AND DISCUSSION

Water pollution can be caused by a number of sources ranging from industrial resources and sewage treatment plants and factories to mining activities, paved roads and agricultural runoff. Such issues have become one of the biggest problems in many developing and developed countries. These pollutants when not treated properly, can cause mutagenic or toxic effects directly on humans, affecting human health, resulting in diseases like cancer, congenital malformations, and cardiovascular diseases (Grover & Kauer, 2009). Siddiqui and his group (Siddiqui et al., 2011) have worked to validate plant-based tests for assessing the toxicity of water in India.

The *Allium* test is advantageous as genotoxicity screening assay, as *Allium* root cells posses the mixed function oxidase system which is capable of activating promutagens or genotoxic chemicals (Odeigah *et al*; 1997a). In the *Allium* test, inhibition of rooting and the appearance of stunted roots indicate retardation of growth and genotoxicty, while root wilting explains toxicity (Odeigah *et al*; 1997b). Both growth retardation and root wilting are accompanied by suppression of mitotic activity and remarkable chromosomal aberration. The present findings provides evidence that effluent and sewage inhibited root growth and caused growth retardation. The reason behind growth inhibition may be due to high rate of chemical oxygen demand which affected certain physiological processes leading to the disturbance in the balance between promoter and inhibitors of endogenous growth regulator (Gill and Saggoo, 2010).

Growth inhibition was most recorded at 50% concentration along with a marked decrease in root length when compared with the control. This is usually accompanied by an increase in chromosome aberrations (Amin and Muzahid, 2009). The suppression of mitotic activity was often used in tracing cytotoxicity (Smaka-Kinel *et al.,* 2013). In our study a decrease in the mitotic index was found as the concentration of effluent increased which indicates the cytotoxic effect of sewage and industrial effluent. Chrosomal aberration were observed to increase as the concentration of effluent and sewage increased. Among the chromosomal aberration observed, IN, EN and CF were most frequent in all concentrations band kept on increasing from concentrations of industrial effluent towards higher concentrations of sewage. Such findings are responsible for the completely decayed roots found in 25% and 50% concentration. The most common abnormalities were c-mitosis and disturbed metaphase. Sticky chromosomes and binucleated cells were recorded in noticeable amount. In addition to the above, at anaphase and telophase bridges, lagging chromosomes and irregular anaphase were also observed. The mitotic index in the root meristems grown in the negative control ranged from 17.3 to 19.8.

Table 2 shows the mitotic index values in root meristems growth in different concentrations of effluent

FIGURE 1. Panchgram Paper Mill

FIGURE 2. Silchar Municipal sewage

FIGURE 3. Sample of an onion bulb showing the normal root growth

FIGURE 4. Organization of the experiment, with *Allium cepa* where groups of 5 onion bulbs were exposed for growth

and sewage from the three concentrations of wastewater treatment. The decrease in the mitotic index of the root tips reached statistical significance only in the highest tested concentrations. The cytogenetic aberrations most commonly observed in anaphase-telophase cells were bridges, fragments and chromosome lagging. Aberrant mitotic cells were counted and expressed as mean and SD of the selected onion bulbs. In the root meristems of the negative control, the percentage of aberrant cells

Table 1. Root length variation of *Allium cepa* after cultivation in different concentrations of paper mill effluent and domestic sewage (10%,25% and 50%)

Treatment gropus	Concentration	Root length in different time interval (mean±std.error)					
		Before treatment			After treatment		
		24 hrs	48 hrs	72hrs	24 hrs	48hrs	72 hrs
Control	---	0.16±0.045	0.8±0.078	3.67±0.136	6.04±1.34	7.57±1.44	9.04±1.65
Positive Control (MMC)	2mg/lit	1.64±0.22	2.97±0.37	4.57±0.93	5.54±0.59	5.7 ±0.55	5.77±0.62
Paper Mill Effluent	10%	0.26±0.075	1.1±0.129	4.44±0.062	5.03^{***}±0.045	5.13^{***}±0.045	6.1^{**}±0.107
	25%	0.2±0.068	1.23±0.091	4.06±0.39	5.05^{***}±0.35	4.86^{***}±0.349	5.36±0.286
	50%	0.36±0.045	1.67±0.169	2.96±0.223	3.9^{***}±0.223	4.53^{***}±0.075	4.93±0.062
Domestic Sewage	10%	0.23±0.062	1.93±0.219	4.13±0.164	4.8^{***}±0.165	5.3^{***}±0.165	5.7±0.186
	25%	0.13±0.068	1.23±0.091	4.33±0.169	4.9^{***}±0.181	5.3^{***}±0.181	5.66±0.198
	50%	0.35±0.062	1.73±0.248	3.73±0.091	3.96^{***}±0.075	4.3(((±0.029	4.5±0.029
Root length unit=cm; n=3.							

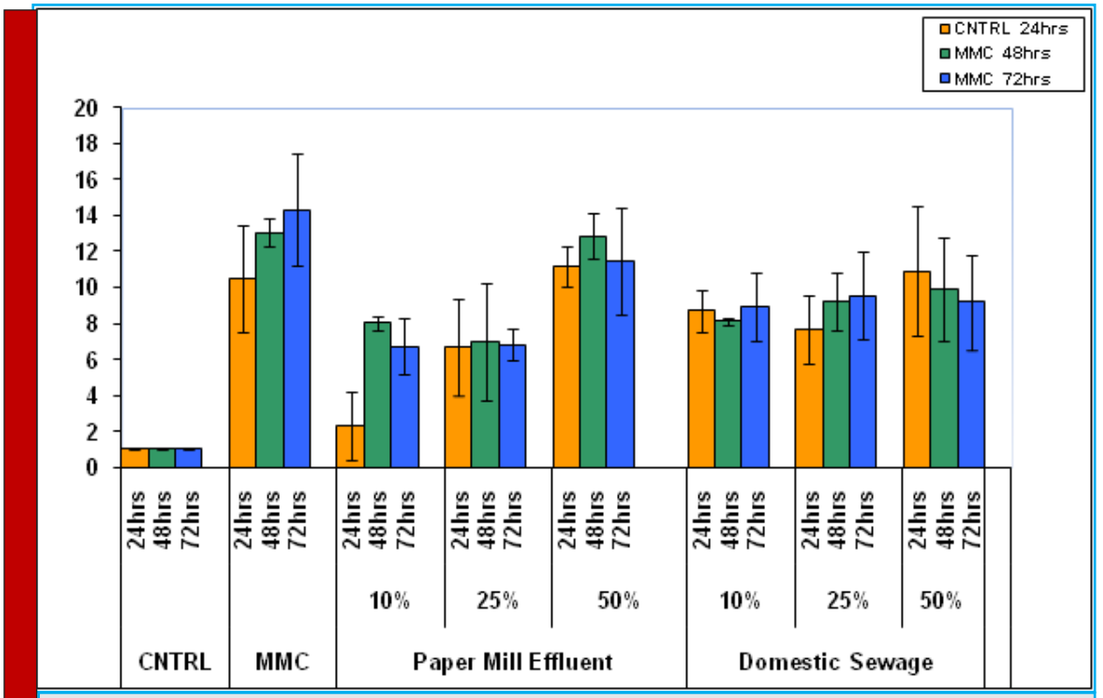

FIGURE 5. Graphical representation of various types of meiotic abnormalities after treatment with different concentrations of Paper Mill Effluent and Domestic Sewage.

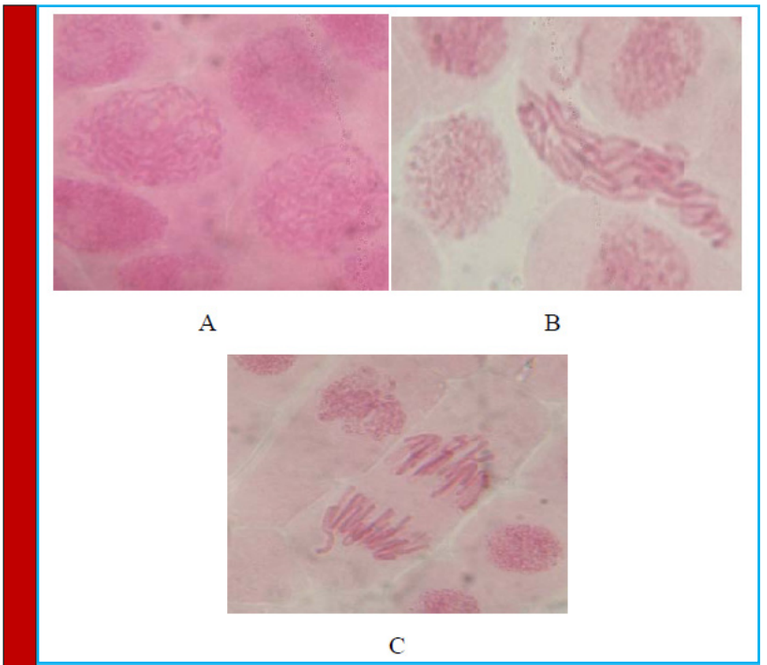

FIGURE 5. *Allium cepa* cells in regular or normal division. A-Prophase = the chromosomes are visible andtangled; B-Metaphase= the chromosomes are arrangedin the equatorial plate; C-Anaphase = the sister-chromatids separatemoving towards the spindle poles.

Table 2. Frequencies of different types of meiotic abnormalities after treatment with different concentrations of Paper Mill Effluent and Domestic Sewage.

Dose/Treatment		Fixn. time	Total no. of cells (n=3)	No. of dividing cells	MI (mean±std. deviation) MN	Cytotoxic effects								Total Aberrant Cells	% Aberrant Cells (mean± std.error)
						STK	IN	EN	VC	BNC	CB	CF			
Contrlo 48h 72h		24h	3060	542	17.34±2.10	0	0	0	0	0	0	0	0	0	1.02±0.02
		3060	610	19.66±1.96	0	0	0	0	0	0	0	0	0	1.03±0.05	
		3060	623	19.8±1.56	0	0	0	0	0	0	0	1	1	1.03±0.05	
Positive control (MMC) 48h 72h		24h	3080	248	8.04±0.44	15	22	85	15	3	2	5	22	323	10.48±2.98
		3099	224	6.8±0.30	26	24	98	18	6	6	16	21	404	13.03±0.78	
		3119	246	7.87±0.28	33	28	101	22	8	8	23	18	447	14.33±5.08	
Paper Mill Effluent	10%	24h	3061	491	16.36±0.14	0	30	93***	15	12	13	17	51	231	2.31**±1.86
		48h	3083	450	14.57±3.48	0	19	133***	6	4	3	11*	71***	247	8.01***±2.42
		72h	3075	472	15.33±2.19	0	16	156***	9	10	4	9	69***	273	6.73**±2.59
	25%	24h	3061	429	14.03±1.08	0	16	80***	20	15	10	17	46	204	6.72**±3.68
		48h	3050	461	15.11±0.11	0	20	122***	18	18	20	13	22	233	6.98**±3.24
		72h	3020	456	15.09±0.22	0	17	85***	20	20	21	22	21*	206	6.82**±0.86
	50%	24h	3072	337	10.97±0.9	0	56***	127***	27	22	17	14	80	343	11.16***±4.13
		48h	3067	408	13.29±0.84	3	59***	156***	39	25*	24	20	82***	408	12.87***±2.27
		72h	3033	437	14.42±1.4	3**	80***	145***	40	37***	19	30	98***	452	11.5***±6.98
Domestic Sewage	10%	24h	3031	359	11.85±1.1	0	0	94***	137***	1	24	5	3	264	8.7**±1.2
		48h	3051	436	14.29±1.49	0	0	92***	110***	1	36	6	3	248	8.12**±0.17
		72h	3063	512	16.34±1.22	0	1	96***	116***	3	36	13	10	275	8.94**±1.89
	25%	24h	3044	551	18.07±3.66	0	15	66*	66**	8	24	26*	34	239	7.65**±1.91
		48h	3089	617	27.56±10.49	0	25	60*	66**	26	34	42***	33	286	9.22***±1.64
		72h	3051	652	21.38±1.28	0	30	67**	72**	32***	30	24	34	289	9.55***±3.44
	50%	24h	3023	431	14.25±0.58	2	13	72***	115***	22	44	33***	30	331	10.91***±5.61
		48h	3046	457	14.66±1.46	1	35*	55*	6**8	26*	31	42***	43	301	9.88***±4.88
		72h	3045	473	15.58±1.57	4	57***	34	16	36***	20	57***	55*	279	9.19***±3.63

MN=Micronucleus, STK=Stickiness, IN=Irregular Nuclues, VC=Vagrant Chromosome, BNC=Bi Nucleated Cells, CB=Chromosome Bridge, CF=Chromosome Fragment.
Control : Dechlorinated tap water, MMC: Mitomycin C was used as positive control, When compared PME and DS with Control P<0.05=* P<0.01=**; P<0.001=***

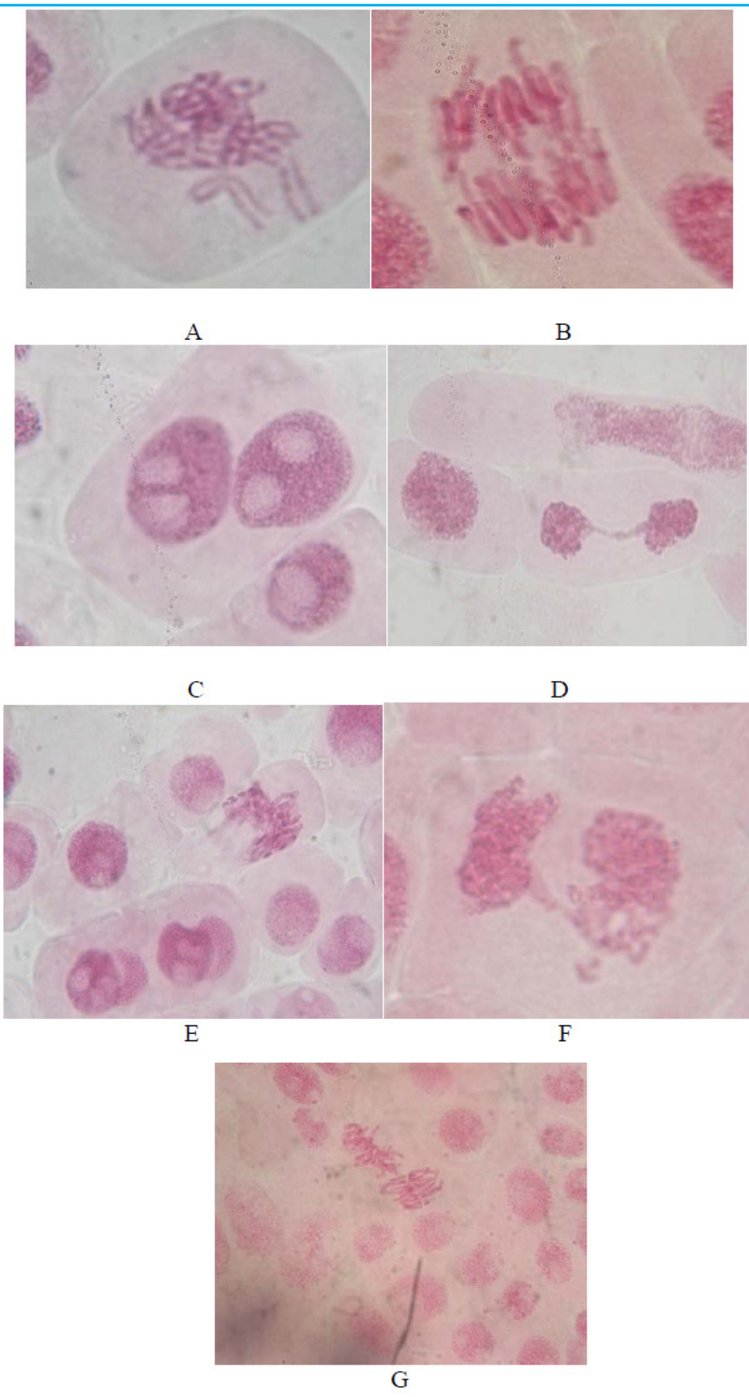

FIGURE 6. *Allium cepa* meristematic cells showing the alterations due to the action of industrial effluent and domestic sewage; A-irregular metaphase, with unorganized chromosome, alsoknown as C-metaphase, showing chromosomes with no orientation on the equatorial plate; B-irregular anaphase,with anaphasicmicro-bridges; C-irregular cell, binucleate, with an elliptical aspect; D-telophase bridge; E-cell with adherent or damaged nucleus, F-irregular cell; G-metaphase with numerical alteration, due to duplication of the number of Chromosomes.

FIGURE 7. Photographs of onion bilbs captured after exposure to 50% concentration of effluent and sewage

represents an avg. of 1.03 which was recorded as 8.34 and 10.09 in effluent and sewages.

Results obtained in our study showed that sewage and effluent induced chromosomal aberration through interactions with DNA and proteins leading to chromosome stickiness, mitotic disturbances and cell damage. Present finding is similar to the results of Odeigah *et al.* (1997b) in which roots of *Allium cepa* exposed to effluent from industrial wastes resulted to cells with chromosomal aberration. It would be beneficial to apply *A. cepa* chromosomal assay as a tool for monitoring the genotoxic effects of industrial effluent and wastewaters thereby providing information on the need for environmental managers to further subject treated industrial effluent to Toxicity Identification Evaluation and Toxicity Reduction Evaluation before they are finally discharged. This will enable proper chemical analysis of industrial effluent in order to identify the constituent that is really genotoxic and its prompt removal from the effluent before discharge.

CONCLUSION

According to the environmental campaign organization WWF," Pollution from toxic chemicals threatens life on this planet. Every ocean and every continent, from the tropics to the once-pristine polar regions, is contaminated. Clearly the problems associated with water pollution can disrupt life on our planet to a great extent. Increasing population and industrialization and direct exposure of sewage in water bodies has resulted in production of huge amount of liquid waste which ultimately finds its way to water bodies like rivers, lakes, streams and oceans. These effluents and sewages if provided to plants, animals and human for consumption, can lead to spreading of different types of hazardous diseases. Hence, it becomes mandatory to analyze the level of pollution caused to water and its flora and fauna.

We can conclude from our investigation that *Allium cepa* test is an excellent biomarker of chromosomal aberrations and has occupied an important place for the prevention and prediction of environmental impact that will be caused by the exposure of effluents and sewages. The above findings revealed the fact that industrial effluent and domestic sewage have almost equivalent capabilities to cause harmful damages to the water bodies. Life is ultimately about choices and so is pollution. We can live with sewage strewn beaches, dead rivers, and consume fish that are too poisonous for us to eat. Or we can work together to keep the environment clean so that plants, animals and people who depend on it remains healthy. It is ultimately up to us to be informed, responsible and involved when it comes to the problem we face with our water. We must become familiar with our water resources and learn about ways for disposing harmful household wastes so that they do not end up in sewage treatment plants that can't handle them or landfills not designed to receive hazardous materials. Hence proper measures should to be taken for the management of water quality and the aquatic life.

ACKNOWLEDGEMENTS

We are grateful to UGC for providing fellowship to RP and BR.

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