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RESEARCH ARTICLE

Genotoxicity assessment of water samples from Gomti river, U.P. India using the *Allium cepa* L. test

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Abstract

The present work was performed using the common onion *(Allium cepa* L.) was used to evaluate the genotoxicity of Gomti river water. The test water was collected at five sampling sites. The samples were analysed for macroscopically and microscopically evaluated. The water samples showed root growth and mitotic inhibition (MI) in *Allium cepa*. However the inhibitory effects were not dose dependent. All water samples increased the frequency of chromosomal abnormalities and two water samples produced alteration in mitotic index of the root cells. Water samples also altered root growth and morphological modifications in the *Allium cepa* roots. Water collected from Nishatganj bridge was most consistently toxic and genotoxic of the samples. The data indicate that Gomti river water contain toxic and genotoxic compounds that potentially may impact this aquatic ecosystem.

INTRODUCTION

Water is an indispensable natural product that finds its use in virtually all aspect of human life. Thus, there is a good need for ensuring that water used by humans does not contain hazardous substances.Urban industrial and agricultural wastes can add significant amount of contaminants to surface river water and sediments and consequently, water pollution is a serious problem for the health of the biota and humans that interact with these aquatic ecosystems. The contamination of water resources by genotoxic compounds is a world wide problem (Buschini *et.al*.,2004 , Akeem *et.al*.,2011, kopliku and Mesi,2013). The pollution of water bodies is a global problem because of the danger,that polluted waters may contain mutagenic and carcinogenic substances, that may cause or promote the occurance of human diseases such as cancer, cardiovascular disease and premature aging (Radic *et.al*., 2010).

The plant assay of *Allium cepa* L. (2n=16) has been used extensively to evaluate the cytotoxicity and genotoxicity of water samples (Rank and Nelson, 1998, Olorunfemi *et.al*.,2011, Olorunfemi *et.al*.,2010, Samuel *et.al*.,2010). This assay is low cost, is easy to use and produces similar results to animal tests because of similarity in their genetic compositions, hence same response to mutagens. The presence of metacentric chromosome in *Allium cepa* cells allows easier and better microscopic assessment.

River Gomti is an important tributary of river Ganga and perennial river of Awadh plains run across the major part of U.P. covering nine district and 940 Km stretch area. The regular pollution of the river through waste water discharge and refuse by residents poses dangers not only to the flora and fauna in it, but also to higher animals including humans along the food chain. This because the river has as its primary producers the plants being watered by this polluted water. However,plant assays, such as *Allium cepa* tests, may have some advantages over microbial and mammalian cell tests for environmental monitoring. Plant assays are highly sensitive to many environmental pollutants, including heavy metals (Fiskesjo,1988). Further more the test plant can be directly exposed to complex mixtures or environmental samples either in the laboratory or in situ (Fiskesjo,1985 , Grant *et.al*.,1992). Because of

the large size of their chromosome, higher plant are suitable for cytological analysis and the responses seen in plant test are highly correlated with those seen in other biological system, making plant tests for evaluating the genotoxicity of environmental samples (Leme, 2009 , Firbas and Amon,2013). In this study, we used the *Allium cepa* test to evaluate the toxicity and genotoxicity of Gomti river water.

Materials & methods:

Plant material- Onion bulbs (*Allium cepa* L.,2n= 16) of the purple variety of average size (15-22 mm diameter) were purchased locally in market of Lucknow city. They were sun dried, and 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 bulbs were than placed in freshly prepared distilled water for 24 hour, three replicate bulbs were used for each water sample and control for examination (Rank and Nielson,1993). The bulbs were removed from the distilled water and placed on a bloting paper to remove excess water.

Test procedure- For evaluation of root growth inhibition, the bulbs were exposed directly in samples of river water of five selected sites(Site-A_1, A_2, A_3 ; Site-B_1, B_2, B_3; Site-C_1, C_2,C_3; Site-D_1, D_2, D_3 and Site-E_1, E_2, E_3 respectively and the control. Each onion bulb was placed in 100 ml glass vials filled with the test or control water for 96 hour time duration. The test were performed at room temperature with a natural light dark regime and protected against direct sunlight. At the end of each exposure period 24 hour, 48 hour, 72 hour and 96 hour, the root of onion bulbs with the best growth at each sample water were removed and their length measured (in cm) with a meter rule. After the exposure period of 24hr, 48hr, 72hr and 96hr. the root fixed in FAA (Formalein acetic alcohol) in the ratio of 90% alcohol: 40% formalin: Glacial acetic acid (85:10:5 ml) for 12 hour and then fixed in 70% alcohol for further examination. Samples roots were hydrolyzed in 1N HCL for 20 minutes after which they were washed in distilled water. After then squashed on slide, stained with haemotoxylin stained for 15 minutes and coverslips carefully lowered on it to exclude air bubbles. The coverslips were sealed on the slide with fingernail polish as suggested by Grant (1982). Two slides were prepared for each sample and the control, and they were analyzed at light microscope for study of chromosomal aberrations. The mitotic index was calculated as the number of dividing cells per 100 observed cells (Fiskesjo, 1997 &1985).

$$MI = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}}$$

Results and Discussion

Statistical analysis: Mitotic index (MI) was calculated by scoring dividing cells. The experimental data is presented as mean ± S.E. of triplicate experiments.

The effect of water samples on the root growth of onions suggest their level of toxicity using growth inhibition as a determining factor. The mean root length of *Allium cepa* grown in river water after time interval of 24 hour, 48 hour, 72 hour and 96 hour are shown in table-1. The mean root length of onion bulbs at site-A and site-B river water increased as time increased as compared to the onion bulbs grown in the control. While at site-D and site-E, root length retard as compared to control. At site-C and site-D, the mean root length increased till 48 hour and then decreased after 72 hour and 96 hour time interval. The mean root length of *Allium cepa* grown in river water were compared to the control length, and expressed as a percentage of the control values. The extent of the cell proliferation and differentiation in the apical meristem could directly correlate with the rate of root growth. Previously, the inhibitory effect of river water on root growth of *Allium cepa* was reported by Akeen Akinboro *et.al.* (2011) in Sangai river in Malaysia, Barberio A *et.al.* (2009) in polluted river water in Brazil. In *Allium* test,there usually seems to be a certain correspondence between root growth retardation (toxicity) and certain chromosomal deviations (genotoxicity) reduction. When chromosomal aberrations occur, there are always some growth restrictions (Fiskesjo, 1985). In *Allium cepa*, whenever there is root growth inhibition, there is always reduction in the number of dividing cells (Bakare,2001, Bakare *et.al.*,2009 ,Olorunfemi and Ogunsanwo,2011). The induction of root malformations in *Allium cepa* has been shown to be useful sign of toxicity in previous studies (Bakare *et.al.*,2009, Olorunfemi *et.al.*,2011). In this study, the onion bulbs induced chromosome aberrations in each river water samples. This corresponds to the degree of pollution of the tested water. In addition multiple damages on chromosome point to serious genotoxicity (Firbas,2011). However the types varied with the source of the onion bulbs. Chromosomal aberrations such as vagrant, stickiness,bridges,laggards were found in the onion bulbs. According to cytotoxic indicative parameter (% MI). Mitotic depression was observed in all water samples as

compared to control (Table- 2 & 3). After 24 hour exposure period, the MI index simultaneously decrease from site-A to site-E. This trend was also similar after 48 hour time duration in all samples. After long exposure time (96 hour), MI decrease in all samples as compared to 24 hour time duration as well as increase in time duration in *Allium cepa* was noticed in river water samples. Chromosomal aberrations like sticky chromosome in metaphase, bridge in anaphase, laggard in metaphase was noticed in some water samples (Plate-1). Further abnormalities, that appear are sticky chromosome in metaphase, laggard in metaphase ,gap damages were reported by various workers in their studies (Al-Sabti,1989, Panneerselman *et.al*.,2012). Positive results in higher plant systems indicate the presence of cytotoxic or genotoxic substances in the river water and indicate the potential for direct or indirect risks for living organism (Fiskesjo,1993). However, in the present study, the data indicate that presence of cytotoxic and genotoxic substances in Gomti river water, that are reduced by water treatment process. The cytotoxic or genotoxic compounds present in river water were not directly identified in this study but were assessed by various workers (Santos *et.al*.,2002 , Lucila *et.al.*,2007). The author suggested that the *Allium cepa* chromosomal assay is a reliable tool for monitoring genotoxic substance present in water. The goal of our research is to give an immediate and important contribution to preserving the health of the most precious life of river water. One to our lack of knowledge and carelessness, we have already polluted some water sources, therefore it is our obligation to correct our mistakes. However positive result in *Allium* test should be considered a signal of warning as this may constitute risk to environment and human health. Therefore it is recommended that river water be treated before they are used as irrigation, that safety of humans would be achieved.

Table-1: Root length of *Allium cepa* L. treated with Gomti river water at different time interval

Sites	Mean root length±S.E.(cm)	Mean root length±S.E.(cm)	Mean root length±S.E.(cm)	Mean root length±S.E.(cm)
	24 hour	48 hour	72 hour	96 hour
Site-A-1	1.9±0.17	3.7±0.13	4.1±0.23	5.3±0.21
Site-A-2	0.6 ±0.20	1.2±0.12	2.2±0.21	2.9±0.11
Site-A-3	0.4±0.11	0.9±0.11	4.4±0.22	5.2±0.14
Site-B-1	0.2±0.16	1.2±0.12	2.0±0.09	2.8±0.17
Site-B-2	0.3±0.13	1.2±0.11	3.2±0.13	3.9±0.16
Site-B-3	1.4 ±0.12	2.7±0.18	3.1±0.08	5.2±0.15
Site-C-1	1.6±0.11	2.3±0.20	3.4±0.03	3.5±0.17
Site-C-2	1.5±0.12	1.6±0.18	3.7±0.04	4.0±0.16
Site-C-3	1.8±0.16	2.8±0.19	4.8±0.07	4.9±0.12
Site-D-1	0.4±0.14	0.9±0.16	1.2±0.09	2.1±0.21
Site-D-2	0.9±0.16	1.1±0.15	1.6±0.07	2.0±0.22
Site-D-3	0.8±0.13	1.2±0.14	1.9±0.11	2.2±0.20

Site-E-1	0.2±0.15	1.4±0.11	1.6±0.12	2.0±0.22
Site-E-2	0.1±0.13	0.4±0.08	1.0±0.15	1.6±0.11
Site-E-3	1.5±0.10	1.9±0.09	1.2±0.13	1.9±0.13
Control	0.8±0.19	1.1±0.21	2.1±0.19	3.2±0.11

Values are mean of three replicates ± SE

Table-2: Cytological effects of Gomti river water on cells of *Allium cepa* L.

Sites	**24 hour**		**48 hour**		**72 hour**		**96 hour**	
	No. of dividing cells	Mitotic index %	No. of dividing cells	Mitotic index %	No. of dividing cells	Mitotic index %	No. of dividing cells	Mitotic index %
Site-A-1	224	22.4	264	26.4	156	15.6	152	15.2
Site-A-2	252	25.2	238	23.8	216	21.6	127	12.7
Site-A-3	244	24.4	216	21.6	176	17.6	123	12.3
Site-B-1	252	25.28	252	25.2	174	17.4	134	13.4
Site-B-2	260	26	176	17.6	178	17.8	114	11.4
Site-B-3	232	23.24	23.4	23.4	198	19.8	102	10.2
Site-C-1	195	19.56	19.6	19.6	232	23.2	92	9.28
Site-C-2	180	18.08	180	18	236	23.6	82	8.20
Site-C-3	174	17.44	168	16.8	244	24.4	86	8.62
Site-D-1	188	18.88	194	19.4	241	24.1	112	11.2
Site-D-2	198	19.84	171	17.1	258	25.8	98	9.85
Site-D-3	192	19.20	184	18.4	250	25.0	97	9.71
Site-E-1	168	16.80	178	17.8	202	20.2	117	11.7
Site-E-2	209	20.9	216	21.6	174	17.4	95	9.57
Site-E-3	180	18.0	15.6	15.6	186	18.6	78	7.85
Control	272	27.2	252	25.2	240	24.0	142	14.2

Table-3: Results from genotoxicity testing of river water of different sites in the *Allium cepa* L. root chromosomal aberration assay after 24 h and 48 h

Sites	24 hour						48 hour					
	No.of dividingcells	Chromosomal aberration				Total abe. Ratio %.	No.of dividingcells	Chromosomal aberration				Total abe. Ratio %
		Lg	Vg	Sc	Bg			Lg	Vg	Sc	Bg	
Site-A-1	224	2	0	0	1	1.3	264	2	2	3	1	3.03
Site-A-2	252	1	0	0	3	1.60	238	3	2	2	0	2.94
Site-A-3	244	2	1	0	1	1.19	216	1	1	0	3	2.31
Site-B-1	252	2	1	0	0	1.19	252	3	2	0	1	2.38
Site-B-2	260	3	0	1	2	2.30	176	1	3	2	0	3.40
Site-B-3	232	2	0	0	4	2.58	234	2	0	3	1	2.56
Site-C-1	195	2	4	4	3	6.66	196	4	3	3	2	6.12
Site-C-2	180	3	1	2	3	5.00	180	2	4	4	0	5.55
Site-C-3	174	4	5	2	1	6.89	168	3	2	3	2	5.95
Site-D-1	188	2	0	0	1	1.59	194	2	1	2	1	3.09
Site-D-2	198	3	0	2	1	3.03	171	0	0	1	2	1.75
Site-D-3	192	2	2	3	0	3.64	184	1	2	0	1	2.17
Site-E-1	168	0	1	1	2	2.38	178	0	1	2	1	2.24
Site-E-2	209	4	2	2	1	4.30	216	1	2	0	1	1.85
Site-E-3	180	3	1	2	0	3.33	156	2	3	1	2	5.12
Control	272	0	1	2	1	1.47	252	2	3	2	1	3.17

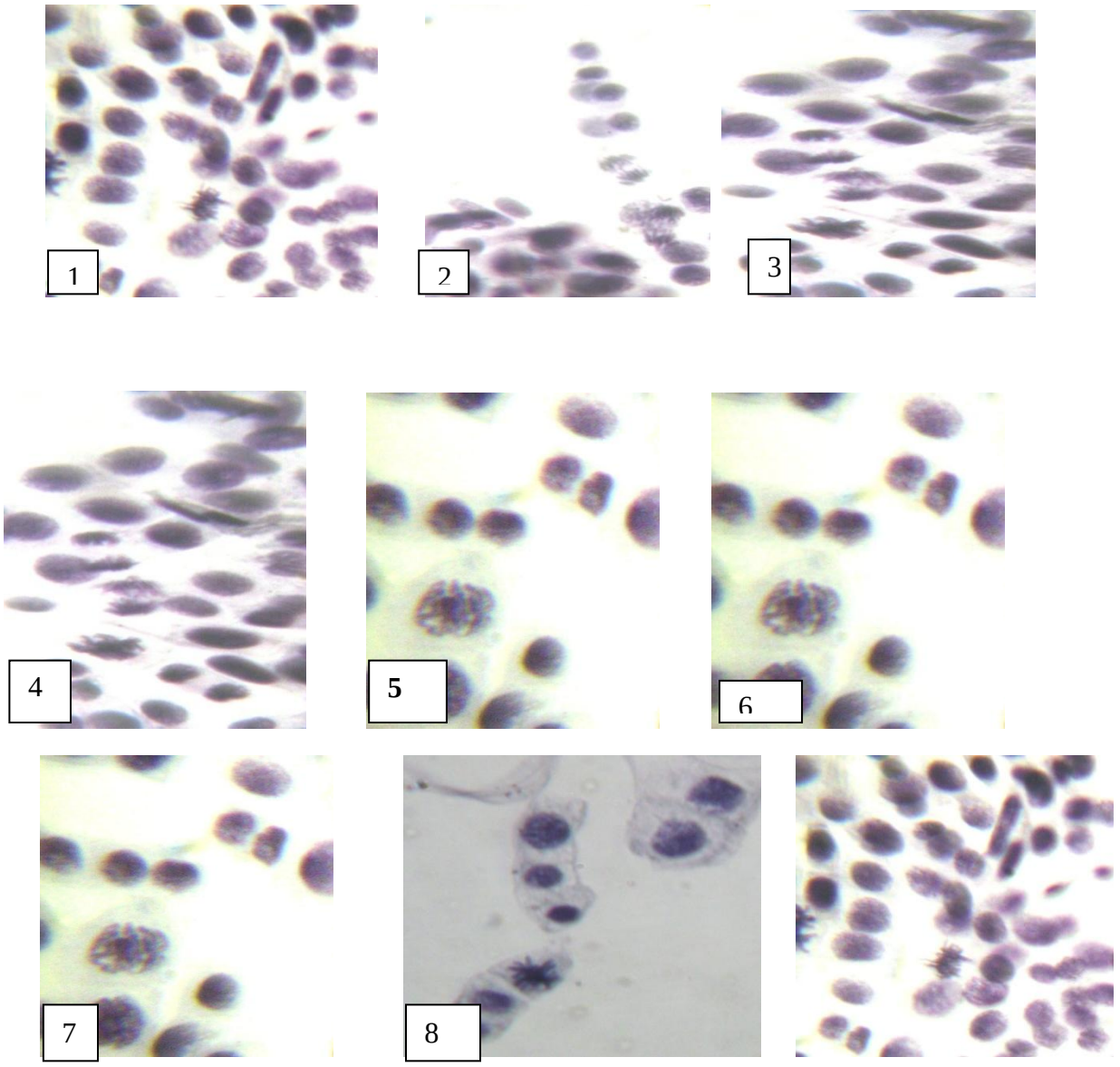
Note: Lg-Laggard, Vg- Vagrant, Sc- Stickness, Bg- Chromosomal bridge

Table-4: Results from genotoxicity testing of river water of different sites in the *Allium cepa* L. root chromosomal aberration assay after 72 hr and 96 hr

Sites	72 hour						96 hour					
	No.of dividingcells	Chromosomal aberration				Total abe. Ratio %	No.of dividingcells	Chromosomal aberration				Total abe. Ratio %.
		Lg	Vg	Sc	Bg			Lg	Vg	Sc	Bg	
Site-A-1	156	2	1	2	0	3.20	152	3	1	0	0	2.63
Site-A-2	216	1	3	1	1	2.77	127	2	0	0	1	2.36
Site-A-3	176	1	0	0	2	1.70	123	1	2	2	0	4.06
Site-B-1	174	1	1	0	3	2.87	134	2	0	0	3	3.73
Site-B-2	178	0	0	1	0	0.56	114	0	1	1	1	2.63
Site-B-3	198	1	3	2	1	3.53	102	0	2	1	1	3.92
Site-C-1	232	2	3	2	1	3.44	92	3	2	1	2	8.69
Site-C-2	236	1	2	0	2	2.11	82	4	1	2	1	9.75
Site-C-3	244	2	1	1	3	2.86	86	3	2	1	2	9.30
Site-D-1	241	3	1	1	0	2.07	112	3	2	1	2	7.14
Site-D-2	258	2	3	2	1	3.10	98	1	0	0	3	4.08
Site-D-3	250	1	2	0	3	2.40	97	2	0	0	4	6.18
Site-E-1	202	2	3	1	2	3.96	117	1	1	2	1	4.27
Site-E-2	174	1	0	0	1	1.14	95	2	1	1	0	4.21
Site-E-3	186	0	0	0	2	1.07	78	3	0	0	1	5.12
Control	240	1	2	0	0	1.25	142	1	0	0	1	1.40

Note: Lg-Laggard, Vg- Vagrant, Sc- Stickness, Bg- Chromosomal bridge

PLATE-1



Stages of mitosis and chromosomal aberrations induced in the cells of onion treated with river water after24h, 48h,72h and 96 h time interval. Abnormalities were observed (1) disturbed metaphase(2) anaphase (3) anaphase bridge(4) anaphase (5) prophase (6) interphase (7) sticky metaphase

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