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Chromosomes damage by sewage water studies in the *Allium cepa* L. and *Zea mays* L.

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Abstract. The effect of sewage water sample of the three locations Khardah (22.7003° N, 88.3753° E), Titagarh (22.7383° N, 88.3737° E), Madhyamgram (22.6924° N, 88.4653° E), and Control (distilled H_2O) in the district of North 24 Parganas (22.6168° N, 88.4029° E), West Bengal, India on the damage of chromosomes in the onion (*Allium cepa* L.) and maize plant (*Zea mays* L.) were investigated by employing mitotic chromosomal aberration assay. Physiochemical analysis of sewage water samples showed the pH is 5.10-5.30 in nature. Few heavy elements: Fe, Mn and Zn in the sample from Khardah (22.7003° N, 88.3753° E) sewage water exceeded the Indian Standard 10500:2012 and WHO's (2006) permissible limits. Whereas Cl, Cu, Pb, Cr, and Cd are more or less within limit of the standard condition. The obtained data exhibited a decline in reproductive capacity of cells and the occurrence of deviation from the normal mitotic cell division. The mitotic index (MI) decreased significantly ($p < 0.05$) in both the cases and is given as Control (57.03 %) > Madhyamgram (41.70 %) > Titagarh (33.85 %) > Khardah (31.57 %) in *Allium cepa* L. and Control (49.33 %) > Titagarh (21.45 %) > Madhyamgram (26.47 %) > Khardah (24.05 %) in *Zea mays* L. The chromosomal aberrations (CAs): Karyorrhexis, Karyolysis, Fragments, Lagging chromosome, Anaphase bridges are present in significant amount in the crops treated with sewage water sample than the one with control condition. Heavy metals act as pollutants in the sewage water sample which has cytotoxic effect on cells, threat to water ecosystem and human health.

Keywords: sewage water, mitotic index, indian standard, heavy metal, cytotoxic, ecosystem.

INTRODUCTION

Rapid industrialization in the last four decades has resulted in the mushrooming of production units even in the vicinity of semiurban and rural areas of the country. Several hazardous chemical industries discharge their untreated effluents into the atmosphere. Water and soil along with the eco-environmental profile of the area are adversely disturbed. The endemic exposure to pollutants causes toxicity, morbidity, early mortality, genetic and cytogenetic damage and various other pathological symptoms in the exposed human and plant populations.

This study was undertaken to evaluate the cytotoxic effects of effluents in sewage water of the three locations Khardah (22.7003° N, 88.3753° E), Titagarh (22.7383° N, 88.3737° E), and Madhyamgram (22.6924° N, 88.4653° E) of North 24 Parganas (22.6168° N, 88.4029° E), West Bengal, India. For more than forty years officially accepted *"Allium test"* is used widely for assessment of the environmental water pollution (Fiskesjo 1985, 1997; Ivanova et al. 2002, 2005; Rank 2003). Various investigators (Al-Sabti 1989; Smaka-Kinkl et al. 1996; Rank and Nielsen, 1998; Moraes and Jordao, 2001) advocate different plant test systems which are useful for studying cytotoxicity of heavy metals. Currently, the physio-chemical and cyto-toxicological evaluation of sewage water discharges from the three locations (T_1, T_2, and T_3) by different ways has not been documented, thereby no information on their hazardous effect on agricultural field and the ecosystem is recorded. With this background, the present work was undertaken to investigate chromosomal damage (cytotoxic) impact of sewage effluents collected from three different locations on root tip meristematic cells of *Allium cepa* L. and *Zea mays* L. with special reference to analysis of physio-chemical parameters of the liquid waste.

MATERIALS AND METHODS

Bluish and blackish sewage water was collected from the three main drains of T_1 = Khardah (22.7003° N, 88.3753° E), T_2 = Titagarh (22.7383° N, 88.3737° E), and T_3 = Madhyamgram (22.6924° N, 88.4653° E), of North 24 Parganas (22.6168° N, 88.4029° E), West Bengal, India at the depth of six inches from three random points within the drain of each location. The sewage water samples were filtered four times by muslin cloth and then stored in a clean plastic jar for chemicals analysis and setting experiment along with distilled water as a control (T_4) on two species namely, *Allium cepa* L. and *Zea mays* L.

Physicochemical parameters were analyzed from the three locations' (T_1, T_2, and T_3) sewage water samples (filtered four times) for a standard physicochemical property (chloride) according to IS:3025 (Part 32): 1988, RA 2003. The eight heavy metals, i.e., Copper (Cu), Chromium (Cr), Nickel (Ni), Iron (Fe), Zink (Zn), Cadmium (Cd), Lead (Pb), Manganese (Mn) were determined in mg/l, following the methods described in APHA 22nd edition 3125B and WHO-2006 limits (Olorunfemi et al. 2014).

Chromosome preparation was made from the treated root tips of both the species

Chromosome preparation was performed in both the species following the protocol adapted by Sharma and Sharma (1980). The root tips of treated and control sets of both species were fixed in Carnoy's fluid-I for overnight followed by treatment with 45% acetic acid for 10 minutes at room temperature. The resultant root samples were stained for 45 minutes with a mixture of 2% Aceto-Orcein:1N HCl (9:1) and warmed lightly at 60°C. The meristematic tip portion (~ 1mm size) of onion and maize roots were cut and placed on a clean grease free slide in a drop of acetic acid (45%) and squashed, later temporarily sealed with paraffin wax. Slides were prepared from five randomly drawn root tips from each treatment of both the species. Five random microscopic fields from each slide were scored under Olympus with the Prog-Res Capture Pro 2.1 photo system. The mitotic indices were calculated for all the treated materials of each treatment.

Statistical Analysis

The experiment was organized according to a randomized complete design (RCD) with three replications. A two-way ANOVA was performed for test of significance at $p<0.05$, employing F-test. Data were expressed as mean ± standard error (SEM) (Gomez and Gomez 1984). The mean mitotic index of each treatment was compared with those corresponding to control employing "t" test for significant difference, if any.

RESULTS

Heavy metals and chloride determination in the sewage water samples

The heavy metal and chloride analysis of the sewage water sample of the three locations have been shown in the Table 1. The sewage water collected from different locations were acidic in nature on pH scale: 5.30 (T_1), 5.15 (T_2) and 5.10 (T_3) during the middle of February, 2017.The sewage water sample from the three locations attained a higher range of iron concentration i.e., 0.34-0.52 mg/l as compared to the limit of 0.001-0.30 mg/l. The contents of copper, chromium, cadmium and lead were found less than the permissible limit (0.001 mg/l) in the sample of T_1, T_2, and T_3 treatments, except copper (0.006 mg/l) in T_3 treatment within limit compared with National (APHA 22nd edition 3125 B and IS: 10500:

Table 1. Contents of Heavy metals, Chloride and pH in the experimental sewage water samples (T_1, T_2 and T_3).

Sl. No.	Different parameters in the water samples of the three locations	Limit	Requirements As per IS 10500: 2012. Maximum	Result of the three locations or treatments (T_1, T_2, & T_3)			WHO (2006) Limit
				T_1=Khardah (22.7003^0 N, 88.3753^0 E)	T_2=Titagarh (22.7383^0 N, 88.3737^0 E)	T_3=Madhyamgram (22.6924^0 N, 88.4653^0 E)	
1	Copper (Cu)mg/l	0.001	Max:1.5	< 0.001	< 0.001	0.006	-
2	Chromium (Cr)mg/l	0.001	Max: 0.05	< 0.001	< 0.001	< 0.001	0.05
3	Nickel (Ni) mg/l	0.001	Max: 0.02	0.006	< 0.001	0.002	0.02
4	Iron (Fe) mg/l	0.001	Max: 0.30	0.340	0.501	0.520	-
5	Zink (Zn)mg/l	0.001	Max: 15.0	0.450	< 0.001	0.002	0.01
6	Cadmium (Cd)mg/l	0.001	Max: 0.003	< 0.001	< 0.001	<0.001	0.003
7	Lead (Pb) mg/l	0.001	Max: 0.01	< 0.001	< 0.001	< 0.001	0.01
8	Manganese (Mn) mg/l	0.001	Max: 0.30	0.321	0.275	0.101	-
9	Chloride (Cl)(mg/l)	N/A	Max: 1000	89.19	79.55	269.98	-
10	pH	-	-	5.30	5.15	5.10	6.5-9.5
11	Colour	-	-	Bluish	Blackish	Blackish	-

Contents of heavy metals & chloride present in the three experimental fields (T_1, T_2, & T_3) done by efrac (Edward Food Research & Analysis Centre Limited, Subash Nagar, P.O. Nilgunj Bazar, Barasat, Kolkata-700121, India. Email: efraclab@cfrac.org, Ph. No.91-3371122800.

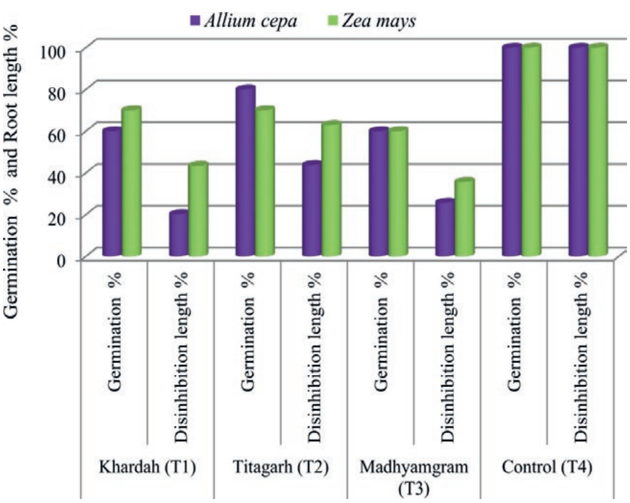

Figure 1. Effect of Sewage water and control samples (T_1, T_2, T_3, and T_4) on Germination % and Disinhibition root length % in *Allium cepa* L. and *Zea mays* L.

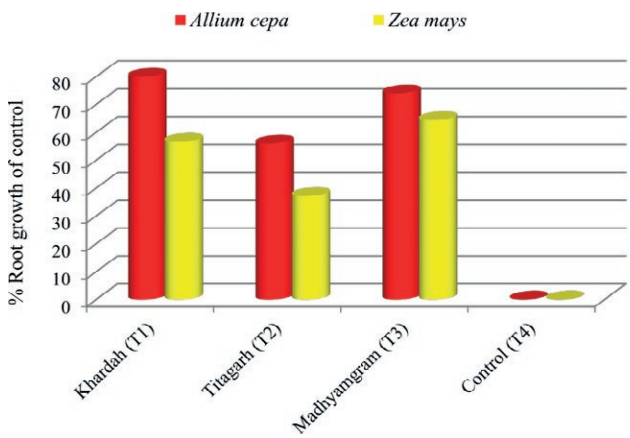

Figure 2. Percentage root growth of *Allium cepa* L. and *Zea mays* L. roots exposed to the test Sewage water and control samples (T_1, T_2, T_3 and T_4).

2012) and WHO's (2006) standards. The result revealed that the concentration of manganese (0.321 mg/l) content is higher in the sample of Khardah (22.7003° N, 88.3753° E) location while it was found in the range of limit (0.001-0.03 mg/l) in the sewage water sample of Titagarh (22.7383° N, 88.3737° E), and Madhyamgram (22.6924° N, 88.4653° E) respectively.

General toxicity-root growth inhibition and deformity of Allium cepa *L. &* Zea mays *L. test*

There was a significant ($p< 0.05$) root growth inhibition of the two species in the wastewater samples of three locations compared with distilled water (Figure 1**).** Root length in distilled water was higher than that in wastewater samples for both of them (Figure 2**).** Sewage water trials were compared with control treatment (distilled water). The mitotic index (MI) signifyingly decreased along with an increase in chromosomal aberrations (CAs) of the root tips meristematic cells of onion and maize were found (Table 2, Figure 5).

Table 2. Frequencies of different types of cells after treatment with different water samples (T_1, T_2, T_3 and T_4) Mean ± SE & ANOVA in both species.

Species	Locations	Total Cells	Dividing Cells	Cells of different stages				Abnormal cells					Mitotic Index (%)	Aberration Frequency (%)
				Prophase	Metaphase	Anaphase	Telophase	Karyorrhexis	Karyolysis	Fragmented	Laggard	Anaphase bridge		
Allium cepa L.	Khardah	608.80± 131.53	192.20± 49.46	173.20± 50.26	7.40± 1.69	8.00± 1.76	3.60± 1.86	29.20± 5.41	30.40± 5.97	3.40± 0.99	5.60± 1.27	5.60± 1.27	31.57	11.27
	Titagarh	582.60± 46.44	197.20± 57.71	174.80± 59.90	6.80± 2.14	12.00± 3.05	3.60± 1.69	65.40± 18.11	29.00± 9.57	2.00± 0.79	8.60± 2.17	8.60± 2.17	33.85	18.02
	Madhyamgram	620.20± 90.70	258.60± 44.17	238.40± 39.23	10.60± 3.20	6.80± 2.88	2.80± 1.83	22.00± 1.76	19.80± 7.09	2.60± 1.69	5.80± 2.77	5.80± 2.77	41.70	8.09
	Distilled water	678.20± 61.68	386.80 ± 33.06	357.00 ± 27.86	17.60 ± 8.92	7.6± 1.86	4.60± 2.68	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	57.03	0.00
	ANOVA	NS	**	**	**	**	NS	**						
Zea mays L.	Khardah	590.40± 114.33	142.00± 34.63	130.80± 33.33	9.60± 2.56	1.40± 1.69	0.40± 0.61	90.80± 24.85	0.00± 0.00	6.40± 2.31	0.80± 0.50	0.80± 0.50	24.05	16.60
	Titagarh	757.20± 40.05	214.60± 66.99	132.80± 29.82	9.80± 4.81	1.80± 1.45	0.80± 0.93	156.40±68.61	0.00± 0.00	5.40± 2.90	0.60 ± 0.61	0.60± 0.61	28.34	21.45
	Madhyamgram	564.60±142.05	149.40± 53.46	137.20± 51.74	7.00± 2.84	1.80± 0.93	3.40± 1.69	53.60± 16.32	0.00± 0.00	2.80± 1.22	0.80± 0.50	0.80± 0.50	26.46	10.13
	Distilled water	654.00± 39.87	322.6 ± 64.73	287.40 ± 53.67	29.80± 13.47	4.00± 2.08	1.40± 2.44	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.0± 00	49.33	0.00
	ANOVA	*	**	**	**	*	*	**						

Analysis of variance (ANOVA): Significant effects are indicated by as * = $p < 0.05$ (5%); ** = $p < 0.01$ (1%) and while non-significant effects are indicated by NS.

Allium cepa *L. test with sewage water samples*

Allium cepa L. meristematic cells in the root tips after 72 hours exposure to the different sewage water treatments exhibited various chromosomal aberrations (CAs) in comparison to distilled water (control) that included karyorrhexis, karyolysis, fragmentation, laggard, and anaphase bridge (Figure 3). T_1 has significantly decreased anaphase bridge (5.60 ± 1.27), laggard chromosome (5.60 ± 1.27), mitotic index (31.57%). T_2 has extensively decreased fragmentation (2.00 ± 0.79) only. T_3 has significantly decreased karyorrhexis (22.00 ± 1.76), karyolysis (19.80 ± 7.09) and aberration frequency (8.09%). The activities of different types of abnormal cells and aberration frequency % were seen to have a higher value in all samples (T_1, T_2, and T_3) as compared to control (T_4) in the onion (Table 2).

Zea mays *L. test with sewage water samples*

Chromosomal aberrations (CAs) induced in *Zea mays* L. root tips meristematic cells after 72 hours exposure to different waste water treatments (T_1, T_2, and T_3) in comparison with distilled water (T_4) were summarized in Figure 4. The treatment T_1 has significantly decreased only mitotic index (24.05%). T_2 has considerably decreased laggard chromosome (0.60 ± 0.61) and anaphase bridge (0.60 ± 0.61). T_3 has drastically decreased karyorrhexis (53.60 ±16.32), fragmentation (2.80 ± 1.22) and aberration frequency (10.13%). No effect on karyolysis was found in T_1, T_2 and T_3 samples. The activities of different types of abnormal cells and aberration frequency % was observed to have higher values in all sewage water samples (T_1, T_2, and T_3) as compared to control (T_4) in the maize (Table 2).

Different types of abnormal cells

The aberration shown at level of nucleus is of two types: (i) Karyorrhexis and Karylysis (ii) Chromosomes.

Karyorrhexis

It is the manner of destructive fragmentation of the nucleus of dying cells where the chromatin is irregularly distributed throughout the cytoplasm (Figure 3A, E, I, and Figure 4A, D, F).

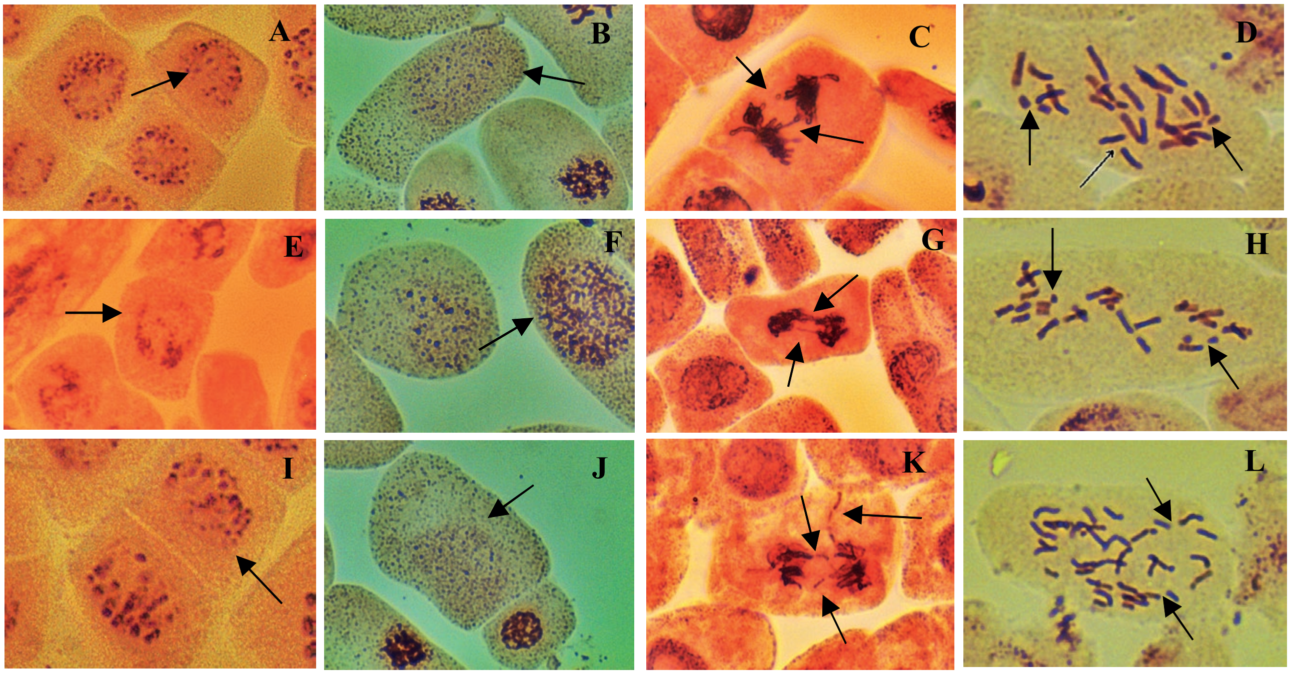

Figure 3. Photomicrographs of cytological aberration in *Allium cepa* L. (2n=16) root tip cells treated with different sewage water samples. (A) Karyorrhexis (B) Karyolysis (C) Anaphage bridge and (D) Fragmented, respectively in Khardah water; E. Karyorrhexis, F. Karyolysis, G. Anaphage bridge and H. Fragmented, respectively in Titagarh water; I. Karyorrhexis, J. Karyolysis, K. Laggard, Anaphase bridges and L. Fragmented in Madhyamgram water.

Karyolysis

Enzymatic dissolution leads to complete suspension of the chromatin in a dying cell. After karyolysis the whole cells will be stained uniformly (Figure 3B, F, J).

Chromosome laggard

Particular concentration of few turbagens which has affinity for thiol groups that induce various types of spindle disturbances at all stages of mitosis division in most of cells were confined to one or more number of chromosomes. The movement of chromosomes deviated from the main mass and were often seen to be lost. Such aberrant chromosomes have been called “laggards” (Figure 3K). It may be present in the location of spindle area or outside of it (Figure 3K).

Anaphase bridge

Chromatin bridge is a mitotic event that forms when telomeres of sister chromatids combine together and fail to completely segregate into their respective daughter cells. This event mostly occurs during the anaphase stage that is why called anaphase bridge (Figure 3C, G, K and Figure 4C, E, G).

Chromosome fragmentation

Chromosome fragmentation results are indicators of a clastogenic action from the numerous breaks in the chromosome arms where there is loss of integrity of the chromosome. Disintegration can range from partial to total breakup of the chromosome (Figure 3D, H, L and Figure 4B).

DISCUSSION

The research survey works (Ma 1999; Fatima and Ahmed 2005, 2006b) on industrial effluent samples that were taken from different parts of the city of Aligarh and Ghaziabad, UP in India. It may be used as a bio indicator for aquatic atmosphere. In our experiment different elements and microorganism present in the wastewater samples of the three locations might have induced cytological effects on the roots of both species i.e., *Allium cepa* L. and *Zea mays* L. It may have direct or indirect risk on their life due to irriga-

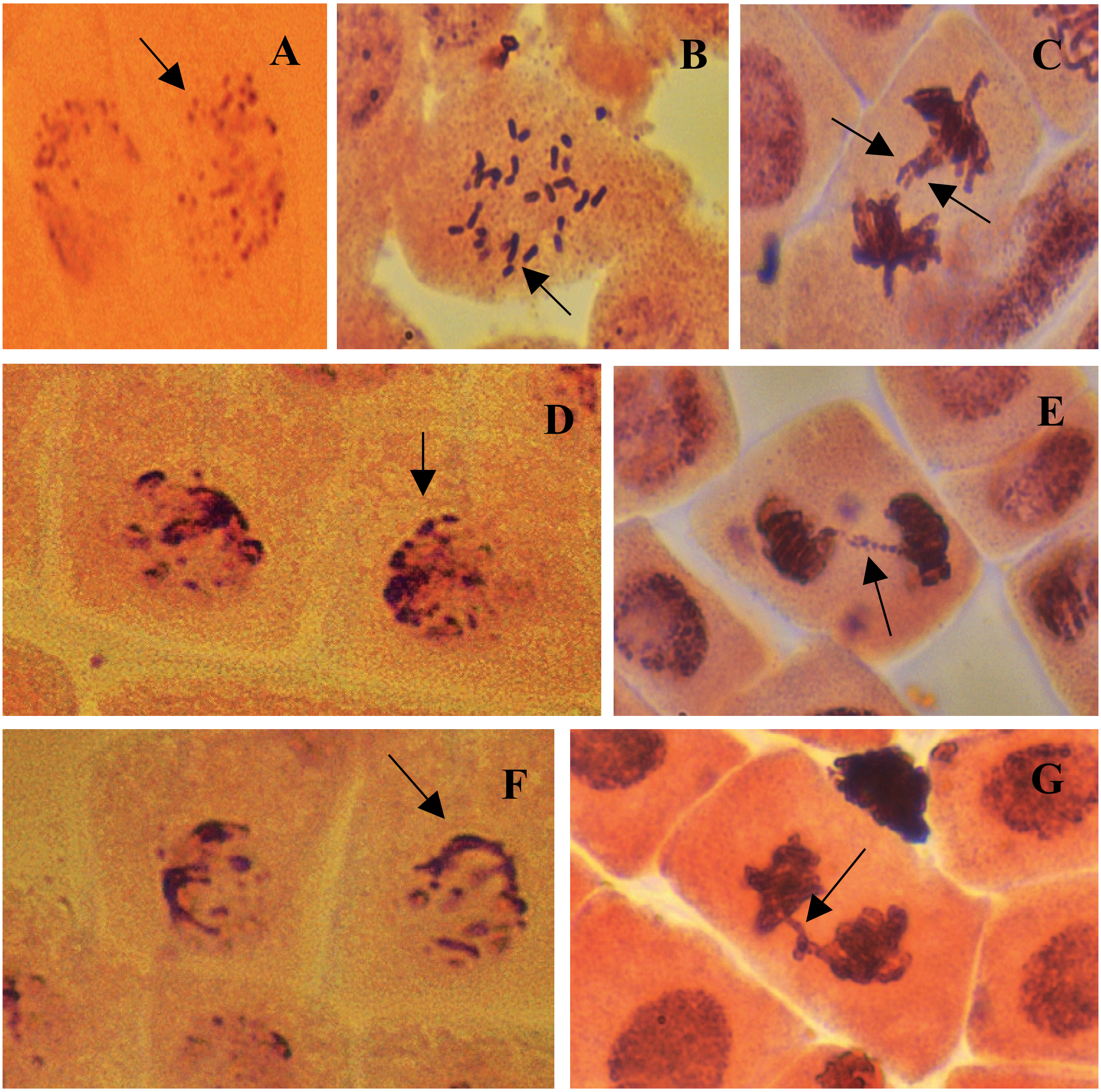

Figure 4. Photomicrographs of cytological aberration in *Zea mays* L. (2n=20) root tip cells treated with different sewage water samples. A. Karyorrhexis, B. Fragmented, C. Anaphage bridge, in Khardah water; D. Karyorrhexis and E. Anaphage bridges in Titagarh water; F. Karyorrhexis, and G. Anaphase bridge in Madhayagram water.

tion with sewage wastewater to the food plants (Iqbal et al. 2016; Chary et al. 2008). Trace elements accumulation in the food chain may harm different organism in the ecosystem along with humans. Several studies have shown that presence of various metal in the Industrial waste water can cause the various nature of chromosomal aberrations like lagging chromosome, fragmented chromosome, anaphase bridge and binucleated cells etc. in the meristematic root tip cells of *Allium cepa* L. and plants with such abnormalities which may induce alterations in the genetic constitution not only the future progenies but correspondingly have triggered further complication in mankind when consumed as nourishment materials (Sabeen et al. 2020). Surveys on sludge samples from thirty-four cities in the USA have reports that there were no effects of chromosomal aberration

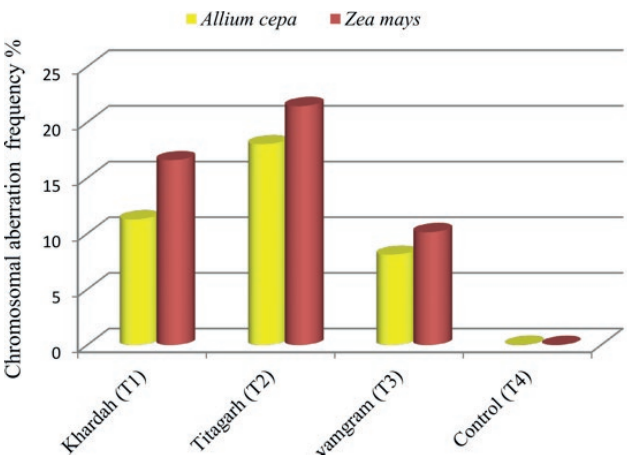

Figure 5. Effect of sewage water and control samples (T_1, T_2, T_3, andT_4) on Chromosomal aberration frequency % in *Allium cepa* L. and *Zea mays* L.

due to the treatment of effluents (Babish et al. 1983). Conversely, there are also studies, revealing cytological effect of extracts from wastewater sludges collected from various American cities on the test of *Salmonella typhimurium* sample (Mumma et al. 1988; Brown et al. 1991; Blevins and Brennan 1990). In 1998, White and Rasmussen demonstrated that in the large areas of metropolis cities, wastewaters of different municipalities are a multifaceted combination of effluent resources from domestic and industrial sewage, containing a widespread series of heavy or light constituents from a source of different varieties. Siddiqui et al. 2011 had strongly recommended that seed germination of different species such as *Brassica oleracea* var. capitata, *Pennisetum glaucum* and *Cucumis sativus* are remarkable living beings for heavy metal toxicological monitoring of industrial effluents and XAD concentrated river water. Furthermore, it was reported that significant quantities of different types chromosomal abnormalities including fragmentation, bridges and stickiness were found by *Allium cepa* test. Cytogenetic effect of the carbon black factory industrial effluents in *Allium sativum* root meristem cells not only retarded germination percentage and radical growth but also induced chromosomal aberrations: karyolysis, fragmentation, laggards (Ray and Saha 1992). In the absence of telomeres, chromosomes turned out to be adhesive in nature which may join the end part of other fragmented chromosomes in the root tip of meristem cells of *Allium cepa* in presence of alprazolam chemical compound (Nefic et al. 2013). The presence of breaking fragments, laggards, chromosome bridges and stickiness with other abnormalities are viewed as mitotic irregularities are due to an-eugenic agents (Zang and Yang 1994; Silveira et al. 2017; Haq et al. 2017). Grant (1982) told that chromosomes stickiness probably occurred due to degradation or de-polymerization of DNA segment of the chromosome. It was also reported that the sticking of chromosomes resulted from DNA compression and adhesiveness of inter-chromosome fibers (Schneiderman et al.1971). One of the abnormalities, which is stickiness and it shows high toxic substances are present along with irreversibility while acentric fragments that appear in anaphase stages are the result of chromatids or chromosome interruptions, representing interference with DNA. Bridges in Anaphase stage are the outcome of the disruptions and joining of chromatids or chromosomes (Turkoglu 2007). It is also described that anaphase bridges occur as an output of adhesiveness of chromosomes, unequal process of translocation or inversion in the segments of chromosome (Gomurgen 2005). Studies by Nagajyoti et al. (2010) and Fashola et al. (2016) indicates that among the heavy metal cadmium (Cd) is known to be carcinogenic and mutagenic in biological system. As per the investigation reported by Adhikari (2019) indicated that lead (Pb) one of heavy metal act as a robust mutagenic mediator on *Lathyrus sativus*. Nickel with magnesium can be the cause for chromatin condensation of the cells (Lee et al. 1995). Whereas it also stated that the combination of Nickel and Chromium affected the cell division of mitotic spindle leading to chromosomal aberration in the root's tips of *Allium cepa* (Anderson 1985). The trace amount of few metals such as Mn, Fe, Zn and Cr combined together or individually has caused the observed cytogenotoxic effects and reported to induce aberrations in the larvae of Newt (Godet et al, 1993). It also reported that heavy metals induced the toxicity and mutagenicity on *Zea mays L.* (Vojtechova and Leblova, 1991). The Mitotic index (MI) inhibition has been accredited to the effect of different environmental substances on DNA and synthesis of protein of the living organism (Chauhan et al. 1998). Nefic et al. 2013 revealed that the occurrence of high concentration of heavyweight metals in the earth sample triggered the downward movement of the Mitotic index of the meristematic root tips cells of *Allium cepa* L. Several heavy metals inhibit the cell division along with reduction of MI in the cortex of the meristematic root tips of *Zea mays* L. (Kozhevnikova 2009).

In this study, the *Allium cepa* L. and *Zea mays* L. roots anaphase-telophase assay at different stages of cells established that all the three wastewater samples had approximately same levels of toxicity. In the Titagarh (22.7383° N, 88.3737° E) samples, the aberration frequency percentage was however higher in both species than the other two samples of wastewater. Even the mitotic index percentage in all three wastewater location samples were half of the control sample (distilled water).

CONCLUSION

The study indicates that the heavy metals present in the wastewater samples in Khardah (T_1); Titagarh (T_2); and Madhyamgram (T_3) in the district of North 24 Parganas, West Bengal, induced chromosomal aberrations: Karyorrhexis, Karyolysis, Fragmented, Laggard and Anaphase bridge, reduced the Mitotic index and morphological structure also such as germination % and root length inhibitions % in *Allium cepa* L. and *Zea mays* L. It may be concluded that presence of heavy metals such as Ni, Ld, Mn, Fe, Cd leads to decrease cell reproduction and increase in the chromosome mutation frequency, posing a great potential threat to water ecosystem and human health as well. Thus, the investigation advocates treatment of wastewater of three locations for decreasing contamination load before releasing for irrigation in the agricultural field or into the rivers.

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