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GENOTOXIC EVALUATION OF INDUSTRIAL AND KITCHEN WASTEWATER USING *ALLIUM CEPA* ASSAY

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ABSTRACT

The genotoxicity of wastewater samples was investigated using *Allium cepa* assay. Wastewater samples were taken from marble cutting factory, cloth dying factory, cooking oil industry and residential kitchen from Karachi, Pakistan. Onion root tips were treated with different concentrations (10, 50 and 100%) of industrial and kitchen wastewater. Three slides per treatment and concentrations were prepared. It was noticed that mitotic index decreased by increasing the concentration of wastewater. The results also showed that wastewater treatments produced various types of chromosomal aberrations, where stickiness was most frequently occurring aberration. It was also observed that effect of marble and kitchen wastewater was drastic, as most of the cells were ruptured at 100% concentration. Frequency of aberrations was different in different samples.

Key words: Wastewater, *Allium cepa,* mitotic index, chromosomal aberrations

INTRODUCTION

Most industries in developing countries (including Pakistan) have incomplete and inefficient sewerage system and discharge their wastewater into land, lakes, rivers and canals (Abu and Ezeugwu, 2008; Grover and Kaur ,1999). and contribute large amount of pollutants (El-Shahaby *et.al.*, 2003). Effluents from industries contain hazardous genotoxic chemicals, many of them do not undergo degradation during wastewater treatment due to high degree of persistence, or end up with wastewater sludge due to lipophilic nature. Industrial as well as sewage wastewater may contain many heavy metals like copper, iron, zinc, cadmium, lead, chromium and mercury (Fiskesjo, 1988; Ukaegbu and Peter, 2009). It is known that pollution lowers the quality of life in various aspects. The subtle danger of pollution lies in the fact that they could be mutagenic or toxic that can cause various human diseases like cancer, cardiovascular disease, and premature aging (Grover and Kaur, 1999). Karachi is the largest industrial city of Pakistan, where different types of industries are in full operation such as textile mills, petroleum refinery, cooking oil industries, dying factories, marble factories, and leather industries. Wastewater from these industries could be the serious threat to the peoples living in Karachi. There are several plant assay systems to monitor effect of genotoxic substances in the environment (Ukaegbu and Peter, 2009). Plant assays are useful and easy in testing complex environmental samples like wastewater (Grover and Kaur, 1999). Allium test using *Allium cepa* L, is convenient and standard test system for estimating the harmful effects of various chemicals on living organisms (Fiskesjo, 1993; Rank and Nielsen, 1998), because its excellent chromosome conditions (Abu and Mba, 2011). Onion roots are very effective to evaluate the genotoxic effect of wastewater, because the root tips are first to be exposed to chemicals (Odeigah *et al.,* 1997). Root tips are the best material for the evaluation of toxic substances because total plant growth and development depends on mitotic process in plant meristematic regions. Allium test has high correlation with other system (MIT-217 cell test with mice, rats or humans in vivo) and could be used as an alternative to laboratory animal in toxicological research (Fiskesjo and Levan, 1993). Chromosomal aberrations in *Allium cepa* seeds exposed to industrial effluent contaminated with azo dyes, such as abnormal anaphases, fragments, loss of chromosomes, C-metaphase, micronuclei and multinucleated cells (Carita and Marin-Morales, 2008). Similarly (Samuel *et al.*, 2010) reported that paint and textile industrial effluents induced chromosomal aberrations in onion root tips, where bridges, fragments and stickiness were most frequently observed.

MATERIALS AND METHODS

Test organism

Healthy and uniform in size onion bulbs were obtained from local market of Karachi and used for this study.

Test agents

Marble cutting, cloth dying, cooking oil industry and kitchen wastewater samples were used as test agents for current study. Samples were collected directly from discharge pipes. Wastewater samples were collected in plastic containers and stored in the refrigerator till dilutions [0 (T1), 10 (T2), 50 (T3) and 100% (T4)] with distilled water. Distilled water served as control.

Cytological study

Cytological experiments were carried out in may 2004 using different wastewater samples in various concentrations along with control. Experiment was performed according to El-Shahaby *et.al.*, (2003) with minor modifications, outer dry scales of onion bulbs were carefully removed and bottom dry plate was scraped away without destroying the root primordia These onions were inverted on beaker containing distilled water (d/w) in such a way that their basal part should dip into distilled water and allowed them to grow for two days. Onion bulbs were transferred on another beaker containing wastewater samples and left for 1 h. For control distilled water was used. Three bulbs per treatment were used. Root tips were cut and treated with Carnoy's solution (alcohol: acetic acid in 3:1 ratio) for 24 h. Root tips were hydrolyzed in a solution of 1N HCl at 60°C for 5 min and stained with 1.8% aceto-orcin stain for 24 h. The squash technique was applied for the study of mitotic index (MI) and chromosomal aberrations. Three slides were made for each treatment and concentrations. Data was collected for number of dividing cells out of 500 cells at 40X objective. All slides were coded and examined for mitotic index and scoring normal and aberrant chromosomes in cells. Photographs were taken. Mitotic index was estimated as number of dividing cells over 500 cells expressed in percentage (Rank *et al.,* 2002). Chromosomal aberrations were examined in 500 cells for each treatment.

RESULTS

Allium test is considered as a standard and quick method to study the effect of genotoxic substances. It was observed that wastewater samples suppressed cell division. When onion roots were treated with different wastewater samples of various concentrations (0, 10, 50 and 100%), MI reduced as concentration was increased (Figure 1). For 10% concentration of marble wastewater sample MI was 6.91% (inhibited to 26.2%), which further inhibited to 53.7% for 50% concentration and 100% inhibited for undiluted sample (Figure 2). There was 6.37% MI (31.94% inhibition) when onion roots were treated with 10% concentration of wastewater of cloth dying industry, which was further inhibited to 45.94% and 53.73% at 50 and 100% concentrations respectively. Similar pattern of MI was observed for cooking oil and kitchen wastewater samples. At 100% kitchen wastewater, there was numerous ruptured cells.

Analysis of variance (ANOVA) showed that there was significant difference between treatment, concentrations, aberrations and interactions (Table 1). Percentage of normal cells was increased in control samples which reduced by the increase in concentration. Result also revealed that various concentrations of different wastewater samples possessed significant effect on percent chromosomal aberrations. Duncan's Multiple range test was applied to test for significant difference ($P<0.05$) in the mean chromosomal aberrations in onion root tip cells exposed to various wastewater samples of different concentrations (Table 2). Chromosomal aberrations observed in this research were stickiness at metaphase and anaphase, dislocated metaphase, dislocated anaphase, fragmented chromosomes, bridges, forwarded chromosomes, multipolar anaphase, lagging chromosomes, and C-mitosis (Table 3). It was noticed that stickiness and dislocated metaphase were the most frequently occurring aberrations for any treatment and concentrations while least frequently observed aberrations were lagging chromosomes and C-mitosis, which was present only at 50% marble and Dying samples respectively.

Marble waste water (10%) was responsible to produce sticky metaphase, dislocated metaphase and Bridges. Where dislocated metaphase was significantly more than bridges which in turn more than dislocated metaphase. When the concentration was 50%, dislocated metaphase was significantly high, but at 100% concentration the difference was significantly high for sticky and dislocated metaphase. Cloth dying wastewater sample at 10% concentration revealed significantly high percentage for sticky metaphase, whereas at 50 and 100% concentration dislocated metaphase was significantly more frequent.

Test agent from cooking oil industry revealed sticky anaphase with significantly highest percentage at 10 % concentration. Fragmented chromosomes were significantly more at 50% concentrated oil wastewater sample while at 100% concentration, sticky metaphase and anaphase were most frequently observed. The effect of kitchen wastewater sample was more drastic on cell cycle at 100% concentration. However at 50% concentration four different types of chromosomal aberrations were detected, where sticky metaphase and dislocated metaphases were at high percentages.

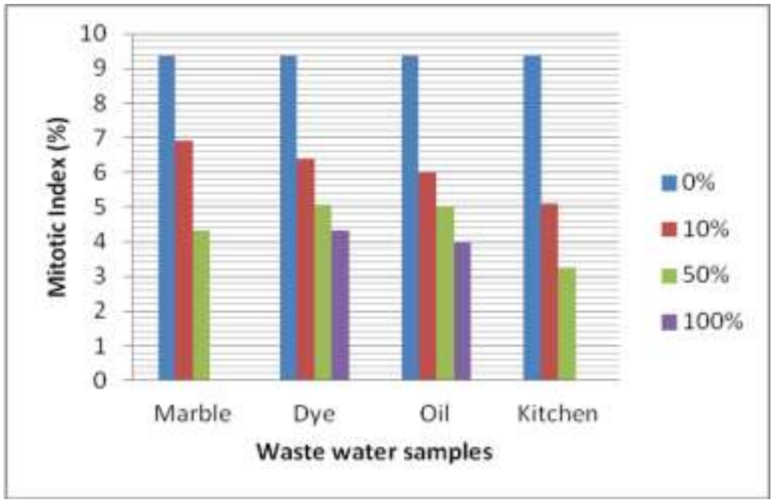

Fig. 1.The effect of control and different concentrations on MI of onion root tips treated with four different wastewater samples.

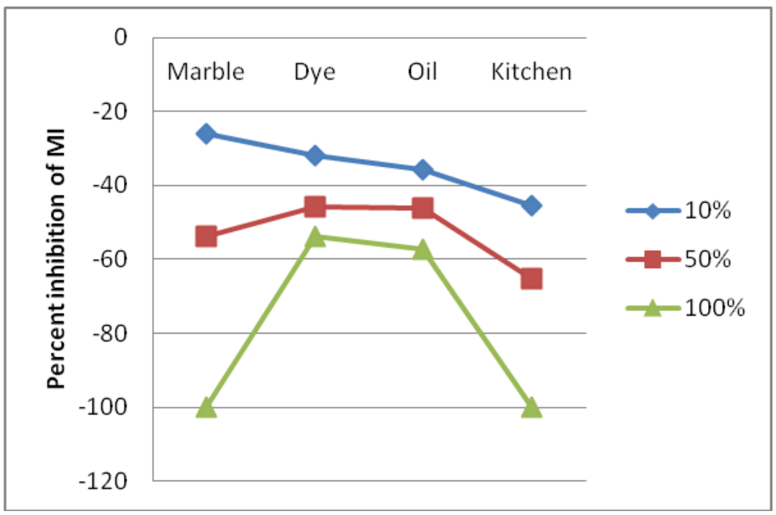

Fig. 2. Percent inhibition of MI on onion root tips treated with different concentrations of four wastewater samples.

Table 1. Mean squares for waste water treatments, concentration, and aberrations in onion root tips.

Sources of Variation	df	MS
Test agent (T)	3	124.01*
Concentrations(C)	3	2031.41*
Aberrations(A)	9	2372.84*
T x C	9	225.81*
T x A	27	211.61*
C X A	27	465.7*
T X C X A	81	215.8*
Error	318	0.234

Table 2. Mitotic index and mean comparison for chromosomal aberrations by Duncan's multiple range test (at 5% Probability) on onion root tips, treated with various waste water test agents of different concentrations.

Test agents	Conc. (%)	Mitotic index (MI)	Percent of normal cells	Chromosomal aberrations Percent: Sticky metaphase	Sticky anaphase	dislocated metaphase	dislocated anaphase	Fragmented chromosomes	Bridges	Forwarded anaphase	multipolar anaphase	Lagging chromosomes	C-mitosis
Control	0	9.36± 0.061	48.5	0	0	0	0	0	0	0	0	0	0
Marble	10	6.91 ± 0.039	26.08	13.44^{c}± 0.53	0	26.42^{a}± 0.60	0	0	17.33^{b} ± 0.29	0	0	0	0
	50	4.33 ± 0.046	25.71	18.41^{b}± 0.68	17.08^{c} ± 0.43	20.3^{a}± 0.93	6.07^{e}± 0.23	11.36^{d}± 0.26	0	0	0	2.51^{f}± 0.187	0
	100	0	0	36.91^{a} ± 0.37	10.87^{b}± 0.196	36.89^{a}± 0.65	0	9.9^{c}± 0.3	5.48^{d} ± 0.36	0	0	0	0
Dying	10	6.37 ±0.022	25.92	30.74^{a} ± 0.56	14.57^{b}± 0.394	11^{c}± 0.06	4.0^{e}± 0.251	7.80^{d} ± 0.23	0	7.23^{d} ±0.12	0	0	0
	50	5.06± 0.0315	12	16.4^{b}± 0.61	11.93^{c}± 0.52	50.26^{a} ± 0.98	0	4.35^{d} ± 0.176	0	0	0	0	4.3^{d} ±0.152
	100	5.06± 0.0316	0	33.03^{b}± 1.56	7.3^{c}± 0.26	46.58^{a}± 1.08	3.8^{d} ± 1.0	0	0	0	0	0	0
Oil	10	5.06± 0.0317	38.46	0	23.5^{a}± 0.27	11.53^{b}± 0.56	0	3.74^{c}± 0.12	12.1^{b} ± 0.32	11.78^{b}±1.3	0	0	0
	50	5.06± 0.0318	14.8	11.53^{c}± 0.48	7.36^{d}± 0.08	21.1^{b}± 0.57	0	59.58^{a} ± 0.38	11.58^{c}± 0.27	0	0	0	0
	100	5.06± 0.0319	4.7	28.97^{a} ± 0.82	19.21^{b} ± 0.46	27.92^{a}± 0.32	0	19.51^{b} ± 0.54	0	0	0	0	0
Kitchen	10	5.06± 0.0320	35.29	28.97^{a}± 0.34	23.67^{b}± 0.163	0	0	0	11.97^{c}± 0.127	0	0	0	0
	50	5.06± 0.0321	0	51.26^{a} ± 0.54	4.1^{d} ± 0.066	47.89^{b} ± 0.057	0	0	8.43^{c}± 0.33	0	0	0	0
	100	5.06± 0.0322	0	-	-	-	-	-	-	-	-	-	-

DISCUSSIONS

It is reported by many researchers that there is presence of heavy metals in effluents of many industries which are hazardous directly or indirectly for any living organism. Genotoxic effect of these hazardous chemicals has been studied for many years using higher plants as biological system (Rank *et al*., 2002). MI is served as bio-monitor to assess the genotoxicity of industrial effluents (Fiskesjo, 1985). Current study showed that the MI decreased as the concentration of wastewater was increased, supported by the work of Samuel *et al*., (2010) found the decrease in MI by increasing the concentration of paint and textile industrial effluent. Olive oil and milk industrial wastewater also reduces MI (Aksoy *et al.,* 2011). This may be due to the increase in concentration of heavy metals or other toxic substances by increasing wastewater concentration (Abu and Ezeugwu, 2008). It indicated that genotoxic effect of wastewater samples and that this mitodipressive effect was dose dependent. At 100% concentration of marble and kitchen wastewater sample, mitosis was completely inhibited. MI decreased as concentration of sewage effluent was increased and at 80% concentration there was no mitosis (Ukaegbu and Peter, 2009).

Presence of heavy metals in wastewater samples could be the reason of chromosomal aberrations in *Allium cepa* root tips, with no aberrations in control. It was noticed that more than one kind of chromosomal aberrations was found per cell and treatment, however the type of aberrations varied. Stickiness at metaphase or at anaphase were most frequently observed aberrations in all treatments and concentrations. Same results were reported by (Ukaegbu and Peter, 2009) in sewage effluent and according to (Fiskesjo, 1985), stickiness are indicative of a highly toxic and usually irreversible toxic effect leading to cell death. It could be responsible for complete decay of roots especially at high concentrations (Ukaegbu and Peter, 2009), probably leading to cell death (Fiskesjo, 1985). Sticky chromosomes could be due to the incomplete replication of chromosomes by defective enzymes (Bennet, 1977). According to (Amin, 2002), chromosomes stickiness is probably due to immediate reactions with DNA during its inhibition periods, creating DNA-DNA or DNA-Protein cross linking. Chromosome stickiness is due to improper folding of chromatin fiber into single chromatid and chromosome, thus unable to freely separate (Rad *et al.,* 2011).

Lagging chromosomes have been reported in *Allium cepa* root tips, treated with wastewater of copper mine (Inceer *et al.,* 2000). *Allium cepa* root tips exposed to sewage effluent (Ukaegbu and Peter, 2009). Formation of laggard chromosome orientation may be due to the failure of spindle apparatus to organize in the normal way (Kaymak, 2005). C-mitosis was detected only in 50% concentration of cloth dying wastewater; however it was reported by many workers studying cytotoxic effect of various types of wastewater. C-mitotic effects indicate risk of aneuploidy (Fiskesjo, 1985) and it is the common sign of spindle inhibition (Abu and Mba, 2011).

Conclusion

In general, chromosomal aberrations indicate that wastewater samples were responsible for genotoxic effects. Use of untreated wastewater for irrigation purposes could be considered as an alarming factor of risk to the environment and human health. It is therefore recommended that these wastewater samples be treated before using for irrigating any crop.

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