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Genotoxicity Screening of Industrial Wastewater Using the *Allium cepa* Chromosome Aberration Assay

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Abstract: Response of the *Allium cepa* genetic material to the presense of potential cytotoxic and genotoxic substances in aquatic environment was used to evaluate the toxicity of industrial wastewater collected from 4 different sites: Shawa, Meet El Akrad, Telbana and Belgay along the new Mansoura drain in Sandub area, Dakahlia Province. For *in situ* monitoring of the cytotoxicity level, the inhibition of mitotic division in meristematic cells, was assayed. For testing the genotoxicity of the collected water samples, two assays were used, chromosome aberration assay in mitotic cells and micronucleus assay (MNC) in interphase cells. The results indicate that all the water samples collected from the drain were highly mutagenic. Concentrations of the heavy metals (Pb, Zn, Co, Cd, Cu) were also determined and could partly be correlated with the toxicity of water and industrial load of Sandub area.

Key words: Genotoxicity, *Allium cepa*, industrial wastewater, chromosome aberration assay, micronucleus assay

Introduction

Pollution is a major problem of some countries. With rapid strides in industrialization of these countries, the problem is being further accentuated. Some of the cities in the world are having incomplete sewerage and therefore discharge its wastewater into large lakes, rivers and canals or drains (Grover and Kaur, 1999). It is well established that pollution lowers the quality of life in various aspects. Besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human afflictions like cancer, cardiovascular diseases and premature ageing (Grover and Kaur, 1999). Most industrial wastewater can be characterized as extremely complex mixtures containing numerous inorganic as well as organic compounds (Nielsen and Rank, 1994). The complexity makes it almost impossible to carry out a hazard assessment based on chemical analysis.

In the search for test systems which combined with the chemical analysis, can be used to provide data as a scientific basis for regulating the discharge of potentially hazardous substances into the environment and suitable for performance of toxicity evaluation, the *Allium* test seems to have some advantages. The *Allium* test for genotoxicity was introduced by Levan (1938) and has been used on wastewater in other studies (Ravindran, 1978; Shanthamurthy and Rangaswamy, 1979; Smaka-Kincl *et al.*, 1996; Mishra, 1993). It was proposed as a standard method in environmental monitoring and toxicity screening of wastewater and river water (Fiskesjo, 1985, 1993; Rank and Nielsen,1993, 1998). The test can be used without any condensation, purification or sterilization of the wastewater. Furthermore the test is easy to handle, it has low cost and it shows good correlation with mammalian test systems (Fiskesjo, 1985).

In recent years, an industrialization movement in several cities, has contributed large amounts of pollutants through effluents being deposited into the water of drains. The new mansoura Drain is considered as one of the main agricultural drainage system in Mansoura district. It receives effluents of factories with different lines of business (e.g. factory of oils and soap, factory of resins and chemicals, Detex factory, etc.). The present work was done to evaluate the genotoxic effects of industrial wastewater of different sites in Sandub industrial region using the *Allium cepa* chromosome aberration and micronucleus (MNC) assays.

Materials and Methods

Sampling sites and analysis of samples: The sampling sites were selected at the industrial Sandub area in Mansoura district. Water samples were collected from an area covering a distance of 6 km along the course of the new mansoura drain from sites 1-4 ((Shawa, Meet El-Akrad, Telbana and Belgay). Shawa and Belgay are upstream of the discharge point and Meet El-Akrad and Telbana are downstream.

At the time of collection the water reaction (pH) and electrical conductivity (EC) of the samples were recorded and the samples were analyzed for Cl, SO_4, CO_3, Na, Ca and Mg. The concentrations of some potentially mutagenic heavy metal ions (Zn, Cd, Co, Cu, Pb) were

assessed too. The procedures followed in the water analysis are according to Piper (1947), the U.S. Salinity Laboratory Staff (1954), Jackson (1962) and Allen *et al.* (1974). Samples were assayed with the *Allium* chromosome aberration assay and *Allium* MNC assay.

Chromosome aberration and MNC assays: The *Allium* test was carried out as described by Fiskesjo (1985) and later modified by Rank and Nielsen (1993). Commercial onion bulbs (*Allium cepa)*, were obtained from the National Institute of Agriculture, Cairo. The onions were not treated with any growth inhibitors. Before use the loose outer scales were carefully removed and the dry bottom plates were scraped away without destroying the root primordia.

For each water sample six onions were set up and allowed to produce roots in tap water for 2 days. The tap water was changed every day. On the second day the bulb with the poorest growth in each set was discarded and the other five transferred to the wastewater samples. Tap water was used as a control and handled alike for all the experiments. After 24 h treatment, the root tips were fixed immediately in aceto-alcohol (1:3). After fixation, the slides were prepared for examination using 5-6 root tips from each bulb. The root tips were macerated in drops of 1N HCl at 60°C for 3 min followed by staining in Carbol Fuchsin stain (Koa, 1975). Root tips were then squashed in a 2% aceto-orcein stain in 45% acetic acid. Slides were made permanent, mounted in canada balsam, examined and photographed.

The mitotic index and chromosome aberrations were determined by examination of 400 cells per slide and calculated as mitotic cells per 1000 cells. Chromosome aberrations were characterized and classified in the following categories: bridges, fragments, laggard chromosomes, micronuclei and other aberrations, e.g. chromosome ring, stickiness and disturbed (anaphase, metaphase and telophase). Micronuclei were scored in interphase cells. All slides were coded and examined blind. Statistical analysis was performed using the χ^2-test (Snedecor and Cochran, 1968; Dougherty, 1990).

Results

The influence of various genotoxic substances from the water samples collected from the study sites was determined by analyzing the types and frequencies of aberrant metaphase, anaphase and telophase cells, as well as by determining the existence of micronuclei in interphase cells (Table 2). Samples showed positive

Table 1: Chemical analysis of water samples collected from different sites along the new mansoura drain of Sandub area in Dakahlia Province

Site	pH	EC (umhos/cm)	Cl (%)	SO_4 (%)	HCO_3 (%)	CO_3 (%)	Na	Ca	Mg	Co	Cd	Zn	Cu	Pb
Shawa	8.01	750	0.06	0.03	0.12	0.0	28	5.7	43	0.40	0.10	0.15	0.20	0.29
Meet El-Akrad	8.18	650	0.06	0.04	0.12	0.0	33	1.2	53	0.20	0.10	0.22	0.40	0.43
Telbana	8.20	800	0.08	0.04	0.23	0.0	35	1.0	50	0.40	0.15	0.10	0.40	0.43
Belgay	8.34	850	0.08	0.03	0.20	0.0	9.1	26.0	7.3	0.40	0.10	0.20	0.40	0.43

Concentrations of heavy metals were measured in mg l^{-1}

Table 2: Chromosome aberration and MNC assays in *Allium cepa* root cells exposed to wastewater samples from different sites along the new mansoura drain. Total frequency of aberrations were expressed as a percentage of the total dividing cells, different types of abnormalities were expressed as a percentage of the number of cells in each phase

	Chromosome aberration assay									MNC Assay	
	Aberrant prophases (%)	Aberrant metaphases (%)		Aberrant anaphases (%)				Aberrant telophases			
Sites	Micronucleus	Disturbed	Chr. ring	Disturbed	Asynchronyony	Fragment	Late *separation*	*Disturbed*	*Bridge*	Total Abn. (%)	MNC (%)
Control	8.0	0.0	0.0	20.4	0.0	1.9	0.0	10.7	0.0	26.2	4.0
Shawa	0.0	0.0	8.7	38.1	0.0	0.0	0.0	14.3	7.1	89.2	52.0
Meet El-Akrad	0.0	8.0	0.0	24.0	4.0	4.0	0.0	21.2	3.0	80.0	24.0
Telbana	4.0	6.7	0.0	25.0	0.0	7.1	3.6	45.5	3.0	104.2	10.0
Belgay	0.0	0.0	0.0	0.0	0.0	25.0	0.0	29.2	0.0	112.8	41.0

Table 3: Results of mitotic inhibition test in *Allium cepa* root cells exposed to wastewater samples from different sites along the new mansoura drain

Sampling sites	Total divided cells Mean±SE	Mitotic index Mean±SE	Phase index (%)±SE Prophase	Metaphase	Anaphase	Telophase
Control	135.29±1.0	13.53±0.29	20.83±0.12	34.58±0.16	22.50 ±0.09	23.33±0.12
Shawa	51.11±1.42*	5.11±0.18*	30.12±0.10*	27.71±0.09*	25.30±0.07*	16.87±0.06*
Meet El Akrad	61.41±1.81*	6.14±0.21*	16.16±0.08N	25.25±0.09*	25.25±0.09*	33.33±0.11NS
Telbana	83.88±1.59*	8.39±0.24*	21.55±0.09*	25.86±0.10*	24.14±0.09	28.45±0.12NS
Belgay	46.17±1.71*	4.62±0.17*	23.21±0.08*	19.46±0.09*	14.29±0.07*	42.86±0.11NS

* Significant at 0.05 level from control, NS: not significant, Total examined cells= 1000

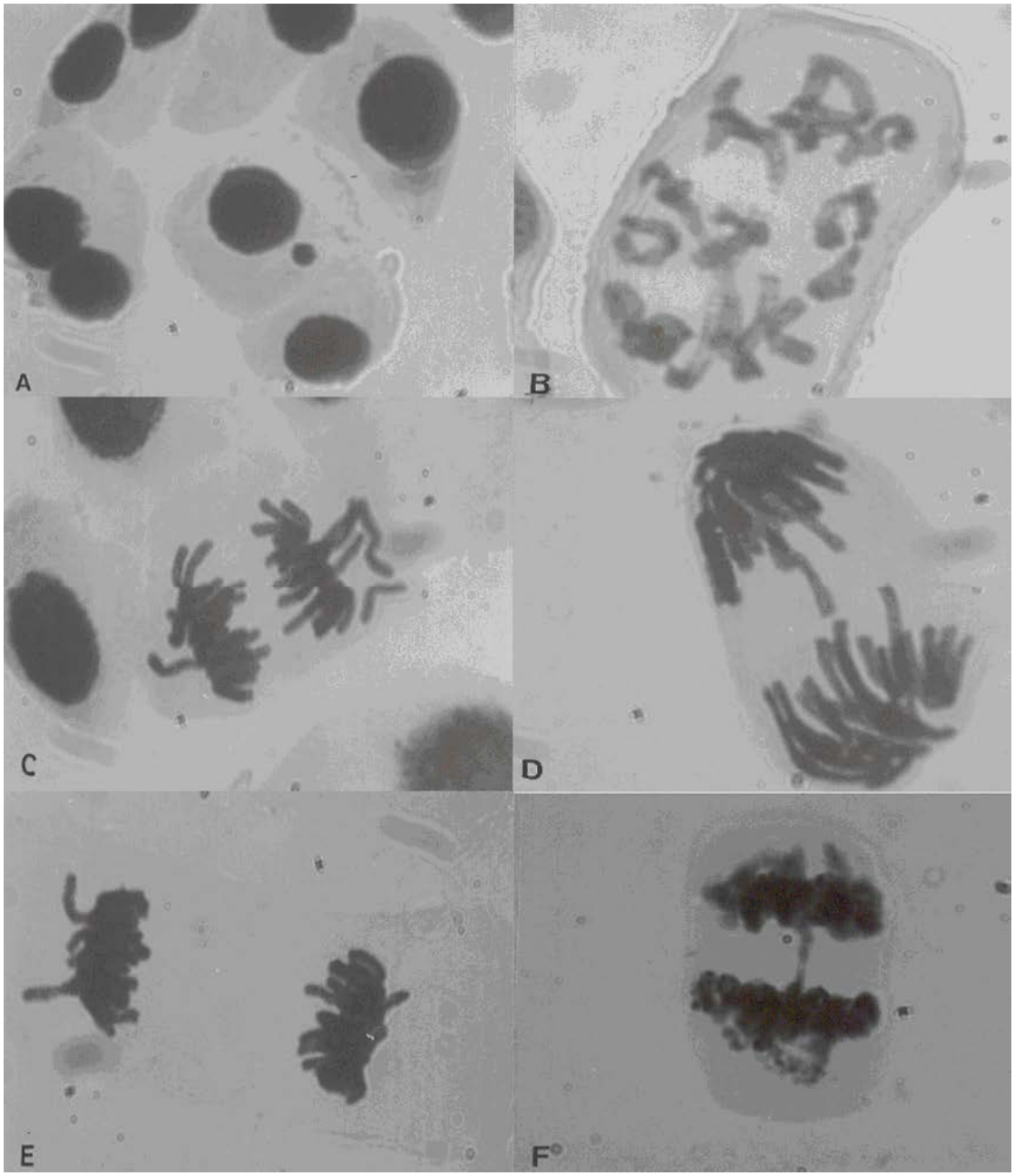

Fig 1: Different chromosome aberrations of treated *Allium cepa* with industrial wastewater in Sandub industrial area. (A) micronucleus at interphase, (B) chromosome ring, (C) disturbed anaphase, (D) late separation of chromosomes at anaphase, (E) disturbed telophase and (F) bridge at telophase (x= 1000).

responses under the *Allium* MNC and chromosome aberration assay when compared with the control. The mitotic index could be another endpoint for the general toxicity. There were significant differences in the mitotic index between all the water samples and the control (Table 3).

Chemical water analysis: Results showed that the water reaction is slightly alkaline with pH values varied from 8.01-8.34 (Table 1). The lowest value of EC (650 umhos/cm) is attained in Meet El- Akrad site while the highest value (850 umhos/cm) is attained in Belgay site. The contents of chloride, sulphate and bicarbonate are obviously comparable in the study sites. Also, it has been shown that the heavy metal contaminations reaches high levels. The concentration of Pb ranges from 0.29-0.43 mg l^{-1}, Zn ranges from 0.10-0.22 mg l^{-1}, Cu ranges from 0.20-0.40 mg l^{-1} , Cd ranges from 0.10-0.15 mg l^{-1} and Co ranges from 0.20-0.40 mg l^{-1} . Pb and Cu concentrations were the same in the three sites: Meet El-Akrad, Telbana and Belgay and Cd concentrations were nearly the same in the three sites: Shawa, Meet El-Akrad and Belgay, whereas, Co-concentrations were the same in the three sites: Shawa, Telbana and Belgay.

***Allium* MNC assay:** The *Allium* MNC assay results show a statistically significant differences in MNC frequency in comparison with the control (Table 2). The contaminated water samples from site 4 (Belgay) and 1 (Shawa) induced cells with a high percentage of MNC (41 and 52%, respectively) and the water samples from site 2 (Meet El-Akrad) induced MNC at moderate level (24%). Whereas the water samples from site 3 (Telbana) induced MNC with a low percentage (10%).

***Allium* chromosome aberration assay:** The most common cytogenetical aberrations in metaphase cells were chromosome ring and disturbed metaphase, in the anaphase and telophase cells disrupt spindles (disturbed). Chromosome fragments and bridges were found in various frequencies. Representative samples of mitotic abnormalities are shown in Fig.1. The genotoxicity of the water samples which revealed by the frequency of aberrant mitotic cells can be ranked in the following order: Belgay> Telbana>Shawa>Meet El-Akrad.

Discussion

The *Allium* test has often been used for the determination of cytotoxic and/or genotoxic effects of various substances (Grant, 1982; Smaka-Kincl *et al*., 1996). It is considered to be a standard procedure for quick testing and detection of toxicity and pollution levels in the environment. Results of the *Allium* test may indicate the presence of certain cytotoxic/genotoxic or mutagenic substances in the environment, which represent direct or indirect risks for all living organisms.

In aquatic environments documentation of genotoxic pollutants contaminated with industrial effluent is becoming a frequent occurrence (Parry *et al*., 1976; Rank and Nielsen, 1994; Jiang *et al.,* 1999). Fiskesjo (1985) has demonstrated the usefulness of root tips of *Allium cepa* as a test system for monitoring the genotoxic effects of contaminated water from a river receiving effluents from a chemical factory in Sweden. The *Allium* test was found to be very useful for evaluating and ranking aquatic toxicities for a number of metals, including mercury (Fiskesjo, 1988; Dash *et al.,* 1988; Rank and Nielsen, 1998). In water samples from sites 1-4 different degrees of mitotic index decrease were determined, which indicates the presence of cytotoxic substances in water causing inhibition of mitotic activities. The cytotoxicity level can be determined by the decreased rate of the mitotic index. A mitotic index decrease below 22% of the control causes lethal effects on test organisms (Antonsiewicz, 1990), while a decrease below 50% usually has sublethal effects (Panda and Sahu, 1985) and is called cytotoxic limit value (Sharma, 1983). In the present study, Shawa and Belgay wastewater samples showed a low mitotic index (5.11±0.18 and 4.62±0.17, respectively). These values represent 34.14 and 37.76%, respectively of the control, giving the water samples from these two sites sublethal effects. The mitotic index decrease in onion root meristem was found to be a reliable means for quick determination of the presence of cytotoxic substances in the environment, for monitoring the cytotoxic pollution level in the natural environments and for evaluations of water pollution levels. This parameter is sensitive enough also to be used for monitoring the pollution levels of slightly polluted water (Smaka-kinel *et al.,* 1996).

Statistical analysis showed that the genotoxic activities of the wastewater samples induced micronuclei in the roots of *Allium*, indicating the efficiency of *Allium* MNC system in detecting clastogenic potential of pollutant water, these data are in disagreement with Ma *et al.* (1995) who found the root micronucleus systems are effective only in detecting the genotoxicity of chemicals and physical agents rather than water pollutants. The induction of micronuclei in root meristems of *A. cepa* or any cell of any other organism is the manifestation of chromosome breakage and disturbance of the mitotic process due to spindle abnormalities (Dash *et al.,* 1988; Grover and Kaur, 1999). Micronuclei were considered as an indication of a true mutation effect (Auerbach, 1962), thus, the high percentage of the micronuclei induced by the studied wastewater samples may indicates the mutagenic effect of them. On the other hand, the percentage of aberrant

metaphase as well as anaphase cells for all samples expressed a statistically significant difference varying only in relevance levels. The increase of percentage of aberrant metaphase, anaphase and telophase cells in onion root tip meristems indicates genotoxic effects of wastewater samples.

The test results of these assays (chromosome aberration and MNC) are the indication of reliability and high sensitivity of these two bioassays. Also, the relatively high genotoxicity of the water samples may be attributed to the effluent from the industrial establishment confirming that the source of mutagens came from the industrial effluent in the study area. Among those mutagens, heavy metals are a potentially mutagenic class of environmental pollutants and some of them are implicated in the induction of tumors in experimental organisms and exposed humans (Minissi and Lombi, 1997). It was shown that these metals could induce clastogenic and aneugenic effects including mitosis and cytokinesis disturbances (Dovgaliuk *et al.,* 2001) and the obtained results showed that the tested water samples inhibit or reduce the mitotic activity of meristem cells and cause the chromosome and nucleus irregularities. In particular, bridges, breaks, stickness and micronuclei were recorded as mitotic irregularities caused by cadmium (Zhang and Yang, 1994) and in a study on *Allium cepa* root cells (Evseeva *et al.,* 2001) it has been shown that these cytogenetic effects of Cd results mainly from its ability to induce genome damages. On the other hand, lead has been tested (Johnson, 1998) and found to be capable of eliciting a positive response in an extraordinarily wide range of biological and biochemical tests, including enzyme inhibition, fiedility of DNA synthesis, mutation and chromosome aberrations. It complexes with many biomolecules, so it is likely to be a selective agent which acts on and influences the genetic structure. Fiskesjo (1988) and Jiang *et al.* (2000) reported a rapid decrease of the mitotic index caused by copper whereas Steinkellner *et al.* (1998) found a moderate increase in MNC frequencies caused by zinc ions. While taking into consideration the frequency of anaphase and telophase damages, frequencies of MNC in interphase nucleus and also the level of heavy metals in the examined water samples, in particular Cd (0.10-0.15 mg l^{-1}) and Pb (0.29-0.43 mg l^{-1}), the effects of them may be considered as clastogenic due to the impairment of spindle function. The malfunction of the spindle mechanism could be attributed to the reactivity of metal ions with the tubulin SH group (Dash *et al.,* 1988).

Our results indicate different degrees of contamination by both chromosome aberration and MNC assays among different sites. The ranking of genotoxic potencies in MNC assay is in the descending order Shawa>Belgay>Meet El-Akrad > Telbana, whereas chromosome aberration assay shows the order Belgay>Telbana>Shawa>Meet El-Akrad. Taking in account the same assessment values of some heavy metal levels in some of the sites, this variation in genotoxicity evaluation is perhaps most likely the result of physico-chemical factors (e.g., pH, salinity, hardness etc.) in the contaminated water which affect the bioavilability of heavy metals and hence the genotoxicity of them.

In conclusion, according to the results of *Allium* test on the new Mansoura drain water samples collected from and around Sandub area, the water pollutants in this drain have reached a relatively high level. Based on the mitotic inhibition test, water samples collected from Shawa and Belgay sites show a high rate of cytotoxicity. The reduction of pollution level of the region which was further down the stream (Telbana and Meet El-Akrad) could be interpreted as a result of dilution factor and the degradations of the pollutants. On the basis of the sample site analysis, most of the pollutants came from the effluent of the industrial wastewater. It is necessary to control the industrial effluent in order to prevent further deterioration of the water quality. *A. cepa* chromosome aberration and MNC assays could be recommended for biomonitoring of the aquatic pollution with regard to the simplicity of the test procedure and its ability to detect genotoxicity of wastewater, therefore, underline the application of plant genotoxicity testing for cost-effective monitoring. Generally speaking, this type of practical work can give a first alert of an environmental hazard and a large scaled monitoring network using the plant bioassays can protect ecosystems, including human beings.

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