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Full Length Research Paper

Genotoxic effects of industrial wastewater on *Allium cepa* L.

Levent Şık[1]*, Okan Acar[2] and Cüneyt Akı[2]

[1]Department of Biology, Science and Art Faculty, Celal Bayar University, Muradiye-Manisa/Turkey.
[2]Department of Biology, Science and Art Faculty, Çanakkale Onsekiz Mart University, Çanakkale/Turkey.

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The aim of this research is to study the effects of different concentrations of water on both incoming and outgoing in central biological and chemical wastewater treatment plant in Manisa (Turkey) organized industrial zone (MOIZ) on the *Allium cepa* L. root meristems, having been rooted in distilled water for 48 h. The union bulbs were kept in the 100% concentrations of the refined water (RW) and of 10, 25, 50 and 100% concentrations of unrefined water (UW). Distilled water was used for the control samples. It was determined that wastewater reduced the rate of the mitotic division of different concentrations and increased the mitotic anomalies. Mitotic index was found to be 33.8, 31.2, 23.6 and 16.7% in the control group, RW, 10% concentration of the UW, and 25% concentration of the UW, respectively. On the other hand, the rates of Mitosis / (Anaphase + Telophase) were 0.23, 0.28, 0.42, 0.71 in the control group, RW, % concentration of the UW, and 25% concentration of the UW, respectively. Plant growth was interrupted in the 50 and 100% concentrations of the UW and the mitotic division was inhibited. No anomalies were encountered in the control group. In the RW, a low rate of anomaly was observed, while in the different concentrations of the UW, chromosomal aberrations such as high frequency of lagging chromosome, irregular distribution, polar slips, horizontal division and sticky chromosome were observed.

Key words: Wastewater, refined water, mitotic index, chromosomal aberrations.

INTRODUCTION

Environmental problems are one of the most important dangers threatening to human, animal health and the ecological balance. To meet the increasing food demand in relation to the rise in the population of the world and to enhance the quality of life brings about many environmental problems. All kinds of waste cause adverse effects mostly on air, water and soil and in turn on the living organisms in such areas. The problems that need to be solved by the industrial plants are similar to those threatening the habitats of the living organisms. An environment-friendly plant can proceed with its production in harmony with its surroundings keeping all the waste refining units operating.

On the other hand, it is reported that the use of wastewater for the irrigation of the agricultural fields harms the mitotic division of plant and in turn wipes out the plant due to some substances contained within this water (Carita and Marin-Morales, 2008). If these plants are consumed as food, it may influence human health adversely. The chemicals profile of plants grown on such fields could give rise to serious consequences such as allergy at early ages, respiratory disorders, coronary and cancer in middle ages.

Many synthetic and natural substances in the environment should be tested with animals due to the carcinogenic potentials of the industrial wastewater. However, such tests take too much time and cost a lot of money. Therefore, to be able to measure the carcinogenic potentials of chemicals and industrial wastewater, *in vitro* tests systems have been developed. These tests, known as short time tests for genotoxic effects measure the effects in the genetic systems by the chemical substances and help to understand a relation between the results obtained and carcinogenic potentials of these substances. In measuring the genotoxic potentials, such values as chromosomal aberrations and their frequencies,

*Corresponding author. E-mail: levents@bayar.edu.tr. Tel.: +902362412151.

sister chromatide shifts, mutation are obtained with short time tests (Carita and Marin-Morales, 2008; Magdaleno et al., 2008).

It is known that *Allium cepa* L. is a favorable material in the chromosomal aberration tests in the environmental pollution of the meristematic cells. Ateeq et al. (2002) determined pentachlorophenol (PCP), 2,4-dichlorophenoxyacetic acid (2,4-D), 2-chloro-2,6-diethyl-N-(butoxymethyl) acetanilide (butachlorine) and c-mitosis with 50% EC_{50}, stickiness, chromosomal breakings and the genotoxic amount of the mitotic index. In the studies carried out by Allard et al. (1946) and Moore (1974), high concentrations of 2,4-D were used as herbicide, whereas the low concentrations of the same substance were determined to be growth-inducing in the same form and in the same appearance as indol-3-acetic acid by Moore (1974) and Hsuesh et al. (1947). It was also determined that 2,4-D has some genotoxicity at chromosomal level (Mohandas et al., 1972). Dovgalyuk et al. (2001) comparatively investigated the cytogenetic effects caused by metal salts such as lead, zinc, aluminum, copper, nickel and cadmium on apical meristem cells of the *A. cepa.*

The effect of the waste caused by industrial plants on environmental pollution is serious. Some plants dispose the solid surplus of the waste by pressing and burying them. The chemicals in these solid wastes contacting with the rain waters penetrating the soil dissolves and diffuses the water and combine with the underground water, causing these waters to get polluted. In a study that investigated the cytogenetic effects of the fluids from pressed waste on the plants, leather industry wastes were analyzed (Chandra et al., 2004). Aybeke et al. (2000) investigated *Triticum aestivum* (wheat) mitotic division anomalies on the root of the meristem cells and the total amount of protein by wastewater of an olive oil plant. Another study carried out in 2003 investigated the effect of the fertilization on the accumulation of toxic element in the union (*A. cepa*) watered via Karakoyun river which flows through the center of Şanlıurfa and where domestic and industrial wastes are disposed of (Doğan, 2003). Katkat et al. (1996) investigated how water wastes of Gemlik fertilizer industry inc. can be made use of in agriculture. In another study, the cytological effect of the storage of the two commercial mineral waters bottled with this material in different environmental conditions on the roots of *A. cepa* was investigated (Evandri, 2000).

MATERIALS AND METHODS

A. cepa bulbs were selected for research material because it is easy to obtain root meristem and have a small number of chromosome (2n = 16). The water incoming (pure) and outgoing (refined) at central biological and chemical wastewater treatment plant in Manisa (Turkey) organized industrial zone (MOIZ) in January 2006 was sterilized and bottled in plastic containers. Then 10, 25, 50 and 100% concentrations of incoming for refinement and 100% concentration of outgoing as refined were prepared.

A. cepa roots were grown at 20 - 22°C. Onion bulbs, when their root length became 1.5 - 2.5 cm (48 h), were transferred to the glass pots containing wastewater prepared at different concentrations and were kept waiting for 48 h. 2 cm long root meristems were cut and fixed in carnoy (ethanol: glacial acetic acid; 3:1). After that, fixed roots are hydrolyzed in 1 N HCl at 60°C for 10 min. Acetoorceine was used as chromosome dye and 10 mitosis samples were prepared from control and treatment groups via squash preparations.

Mitotic index [(mitotic Index %) = (number of dividend cell/number of total cell) x 100] and mitosis / (anaphase+telophase) [M/(A+T)] was calculated. The mitotic cell number, the % of anomaly cells, total anomaly cells and types of anomalies for every area were counted in microscopic analysis. The % of different mitotic aberrations was calculated from the ratio of number of total cells dividing to number of normal mitotic cells. The % of different mitotic deviations was calculated from randomly selected cells from among all the samples, whereas mitotic division was calculated by the proportion of the normally divided cells to the total cells. The microscopic observations and the photographs were received using SOIF XSZ – H investigation microscope.

RESULTS AND DISCUSSION

The research revealed that 10, 25, 50 and 100% concentrations of the wastewater (pure) reduced the mitotic division rates due to the concentrations in the root meristem and created various chromosomal anomalies. Among the anomalies, the most distinctive ones and those having the highest observation frequency are polar spindles at anaphase, lagging chromosome, formation of fragments, and irregular distribution of chromosome (Figures 1 and 2). The mitotic index, which is 33.8% in the control samples, was found to be 31.2% in the samples germinated in refined water, 23.6% in 10% concentration of the unrefined water and 16.7% in 25% concentration of the unrefined water. The rates of M/(A+T) were 0.23 in the control group, 0.28 in refined water, 0.42 in 10% concentration of the unrefined water and 0.71 in 25% concentration of the unrefined water.

Almost no anomalies were encountered in the control samples, but a low frequency of polar spindle (0.002) was found at anaphase and metaphase. However, this frequency is 4 times higher in the samples obtained from refined water. Among the chromosome anomalies caused by unrefined water were lagging chromosome, irregular distribution of chromosome, polar spindles, a high level of horizontal division, but in 25% concentration sticky chromosomes were also found. It was determined that 50 and 100% concentrations of the incoming wastewater completely inhibited the plant growth in *A. cepa* and prevented the formation of mitotic phases. These conditions show that there is a danger for the plants found in the area where sampling was made. The inhibition of the mitotic efficiency of the plants watered through the water containing abundant wastewater and the evolution of the deviations influence the efficiency of the watered plants adversely. In addition, mitotic anomalies will create plants having chromosome deviations in the number and the structure of the chromosomes. In

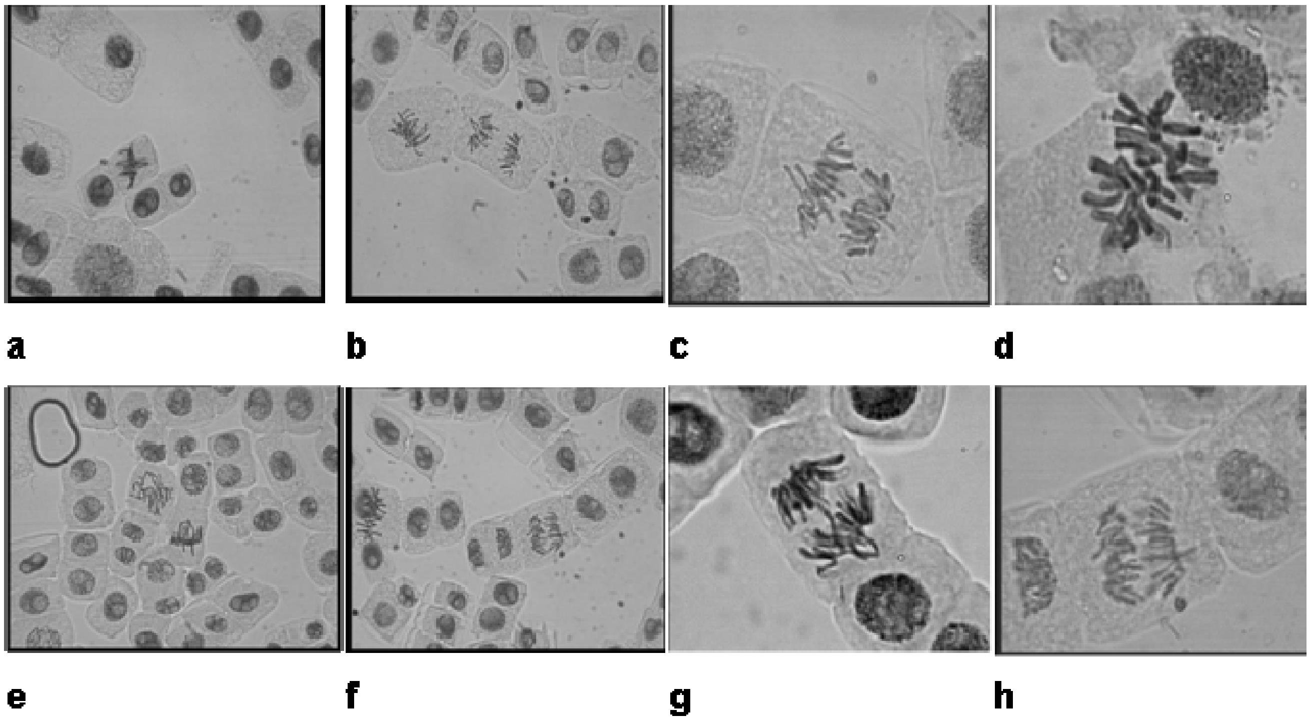

Figure 1. Samples of the anomalies observed in the mitosis following 10% pure water treatment: (a) polar slip, (b) polar slip, (c) irregularities at anaphase, (d) irregular distribution of chromosomes in metaphase, (e) polar slip, irregular distribution of chromosomes, (f) distortion of chromosome structure, (g) lagging chromosome, and (h) irregular distribution of chromosomes.

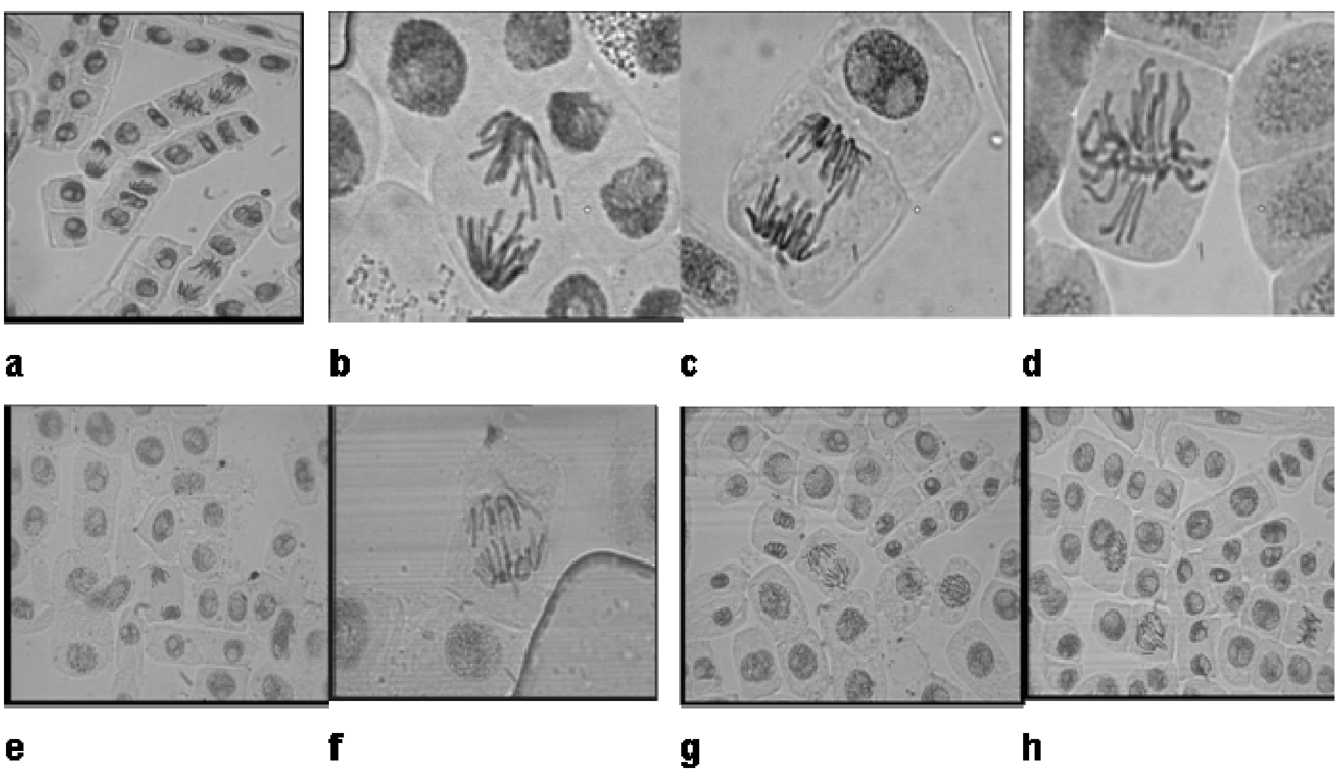

Figure 2. Samples of the anomalies observed in the mitosis following 25% pure water treatment: (a) drifting away from the metaphase plate, (b) irregular chromosome splits, (c) chromosome bridge at anaphase, (d) irregular metaphase, (e) chromosome stickiness, (f) polar slip, (g) irregular distribution of chromosomes at anaphase, and (h) lagging chromosome.

Table 1. The water analyses values of January 2006.

Water	COD	Suspended solids	T. Cr	Pb	Cu	Zn	pH
Incoming water (I)	1418	270	0.625	1.75	2.313	1.813	8.6
Outgoing water (O)	111	20.4	0.09	0.2	0.29	0.28	7.8
STD Value (I)	4000	500	5	3	2	10	6.5 - 10.0
STD Value (O)	400	200	1	1	3	5	6.0 - 9.0

Values (except for pH) are in mg/l. Source: MOIZ.

addition to the plants, similar influences are likely to be seen in other organisms using this water directly or indirectly.

Kara et al. (1994) determined that mitotic index decreased in the root meristem samples of *A. cepa* germinated in the water samples obtained from the organized industry area near the Gediz River. For example, mitotic index, which is 30.6% in the control group, reduced to 26.82% in the first water sample to 23.55% in the second. These results demonstrated that wastewater had the antimitotic agent effect. Similar results were found in the studies carried out to determine the mutagenic effects of chemicals. For example, İnceer and Beyazoğlu (2000) reported that $CuCl_2$ and Al^{3+} reduced the mitotic efficiency of the root meristem of *Vicia hirsut*a, *A. cepa* and *Allium sativum* L.

Though the effects of mechanisms for various chemicals are not fully known, it is thought that they affect DNA synthesis or various enzymes involved in cell energy systems. Accordingly, it was reported that if ATP level in the cell decreases by 50%, mitosis can completely be prevented (Kara et al., 1994). Therefore, it can be thought that the displacement of the chromosomes by the chemical substances affecting ATP synthesis can be inhibited by anoxia conditions or other ways because it is claimed that insufficient oxygen causes a delay in division by inhibiting the metaphase. Similarly, cell division is inhibited by agents repressing the energy producing processes such as glucose respiration and phosphorylation. The energy emitted through respiration is accumulated as organic compounds and employed in the process of cell division.

A number of studies investigated the chromosomal effects of the chemical substances on plants, animals and humans using different chemical substances. The same chromosomal defects were observed in the lymphocytes of people exposed to many heavy metals and chemical compounds including mercury due to their occupations (Lerda, 1992). Mercury compounds inhibit DNA replication linking the nucleus membrane and organelles in cells and also cause fragmentation in DNA (De Flora, 1994). In addition, the mercury link itself to the sulpyhydrile groups found in the enzymes and protein groups, functions as an inhibitor preventing the synthesis of protein necessary for the normal cell division and delaying mitotic cycle.

Ağar and Uysal (1997) investigated the effects of $HgCl_2$ on *A. cepa* root meristem cells. $HgCl_2$ applied to *A. cepa* root cells at different dosages caused chromosomal defects to occur in the cells. Chromosomal anomalies demonstrate differences due to the division phases. These anomalies were irregular distribution of chromatin and granulation at prophase and clustering of chromatides, lagging chromosomes and chromatides breakage at metaphase. Irregular distributions between sister chromatides and bridge formation were observed at anaphase.

The incoming and outgoing water employed in this study is regularly analyzed by central biological and chemical wastewater treatment plant in MOIZ compatible with the standards required by ministry of environments. In these analyses, the determinations of chemical oxygen demand (COD), suspended solids, total Cu and Zn were carried out. According to the average data obtained in January 2006, suspended solids ranged from 270 mg/l in unrefined water to 20.4 mg/l in refined water, and COD from 1418 mg/l in unrefined water to 111 mg/l in the refined water (Table 1). There is no significant difference when the total values of Cr, Pb, Cu and Zn are compared to the standards. It can be thought that the decrease in COD had a positive influence on the mitotic index of the refined water because the decreasing amount of oxygen is naturally likely to influence the cellular energetic events adversely. It is also thought that this may be closely related to the inability to determine the mitotic division in 50 and 100% concentrations of the unrefined water.

The water refined at central biological and chemical wastewater treatment plant in MOIZ is discharged into Sarı çay. This water released in this way is used in the irrigation of the fields in the plain covering a large area. All the chemical agents with the mutagenic effects on the plants are transferred to plants via irrigation. Today, wastewater is used for irrigation in many places. For the mutagenesis to be decreased, these waters should be decontaminated from chemical and biological pollutants, which might be a good solution to the problem. Agricultural biotechnology, with the transmission of the genes creating resistance to the diseases and the detriments, can be employed both to decrease the amount of the pesticide and to provide an increase in the yield and to improve the plants' tolerance to the pollution sources.

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