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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(2): 184-188, 2002

Cytotoxicity Testing of Sewage Water Treatment Using *Allium cepa* Chromosome Aberrations Assay

Amal W. Amin
Botany Department, Faculty Of Science, Alexandria University, Egypt

Abstract: The genotoxicity of sewage water samples was investigated using the *Allium* root chromosomes assay. Sewage influent (untreated) and effluent (primary treated) were used to treat *Allium cepa* bulbs in 1995. The treatment with both sewage waters and their dilutions decreased the mitotic index and increased the aberration rate of dividing and nondividing cells significantly than the control. Sewage water from the West Plant caused the highest rates of chromosomal abnormalities due to its pollution from industrial activities. It was recommended that sewage water needs further treatments to remove or lower the pollutants as heavy metals before being used.

Key words: Influent and effluent sewage water, cytotoxicity, genetic damage

Introduction

Wastewater has been used to support agricultural production in many countries over a considerable period of time. The area of land under irrigation with wastewater has increased significantly over the past two decades due to constrains on water supply and increasing concerns over the environmental implication of previous disposal routes. Effluent reuse can provide considerable social, economic and environmental benefits when used under controlled conditions to establish the protection of health of both farm workers and consumers of the produce.

The decision to use treated sewage water (effluent) for irrigation of crops has been adopted in many Mediterranean and Middle East countries and in much other semi arid areas across the world. In Egypt, there are many examples of planned reuse of sewage effluent e.g. Gabal-El Asfar Old farm, El-Saff and Nubaria areas (Hall and El-Hakeh, 1999 and El- Bagouria, 1999). In certain countries sewage effluent and influent waters are mixed with certain surface water sheds to be used in irrigation as in the case of Ghota area in Syria and Kherbet Al- Samarah in Jordan (El-Bagouri, 1999).

The main components of sewage effluent (SE) that warrant particular consideration to irrigation include: a- The contents of major and minor nutrients (N, P, K, Ca and Fe, Zn, Cu, Mn, B) as well as heavy metals (Cd, Pb, Cr, Hg). b- The concentration of total dissolved solids (TDS) as well as that of certain cations and anions which might cause adverse effects on soil properties and plant growth. c- Chemical oxygen and biological demands (COD and BOD). d- The presence of certain chemical pollutants (El- Bagouri, 1999).

Increased growth of olive trees and fodder grasses was observed for irrigation using sewage influent (SI) and effluent (Saavedera *et al.*, 1984; Karunaichamy *et al.*, 1989). However, the accumulation of heavy metals from sewage effluent in surface layers of the soil and in some plant roots, was suggesting to cause some genotoxic effects in corn (Narwal *et al.*, 1990; Narwal *et al.*, 1991; Grover and Kaur, 1999; Cabrera and Rodriguez, 1999).

Alexandria General Organization for Sanitary Drainage (AGOSD) developed two stations for sewage water treatment, one in the east and the other in the west of Alexandria city. Up till now, the primary treatment is carried out by removing the settled, floating and suspended solids from sewage influent. The resulting liquid portion varies in composition according to human behaviour and industrial activity of the two stations. The use of this primary treated sewage water in agriculture has come into consideration to overcome the problem of low water budget.

The principal objective of this study is to qualify the effect of untreated and primary treated sewage water on the genetic material of *Allium cepa*. This will give an insight to use this wastewater after the proper dilution with ordinary fresh water in irrigation of newly reclaimed lands used for cultivation of crops.

Materials and Methods

Untreated and primary treated sewage water (SI and SE respectively) were obtained from the East and West Treatment Stations of Alexandria General Organization for Sanitary Drainage (AGOSD) in 1995. Samples were collected every one-hour and pooled for daily use. The pH, temperature, settable, suspended substances and biological and oxygen demand (BOD and COD) were estimated in AGOSD laboratories. Homogeneous onion (*Allium cepa*) bulbs were procured from the local market and used to study the efficiency of SI and SE for irrigation. The old roots and loose scales of onion bulbs were removed, and then suspended in different concentrations of the tested water (SI, four concentrations of SE: 100%, 75%, 50%, 25% and the control as distilled water) and incubated at 21± 1°C. The solutions were changed every day during the experiment. The root lengths were measured after 3, 7, 10 and 12 days and the means of 5 roots were calculated. For cytological study the emerged roots were fixed in aceto-alcohol (1:3) after three and seven days of starting the treatments. The conventional Feulgen squash method (Sharma and Dphil, 1980) was used to prepare permanent slides of root meristems. The mitotic activity and rate and kinds of aberrations were recorded using four replicas for each treatment.

The results were given as arithmetic means and statistically evaluated by applying the analysis of variance and LSD tests, using the COSTAT program.

Results

The quality and the concentrations of potentially toxic elements in sewage influent and primary sewage effluent are summarized in Table 1. The pH of different sewage waters of the two treatment plants was similar. The settable and suspended solids were about 4.5 times higher in the SI of East Plant than that of the West one. Both BOD and COD of the West Plant were higher than that of the East one. But, the SI of the East Plant showed the lowest BOD: COD ratio.

Generally, it was observed that the sewage water of both Plants reduced the mean number of emerged roots per bulb and suppressed roots than the control (Table 2). Signs of wilting started to appear on few of the treated roots after 3

Table 1: Quality of sewage influent and effluent from the East and West treatment plants of Alexandria.

Character		East plant		West plant	
		Influent	Effluent	Influent	Effluent
pH		7.40	7.40	7.35	7.31
Temperature	(° C)	28.92	28.74	27.50	27.50
Settable substances	(mg/l)	10.52	0.78	18.50	0.50
Suspended substances	(mg/l)	532.40	98.40	155.00	8.50
Dissolved Oxygen	(mg/l)	0.73	1.67	-	-
BOD	(mg/l)	175	115	475	258
COD	(mg/l)	1758	398	1848	430
BOD/COD	(ratio)	0.10	0.29	0.26	0.60

BOD = Biological oxygen demand COD= Chemical oxygen demand

Table 2: Root length of treated 3, 7, 10 and 12 days *Allium* bulbs using different concentrations of sewage water from the East and West treatment plants. The mean number of emerged roots/bulb was calculated at the end of the experiment. (SI: sewage influent, SE: Sewage effluent)

Treatment		Duration time (Days)				Emerged roots/ bulb (no.)
		Three	Seven	Ten	Twelve	
East treatment plant						
Control		1.50 b	1.55 ab	2.40 a	2.41 a	19.67
SE	25%	1.60 b	1.66 ab	1.84 ab	2.03 ab	17.33
SE	50%	1.57 b	1.66 ab	1.80 ab	2.11 ab	16.33
SE	75%	0.90 b	0.93 b	0.93 b	0.93 b	4.67
SE	100%	1.93 a	2.14 a	2.20 a	2.22 a	10.67
SI		0.68 b	1.07 b	1.30 ab	1.50 ab	9.33
	P	0.0022**	0.0183*	0.2258 ns	0.0389*	
	LSD	0.73	0.67	1.73	1.18	
West treatment plant						
Control		1.50 a	1.55 ab	2.40 a	2.41 a	19.67
SE	25%	1.38 a	1.50 ab	2.36 a	2.40 a	13.67
SE	50%	1.10 a	1.11 ab	1.20 ab	1.20 b	12.67
SE	75%	1.20 a	1.53 a	1.90 a	2.06 a	7.67
SE	100%	1.03 a	1.05 ab	1.30 ab	1.35 b	11.67
SI		0.87 a	0.90 b	0.90 b	0.90 b	5.00
	P	0.1877 ns	0.0292*	0.0092***	0.007***	
	LSD	0.818	0.576	1.142	0.805	

Figures with similar letter are not significantly different at 0.05. * Significant at 0.05 and *** Significant at 0.01

Table 3: Cytological parameters of 3 and 7 days old roots emerged after *Allium* bulbs treatment with different sewage water concentrations from the East and West treatment plants of Alexandria. (MI: mitotic index, P: prophase, M: metaphase, A+ T: anaphase-telophase, ADC's: abnormal dividing cells, ANDC's: abnormal non dividing cells).

Treatments		Total cells (no.)	MI (%)	Mitotic distribution (%)			M/P (ratio)	ADC's (%)	ANDC's (%)
				P	M	A+ T			
After 3 days treatment									
Control		10158	11.31 c	74.70	8.72	13.60	0.12	0.77 c	0.16 b
SEE	25%	9908	4.73 ab	55.72	13.76	29.47	0.25	13.02 a	0.67 a
SEE	50%	9976	4.73 ab	78.80	5.25	13.48	0.07	7.16 b	0.48 ab
SEE	75%	11201	6.46 ab	76.68	9.17	14.15	0.12	8.10 a	0.75 a
SEE	100%	8728	2.90 a	91.91	0.96	7.13	0.05	1.94 c	0.58 ab
SIE		7053	2.50 a	89.22	4.23	6.55	0.05	5.50 bc	0.29 ab
Control		10158	11.31 d	74.70	8.72	13.60	0.12	0.77 b	0.16 b
SEW	25%	11654	5.12 c	75.24	6.42	18.04	0.09	8.89 ab	0.70 ab
SEW	50%	9658	2.23 b	77.24	13.28	9.49	0.17	13.78 ab	0.46 ab
SEW	75%	2644	0.22 a	87.50	0.00	12.50	0.00	0.00 b	0.69 ab
SEW	100%	10073	1.61 ab	37.63	26.03	36.05	0.69	23.77 a	0.63 ab
SIW		7861	0.22 a	75.21	2.56	22.22	0.03	13.77 ab	1.76 a
After 7 days treatment									
Control		8172	8.81	61.55	8.82	29.63	0.14	1.11	0.00
SEE	25%	3571	3.03	87.71	2.49	9.80	0.03	1.87	0.26
SEE	50%								
SEE	75%					nondividing and faintly stained cells.			
SEE	100%								
SIE		5331	7.17	53.12	13.66	33.23	0.26	3.65	0.28
SEW and SIW		nondividing and faintly stained cells.							

Similar letters are not significantly different at 0.05.

SEW = Sewage effluent of west treatment plant SIW = Sewage influent of west treatment plant

SEE = Sewage effluent of east treatment plant SIE = Sewage influent of east treatment plant

Table 4: Types and percentages of abnormal dividing and nondividing cells in meristems of 3 and 7 days old roots after *Allium* bulbs treatment with different sewage water concentrations from the East treatment plant. (treatments using 100, 75 and 50% contain faintly stained nondividing cells).

Treatment time	After three days						After seven days		
Treatments	Control	SI	SE 100%	SE 75%	SE 50%	SE 25%	Control	SI	SE25%
Total dividing cells (no.)	1142	116	148	752	471	484	729	385	135
Aberration %*	0.79	5.42	2.70	6.91	7.43	12.40	1.10	3.9	4.44
Aberrations/ cell	1.00	1.56	1.75	1.40	1.29	1.07	1.10	1.00	1.50
Kinds:									
*abnormal P %**	0.00	0.00	25.00	5.77	5.71	0.00	0.00	0.00	0.00
binucleate	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00
sticky	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00
*abnormal M %**	11.11	100.00	25.00	78.85	71.43	61.67	75.00	53.33	83.33
sticky**	0.00	4.22	1.36	5.45	4.67	7.64	0.55	1.56	2.96
c-metaphase	0.00	4.22	0.68	2.93	1.91	0.41	0.14	0.00	0.00
multigroups	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.26	0.00
abn. chrom. orientation.	0.09	0.00	0.00	0.00	0.00	0.00	0.14	0.26	1.48
others***	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.74
*abnormal A+ T %**	88.89	0.00	50.00	17.31	22.86	38.33	25.00	46.67	16.67
sticky	0.00	0.00	0.68	0.40	0.42	3.31	0.00	0.00	0.74
abn. chrom. orientation.	0.61	0.00	0.68	0.66	0.64	0.62	0.14	0.26	0.00
abn. chrom. Movement****.	0.90	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00b
binucleate	0.00	0.00	0.68	0.13	1.06	1.24	0.14	1.56	0.74
Total nondividing cells (no.)	9016	6887	2078	11201	99505	9427	7443	4946	3571
Aberration (%)	0.16	0.13	2.36	0.72	0.47	0.69	0.00	0.28	0.26
Kinds:									
Micronucleus	0.13	0.07	0.00	0.00	0.00	0.01	0.00	0.00	0.23
residual bridge	0.02	0.00	0.00	0.00	0.20	0.02	0.00	0.00	0.00
Multinucleate	0.00	0.04	2.36	0.72	0.45	0.52	0.00	0.28	0.03
others*****	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00

* calculated from total dividing cells *** polyploidy, binucleate and contracted chromosomes.
** sticky, clumped and ring figures **** disturbed and precocious chromosomes ***** large nucleus and nucleolus

Table 5: Types and percentages of abnormal dividing and nondividing cells in meristems of 3 days old roots after *Allium* bulbs treatment with different sewage water concentrations from the West treatment plant

Treatments	Control	SI	SE 100%	SE 75%	SE 50%	SE 25%
Total dividing cells (no.)	1142.00	19.00	160.00	5.00	216.00	619.00
Aberration %*	0.79	10.53	18.13	0.00	12.96	8.72
Aberrations/ cell	1.00	1.50	1.07	0.00	1.36	1.07
Kinds:						
*Abnormal P %**	0.00	0.00	0.00	0.00	3.57	5.56
binucleate	0.00	0.00	0.00	0.00	0.46	0.48
*Abnormal M %**	11.11	50.00	82.76	0.00	89.29	51.85
sticky**	0.00	5.26	13.75	0.00	12.96	4.68
c-metaphase	0.00	5.26	0.63	0.00	2.31	0.32
multi group	0.00	0.00	1.25	0.00	2.31	0.32
abn. chrom. orientation.	0.09	0.00	0.63	0.00	0.00	0.32
others***	0.00	0.00	0.00	0.00	0.46	0.00
*Abnormal A+ T %**	88.89	50.00	17.24	0.00	7.14	42.59
sticky	0.00	0.00	0.63	0.00	0.00	0.16
abn. chrom. orientation.	0.61	5.26	0.63	0.00	0.46	2.10
abn.chrom.movement****.	0.09	0.00	0.63	0.00	0.46	0.48
binucleate	0.00	0.00	0.00	0.00	0.00	0.00
bridges	0.00	0.00	1.25	0.00	0.46	0.97
Total nondividing cells (no.)	9016.00	6842.00	9913.00	2638.00	9442.00	11035.00
Aberration percent	0.16	3.32	0.67	1.40	0.46	0.72
Kinds:						
Micronucleus	0.13	0.09	0.01	0.00	0.02	0.00
Residual bridge	0.02	0.00	0.05	0.00	0.00	0.01
Multinucleus	0.00	3.19	0.61	1.40	0.39	0.71
others*****	0.00	0.04	0.00	0.00	0.04	0.00

* calculated from total dividing cells. ** sticky, clumped and ring. *** binucleate and contracted chromosomes.
**** disturbed and precocious chromosomes. ***** large cell and large fragmented nucleus.

days by using SIE and after 7 days using all treatments except SIE and 25% of SEE.

The treatments with SI and SE from both Plants changed the mitotic phase distribution. This is expressed by lower M/P ratios than the control (Table 3). However, higher M/P values than the control was found after 3 days treatment with 25% SEE, 50% and 100% SEW and after 7 days using SIE.

Treatment with SI and SE of the East and West Plants for 3 days lowered the mitotic activity significantly than the control. This was expressed by decreasing the mitotic index (MI) with increasing the concentration of SE (Table 3). But 75% SEE treatment was an exception giving the highest MI value (6.5%), which was still less significantly lower than the control and was accompanied by the shortest roots (0.9). However, the same concentration of the SEW as well as the SIW treatments decreased MI to 0.22. No dividing cells were found after seven days treatments with all sewage waters from the West Plant and after the 100, 75 and 50% concentrations of sewage effluent of the East Plant and all nondividing nuclei lost their ability to be stained by Feulgen.

The aberration rate of nondividing cells increased significantly using SI and SE of both Plants (Table 3). But the SIE treatment induced lower aberration rates than that of SIW. The frequency of abnormalities increased significantly by SEE and SEW concentration and using SIW. The most frequent types of aberrant nondividing cells included binucleate cells with or without complete or residual bridge and multinucleate cells (Table 4 and 5).

The rate of aberrant dividing cells increased (Tables 4 and 5) by decreasing the concentration of SEE, while SI induced aberration rates in between those induced by 75 and 100% treatments. Contrary, increase in SEW concentration increased the aberration percentage, while SI induced about the same value (of aberrations) as that induced by 50% SEW.

More than one kind of aberrations were found per cell (Tables 4 and 5). The highest values were recorded in 100% SEE and SIW treatments (1.75 and 1.5, respectively), compared to one aberration per cell in the control.

Sticky metaphase chromosomes were the most frequent kind of aberrations induced by SE and SI treatments from the two Plants. C-metaphase was the second more frequent kind of aberration. Abnormal chromosome orientation and movement (disturbed and precocious chromosomes) were also recorded (Table 5).

Discussion

Parameters such as root shape and growth, frequencies of mitosis and abnormal cell divisions can be analyzed to estimate the cytotoxicity, genotoxicity and mutagenecity of environmental pollutants (Grant, 1982; Nielsen and Rank, 1994; Kovalchuk *et al.*, 1998). In the *Allium* test, inhibition of rooting and the appearance of stunted roots indicates retardation of growth and cytotoxicity, while root wilting explains toxicity. Nevertheless, both growth retardation and root wilting are accompanied by suppression of mitotic activity and occurrence of chromosomal aberrations. The present study provides evidence that influent sewage water (SI) from the two Plants inhibited root growth after its emergence, while the effluent sewage water (SE) caused growth retardation. The inhibition of growth may be due to the high rate of chemical oxygen demand (COD) of the SI (El-Bagouri, 1999), which affected certain physiological processes leading to the disturbance in the balance between promoters and inhibitors of endogenous growth regulators (Grover and Tejpaul, 1981). In addition, the appearance of sings of wilting on emerged roots using SI and some concentrations of SE could be attributed to lower resistance of the cellular membranes to pollutants (Amin, 1991) through the disturbance in the osmoregulatory system (Webbe, 1961).

The suppression of mitotic activities was often used for tracing cytotoxicity (Smaka-Kinel *et al.*, 1996). This is usually accompanied by an increase in the fraction of cells with c-mitosis, multigroups, sticky and abnormal chromosome orientation (Amin and Migahed, 2000). In present study, a decrease in the mitotic index was found to be significant with an increase in the concentration of SE and the use of SI from both East and West Plants. This indicates the cytotoxic effect of the untreated and high concentrations of sewage water. The suppression of mitotic activity was probably due to either the blocking of G_1, suppressing cells from DNA synthesis (Schneiderman *et al.*, 1971) or the blocking of G_2, preventing cells from entering mitosis (Vant Hof, 1968). This occurred probably through the uncoupling of respiration processes and carbohydrate metabolism (Gönnert and Stufe, 1961; Shoeb, 1975; Andrews *et al.*, 1983) leading to low ATP content, which is essential for progress of mitosis. However, the occurrences of low M/P ratios in most root cells of sewage water treated bulbs indicate that the dividing cells (in spite of being few) are in the prophase stage. This indicates that after the mitotic suppression, the surviving cells started to divide again, but did not pass to the metaphase stage. Since sewage water contain an appreciable amount of Ca^{++} (El-Bagouri, 1999), the impairment of nuclear membranes could be the cause for the failure of sequestering Ca^{++} needed for the transition from prophase to metaphase (Aarmeda *et al.*, 1984).

The rate of aberrant dividing cells increased by sewage water treatment. However, it increased by decreasing the SE concentration. The high content of organic matter of SI and SE acted as chelating agent to heavy metals (Das *et al.*, 1997) lowering the aberration rate. Decreasing the concentration of the organic matter by dilution (of SE) might enhance the rate of translocation (transfer) of heavy metals (found in SE) to root cells (Rank and Nielsen, 1998) causing this increased rate of aberrant dividing cells by decreasing SE concentration. However, the SIW induced twice the aberration percentage induced by SIE treatments, and this percent decreased by decreasing the concentration of SEW. This could be due to the increased content of heavy metals of this industrial area than the organic matter molecules chelating them. Accordingly, the rate of soluble and available metals in sewage water increased and subsequently accumulated in root cells depending, to a great extent, on its transfer coefficient value (Amin and Sherif, 2001). The presence of other toxic compounds resulting from industrial activation might participate in the production of some aberrations.

Chromosomal aberrations occur due to lesions in both DNA and chromosomal and spindle protein causing genetic damage. The different types of aberrations occurred in present study were probably due to the effect of genotoxic compounds found in sewage water (as household chemicals, chlorophenols, trace elements, heavy metals etc.). This caused drastic changes in chromatin, spindle apparatus and centromeres, leading to impairment of chromosome alignment onto metaphase plate, abnormal spindle orientation, abnormal chromosome movement and c- mitosis. The abnormal orientation of some chromosomes was found to be due to altered quality and quantity of kinetochore heterochromatin (Jannifer *et al.*, 1988). In the present study, c-mitosis occurred in high frequency after sewage water treatment. It included partial c-mitosis, poorly developed metaphase plates and star metaphase, which were recorded also by Önfelt (1987a). The mechanistic background to spindle disturbances with compounds might be partially based on the partitioning (sharing) of some household chemicals into hydrophobic components of the cell (Önfelt, 1987b). In addition, the presence of Ca^{++} (El-Bagouri, 1999) as well as chlorophenols (UNESCO, 1996) in sewage water could be the cause of the occurrence of c-mitosis after 3 days. However, Önfelt (1987b) found that chlorophenols could increase the level of cytoplasmic Ca^{++} to the extent of causing a reversible c- mitotic effect, which would explain the disappearance of c-mitotic figures after 7 days of treatment. In the meantime, the increase in Ca^{++} neutralizes the negative charges on chromatin fibrils, which would increase chromosome repulsion and chromatin condensation causing c-mitotic chromosomes (Dipankar and Crothers, 1986).

Chromosome stickiness is caused probably through immediate reactions with DNA during its inhibition period causing DNA-DNA or DNA-protein cross linking (Ashour, 1988 and Amin, 1991), or through reactions with the lysosomal system altering the physicochemical properties of nucleic acids and/or nucleoproteins (Mertz, 1969), liquification of the chromatin material (Adam and Farah, 1989), which consequently lead to the hindrance of normal chromosome separation at anaphase giving sticky chromosome bridges which were recorded in appreciable percentage in this study. Sticky bridges might be also the result of incomplete replication of chromosomes by defective or less active replication enzymes (Sinha, 1979) or late replicating DNA sequences of the telomeric heterochromatin (De-Faria and Jaworska, 1972 and Bennet, 1977). If heterochromatin blocks did not finish DNA replication when the nucleus is ready to divide, bridge formation would occur (Kaltsikes *et al.*, 1984).

In conclusion, sewage water either untreated or primary treated induced chromosomal aberrations through the interaction with DNA and proteins leading to chromosome stickiness, mitotic disturbances and/or cell damage. In addition, sewage water of the West Treatment Plant contained more pollutant compounds (from industrial activities) causing higher rates of chromosomal abnormalities. Therefore, it is highly recommended that sewage water need more treatments and heavy metals should be lowered before being used for irrigation. So, we can achieve safety results for irrigated soil, drainage canals, underground water, farm animals and consequently human safety and welfare.

Acknowledgment

The author greatly appreciated Dr. Laila M. El-Sadek, Professor of Genetics and Cytology, Botany Department, Faculty of Science, Alexandria University, Egypt for the critical review of the manuscript. Thanks to Eng. Hassan El-Hakeh, ex- chairman, Alexandria General Organization for Sanitary Drainage for his help in providing us with tested water.

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