CHEMICAL COMPONENTS AND CYTOTOXIC EFFECTS OF FOUR DIFFERENT TYPES OF WASTEWATER

SOLTAN, H. A. H. [1*], A. M. ATA[2] and E. A-K EL-MAMLOUK[1]

1. *Central Lab of Organic Agriculture, ARC, Ministry of Agriculture, Giza, Egypt.*
2. *Department of Genetics, Faculty of Agriculture, Minia University, Egypt.*

* Corresponding author, soloo2525@gmail.com

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Abstract

The present study aimed to estimate the chemical components and cytotoxic effects of four types of wastewater such as industrial wastewater (IWW), agriculture wastewater (AWW), sewage wastewater (SWW) and mixed wastewater (MWW) on meristematic tissues and cells of faba bean, *(Vicia faba*) root tips. Some physio-chemical characteristics as well as heavy metals contents were measured in these four wastewater samples compared with that of tap water using values of World Health Organization (WHO) as standard parameters. Mitotic index (MI), phase index and chromosomal abnormalities were calculated as toxicity indicators. The results revealed that MI values of almost all seeds treated with the different concentrations of various types of wastewater were significantly lower than those of control treatment. Different types of mitotic aberration were observed such as chromosome bridges, lagging chromatid and chromosomes, chromatin fragments, micronuclei and tripolar cells. It was clearly observed that waste water increased chromosomal aberrations and induced disturbances in mitotic phases. Therefore, it could be recommended that all wastewater should be treated according to WHO standards to avoid and minimize the pollutant effects before using for irrigation purpose, so that safety of humans would be achieved.

Key wards: cytotoxicity, *Vicia faba*, mitotic index, wastewater, chromosomal aberration

INTRODUCTION

Water pollution is a serious problem worldwide for the health of the biota and humans that interact with these aquatic ecosystems because of the presence of genotoxic compounds (Vargas *et al*., 2001; Ohe *et al*., 2003 and Buschini *et al*., 2004). These pollutants came from various sources such as industrial effluents (Ohe *et al.,* 2004) and treated and/or untreated domestic wastewater discharge including sewage (Terzic *et al.,* 2008 and Bolong *et al.,* 2009), and non-point source from agricultural land and urban runoff (Brett *et al.,* 2005 and Santhi *et al.,* 2001).

Agriculture expansions in Egypt depend mainly on irrigation however, water supply from irrigation canals is not sufficient enough. Therefore, farmers in many parts of the Nile urgently use drainage of water for irrigating their fields, (Khalifa *et al.,* 2003). Estimation indicates that more than fifty countries of the world with an area of twenty million hectares are treated with polluted or partially treated polluted water (Mahmood, 2006). Plants grown in contaminated soils when consumed by peoples can result in health problems (Wahid *et al.*, 2004) like diarrhea, mental retardation, liver and kidney damage (Matsuno *et al*., 2004 and Uzair *et al*., 2009).

Because of the importance of water quality to health, many types of genotoxicity and mutagenicity assays employing microorganisms and mammalian cells are used for monitoring of complex environmental samples (Verschaeve, 2002; Isidori *et al*., 2004; Reinecke and Reinecke, 2004 and Russo *et al*., 2004). However, plant assays, such as *Vicia faba* test, may have some advantages over microbial tests for environmental monitoring (Ata *et al*, 2008 and Nassif *et al*, 2009). Plant assays are highly sensitive to many environmental pollutants, including heavy metals (Fiskesjo, 1988), and have been used for monitoring the potential synergistic effects of mixtures of pollutants, including hydrophilic and lipophilic chemicals (Grover and Kaur, 1999; Ateeq *et al.*, 2002; Rank *et al*., 2002 and DeCampos and Viccini, 2003). Furthermore, the tested plants can be directly exposed to complex mixtures or environmental samples either in the laboratory or *in situ* (Rank, 2003). *Vicia faba,* offer a wide range of possibilities of cytogenetic analyses (Ata *et al*, 2003), because the chromosomes of this species are large and few in number (2n = 12) and thus, easy to study (Villalobos-Pietrini *et al*., 1978., Ata *et al*, 2008 and Nassif *et al*, 2009). On the other hand, Faba bean (*Vicia faba*) root-tip cells can be used to measure a variety of cytogenetic factors that can be used as toxicity indicators, such factors including mitotic index determination, chromosome and nuclear abnormalities. The aim of the present investigation was to evaluate the cytotoxic effects of four various types of wastewater (IWW, AWW, SWW and MWW) on *Vicia faba* root tips.

MATERIALS AND METHODS

This study was carried out at the Cytological Lab. of Genetics Department, Faculty of Agriculture, Minia University.

Plant materials

Pure strain seeds of the broad bean, *Vicia faba* (v.Masr1) were used as biological materials and had been brought from the Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

Wastewater

Four different types of wastewater called industrial wastewater (IWW), agriculture wastewater (AWW), sewage wastewater (SWW) and mixed wastewater (MWW) with three concentrations 25%, 50%, and 100% in comparison with control (distilled water) were used in this investigation. Samples of industrial wastewater

(IWW) were collected from the canal bank behind the sugar factory in Abu-Qurqas, region (southern of Minia city, Minia Governorate), before interring into effluent treatment plant. agriculture wastewater (AWW) were taken from agricultural drainage water near Rida Village, west Minia city, (the major producer of vegetables of the local city demand). MWW samples were brought from Al-Moheet water bank, which runs west Minia city, El-Minia Governorate, Egypt while, the SWW samples have been obtained from Sewage purification station in Beni Ebeid, Abu-Qurqas.

Estimation of heavy metals in wastewater samples

Some physic-chemical parameters and heavy metal were determined in wastewater samples and tap water using World Health Organization (WHO) values as standards. Fe^{++}, Mn^{++}, Co^{++}, Pb^{++}, Hg^{++}, Cd^{++}, Cu^{++} and Mo^{++++} heavy metals were estimated in part per million (ppm) of water samples using an Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst A 800) as per the standard protocols of APHA (2005) at Agricultural Research Center (ARC), Regional Centre for Food and Feed, Cairo, Egypt. The source of WHO standered values was Olajire and Imeokpara (2000).

Cytological studies

Seeds of the broad bean were soaked in each water source for 12 hours and then placed on filter paper in petri dishes. Three petri dishes were used for each water source as replicates with six seeds sown in each dish. The irrigation was continued till roots appearance. Roots of 1-2 cm long for each examined plant were cut and fixed in freshly prepared farmer's fixative (absolute ethyl alcohol: glacial acetic acid, 3:1 v/v) for 24 hours. Fixed roots were kept in 70% ethyl alcohol in a refrigerator until use. Treated roots were washed with distilled water, hydrolyzed in 1 N HCl at 60 ^{o}C for 10 minutes, washed by distilled water. The aceto-carmine squash preparation was used for mitotic studies. At least 10000 cells were examined for each treatment (consisting of three seedlings). Photographs were taken wherever necessary using Olympus BX51 microscope with a C-4040 zoom digital camera. Mitotic index, phase index and chromosomal aberrations were noted in each concentration and mitotic index was calculated using the formula:

Mitotic index (MI) = (Number of Dividing Cells/Total Number of Cells) X 100.
Percentage of abnormality of each stage of mitosis was counted for each slide. Percentage of Abnormality = (Number of Abnormal Cells/ Total Number of Cells) X 100 .

Statistical analysis

The statistical analysis was carried out using MSTAT program (Version 4) according to Gomes and Gomes (1984).

RESULTS AND DISCUSSION

Physicochemical parameters and heavy metals in wastewater samples

Physicochemical characteristics and heavy metal concentrations of samples taken from different wastewater and tap water (TW) (which used as control) with WHO permissive standards are shown in Table (1). Physical data clearly showed that IWW samples were strongly acidic with black color and molasses smell, while those of SWW and MWW were slightly acidic (6.8 and 6.4, respectively) with brown color and impurities. AWW samples were nearly neutral value (ph=7.4), similar to the value of tap water and colourless with turbidity. The obtained pH value (4.4) of IWW is outside the WHO recommended for drinking water which made the IWW potentially hazardous especially if it used in irrigation or drinking without proper treatment (Ozoh and Oladimeji, (1984). The low pH value of this IWW may be due to the acidic materials used during the manufacturing process, and the dark (black) color may due to the molasses backward from the manufacturing process. IWW samples showed high concentrations of Fe, Pb, Hg and Mo than those of international maximum permissible levels, while Mn, Cd, and Cu were below. Heavy metal toxicity may result from consumption of the IWW under consideration. Moreover, the long term use of this sugar effluent IWW for irrigation which contaminates soil and crops to such an extent that it becomes toxic to plants and causes deterioration of soil (Fakayode, 2005).

Regarding AWW, It was found that all heavy metals in general, were higher than international maximum recommended levels except Cu which was in the permissible limits. As shown in Table (1), in AWW, Fe content was four folds and Pb was three folds higher than those of the permitted level globally. The rest of the heavy metals recorded a relative increase when compared with the allowed values or fresh tap water. Therefore, the vegetables irrigated with such drainage water are not safe for human and animal consumption. The obtained results are in good accordance with those of Nasr and Zahran, (2015) and El-Ameen *et al.*, (2005) which concluded that the recycling of an agricultural drainage water for drinking or irrigation of agricultural crops, must be accompanied with a proper treatment to avoid higher accumulation of heavy metals and any toxic substances in agricultural fields and ultimately food chain and drinking water.

Heavy metal contents of SWW were higher than those of international maximum recommended levels except Cu and Mo which were in the permissible limits. There were high levels of potentially toxic metals such as Pb, Hg and Cd (0.91, 0.92 and 0.01 ppm, respectively) when compared with the levels of the same metals in tape water (TW). Several studies on plant assays have indicated that the heavy metals present in such sewage water either in its treated or raw sewage forms may accumulate in the soil or could be taken up by the roots of the cultivated plant organs. Therefore, they making plant organs hazard for use in feeding animals and /or human beings (Bohec and Bohec, 1990).

The mixed wastewater (MWW) exhibited real varying concentrations of all eight analyzed heavy metals. It was found that, Fe, Pb, Cd, Mo and Hg were higher than their levels being international maximum recommended, while Cu and Mn were in WHO permissible limits. Heavy metal obtained results indicated that water of Al-Moheet bank is polluted. Similar results, particularly for agricultural drainage polluted Nile was obtained by (Amira, 1997).

Table 1. Some physico-chemical parameters and heavy metal content estimated in part per million (ppm) of different wastewater samples and tap water (TW) using WHO values as standards.

Water sample and WHO standards		physico-chemical parameters				Metal concentration (*ppm*)							
		Odour	**Colour**	**pH**	**Temp**	**Fe**	**Mn**	**Co**	**Pb**	**Hg**	**Cd**	**Cu**	**Mo**
IWW		Smell of molasses	Black with impurities	4.4	25	7.62	0.27	0.08	0.90	0.19	0.01	0.26	0.09
AWW		Odourless	Colourless with turbidity	7.4	20	2.59	0.46	0.22	1.33	0.14	0.04	0.92	0.25
SWW		Bad smell	Brown with impurities	6.8	25	2.11	0.61	0.01	0.91	0.92	0.01	0.05	0.04
MWW		Bad smell	Brown with impurities	6.4	25	2.00	0.38	0.02	0.88	0.20	0.66	0.02	0.36
TW		Odourless	Colourless	7.4	20	0.29	0.18	0.10	0.47	0.00	0.002	0.02	0.89
WHO* Values for tap water	**MPC**	--	--	8.0	--	1.30	0.50	ND	0.10	0.010	0.003	1.50	0.07
	RMC	--	--	6.5	--	0.30	0.10	ND	0.05	0.001	0.005	1.00	0.01

Key: WHO = World Health Organization, MPC = Maximum permissible concentration, RMC = Recommendable minimum concentration, ND = not determined.

Mitotic index (MI)

Mitotic index (MI) and phase index values of meristematic cells treated with different wastewater concentrations are shown in Table (2). MI values of almost all seeds treated with different concentrations of four types of wastewater were significantly lower than that of the control treatment (8.14%).. The lowest value was recorded by 50% SWW treatment (5.83%), whereas the highest value of MI in treated plants was recorded by 25% MWW treatment (7.22%). The prophase index for all treatments exhibited low values when compared with control (59.59%). The lowest value of prophase index was recorded after treatment with 100% mixed wastewater (45.46%). The metaphase index values of all treated seeds were insignificantly changed when compared with that of control treatment. Data in Table (2) showed significant reduction of anaphase and telophase index values of seeds with different wastewaters types in comparison with the control treatment (19.65%).

Table 2. Mitotic indexes obtained from treatments with three concentrations of four wastewater types on faba bean root tips.

Treatments	Conc. %	Total no. of cells	Prophase	Metaphase	Ana&telophase	MI
Control	**Zero**	11793	59.59	20.76	**19.65**	8.14
IWW	**25%**	15317	46.59	25.86	27.56	6.17
	50%	17414	57.45	**17.72**	24.83	6.10
	100%	21343	45.46	24.64	29.90	5.94
AWW	**25%**	13663	46.60	21.57	**31.83**	5.98
	50%	13407	52.67	18.74	28.59	7.17
	100%	14251	47.87	24.00	28.14	7.07
SWW	**25%**	14907	57.64	19.80	22.56	7.22
	50%	17400	55.85	20.17	23.98	6.02
	100%	17564	52.22	19.96	27.82	5.97
MWW	**25%**	14612	49.13	**29.70**	21.17	6.87
	50%	16731	47.34	29.28	23.38	5.83
	100%	17777	53.47	23.88	22.65	6.62
L.S.D. (0.05)			**4.65**	**7.26**	**3.84**	**0.82**

Not surprisingly that irrigation by different types of wastewater cause damaging effects on cells and tissues and induced mitotic changes in root tips of *Vicia faba* when compared to control. These changes were shown as reduction of mitotic index and changes in phase index (Mattar *et al.,* 2014). Mitotic index is considered a parameter that allows to estimate the frequency of cellular division (Marcano *et al.*, 2004; Leme and MarinMorales, 2009; Gadel-Kareem *et al*, 1998, Ata *et al*, 2008). It is an acceptable measure of cytotoxic effect on all living organism, so that the cytotoxicity was defined either a decrease or increase of the mitotic index values (SmakaKinel *et al.,* 1996). The significant reduction in mitotic index reported herein by polluted water may due to one or all of the followings: a- the effects of heavy metal or pollutants of wastewater components on microtubule configuration during cell proliferation (Armbruster *et al.*, 1991), b- disturbance of the mitotic cycle during interphase stage (Mohandes and Grant, 1972), c- inhibition of DNA synthesis (Chand and Roy, 1981) which could be due to blocking of G1 and suppressing DNA synthesis (Schneiderman *et al.*, 1971), d- blocking G2 leading to prevent entering mitosis (Van't Hoff, 1968) and e- inhibits nuclear protein synthesis (Kim and Bendixen, 1987) which leads to inhibit the formation of various metabolic events necessary for mitosis (Rost and Marrison, 1984).

Mitotic aberrations

The percentage of abnormal cells recorded at various stages is given in Table (3) and represented micro-photographically in Figure (1). Some types of mitotic aberrations were clearly observed such as lagging chromosomes, chromatid bridge, chromatin fragments, micronuclei and cells with tripolar. It was clearly observed that all treated seeds exhibited high significant of total percentage of chromosomal aberrations as compared with control treatment (3.3%). The treatment of 100% sewage wastewater treatment induced the highest value of chromosomal aberrations (22.3%) when compared with that of control treatment, whereas the lowest value was recorded for agriculture 25% wastewater treatment (11.7%) when compared with the other wastewater treatments as shown in Table (3).

Table 3. The mean percentages of obtained mitotic irregularities in faba bean root tips treated with three concentrations of four wastewater types.

Treatments	**Conc.**	**Total no. of cells**	**Laggard**	**Bridge**	**Frag.**	**Micro**	**Tripolar**	**T. abnormalities**
control	**Zero**	11793	1.7	0.7	0.3	0.9	0.0	3.3
IWW	**25%**	15317	6.0	3.7	3.7	0.7	1.7	15.7
	50%	17414	7.7	3.3	3.0	1.0	2.7	17.7
	100%	21343	8.0	4.0	3.3	1.0	5.3	21.7
AWW	**25%**	13663	2.3	4.0	3.0	0.7	0.7	11.7
	50%	13407	5.0	2.0	4.7	1.2	0.8	14.0
	100%	14251	10.0	3.3	4.7	3.3	0.7	21.7
SWW	**25%**	14612	7.0	3.3	5.3	3.8	0.7	20.0
	50%	16731	7.3	2.0	3.7	2.3	1.9	17.3
	100%	17777	9.3	3.7	2.7	3.6	2.8	22.3
MWW	**25%**	14907	4.3	4.3	3.3	2.5	1.1	16.0
	50%	17400	6.0	0.7	3.7	1.2	0.6	12.0
	100%	17564	6.7	2.7	2.7	2.4	0.7	14.7
L.S.D. (0.05)			**1.08**	**0.8**	**1.0**	**0.5**	**0.61**	**2.4**

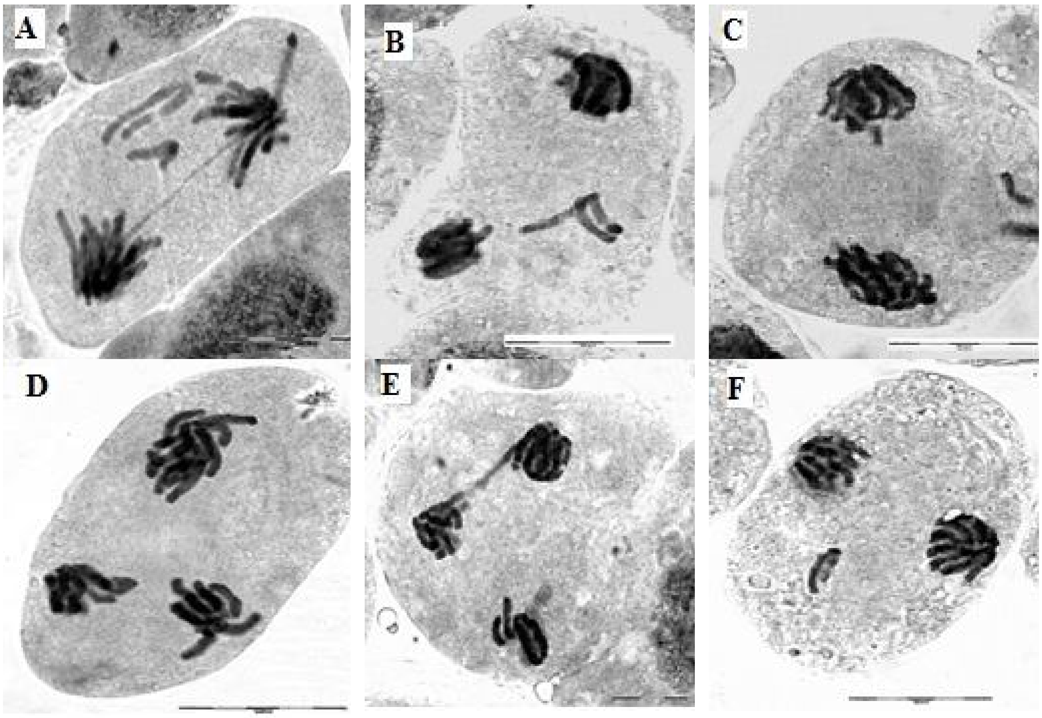

Fig 1. Some of chromosomal abnormalities at different mitotic stages of *Vicia faba* meristemic cells treated with four different wastewater: (A) fragment and bridge at anaphase, (B)lagging chromosome at telophase,(C) fragments at telophase, (D) cell with tripoler telophase, (E) bridge at tripoler cell, (F) lagging chromosome at telophase. Scale bar = 20 microns

The percentage of laggard chromosome reached a maximum of (10.0%) after treatment with 100% agricultural wastewater (AWW). It has been observed that percentages of laggard chromosomes were significantly increased after treatments with all wastewater when compared with control treatment (Table 3). In general, the percentages of bridges, fragments, micronuclei and cells with tripolar were higher after treatments by all types of wastewater than those of control treatment.

Chromosomal abnormalities recorded in this study clearly indicate the existence of cytotoxic effects of wastewater as previously stated by (Amin, 2002). Swage wastewater (SWW) treatment recorded the highest value of mitotic abnormalities compared to control. This was clearly associated with mitotic index showing negative correlation between mitotic index and mitotic abnormalities as previously stated by (Kovalchuk *et al.,* 1998, Bushra *et al.*, 2002). Laggards and bridges represented the most common types of mitotic abnormalities. The induction of laggard could be attributed to the failure of the spindle apparatus to organize and function in a normal way rather than inhibition of these spindle fibers and this may lead to irregular orientation of chromosomes (Grant, 1978; Mansour, 1984 and Patil and Bahat, 1992).

The treatment with all tested wastewater showed high number of breaks and bridges compared to control. The formation of bridges could be attributed to chromosomal breakage and reunion (Haliem, 1990, Gadel-Kareem *et al*, 1998, Ata *et al*, 2008). Furthermore, Kovalchuk *et al.,* (1998) and Nassif *et al*, (2009) attributed bridges and fragments to clastogenic effects which resulting from chromosomal and chromatin breaks. Chromosomal breaks cannot be repaired and are indicative of permanent genetic damage (Bickham *et al*., 2000 and Shugart *et al*., 2003). Micronuclei are true mutagenic aspects with many lead to a loss of genetic material. The micronuclei forms in two ways: One is, the chromosomal fragments formed in the last G2 which could not act in phase with normal chromosomes, and are rejected to the outside of nuclei in interphase. The other is the occurrence of various forms of lagged chromosomes, non-equatorial plane aggregated chromosomes, and the chromosomal grouping (Li, 1997). In general, the induction of micronuclei in root meristmic cells is the manifestation of chromosome breakage and disturbance of the mitotic process due to spindle abnormalities (Dash *et al.,* 1988 and Grover and Kaur, 1999).

The present investigation concluded that all treated wastewater can cause negative effect on *Vicia faba* root tips. All parameters studied such as mitotic index, phase index are negatively affected when compared to control. On the other hand, the percentage of mitotic irregularities was found high for all types of wastewater used compared to control. Many types of mitotic chromosomal abnormalities were observed such as laggard, bridges, fragments, micronuclei and tripolar were observed insure the cytotoxicty of this wastewater which used in this study. Therefore, it could be recommended that all wastewater should be treated according to WHO standards to avoid and minimize the pollutant effects before using for irrigation purpose, so that safety of humans would be achieved.

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التركيب الكيماوى وتأثير السمية الخلوية
لاربعة أنواع مختلفة من المياه الملوثة

حسن احمد حسن سلطان[١] – عبدالتواب محمد عطا [٢] – عماد عبد القادر المملوك[١]

١. *المعمل المركزى للزراعة العضوية بمركز البحوث الزراعية.*
٢. *قسم الوراثة – كلية الزراعة –* جامعة المنيا.

تهدف هذه الدراسة الى تقدير التركيب الكيماوى وتاثير السمية الخلوية لاربعة انواع مختلفة من المياه الملوثة وهى كالتالى مياه الصرف الصناعى، مياه الصرف الزراعى، مياه الصرف الصحى واخيرا مياه الصرف المختلط على الخلايا والانسجة المرستيمية للقمم النامية لنبات الفول البلدى. تم قياس بعض الصفات الفسيوكيماوية وكذلك المحتوى من العناصر الثقيلة لجميع المياه الملوثة ومقارنتها بمياه الصنبور (الكنترول) طبقا للمستويات المحدد بواسطة منظمة الصحة العالمية (WHO). كان الدليل على السمية الخلوية للمياه الملوثة هو حساب معدل الانقسام الميتوزى Mitotic index وكذلك الشذوذات الكروموسومية Chromosomal Aberrations . أشارت نتائج الدراسة ان معاملة البذور بالتركيزات المختلفة من جميع المياه الملوثة ادت الى خفض معدل الانقسام الميتوزى MI بدرجة معنوية بالمقارنة بالكنترول (مياه الصنبور).

خلال الدراسة تم رصد العديد من اشكال الشذوذات الكروموسومية الميتوزية مثل الكبارى الكروموسومية Chromosomal bridges ، الكروموسوم والكروماتيد المتلكئه Chromosome and chromatid laggard ، النويات الصغيرة Micronuclei ، الشظايا الكروموسومية Chromosomal fragments وكذلك الخلايا ثلاثية الاقطاب Tripolar cells . كان من الواضح ان استخدام التركيزات المختلفة لجميع انواع المياه الملوثة احدث زيادة ملحوظة فى الشذوذات الكروموسومية وكذلك على زيادة الاضرار بالمراحل الميتوزية Mitotic phases .

لذلك فانه يمكن ان يوصى بانه لابد ان تتم معاملة جميع انواع المياه الملوثة لكى تصل الى الحدود الامنة التى تسمح بها منظمة الصحة العالمية قبل استخدامها فى مياه الرى للحد من تاثير التلوث وبالتالى المحافظة على صحة الانسان.