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RESEARCH ARTICLE

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RESUMPTION OF MUTAGENICITY IN *VICIA* AFTER SECONDARY TREATMENT OF SEWAGE WATER

ABSTRACT:

The objective of this study was to evaluate the mutagenic potentialities of two samples of water from different sources namely: raw sewage, treated sewage. Genotoxicity test was conducted using two varieties of *Vicia faba* var; Giza 3 and var. Giza Blanca). Several endpoints were used to estimate the mutagenic activity in *Vicia* cells such as: 1-mitotic activity 2-nuclear abnormalities and 3-chromosomal aberrations. Chemical analysis revealed the inefficient treatment of sewage where, many elements increased after secondary treatment, and even those elements which decreased did not reach the safe limits for use. Genotoxicity test exhibited change in mitotic activity with all water samples, either by decrease in the mitotic index in *Vicia* var. G. 3 or increase in case of *Vicia* var. G. Blanca. Many types of chromosomal aberrations and nuclear irregularities were also produced after all treatments. The most frequent aberration was chromosome stickiness among different types of abnormalities such as: micronucleus, banded chromosomes, bridges, laggard chromosomes and ring chromosome. The higher percentage of chromosomal aberrations is exhibited by raw sewage. The current data demonstrated the occurrence of mutagenic compounds in sewage water after secondary treatment and this requires further treatment to remove them. Moreover, their application to irrigate agricultural land must be stopped because of the mutagenic effects of plants irrigated by such waters.

KEY WORDS:
Sewage water, genotoxicity, Vicia test.

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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; Buschini *et al*., 2004). Because of the importance of water quality to health, many toxicity and genotoxicity tests have been used in combination with physical and chemical analysis in order to evaluate both water and environmental quality (Vargas *et al*., 2001; Umbuzeiro *et al.*, 2001; Isidori *et al*., 2004). Water quality is an important risk factor in causing cancer that has been estimated in areas supplied with polluted drinking water. The observed exposure-response relationship indicated a relative risk for lymphomas, pancreatic cancer and esophageal cancer compared with areas in which non-mutagenic drinking water was consumed (Koivusalo *et al*., 1995; Tao *et al.,* 1999).

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 (El-Bagouri, 1999; Hall and El-Hakeh, 1999). However, many researchers proved that, sewage water may contain a wide variety of chemicals, detergents, pesticides, fats and heavy metals (Katyal and Satake, 1996; Rogers, 1996; Sheded and Soliman, 2001). Although, some of the toxic elements may be removed during the treatment process, others will persist and could present phytotoxic problems. Different systems have been used for wastewater treatments. The most appropriate wastewater treatment to be applied before effluent use in agriculture is one which produces an effluent meeting the recommended microbiological and chemical quality guidelines both at low cost and with minimal operational and maintenance requirements (Arar, 1988). Either preliminary, primary, secondary,

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tertiary and/or advanced wastewater treatment.

Many types of genotoxicity and mutagenicity assays employing microorganisms and mammalian cells have used for monitoring of complex environmental samples (Verschaeve, 2002; Isidori *et al*., 2004; Reinecke and Reinecke, 2004; Russo *et al*., 2004). However, plant assays, such as the *Vicia faba* test, may have some advantages over microbial and mammalian cell tests for environmental monitoring. 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; DeCampos and Viccini, 2003). Furthermore, the test plants can be directly exposed to complex mixtures or environmental samples either in the laboratory or *in situ* (Rank, 2003). Because of the large size of their chromosomes, higher plants are suitable for cytological analysis and the responses seen in plant tests are highly correlated with those seen in other biological systems, making plant tests good candidates for evaluating the genotoxicity of environmental samples (Grant, 1999; Sadowska *et al*., 2001; Majer *et al*., 2005). 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 main goal of this study was to assess the efficiency of the secondary treatment process of sewage water for use as an alternative source of irrigation of the cultivated crops and plants especially in newly reclaimed areas, by using cytogenetic approach.Last paragraph

MATERIAL AND METHODS:

Sampling:

Sewage water (treated and raw) was collected from Mansoura station of water sanitation regularly every two weeks using non metallic sampler. The samples were kept in the dark until reaching the laboratory.

Chemical analysis:

On the same sampling day, all water samples were filtered (except those which used for estimation dissolved oxygen and biological oxygen demand). Physicochemical analyses of water samples were carried out for all different stages of wastewater treatment. For each water sample collected for the mutagenicity studies, measurements of Electric conductivity (EC), pH, % salinity, dissolved oxygen (D.O.), total alkalinity, total hardness, biochemical oxygen demand (BOD), chemical oxygen demand (COD), were measured. Elements such as: sodium, potassium, calcium, sulphate, phosphorus, and chlorides were also measured. Moreover, concentrations of some heavy metals such as copper, cadmium, cobalt, lead, zinc and iron using atomic absorption spectro -photometer were estimated (Perkin Elmer, 2380). The parameters analyzed represent the removal efficiency of the nutrients in sewage.

Field experiments:

For cytogenetic analysis two varieties of broad bean (*Vicia faba*, var. Giza 3 and var. Giza Blanca), were obtained from Agriculture Research Center, Legume and Onion, Research Sections, Giza, Egypt.

In situ assay was carried out in the garden of Faculty of Science, Mansoura University. The selected seeds of the two *Vicia* varieties were surface sterilized in 1% $HgCl_2$ solution, and soaked in tap water for 24 hours before being planted. Big pots were filled with a mixture of clay and sand at a ratio of 2:1 (v/v). This soil was cleaned from grasses and other plant debris and left exposed to air for a week before being distributed into the experimental pots. The presoaked seeds were planted in pots (about 10 seeds per pot), irrigated with tap water for two weeks until seedlings appeared, and seedlings were thinned into 4 or 5 plants per pot. The planted pots were separated into 3 groups one for each treatment: raw sewage, treated sewage) and tap water as a control set. Six replicate pots were used for each treatment and follow the changes and variations in each treatment (growth, flowering and fruiting). After maturation, M1 generation harvest seeds were used for laboratory cytogenetic analysis.

Genotoxicity test:

Three sets of *V. faba* (parental and M1 generation) seeds were grown in 6 replicate pots as described above. The plants were irrigated by water samples. Tap water was used as control. Radicals (about 1 cm long) were excised, fixed, stained and squashed as described by Kihlman (1971) and Panda and Sahu (1985). The slides were made permanent by using Canada balsam and microscopically examined under to calculate the mitotic index and score the chromosomal aberrations and nuclear (interphase) irregularities.

Statistical analysis of data:

SPSS program was used to determine means and standard deviations from repeated samples of each of the experimental groups. Least Significance Difference (L.S.D.) was used to determine significance (p=0.05) in the data compared to the control.

Results and Discussion

Chemical Analysis:

Physicochemical analysis of the studied water samples is illustrated in figures 1&2. It included values for E.C., pH, % salinity, D O, C O D, total alkalinity, total hardness and B.O.D., sodium, potassium, calcium, sulphate, phosphorus, total nitrogen and chlorides and the concentrations of copper, cadmium, cobalt, lead, zinc and iron.

In general, chemical analysis of the water samples revealed that the concentrations of many estimated elements and parameters exceeded the safety limits for raw and treated sewage. The values of some physical and chemical parameters increased as well as after the secondary treatment of raw sewage. The data showed that treatment of raw sewage led to increase of concentrations of some elements such as chlorides, total nitrogen, cobalt and iron. pH was also increased after treatment. Whereas, concentrations of other elements decreased after secondary treatment such as sulphate, potassium and cadmium but they were still above the safety permissible range. This indicated the inefficiency of the treatment process and further treatment needed before use in agriculture.

Among the physical parameters, DO values decreased in both raw and treated sewage indicating the organic wastes contamination (Juttner *et al*., 1996). Also, COD in raw sewage was very high which was found to be correlated with genotoxicity in *Vicia faba* (Sang and Li, 2004). The obtained data showed an increase of pH (i.e. increase in alkaline wastes) after secondary treatment of sewage as previously reported by Pratt (1972). On the other hand, agricultural wastes lead to increase in phosphorus level which is reported in case of raw sewage (Shaaban-Dessouki *et al*., 1993). Chlorination is a common water disinfectant method which is able to reduce microbial water pollution, but which can also produce genotoxic and toxic compounds if precursors are present in the water to be treated and the level of chlorine is high (Komulainen, 2004). Most of chlorination by-products are known carcinogens (Komulainen, 2004). Some authors have also used plant assay to assess the genotoxicity induced by the by-products of disinfection, with positive results having been obtained in *Allium cepa* and *Tradescantia* assays (Monarca *et al.,* 2005). In the present study chlorides were found in unsafe limits in all water samples with higher percentage in treated sewage (Fig. 1).

The concentrations of heavy metals showed that there were high levels of potentially toxic metals such as zinc, cobalt, lead, cadmium, copper and others in both sewage water samples (Fig. 2). In particular, high concentrations of potentially toxic metals were detected in the treated sewage water which displayed consistently high levels of genotoxicity. Copper and cadmium were higher in raw sewage whereas treated sewage showed higher cobalt, Zinc and iron. Surprisingly, control samples showed higher concentration of lead compared to the other samples (This may be due to the leakage of lead from lead pipe). 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 man (Bohec and Bohec, 1990).

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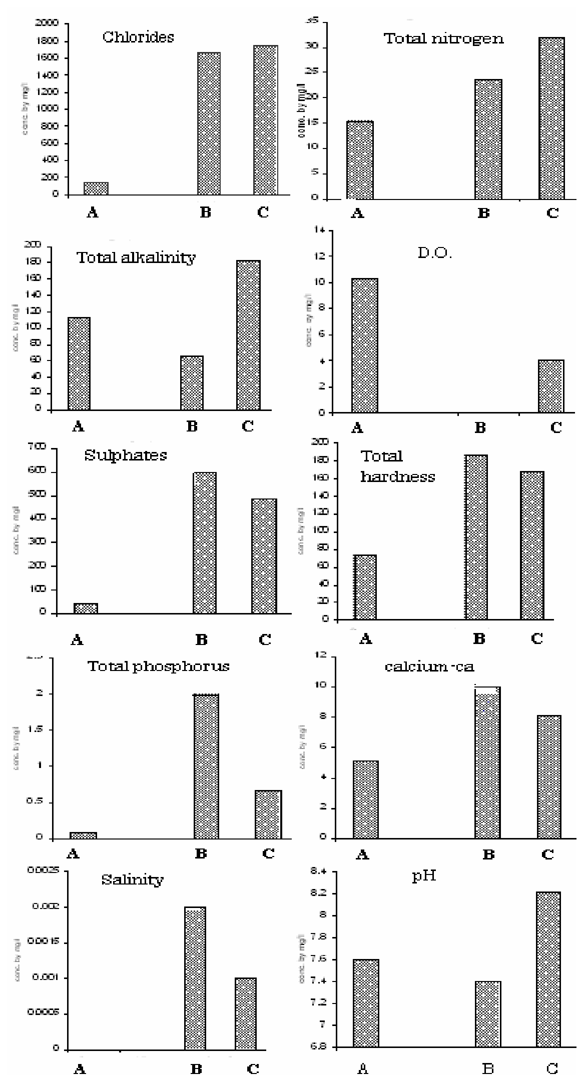

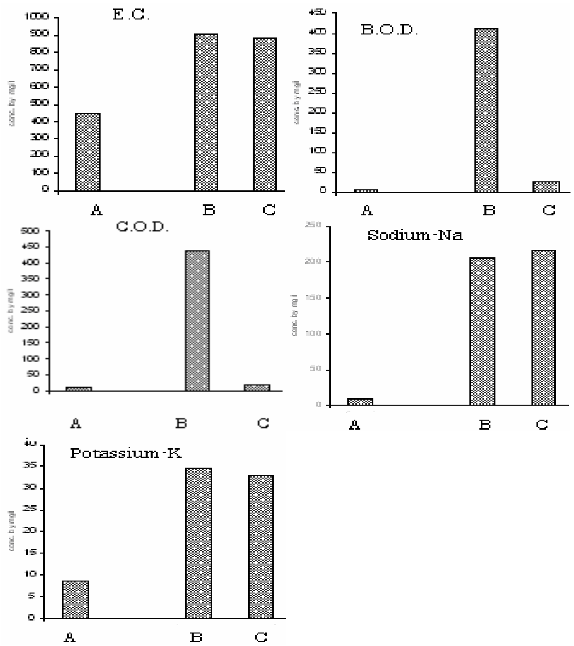

Fig. 1. Physicohemical analysis of the studied sewage samples. A: control; B: untreated sewage; C: treated sewage (Conc. by mg/l).

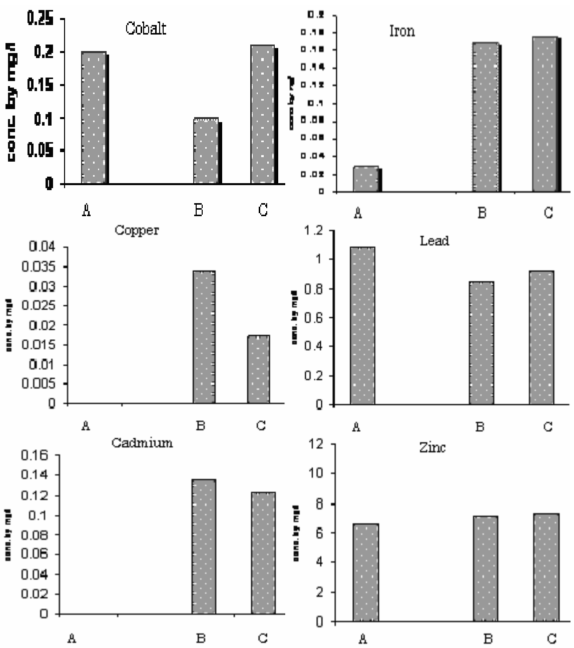

Fig. 2. Chemical analysis of heavy metals in sewage samples. A: control; B: untreated Sewage; C: treated sewage (conc. by mg/l).

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Genotoxicity Test:

Among the assays that have been used for evaluating water quality, plant systems are recognized as sensitive biomonitors of the cytotoxic and genotoxic effects of environmental chemicals and can be used for the detection of environmental mutagens both *in situ* and in the laboratory (Grant, 1999). In this study, toxic effects are evaluated by analyzing physicochemical parameters while both genotoxicity and cytotoxicity in *Vicia faba* test can be monitored through cytological parameters such as mitotic index, micronuclei, and mitotic damage. Positive results in higher plant systems indicate the presence of cytotoxic and/or genotoxic substances in the environment, and indicate the potential for direct or indirect risks for living organisms (Fiskesjo, 1993). In the present study, the treatment of *V. faba* roots with water samples caused alterations in mitotic index, chromosome and mitotic damage and/or changes in the frequency of micronuclei. The water sample obtained after treatment of the sewage water produced a statistically significant decrease in mitotic index and increase in chromosomal abnormalities but was less toxic and genotoxic than the raw sewage water samples. These data indicate the presence of cytotoxic and genotoxic substances in the raw sewage water that are reduced by the water treatment process, but not completely eliminated.

Mitotic index (MI):

Both varieties of *Vicia* species were affected by the studied water in such a way that mitotic index, was altered. The data (Table1) revealed that the tested water samples affect the mitotic index in both generations in *Vicia* (parental and M1 seeds), either by decrease (in *Vicia* G. 3 var.) or increase (in *Vicia* G. Blanca var.). Mitotic inhibition has been attributed to the inhibiting effect of chemicals on the formation of various metabolites which are necessary for normal sequence of mitosis **(**Ghoneim, 2003**).** The suppression of mitotic activity was probably due to either the blocking of G1, suppressing cells from DNA synthesis or the blocking of G2, preventing cells from entering mitosis. Increase in mitotic activity may be explained on the basis that the chemical compounds in sewage did not alter processes regulating the entry of cells into mitosis. Similar results were obtained in the root tips of *Vicia faba* due to sewage irrigation (Hossni and EL Tarras, 1997). M1 seeds exhibited reduction in mitotic index following all treatments indicating the persistence of cytotoxicity effect in this generation due to the presence of chemical compounds in water, partially heavy metals (Malik and Ahmad, 2002; Aleem *et al.,* 2003).

Table 1. Comparison between original (parental) and M1 *Vicia* seeds by % mitotic index (Mean ± S.D., * = significant at p=0.05)

TREATMENT	%M.I. OF *VICIA* VARIETIES			
	Giza B.		Giza 3	
	Parental	M1	Parental	M1
Control	15.7±1.03	19.9±0.96	20.2±0.76	21.3±1.04
Treated	17.2±1.52	16.0±0.79*	17.1±1.67*	17.0±0.79*
Raw sewage	18.0±1.7*	14.2±0.75*	16.7±0.57*	14.9±0.96*

Nuclear Irregularities:

Nuclear abnormalities in interphase cells of parental seeds in both varieties were recorded in table 2. Generally, the total nuclear abnormalities increased in both varieties after both treatments. In case of var. G.3., two types of abnormalities were recorded, micronucleus and binucleated cells (plate 1). Raw sewage induced a significant percentage of micronuclei (5.4 %), whereas non significant percentage exhibited by treated sewage (2.4 %). Nuclear abnormalities in cells of M1 seeds are recorded in table 3. The highest significant increase of total nuclear abnormalities occurred in seeds of var.G.B. treated with treated sewage (4.51 %) whereas raw sewage exhibited less increase (2.79 %) compared to control (2.12 %). The formation of micronuclei is a true mutagenic effect and is a reliable parameter for chromosomal aberration in *Vicia faba* (Sang and Li, 2004**).** An increase in the frequency of micronucleated cells is considered a result of chromosomal and genomic damage caused by clastogens or spindle poisons **(**Russo *et al*., 2004**).** The formation of multinucleated cells may be the result of a preceding multipolar mitosis or the failure of cell plate formation, (Grover and Kaur, 1999).

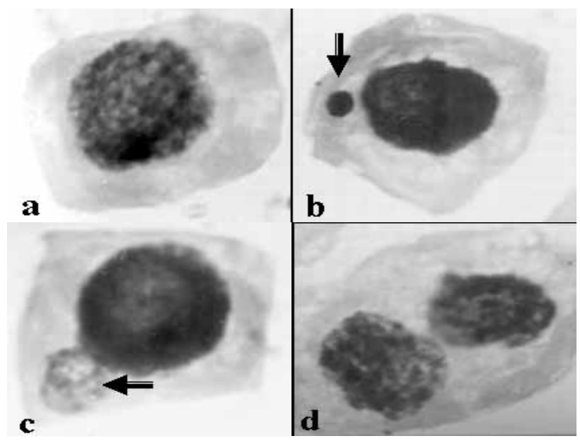


Plate 1. Nuclear abnormalities in Vicia faba,a-normal, b-c-micronucleus, d- binucleated cell.

Table 2. Different types of abnormalities in interphase of *Vicia faba* parental seeds. (Mean ± S.D., * = significant at p=0.05).

TREATMENT	%ABNORMALITIES				% TOTAL ABNORMALITIES	
	Micronucleus		Binucleated cell			
	G.3.	G.B.	G.3	G.B.	G.3.	G.B.
Control	1.2	2.2	0.2	1.0	0.88±0.34	1.9±0.87
Treated sewage	2.4	3.8	1.6*	1.6	2.42±1.22*	3.38±0.85*
Raw sewage	5.4*	3.6	0	0.4	3.24±0.53*	3.9±2.29*

Table 3. Different types of aberrations in interphase of *Vicia faba* M1 seeds. (Mean ± S.D., * = significant at p=0.05).

TREATMENT	% ABNORMALITIES				% TOTAL ABNORMALITIES	
	Micronucleus		Bi/multinucleated			
	G.3.	G.B.	G.3	G.B.	G.3.	G.B.
Control	3.8	2.8	0	0.6	2.42±0.83	2.12±1.3
Treated sewage	3.8	6.0*	0.8	1.6	2.76±0.53	4.51±1.69*
Raw sewage	3.4	4.4	1.0	0.4	2.58±1.05	2.79±0.76

Chromosomal Aberrations in mitosis:

Results of mitotic chromosomal aberrations are recorded in table 4 and shown in plates 2-6.

Total percentage of chromosomal aberrations is significantly increased in mitotic cells of parental and M1seeds in both varieties after all treatments. Chromosomal aberrations included: micronuclei, banded chromosome, stickiness, splitting metaphase, bridges, laggard chromosome, ring chromosome, C-metaphase and disturbed metaphase.

Table 4. The frequencies of chromosomal aberrations for parental/M1 seeds in G.B. and G3 varieties of *Vicia* (Mean ± S.D., * = significant at P= 0.0.

Treatment	Chromosomal aberrations%			
	G.B.		G.3	
	M1	Parental	M1	Parental
Control	25.66±5.92	13.91±6.67	16.34±5.56	12.06±6.39
Treated sewage	46.48±9.88*	37.77±10.16 *	50.98±10.13*	40.32±7.54*
Raw sewage	52.78±14.73*	40.48±9.13*	75.38±9.48*	48.56±11.96*

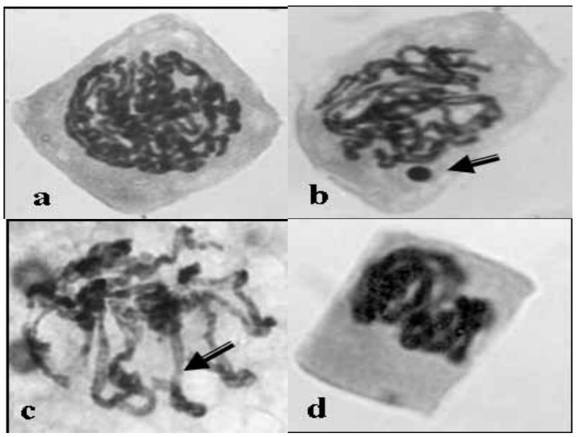
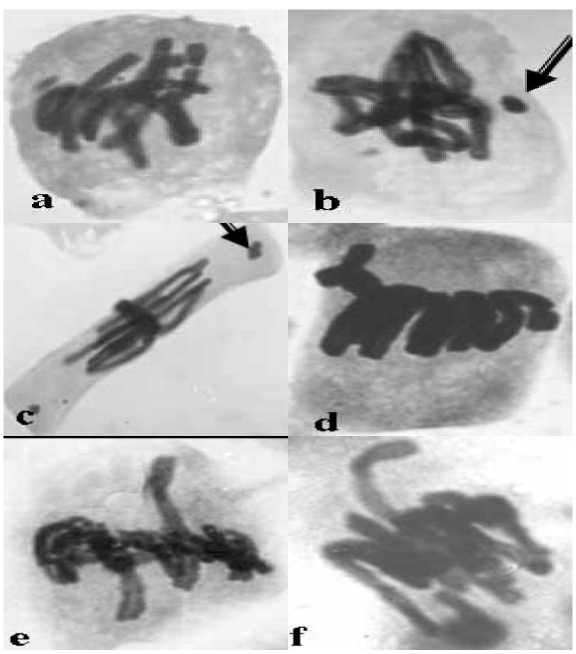


Plate 2. Prophase of *Vicia faba*, a-normal, b- micronucleus, c-banded chromosome, d-stickiness.



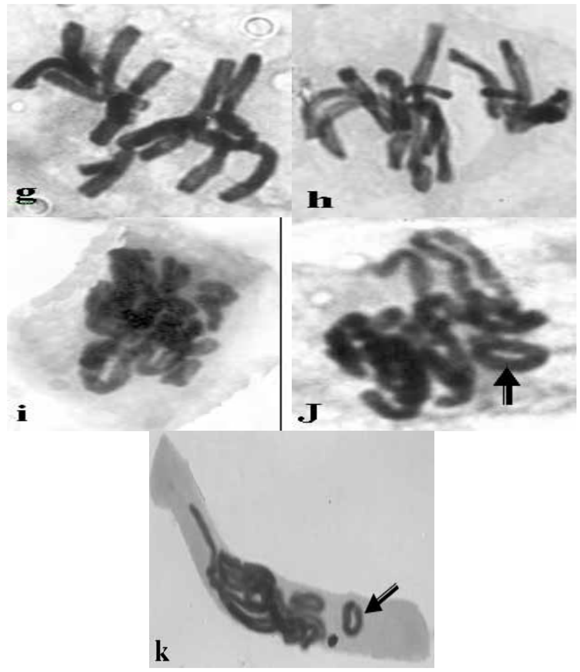
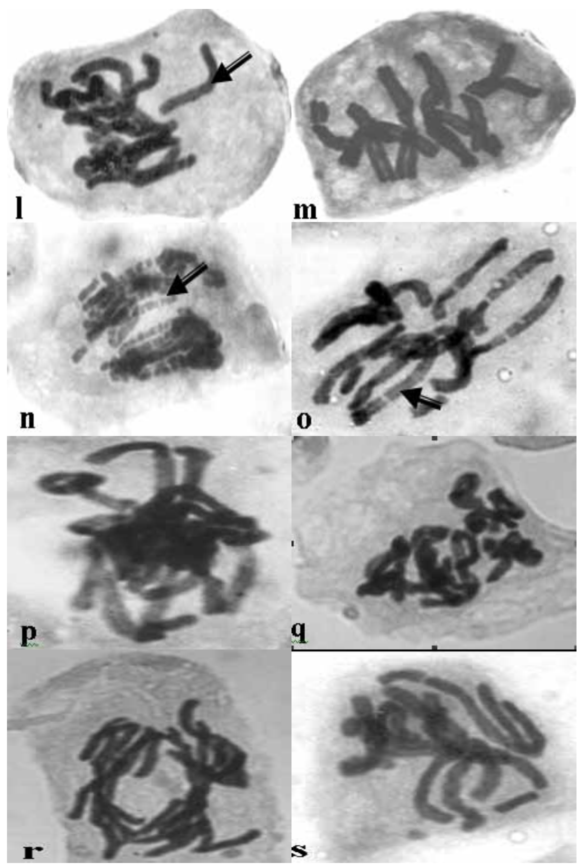


Plate 3. *Vicia faba* metaphase, a-normal, b-c-micronucleus, d-e-f- misorientation, g-h-split, i-stickiness, j-k-ring chromosome.



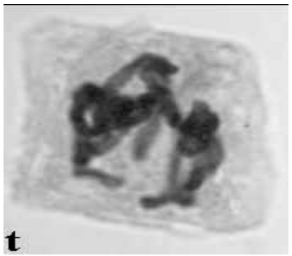


Plate 4. *Vicia faba* metaphase, i-forward chromosome, m-C- metaphase, n-o-banded chromosome, p-t-disturbed metaphase.

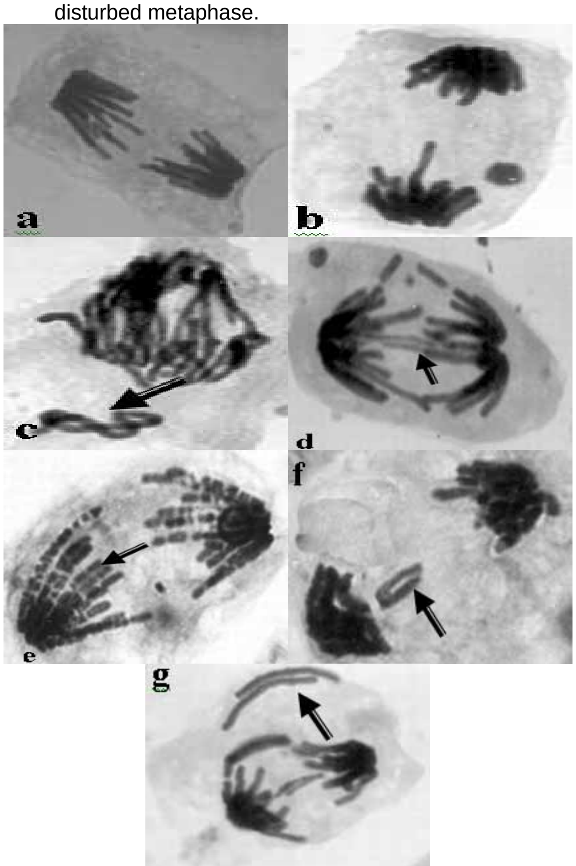
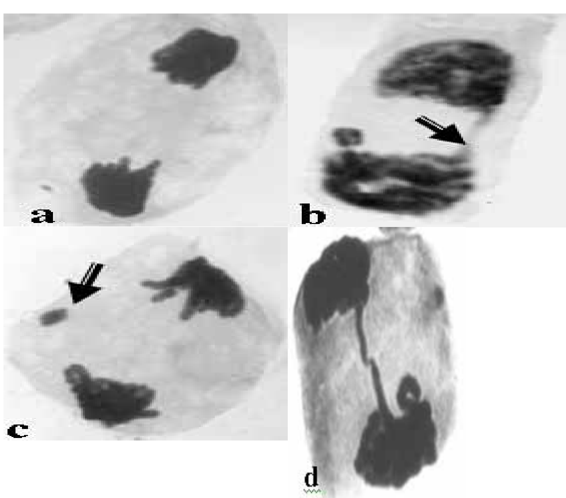


Plate 5. *Vicia faba* anaphase, a-normal, b-micronucleus, c-f-g-laggard chromosome, d-bridge with micronucleus, e-banded chromosome.



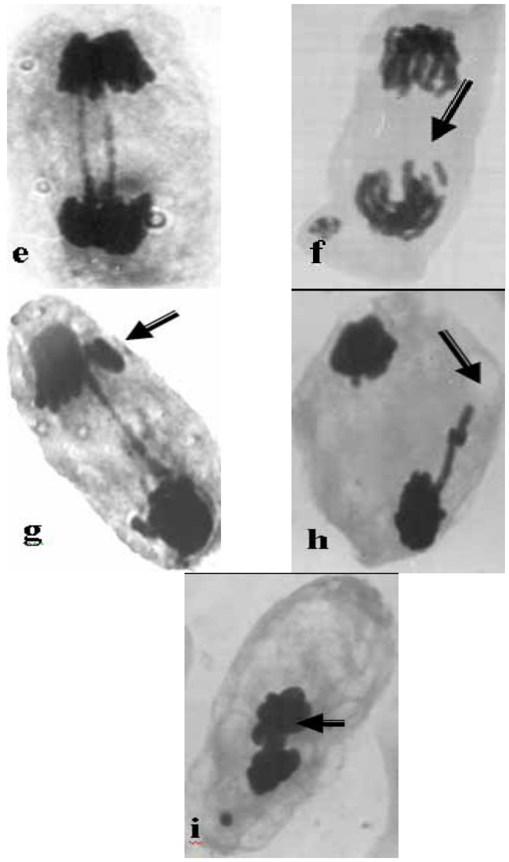


Plate 6. *Vicia faba* telophase, a-normal, b-micronucleus with bridge, c-micronucleus, d-bridge, e-dibridges, f-laggard, g-bridge with laggard, h-broken bridge, i-sticky bridge.

In the present study, different types of the observed aberrations in mitotic cells were probably due to the effect of genotoxic compounds found in the studied sewage water samples. Such toxic compounds (household chemicals, detergents, chlorophenols, trace elements, heavy metals etc.) caused drastic changes in chromatin, spindle apparatus and centromeres, leading to impairment of chromosome alignment on to metaphase plate, abnormal spindle orientation, abnormal chromosome movement and c- mitosis.

Potentially toxic metals, resulting from some agricultural and industrial activities are known to induce chromosome breaks, fragments and micronucleus formation in plants and mammalian test systems (Knasmuller *et al.,* 1998). Among the effects induced are formation of DNA – DNA and DNA – protein cross-links, alterations of mitotic spindles formation producing aberrant mitotic stages, and induction of sister chromatid exchanges (SCE). The induction of micronuclei is usually caused by chromosome breaks or fragments or spindle poisoning, which is an anomalous disjunction of chromosomes during anaphase. Several metals have been shown to be mutagenic and/or carcinogenic in both human and animal studies. They increase the risk of cancer, especially lung and skin cancers (Harris and

Shi, 2003; Leonard *et al*., 2004). Plant assays have been considered useful tools for evaluating and ranking the toxicity and genotoxicity of metals (Evseeva *et al*., 2003; Chandra *et al*., 2005; Unyayar *et al*., 2006). Metals such as iron and copper can also cause indirect effects due to increased oxidative stress leading to cytotoxicity and DNA damage (Costa *et al*., 2002), (Waisberg *et al*., 2003; Unyayar *et al*., 2006). These finding are confirmed by the results obtained in this study where metals are recorded in unsafe concentration in sewage (raw and treated). Some of them such as zinc exert toxic action resulting in mutations that persist in the M2 generation (El Ghamery *et al*., 2003). Although all the treated water samples showed some degree of genotoxicity compared to the raw sewage water, raw sewage appeared to be the most damaging as evidenced by its capacity to induce an increased frequency of chromosome abnormalities. The water sample obtained after treatment of the sewage water produced a statistically significant increase of abnormal mitotic cells frequencies. However, overall it was less toxic and genotoxic than the raw water samples. These data indicate the presence of cytotoxic and genotoxic substances in the raw sewage water that are reduced by the water treatment process.

CONCLUSION:

The toxic effects of the studied water samples, particularly, sewage water (raw and treated), on the mitotic activity paralleled their effects on nucleus and chromosomal behavior. The treatments caused an increase in the percentage of the total abnormalities in the meristematic cells of *Vicia* roots. Although some treatments did not produce a significant genotoxic response, there was some indication of genotoxic potential. This proof their cytotoxicity and mutagenicity especially if they are used in irrigation in case of treated sewage. It is highly recommended that sewage water need more advanced treatments and heavy metals should be lowered before being used for irrigation, where these pollutants might enter into the food chain and cause health hazards to humans.

REFERENCES:

Aleem A, Isar J, Malik A. 2003. Impact of long-term application of industrial wastewater on the emergence of resistance traits in *Azotobacte*r *chroococcum* isolated from rhizospheric soil. Biores. Technol. 86, pp.713.

Arar A. 1988. Background to treatment and use of sewage effluent. Ch. 2, Treatment and Use of Sewage Effluent for Irrigation, (Pescod MB,d Arar A. eds). Butterworths, Sevenoaks, Kent.

Ateeq B, Farah MA, Ali MN, Ahmad W. 2002. Clastogenicity of pentachlorophenol, 2,4-D and butachlor evaluated by *Allium* root tip test. Mutat Res., 514: 105-113.

Bohec J Le, Le- Bohec J. 1990. The use of urban composts for vegetable crops. Infos-Paris. 61: 23- 28.

Buschini A, Martino A, Gustavino B, Monfrinotti M, Poli P, Rossi C, Santoro A, Dorr AM, Rizzoni M. 2004. Comet assay and micronucleus test in circulating erythrocytes of *Cyprinus carpio* specimens exposed in situ to lake waters treated with disinfectants for potabilization. Mutat. Res., 557: 119-129.

Chandra S, Chauhan LK, Murthy RC, Saxena PN, Pande PN, Gupta SK. 2005. Comparative biomonitoring of leachates from hazardous solid waste of two industries using the *Allium* test. Sci. Total Environ., 347: 46-52.

Costa M, Salnikow K, Sutherland JE, Broday L, Peng W, ZhangQ , Kluz T. 2002. The role of oxidative stress in nickel and chromate genotoxicity. Mol. Cell Biochem., 235: 265-75.

De Campos JMS, Viccini LF. 2003. Cytotoxicity of aluminum on meristematic cells of *Zea mays* and *Allium cepa*. Caryologia, 56: 65-73.

El-Bagouri IH. 1999. Potentials and constraints of the reuse of waste water in desert agriculture. Final report of land and water. Management Programmed, Center for Environmental And Developmental for The Arab Region and Europe (CEDARE). pp: 1-16.

El-Ghamery AA, Mansour MM, Abou EL-Yousser MA. 2003. Effect of some heavy metals on mitotic activity, nucleic acids content and protein banding pattern in meristematic roots of *Nigella sativa* and *Triticum aestivum,* Egypt J. Biotechnol., 11: 266-281.

Evseeva TI, Geras'kin SA, Shuktomova II. 2003. Genotoxicity and toxicity assay of water sampled from a radium production industry storage cell territory by means of *Allium*-test. J. Environ. Radioactiv., 68: 235-248.

Fiskesjo G (1988). The *Allium* test – An alternative in environmental- studies The relative toxicity of metal-ions. Mutat Res 197:243-260.

Fiskesjo G. 1993. The *Allium* test in wastewater monitoring. Environ. Toxicol. Water Qual., 8: 291-298.

Ghoneim GT. 2003. Cytological and physiological studies on the effect of some herbicides on Flax. M. Sc. Thesis, Fac. Sci.(Damietta), Mansoura Univ. Mans. Egypt.

Grant WF. 1999. Higher plant assays for the detection of chromosomal aberrations and gene mutations -A brief historical background on their use for screening and monitoring environmental chemicals. Mutat. Res., 426: 107-112.

Grover IS, Kaur S. 1999. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the *Allium* root anaphase aberration and micronucleus assays. Mutat. Res., 426: 183-188.

Hall J, El-Hakeh H. 1999. The Alexandria Effluent and sludge reuse study. In Fourth International water Technology Conference (IWTC) 99, Alexandria, Egypt, pp: 313-323.

Harris GK , Shi X. 2003. Signaling by carcinogenic metals and metal-induced reactive oxygen species. Mutat. Res., 533: 183-200.

Hossni YA, El-Tarras A. 1997. Safety of sewage water in the irrigation of plants, Egypt. J. Hortic., 24(2): 261-270.

Isidori M, Lavorgna M, Nardelli A, Parrella A. 2004. Integrated environmental assessment of Volturno River in South Italy. Sci. Total Environ., 327: 123-134.

Juttner I, Rothfritz H, Ormerdo SJ. 1996. Diatoms as indicators of river quality in Nepalese Middle Hills with consideration of the effects of habitat-specific sampling. Freshwater Biol., 36: 475-486.

Katyal T, Satake M. 1996. Environmental pollution. Animal Publications Pvt. ltd New Delhi, pp. 65- 160.

Kihlman BA. 1971. Root tips for studying the effect of chemicals on chromosomes. In: “Chemical mutagens, (Hollander A. ed.)”, Plenum. New York, 2: 489-514.

Knasmuller S, Gottmann E, Steinkellner H, Fomin A, Pickl C, Paschke A, God R, Kundi M. 1998. Detection of genotoxic effects of heavy metal contaminated soils with plant bioassays. Mutat. Res., 420: 37-48.

Koivusalo M, Vartiainen T, Hakulinen T, Pukkala E, Jaakkola JJK. 1995. Drinking-water mutagenicity and leukemia, lymphomas, and cancers of the liver, pancreas, and soft tissue. Arch. Environ. Health, 50: 269-276.

Komulainen H. 2004. Experimental cancer studies of chlorinated by-products. Toxicology, 198: 239-248.

Leonard SS, Bower JJ, Shi XL. 2004. Metal-induced toxicity, carcinogenesis, mechanisms and cellular responses. Mol. Cell Biochem., 255: 3-10.

Majer BJ, Grummt T, Uhl M, Knasmüller S. 2005. Use of Plant Bioassays for the Detection of Genotoxins in the Aquatic Environment. Acta hydrochim. hydrobiol., 33(1): 45-55.

Malik A, Ahmad M. 2002. Seasonal variation in bacterial flora of the wastewater and soil in the vicinity of industrial area. Environ. Monit. Assess., 73: 263-273.

Monarca S, Feretti D, Zani C, Rizzoni M, Casarella S, Gustavino B. 2005. Genotoxicity of drinking water disinfectants in plant bioassays. Environ. Mol. Mutagen., 46: 96-103.

Ohe T, White PA, DeMarini DM. 2003. Mutagenic characteristics of river waters flowing through large metropolitan areas in North America. Mutat. Res., 534: 101-112.

Panda BB, Sahu UK. 1985. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by organophosphorus insecticide fensulfothion. Cytobios, 42: 147-155.

Pratt PF. 1972). Quality criteria for trace elements in irrigation waters. California Agricultural Experiment Station. 46 p.

Rank J. 2003. The method of *Allium* anaphase-telophase chromosome aberration assay. Ekologija, 1: 38-42.

Rank J, Lopez LC, Nielsen MH, Moretton J. 2002. Genotoxicity of maleic hydrazide, acridine and DEHP in *Allium cepa* root cells performed by two different laboratories. Hereditas, 136: 13-18.

Reinecke SA, Reinecke AJ. 2004. The comet assay as biomarker of heavy metal genotoxicity in earthworms. Arch. Environ. Contam. Toxicol., 46: 208-215.

Rogers HR. 1996. Sources, behavior and fate of organic contaminants during sewage treatment and in sewage sludges. Sci. Total Environ., 185: 3–26.

Russo C, Rocco L, Morescalchi MA, Stingo V. 2004. Assessment of environmental stress by the micronucleus test and the Comet assay on the genome of teleost populations from two natural environments. Ecotox. Environ Safety, 57: 168-174.

Sadowska A, Pluygers E, Niklinska W, Maria MR , Obidoska G. 2001. Use of higher plants in the biomonitoring of environmental genotoxic pollution. Folia Histochem. Cytobiol., 39: 52-53.

Sang N, Li G. (2004. Genotoxicity of municipal landfill leachate on root tips of *Vicia faba*. Mut. Res., 560(2): 123-134.

Shaaban-Dessouki SA, Soliman AI, Deyab MA. 1993. Environmental characteristics and nutrients distribution in Damietta Estuary of the River Nile J. Environ. Sci., 6: 159-177.

Sheded MG, Soliman MSA. 2001. Environmental Impact of Sewage Water on Soil Variables, *Ricinus communis* and *Helianthus annuus* Protein and Associated Species. OnLine Journal of Biological Sciences 1(11): 1046-1050.

Tao XG, Zhu HG, Matanoski GM. 1999. Mutagenic drinking water and risk of male esophageal cancer: A population based case-control study. Am. J. Epidemiol., 150: 443-452.

Umbuzeiro GD, Roubicek DA, Sanchez PS, Sato MIZ. 2001. The *Salmonella* mutagenicity assay in a surface water quality monitoring program based on a 20-year survey. Mutat Res. Genet. Toxicol. Environ. Mutagen., 49: 119-126.

Unyayar S, Celik A, Cekic FO, Gozel A. 2006. Cadmium induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis, 21: 77-81.

Vargas VMF, Migliavacca SB, de Melo AC, Horn RC, Guidobono RR, Ferreira I, Pestana MHD. 2001. Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. Mutat Res., 490: 141-158.

Verschaeve L. 2002. Genotoxicity studies in groundwater, surface water, and contaminated soil. Environ. Toxico., l2: 1247- 1253.

Waisberg M, Joseph P, Hale B, Beyersmann D. 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology, 192: 95-117.

الكشف عن القدرة المطفرة لمياه الصرف الصحي المعالج باستخدام اختبار نبات الفول

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نتيجة لنقص الموارد المائية العذبة المطلوبة للاستخدام الادمى و في الري يتم استخدام مياه الصرف الصحي المعالجة في ري المحاصيل واستهدفت هذه الدراسة تحديد:القدرة المطفرة لعينات من مياه الصرف الصحي من مصدرين مختلفين احدهما غير معالج والآخر مياه صرف معالج. وتم عمل اختبار السمية الوراثية باستخدام صنفين من نبات الفول *Vicia faba* (var. Giza 3 , var. Giza Blanca). وقد تم إجراء التحليل الكيميائي لقياس العديد من العناصر الثقيلة (الحديد و الكادميوم والكوبلت و الزنك و الرصاص و النحاس) بالاضافه للعديد من العناصر الأخرى مثل ا لكلوريدات والكالسيوم وبعض القياسات مثل درجه الحموضة ومعامل التوصيل الكهربي وتأثيرها على النباتات كما تم عمل التحليل الوراثي الخلوي لتقدير معدل الانقسام الميتوزى والشذوذ الكروموسومي والنووي في قمم جذور نبات الفول. وقد أظهرت نتائج التحليل الكيميائي عدم فاعلية عملية المعالجة لمياه الصرف حيث زادت بعض العناصر بعد المعالجة عن حدود الأمان المطلوبة لاستخدامها في الري. كما أدت معالجه قمم الجذور النامية بعينات من المصادر المختلفة إلى:1— نقص واضح في معدل الانقسام في نبات الفول صنف (Giza 3) بينما زاد معدل الانقسام في نبات الفول صنف Giza Blanca), 2- زياده في نسبه الشذوذات الكروموسومية والنووية .وقد أظهرت مياه الصرف غير المعالج أعلى نسبة من الشذوذات الكروموسومية. ونظرا للتأثير الواضح يوصى بعدم استعمال مياه الصرف الصحي سواء المعالجة ثانويا أو مياه الصرف الصحي غير المعالجة التي أثبتت النتائج وجود مركبات مطفرة بها في الاستخدام لري النباتات التي تؤكل. ولذلك فانه يجب أن تشمل الدراسات اللاحقة الدرجات المختلفة من عمليات المعالجة ومحاولة استخدام مؤشرات أخرى مثل دراسة point mutation.

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