Journal of Agricultural Chemistry and Biotechnology

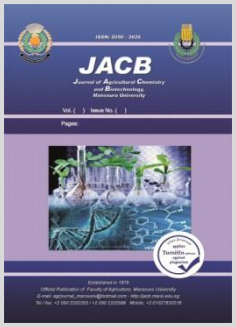
Journal homepage: www.jacb.mans.edu.eg
Available online at: www.jacb.journals.ekb.eg

Genotoxicity Evaluation of Agricultural Drainage Water and Industrial Effluents using Cytological Bioassay

Mervat I. Kamal*; K. A. Zaied; A. H. Abd El – Hady and A. S. Abd El- Mohsen

Depatment of Genetic, Faculty of Agriculture, Mansoura University.

Cross Mark



ABSTRACT

The impact of drainage water pollution on the genetic material of bioindicator *Allium cepa* L. was assessed by cytogenetic analysis. *Allium cepa* L. was germinated in three samples of drainage water in addition to the Nile water as a control. Waste water used in this study included agriculture drainage water and liquid industrial wastes resulted from chemical fertilizer industry. This study aimed to investigate the cytological effects of drainage water from different resources on root meristems cells of *Allium cepa* L. Exposure of onion roots to the waste water showed chromosomal abnormalities which was pollution–dependent. Drainage water induced a variety of chromosomal abnormalities which gradually increased in Kfr El – Sheikh drainage water, Menyet El – Nasr drainage water and industrial waste water, respectively. The drainage water discharged in Menyet El – Nasr center and that discharged from chemical fertilizer industry was effective in forming micronuclei, binucleated cell and disturbance of the spindle fibre apparatus due to high concentrations of chemicals present in these drainage wastes. The toxic chemicals present in drainage water were responsible for the observed genotoxic effects. Laggards, sticky chromosomes, anaphase bridge and fragmented chromosomes being the most frequently seen in all treatments with drainage water. Treatment with drainage water decreased mitotic index and increased the frequency of chromosomal abnormalities compared with the control. To conclude, the untreated drainage water mostly contain toxic chemicals leading to mutagenicity. Based on these findings, the bio monitoring investigation and treatment of drainage water before discharging into the environment are needed. Therefore, mutagenicity/ genotoxicity assays must be considered in water quality monitoring programs to avoid the mutagenic hazards of waste waters.

***Keywords*:** Genotoxicity, chromosomal abnormalities, *Allium cepa* L., cytogenetic analysis, biomonitoring, drainage water, mitotic index.

INTRODUCTION

Chemical fertilizer industrial plants generate waste water characterized by chemicals including nitrite, nitrate, heavy metals, organic and inorganic compounds which are assimilated by aquatic species, pass through the food chain and bio accumulate upon long – term exposure (Sang and Li 2004). These wastes contain environmental mutagens such as heavy metals and reactive oxygen species (ROS) and could be serious risk hazard for human health (Bakare *et al.* 2007). Industrial and agricultural practices are ones of the key sources of thousands of chemicals that enter the environment each year which affect the surface water, ground water and land. Effluent from industries and agricultural practices threating the aquatic system, as well as the genetics of living beings. Carcinogenic and mutagenic compounds have been found in the wastewater (Alam *et al.* 2009). Due to these environmental pollutants,organisms undergo multiple types of damage (Sisman 2014). Different pollutants entered the rivers enter the food chain and cause mutations and disease (Erchull and Fisher 2016). The harmful contaminants enter the food chain due to the use of polluted water in agricultural practices (Brooks *et al.* 2016). *Allium cepa* is an efficient plant for testing genetic alterations caused by the toxic substances, due to its kinetic proliferation properties and low number of chromosomes (2n = 16), making it easy to characterize chromosomal aberrations or damage in the DNA structure (Gomes *et al.*2013). The pollution of water resources is a worldwide problem (Monte Egito *et al.* 2017). Pollutants also pose subtle dangers to human health. To assess the genotoxic effects of such waste waters, plant cells are used in this study for several advantages over microbial and mammalian systems. These advantages include the similarity in the chromosomal morphology with mammals, as well as the fact that plants and mammals have a similar response to the mutagens. In addition, plant cells are less expensive than mammalian systems because of small number of their chromosomes. *Allium cepa* root – tip cells are used to assess a variety of cytogenetic traits that can serve as genotoxicity indicators, including micronuclei induction and chromosomal aberrations (Leme and Marin – Morales 2009). Genotoxicity testing are currently an integral part of the water quality testing (Kungolos *et al.*2006). Mitotic index of cell population are regarded as an important criterion of the growth and multiplication of tissues (Walker and Yates 1652 a and b). 2009).

This study aimed to examine the cytotoxic and genotoxic effects of irrigated wastewater and liquid industrial effluents on *Allium cepa* root meristems via chromosomal abnormalities induced.

* **Corresponding author.**
E-mail address: mervat _ y2007@yahoo.com - Tel : 002 – 01008665560
DOI: 10.21608/jacb.2021.160402

MATERIALS AND METHODS

Materials

Onions bulbs (*Allium cepa* L., Family Amaryllidaceae) were collected commercially from the local market in Mansoura city, Dakahlia Governorate and sun-dried for two weeks. Thereafter, the healthy dry bulbs were used for the genetic test.

Fixative solution

This is carnoys solution which is the most common fixative solution .It consist of one part glacial acetic acid and three parts absolute ethanol . (Mirzaghaderi 2010).

Acetocarmine staining.

Acetocarmine was used to stain plant chromosomes..It was prepared by dissolving 10 g carmine in one litre of 45% glacial acetic acid, then boiled, and cooled for 24h .Filter into dark bottles and store at 4C .10%ferric chloride solution was added to 100ml of acetocarmine solution (Mirzaghaderi 2010).

Waste water of fertilizer industry

The industrial wastewater is discharged via a main pipe into a piece of land at the back of the site. The effluent was collected in January 2018 at the point of discharge and all the water samples used in this study were chemically analyzed for the presence and concentrations of some potentially mutagenic heavy metals in the central Laboratory, Faculty of Agriculture, Mansoura University .

Collection of driange water

Agriculture drainage water was collected in January 2018 from Menyet EL-Naser drainage system located in Dakahlia governorate. The irrigated water incoming (pure) and outgoing (refined) at Menyat El Nasr center and wastewater of fertilizer industry in Talkha centere was collected in January 2018, and bottled in plastic containers.

Methods

Bioassay technique

Onion (*Allium cepa* L.) roots were used for genetic bioassay. Root meristem raised in water was treated with different samples of water including river water as a control, wastewater of chemical fertilizer industry, as well as, agriculture drainage water. Root tips excised from treated and control materials were fixed in 1: 3 acid to alcohols for 24 hr and preserved in 70% ethyl alcohol. Root tips were squashes in 2% acetocarmine stain. Different phases of mitosis were counted and chromosomal abnormalities were observed. Mitotic index, phase indices and total abnormality percentage were calculated at different phases.

Slides were examined microscopically and counts for dividing cells, non-dividing cells, cells at each mitotic phase and aberrant cells were recorded Photomicrographs of typical aberrant cells were documented.

Chromosome squash technique were fowlled according to Busch *et al.* 1996,

RESULTS AND DISCUSSION

Mitotic abnormalities

As shown from the results presented in Table (1) and Figure (1) treatment with drainage water on the root tip cells of *Allium cepa* induces chromosomal abnormalities. Mitotic abnormalities increased by the treatment with chemical fertilizer industrial effluents followed by the drainage water of Menyet El- Nasr. Chromosomal abnormalities observed in this study included micronuclei, fragments, stickiness, binucleated cells, laggard and. Micronuclei was more frequently found in the treatment with industrial drainage water followed by the percentage of diturbed chromosomes. Drainage water induced chromosomal aberration via the interactions with DNA and proteins leading to stickiness of chromosomes and mitotic disturbances (Teena *et al.* 2016). These results agreed with Darlington (1942) who reported that stickiness of chromosomes resulted from the breakdown of DNA. Stickiness was considered as a common sign of toxic effects on the chromosomes probably leading to cell death (Fiskejo 1997). The results obtained herein are in harmony with pratibha (1987) who demonstrated that chromosome bridges and fragments may be induceed through the genotoxicity of starch factory effluents. In this study the spindle abnormalities were shown, if the orientation of spindle was, shifted to the corners of the cell. This could be considered as a signal of warning which may constitute risk to human health. Therefore, this observations demonstrated that drainage water from industrial or agricultural activities must be treated before discharged to the soil or other water bodies. The highest laggard percentage of chromosomes was obtained from the treatment with industrial waste waters which resulted from the failure of the chromosomes to move to either of the poles. In addition, acentric chromosome fragments are considered as laggards (Turkoglu 2007). Stickiness of chromosomes may occurs due to degradation or depolymerization of chromosomal DNA (Grant 1982) or as a result of DNA.

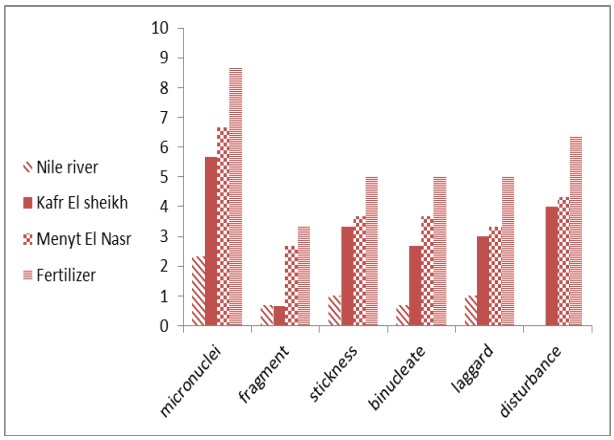


Figure 1. Chromosomal aberrations induced in *Allium cepa* by drainage water resulted from agricultural activities and chemical fertilizer industry

Condensation, as well as stickiness of inter – chromosome fibers (Schneiderman *et al.* 1971), which indicates high toxicity of the tested substance that demonstrate genotoxic effects in plant cells depend on the concentration of pollutants. The positive results of genotoxicity in plant cells confirm that there are microsomal enzymes and peroxidase in higher plants leading to forming reactive intermediates which may induce covalent binding and fragmentation of DNA molecules (Fiskesjo and Lassen 1982).

Table 1. Effects of drainage water on the percentage of aberrant cells in *Allium cepa* root tips.

Treatments	Total no.of studied Cells	No.ofmicronuclei types		Percentage of micronuclei %	Percentage of types of chromosomal aberration				
		Compact %	Non compact %		Fragments %	Stickiness %	Binucleate Cells %	Laggard %	Disturbance chromosome %
Nile river	300	1.33	1.00	2.33	0.67	1.00	0.67	1.00	0.00
Kafr El-Sheikh	300	3.00	2.67	5.67	0.67	3.33	2.67	3.00	4.00
Menyet El-Nasr	300	3.33	3.33	6.67	2.67	3.67	3.67	3.33	4.33
Fertilizer Factory	300	4.00	4.67	8.67	3.33	5.00	5.00	5.00	6.33

Mitotic index

Data presented in Table (2) and Figure (2) indicate that higher mitotic index was obtained from the treatment with Nile water followed by industrial waste water from fertilizer industry. Mitotic index ranged between 15.65–– 34.98. The highest mitotic abnormalities was observed in the treatment with factory effluents followed by the drainage waters from Menyet El- Nasr and Kfr El – Sheikh Governorate, respectively. Mitotic aberrant cells are registered in drainage waters three times at least increase above the control, indicating dependence to concentration of pollutants which reflects water quality. Dropping of mitotic index proved that drainage water carrying pollutants interferes with normal sequences of mitosis acting in inhibiting manner. This should be looked as the blockade of G_2 phases of cell cycle which leading to inhibition of DNA / protein synthesis (Turkoglu 2007). The blockade of glucose metabolism and cell impoverishment of ATP which resulting in deteriorated entry of cells into cell cycle (Amin 2002). The results obtained in this study indicated that drainage waters carrying pollutants induces chromosomal abnormalities depending on the level of pollutants which reflected the quality of testing water.

Natural water from the Nile river appeared the highest percentages of prophase and telophase cells. Hughes 1952). The results obtained herein agreed with Nielson and Rank (1994), who reported that the heavy metals present in the waste waters induced significant chromosomal aberrations. Industrial effluents has the highest genotoxic effect indicated by increased mitotic abnormalities approximately five times above the control, thereby providing it more toxic than agriculture drainage water. The number of total chromosomal aberrations was observed to be the highest in the treatment with industrial waste water, as high genotoxicity which is due to disorganization of chromosome structure in the root meristem cells of *Allium cepa*. This indicated that a high concentration of pollution led to increase in the rate of chromosomal abnormalities and decline the rate of cell division than the control treatment. These findings agreed with Pesnya *et al*.(2017), who reported that mitotic index as a parameter of cytotoxicity has been used to check the cytotoxicity level of the tested substance. Drainage waters used in this study induces genotoxic effects in onion cells including mitotic index and various chromosomal abnormalities, whereas high mitotic abnormalities was shown from the treatment with industrial waste water which containing higher concentration of pollutants. Dulta *et al*.(2018) reported that if the value of mitotic index declines below 22% of the control this indicated a fatal effects on the tested organism. On the other hand, the same authors reported that a sub- lethal effect was shown if the value declines from 50% which known as cytotoxic limit value. This enables us to assess the level of water contamination. This technique indicates a high sensitivity tool which helps to observe the level of contamination contaminated water. Pollution concentration – dependent was shown in the treatment with industrial effluents which appeared mitotic depression than the control. The highest number of the cells carrying abnormalities were achieved in industrial effluents. Thus the mitotic index could be another endpoint for assessment of the genotoxicity of drainage water. Lower percentage of mitotic index observed in this study indicates cell cycle disturbances or chromatin dysfunction because of pollutants interactions with DNA.

Table 2. Percentage of mitotic abnormalities and mitotic index in root meristems cells of *Allium cepa* treated with drainage water from different resources.

Treatments	Total no.of counted cells	% Prophase	% Metaphase	% Anaphase	% Telophase	% Mitotic abnormalites	% Mitotic index
Nile river	300	26.33	3.66	3.66	1.33	8.00	34.98
Kafr El-Sheikh	300	2.66	7.00	5.66	0.33	27.0	15.65
Menyet El-Nasr	300	3.66	6.66	6.66	0.66	31.0	17.64
Fertilizer Factory	300	5.00	10.33	9.00.	0.33	42.0	24.66

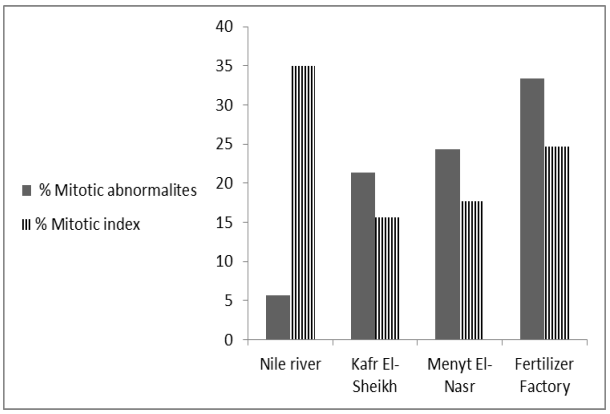


Figure 2. Comarism between different water resources for mitotic abnormalities induced and mitotic index percentage.

Abnormal chromosomal behavior

Chromosomal behavior observed in Figure (3) illustrates some chromosomal aberrations in the root tip cells of *Allium cepa* exposed to the natural resource of Nile water. These aberrations including stickiness of chromosomes in metaphase cells, as well as chromosomal bridge in anaphase cells. These aberrations may be due to the high concentration of ammonia in Nile water above the standards of WHO. The occurrence of sticky chromosomes agreed with Ping *et al*. (2012), who demonstrated that stickiness as a physiological aberration is a type of physical adhesion which containing mainly the proteinaceous matrix of the chromatin material. This may be due as aconsequence of DNA depolymerization, partial dissolution of nucleoproteins, and stripping of protein covering chromosomal DNA (Mercykutty and Stephen 1980). Stickiness of chromosomes

indicated the presense toxic substance which affected on the physical state of chromatin (Fiskesjo 1985). These results agreed with James *et al*.(2015), who observed sticky chromosomes, bridges and vagrants in the Allium cepa cells treated with pharmaceutical effluents, as a result of genotoxicity. Jadhav *et al*.(2011) reported that chromosomal aberrations is an important method for assessment the genotoxicity capacity of textile effluents. Mercykutty and Stephen (1980) stated that sticky chromosomes may arise due to breakage and exchange in basic folding fibre units of chromatids and stripping of the protein covering chromosomal DNA. Dulta *et al*.(2018) stated that stickey chromosomes has an irreversible genotoxic effect leading to cell cessation which are even found to be associated which chromosomal bridges. Therefore, the Nile water induced chromosomal stickiness associated with anaphase chromosomal bridges. Chromosomal bridges shown in this study resulted from chromosome or chromatid breaks. The increased of chromosome stickiness may leading to the formation of sticky bridges in anaphase and telophase cells which leading thereby prevents of normal cytokinesis. Stiky chromosomes reflected that pollutant was affected on chromatin organization. This was related to the disturbed balance in the histones quality or other proteins which responsible for controlling the proper structure of nuclear chromatin (Kurs 2004). Stickiness was common sign of genotoxic effects on chromosomes which may probably leading to cell death (Fiskesjo 1997).aberrations should be indicated by arrows in all figures

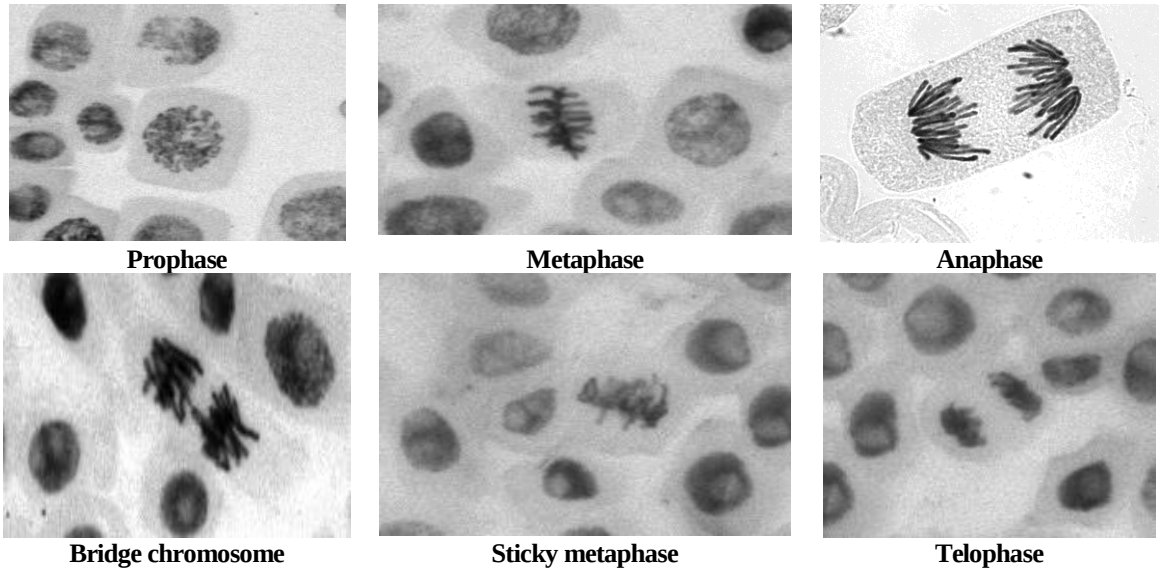


Figure 3. Cytogenetic effects of nile river water on root meristem cells of *Allium cepa*.

The drainage water resulted from agriculture activities in Kfr El – Sheikh Governorate can achieve different cytological effects on root meristem cells of *A.cepa* (Figure 4). The occurrence of chromosomal abnormalties observed including; disturbance chromosomes, telophase, bridge, sticky metaphase, lagging chromosome, sticky anaphase and fragment chromosomes. These abnormalities have been considered as indicators of clastogenic effects (Radic *et al*. 2010). Lagging chromosomes resulted from abnormal spindle formation which leads to the failure of spindle fibers to carrying the respective chromosomes to the polar site resulting in lagging chromosomes. Fragmented chromosomes formed multiple breaks of chromosomes which may be due to loss of chromosomal integrity. This agreed with Grant (1994), who reported that fragmentation occurs in prophase, metaphase and anaphase. In addition, unequal distribution of chromosomes observed herein may be due to the toxic chemicals presented in drainage water. Chromosomal abnormalities observed in this study could be considered a standard procedure for quick testing and assessment of genotoxicity of pollution levels in drainage water, because these wastewater containing a complex mixture of chemical substances that can persist and accumulate in exposed organisms and thus potentially pose a hazard to human health. It was documented that the higher chromosomal abnormalities represent the cytotoxic effects of drainage water on cell division which showed various types of aberrations. Aberrant mitosis cells may occur as a result of spindle poisoning which leading to chromosome disruptions during mitosis. Based on these chromosomal abnormalities this study confirm the genotoxic effect of drainage water carrying pollutants of chemical substances. The occurrence of several types of chromosomal abnormalities in root tip cells exposed to drainage water shows the genotoxic effect of waste water carrying chemical substances resulted the inactivation of spindle formation and deformation of non- histones chromosomal proteins (Olorunfemi *et al.* 2015). Mesi and Kopliku (2015) stated that bi- nucleated cells was another evident for genotoxic effect which is a consequence of prevention of cell plate formation and cytokinesis. The same authors reported that genotoxicity was closely related to carcinogenicity. The high frequent chromosomal abnormalities in anaphase was irregular separation with laggard chromosomes and anaphase bridges. In addition, laggard chromosomes resulted from the failure of chromosomes to move to either of the two poles as a consequence acentric fragments remain laggards. The disturbance of chromosomes observed in this study indicated the accumulated effect of drainage water results in the inactivation of spindle formation.

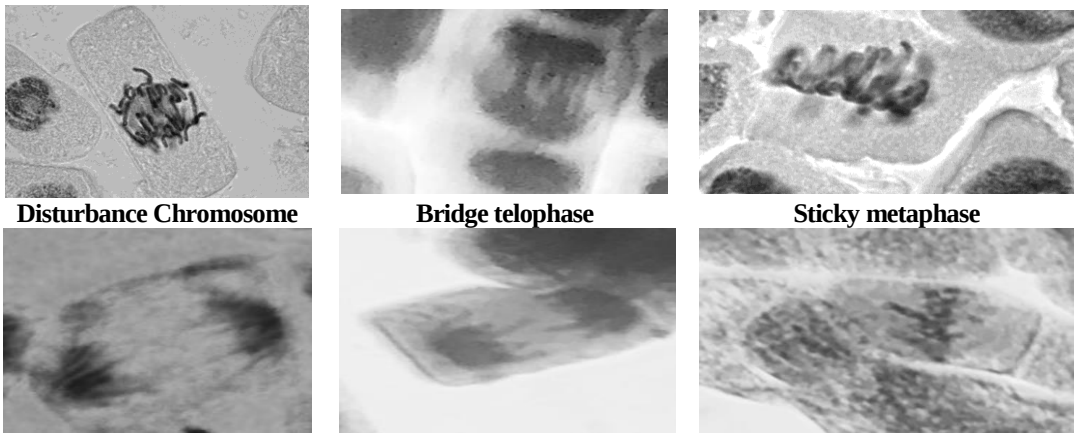


Figure 4. Cytogenetic effects of drainage water from Kfr El Sheikh Governorate on root meristem cells of *Allium cepa*.

As shown from the results presented in Figure (5) *Allium cepa* was used as biosensors for genetic toxicity of drainage water carrying chemical pollutants. Chromosomal abnormalities shown herein including; disturbed chromosome, fragmentation, bimetaphase cells, lagging chromosomes and anaphase bridge, were considered as biomarkers of genetic toxicity. Genotoxicity research is an important in environmental protection policies which allows us to assessment the impact on genetics of water quality (Walia *et al*. 2015). Bimetaphase cells shown herein arise from binucleate cells. The later usually arises as a consequence of the suppression of cell plate formation (Grant 1978), which may be due to the inhibitition of phragmoplast formation in the early telophase (Borooach 2011). In addition chromosome fragmentation generated from different kinds of chromosome abnormalities associated with a loss of genetic material. These results showed that chromosomal aberrations increases with increasing the concentrations of chemical substances in drainage water. Therefore, drainage water from Menyet El- Nasr center showed high values of aberrations compared to Kfr El- Sheikh drainage water. This agreed with Qian (2004) who reported that chromosomal aberrations rate goes up with the concentration of pollutants which consequently increasing genotoxicity, there was an inhibitory effect on cell division. Toxic chemical may prevent the cells to entering prophase resulting in a high frequency of prophase cells. Prophase – arrest could explain the decline of chromosomal aberrations without any decline affect on mitotic index (Odeigah *et al*.1997). The appearance of these abnormalities in chromosomes indicates that the organisms exposed to these pollutants may suffer from the cell death or may have a risk of non- disjunction chromosomes. Chromosomal abnormalities shown herein were considerably higher than that from the drainage water of Kfr El – Sheikh Governorate, which represents lower values of pollutants than that from Menyet El – Nasr center. - Nasr center. It was found that a high concentration of pollutants led to increase the rate of chromosomal abnormalities which was used as a parameter of genotoxicity to check the level of genotoxicity of the tested substances (Pesnya *et al*.2017).

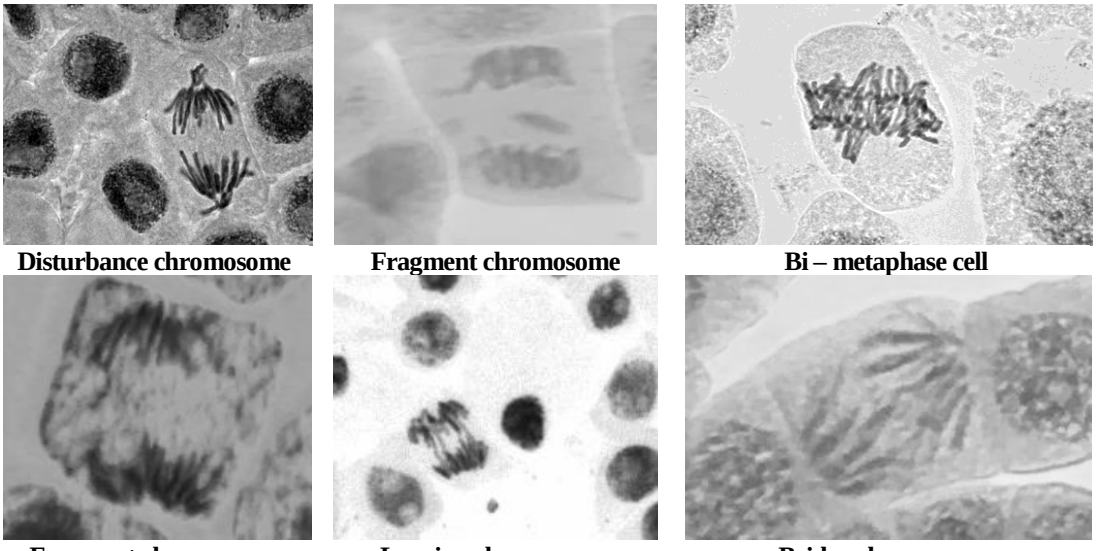


Figure 5. Cytogenetic effects of drainage water from Menyet El- Nasr center on root meristem cells of *Allium cepa*.

As shown in Figure 6 industrial drainage water resulted from chemical fertilizer industry generated different kinds of chromosomal aberrations including; disturbance chromosomes, anaphase bridge, sticky anaphase, binucleate cell, micronuclei, stickey metaphase and fragmentation of chromosomes. Looking into the effect of these effluents, it was noticed that these effluents induce more genotoxicity than the other water resources used in this study. These results reflect the severity of

this water resource in terms of higher kinds of chromosomal abnormalities induced which showed more aberrations than the other water resources tested in this study for their genotoxicity.

This study showed a gradual increase in chromosome abnormalities as the dose of pollutants increased in drainage water, as high genotoxicity generated disorganization of chromosomes in the meristem cells of root tips in *Allium cepa*. The high sensitivity of this technique helps water purification stations to monitor the contamination levels.Therefore,research of testing water quality in the environment protection policies is of great significance, since it enables the specialists to understanding the consequences of genotoxic chemicals present in water resources.

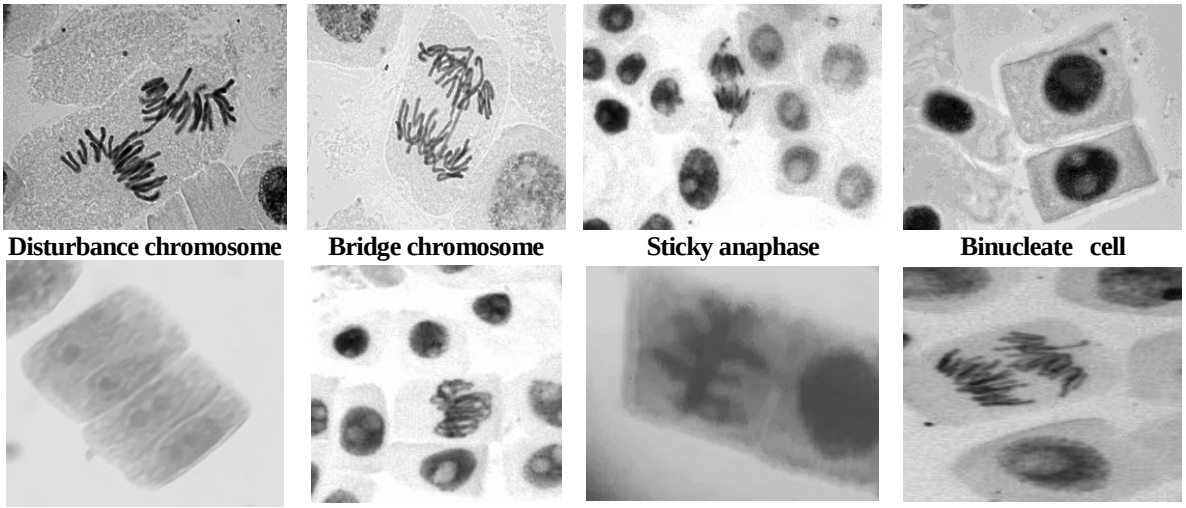


Figure 6. Cytogenetic effects of drainage water from chemical fertilizer industry on root meristem cells of *Allium cepa*.

Chemical analysis of drainage water

AS shown in Table 1 the concentration of most metals in all drainage waters is greater than that in the Nile river water. The total amount of most metal pollution was increased gradually from drainage water of Kfr- El Sheikh governorate, Menyet El- Nasr drainage water, fertilizer factory effluents and oils and soap industry, respectively. It was observed that most metals were increased in industrial effluents than in agriculture drainage waters. Orainage water of Menyet El- Nasr, factory effluents from fertilizer industry, as well as from oils and soap industry, showed high concentration of Aluminum exceeded the standard value of WHO. In addition, the concentration of Mercury in all agricultural and industrial drainage waters was exceeded the standard value of WHO. On the other hand, the concentration of chromium in industrial wastes of fertilizer industry was exceeded the standard value of WHO. So aluminum and mercury in both industrial wastes was exceeded the limit value of WHO. Therefore, drainage water from agriculture and industrial activities have a high impact on the water quality. Although, the values of some metals were lower than the standard limits, the continued discharge of drainage water in the environmental may results in severe accumulation of the contaminants which may effect the lives of the people. The results indicated that drainage water was not good for irrigation. It is therefore recommended that treatment process of drainage water before reusing it in irrigation must be considered to ensure no adverse effect on the ground water quality. Though the ground and the river water quality in and around the factorial sites should be chacked periodically to ensure from their impact on the ground water quality. The results obtained herein agreed with Islam *et al*. (2010), who reported that the river water polluted with industrial effluents are not good for human consumption and the disposal of treated or untreated wastes into the river should be stopped to save the water quality. In addition, Fakayode (2005) showed that the chemical parameters studied in industrial effluents were above the allowable limits. Therefore, drainage water before treatment generally have poor quality water in the affected areas. IN agriculture areas, the routine fertilization in the major source of contamination (Emogor *et al*. 2005).In urban areas, the disposal of industrial waste waters may greatly contribute the contamination of water. So heavy metals discharged from industrial and agriculture activities has a large impact on water quality.

In conclusion, drainage water from different resources generated different cytological changes in root meristems of *Allium cepa* depending on the concentration of pollutants present in the waste water. Higher concentration of chemical pollutants are toxic to the cells. The present study revealed genotoxic and clastogenic properties of drainage water carrying chemical pollutants. Industrial effluents and drainage water from Menyet El- Nasr center induced the highest kinds of chromosomal aberrations if compared with the other water resources. Therefore it is recommended that drainage water must be treated before they are used for irrigation purposes to be safe for humans. Therefore, sustained research efforts are needed to transform drainage water to other value added products via reduce its pollutant constituents to be safe limits before discharging into river stream. If the toxic materials are not treated before discharging, then theses toxic substances are incorporated in organisms via entering the food chain and induce adverse effects on human health. They may lead to cell death and kill the whole organism. Additional studies are required to evaluate the potential risks of genotoxic agents that may be discharged directly or indirectly to the environment causing severe threat to aquatic bodies and generating genetic changes in living organisms. *Allium cepa* test may be useful tool for assessment cytological effects of chemical substances that may be present in drainage water for monitoring environmental pollutants.

Table 3. Chemical analysis of drainage water from different resources as measured by ppm.

Metal		drinking water directives (EU) (mg/L)	Who guidelines drinking water (mg/L)	Nile river water	Drainage water kafer Elsheikh governorate	Drainage water Menyet Elnasr center	Fertilizer factory effluents	Soap and oil factory
Titanium	Ti			0.0	2.769	5.635	7.556	8.862
Aluminum	Al	0.2	0.2	0.104	0.107	1.233	10.3211	2.802
Bismuth	Bi			0.0	0.004	0.014	0.059	0.029
Mercury	HG	0.001	0,006	0.028	0.047	0.123	0.433	0.664
Silver	AG		0.05	0.001	0.013	0.017	0.032	0.018
Boron	B	1.0	2.4	0.028	0.044	0.068	0.095	0.094
Barium	BA		0.7	0.003	0.021	0.039	0.035	0.023
Calcium	CA		75	8.996	29.245	31.376	48.654	35.308
Cadmium	CD	0.005	0.003	0.0	0.0	0.001	0.001	0.001
Cobalt	CO			0.001	0.001	0.001	0.004	0.002
Chromium	CR	0.05	0.05	0.005	0.006	0.008	0.633	0.005
Copper	CU	2.0	2.0	0.006	0.011	0.024	0.033	0.022
Iron	FE	0.2	0.3	0.004	0.014	0.006	0.036	0.122
Gallium	GA			0.0	0.004	0.009	0.011	0.014
Indium	IN			0.0	0.0	0.416	0.0	0.014
Potassium	K		12	5.307	10.627	9.110	6.980	5.560
Lithium	LI			0.0	0.974	0.0	0.0	0.0
Magnesium	MG		30	13.203	14.664	19.096	16.524	11.049
Manganese	MN	0.5	0.4	0.0	0.001	0.002	0.004	0.366
Nickel	NI	0.02	0.07	0.0	0.0	0.003	0.0	0.0
Lead	PB	0.01	0.01	0.0	0.006	0.0	0.0	0.0
Strontium	SR		178.75ug/l	0.037	0.317	0.453	0.156	0.218
Zinc	ZN		3.0	0.008	0.012	0.568	0.041	0.105

Conflicts of interest

The authors declare that no conflicts of interest exist.

REFERENCES

Alam M. Z.; S . Ahmad and A .Malik. 2009. Genotoxic and mutagenic potential of agricultural soil irrigated with tannery effluents at Jajmau (Kanpur), India. Arch Environ Contam Toxicol. 57(3):463– 476.

Amin, A.W. 2002. Cytotoxicity Testing of Sewage Water Treatment Using *Allium cepa* Chromosome Aberrations Assay. Pak. Journ. Biologic Sci, 5(2): 1884-1888.

Bakare, A.A.; A.K.Pandey; M. Bajpayee; D.Bhargav; D.K. Chowdhuri; K.P. Singh; R.C. Murthy, and A. Dhawan, 2007. DNA damage induced in human peripheral blood lymphocytes by industrial solid waste and municipal sludge leachates, Environmental and Molecular Mutagenesis, 48: 30-37 .

Borooah, D.D. 2011.Genotoxicity assessment of water extract of OcimumgratissimumL. using the *Allium cepa*assay. International Journal of Plant, Animal and Environmental Science.1(2): 185-188.

Brooks, B.W.; J. M Lazorchak; M. D Howard; M. V Johnson; S.L Morton; D. A Perkins; E. D Reavie; G. Scott; S. A Smith and J. A Steevens. 2016. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? Environ Toxicol Chem. 35(1):6–13.

Darlington, C.D. 1942. Chromosome chemistry and gene action nature. *Nature*, 149: 66-69.

Duttaa , J.; A. Ahmada and J. S. Caryologia . 2018. Study of industrial effluents induced genotoxicity on Allium cepa: International Journal of Cytology, Cytosystematics and Cytogenetics.

Erchull, C. and L .Fisher. 2016. Remedying and regulating the unintended consequences of subtherapeutic dosing of livestock with antibiotics: can the EPA's implementation of the clean water act reign in the problem. W New Eng L Rev. 38:397–423.

Fakayode, S. O. 2005. Impact of industrial effluents on water quality of the receiving Alaro River in Ibadan. Nigeria, Ajeam-Ragee, 10: 1-13.

Fiskesjo, G and C. Lassen .1982. Benzo (a) pyrene andnitrosoguanidine in the *Allium* test. Mut. Res, 97(3): 188.

Fiskesjö, G. 1985. The Allium test as a standard in environmental monitoring. Hereditas. 102(1):99–12.

Fiskesjö, G. 1997. *Allium* test for screening chemicals: evaluation of cytological parameters. Plants for environmental studies. New York, NY: Lewis Publishers; p. 308–333.

Gomes, K.M.; M.V Oliveira; F. R Carvalho, C.C Menezes and A. P Peron 2013. Citotoxicity of food dyes sunset yellow (E-110), bordeaux red (E-123), and tatrazine yellow (E-102) on *Allium cepa* L. root meristematic cells. Food Sci Tech, 33(1):218-223.

Grant, W.F.1978. Environmental Health Perspectives, 27: 37–43.

Grant, W.F. 1982. Chromosome aberration assays in Allium. A report of the United States Environmental Protection Agency Gene Toxicity Program. Mutat Res. 99(3):273–291.

Grant, W.F. 1994. The present status of higher plant bioassay for the detection of environmental mutagens. Mutation Research, 310: 175-185.

Hughes, A. F. 1952. The Mitotic Cycle. London: Butterworth.

Islam, M.; M. Rahman and F.U. Ashraf.2010. Assessment of water quality and impact of effluents from fertilizer factories to the Lakhya River. International Journal of Water Resources and Environmental Engineering, 2(8): 208-221.

Jadhav, S.B, S.S Phugare; P.S Patil and J.P Jadhav. 2011. Biochemical degradation pathway of textile dye remazol red and subscequent toxicological evaluation by cytotoxicity, genotoxicty and oxidative stress studies. Int Bio deteriorates. 65(6):733–743.

James, O.O.; S.E. Oluwaleye ; A. E .Olufunmilayo and O. A. Adebiyi. 2015. Cytotoxic effects and genotoxic screening of pharmaceutical effluents using onion bulbs (Allium cepa L.).J Adv Bio Biotechnol, 2(1): 51 – 58.

Kungolos, A.G.; C.A. Brebbia; C.P Samaras and V. Popov 2006.Environmental Toxicology. Southampton: WIT Press: 362 pp.

Kurás, L. 2004. Characterization of protein–DNA association in vivo by chromatin immunoprecipitation. In: Dickson RC, Mendenhall MD, editors. Signal Transduction Protocols, Methods in Molecular Biology, 284. Totowa: Humana Press Inc. p. 147–62.

Leme, D. M and M. Marin. 2009. Allium cepa test in environmental monitoring: A review on its application. Mut Res 682: 71–81.

Longford, K.H.; and K.V.Thomas.2009.Determination of pharmaceutical compounds in Hospital Effluents and Their contribution to Waste water Treatment Works .Enviroment International, 35(5) : 766- 770.

Mercykutty, V.C and J. Stephen 1980. Adriamycin induced genetic toxicity as demonstrated by the Allium test. Cytologia. 45(4):769–777.

Mesi , A and D. Kopliku.2015. The use of *Allium cepa* L. assay for toxicity bio-monitoring of hospital effluents - an albanian case. european journal of toxicological sciences, 1:1-15.

Mirsaeedghazi, H.; Z . Emam-Djomeh and S. M. A. Mousavi. 2008. Rheometric measurement of dough rheological characteristics and factors affecting it. International Journal of Agriculture and Biology, 10(1):112–119.

Monte Egito L. M.; M. d. G. Medeiros; S.R de Medeiros and L. F Agnez-Lima. 2007.Cytotoxic and genotoxic potential of surface water from the Pitimbu river, northeastern/RN Brazil. Gen Mol Biol, 30:435- 431.

Nielsen, M.N and J. Rank. 1994. Screening of toxicity and genotoxicity in waste water by the use of Allium test. Hereditas.

Odeigah, P.G.C.; O. Nurudeen and O.O. Amund.1997. Genotoxicity of oil field wastewater in Nigeria. Hereditas,126: 161 – 167.

Olayinka, K. O. and B. Alo,. 2004. Studies on industrial pollution in Nigeria, the effect of textile effluents on the quality of ground water in some parts of Lagos, Nigerian journal of Health and Bio Medical Sciences, 3(1) : 44-55.

Olorunfemia , D. I.; L. Durua and O. Poise . 2015. Genotoxic effects of bilge water on mitotic activity in Allium cepa L. International Journal of Cytology, Cytosystematics and Cytogenetics,68(4): 265-271.

Pesnya, D.S.; A.V Romanovsky; D. A Serov and N.Y. Poddubnaya. 2017. Genotoxic effects of Heracleum sosnowskyi in the Allium cepa test. Caryologia, 70(1):55–61.

Ping, K. Y.; I. Darah ; U. K. Yusuf; C.Yen and S. Sasidharan 2012. Genotoxicity of Euphorbia hirts: An *Allium cepa* assay. Molecules. 17(7):7782-7791.

Pratibha, D. 1987. Cytological effects of starch factory effluents on *Allium cepa*. *Cytology and Genetics*, 23, 132-134.

Qian, X.W. 2004. Mutagenic effects of chromium trioxide on root tips of *Vicia faba*. Journal ofZhejiangUniversity of Science, 5(12): 1570 – 1576.

Radić, S.; D. Stipanicev; V. Vujcić; M. M. Rajcić; S. Sirac and B. P.Kozlina. 2009. The Evaluation of Surface and Wastewater Genotoxicity Using the Allium Cepa Test. 408(5):1228-1233.

Sang, N. and G. Li, 2004. Genotoxicity of municipal landfill leachate on root tips of *Vicia Faba* , Mutation Research, 560: 159– 165.

Schneiderman, M. H; W. C Dewey and D. P Highfield. 1971. Inhibition of DNA synthesis in synchronized Chinise hamster cells treated in G1 with cyclohexamid. Exp Cell Res, 67: 147-155.

Sisman, T. 2014. Genotoxic effects of water pollution on two fish species living in Karasu River, Erzurum, Turkey. Environ Monit Assess. 186(11):8007–8016.

Teena, M.T; K.R .Soumya and K. S .Sudha .2016. Cytotoxic effect of sewage effluent on root tip cells of *Allium cepa* L. South Indian Journal Of Biological Sciences, 2(1): 18-23.

Turkoglu, S. 2007. Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat Res. 626 (1-2):4–14.

Walia, G.K.; D. Handa; H . Kaur and R .w Kalotra. 2015. Ecotoxicological studies on fish, *Labeo rohita* exposed to tannery industry effluent by using micronucleus test. The Nucleus, 58(2):111–116.

Wauker.P.M. B. and H. B. Yates.1952a. Ultraviolet absorption of living cell nuclei during growth and division. Symp. Soc. Exp. Biol. 6, 265—76. WAUCER, P. M. B. & YATES, H. B. (19526). Nuclear components of dividing cells. Proc. Roy. Soc. B, MO. 274-99.

تقييم السمية الوراثية لمياة الصرف الزراعى والصناعى بإستخدام إختبار التقدير الحيوى سيتولوجياً

ميرفت إبراهيم كمال*، خليفه عبد المقصود زايد ، أشرف حسين عبد الهادى و عبدالله سمير عبد المحسن

قسم الوراثة – كلية الزراعة – جامعة المنصورة

تم فى هذه الدراسة تقدير تصادم مياة الصرف سواء الزراعى أو الصناعى الملوثة بالمادة الوراثية لنبات البصل كدليل حيوى بإستخدام التحليل السيتولوجى . تم إنبات البصل فى ثلاث عينات مختلفة من مياه الصرف بالإضافة إلى مياه النيل للمقارنة . شملت المخلفات المائية المستخدمة فى هذه الدراسة مياه الصرف الزراعى من محافظتى الدقهلية وكفر الشيخ ومياه الصرف الصناعى الناتجة عن صناعة الأسمدة الكيميائية. يهدف هذا البحث إلى دراسة التأثير السمى لمياه الصرف من مصادر مختلفة على خلايا القمم النامية لجذور البصل. أظهر تعريض جذور البصل للمخلفات المائية تغيرات كروموسومية مختلفة تعتمد بشدة على درجة تلوث المياة المياة المختبرة. أحدثت مياة الصرف أنواع مختلفة من التغيرات الكروموسومية والتى زادت بشكل تدريجى فى مياه الصرف الزراعى لمحافظة كفر الشيخ ، مياه الصرف الزراعى من مركز منية النصر لمحافظة الدقهلية ، المخلفات الصناعية لمصنع الأسمدة الكيماوية، على الترتيب. أوضحت النتائج أن مياه الصرف الزراعى لمركز منية النصر ومياه الصرف الصناعى كانتا أكثر قوة فى تكوين النويات الصغيرة ، الخلايا المحتوية على نواتين ، إحداث خلل فى تكوين خيوط المغزل ويرجع ذلك إلى محتوى هذه المياه المرتفع من المواد الكيميائية. الكيماويات السامة الموجودة فى مياه الصرف هى المسئولة عن التأثيرات الوراثية السامة التى تم مشاهدتها. إن التلكؤ الكروموسومى ، اللزوجة الكروموسومية ، القنطرة الكروموسومية فى المرحلة الإنفصالية، الشظايا الكروموسومية تعتبر من أكثر التغيرات الكروموسومية مشاهدة وتكراراً فى كل المعاملات بمياه الصرف تحت الدراسة . لقد ترتب على المعاملة بمياه الصرف إنخفاض فى دليل الإنقسام الميتوزى وزيادة فى تكرار التغيرات الكروموسومية المستحدثة بالمقارنة بمياه النيل العادية. لذا خلصت نتائج هذه الدراسة إلى أن مياه الصرف الغير معالجة تحتوى فى الغالب على مواد كيماوية سامة محدثة للطفرات. على أساس ذلك الإتجاه ، فإن هناك ضرورة للدراسات المتعلقة بالتقييم الحيوى للمياه ومعالجة مياه الصرف قبل التخلص منها فى البيئة . ولذلك فإن تقديرات السمية الوراثية والمقدرة الطفرية يجب أن تؤخذ فى الإعتبار فى برامج إختبار وتقدير جودة المياه للتخلص من الآثار الطفرية الضارة الموجودة فى المخلفات المائية.