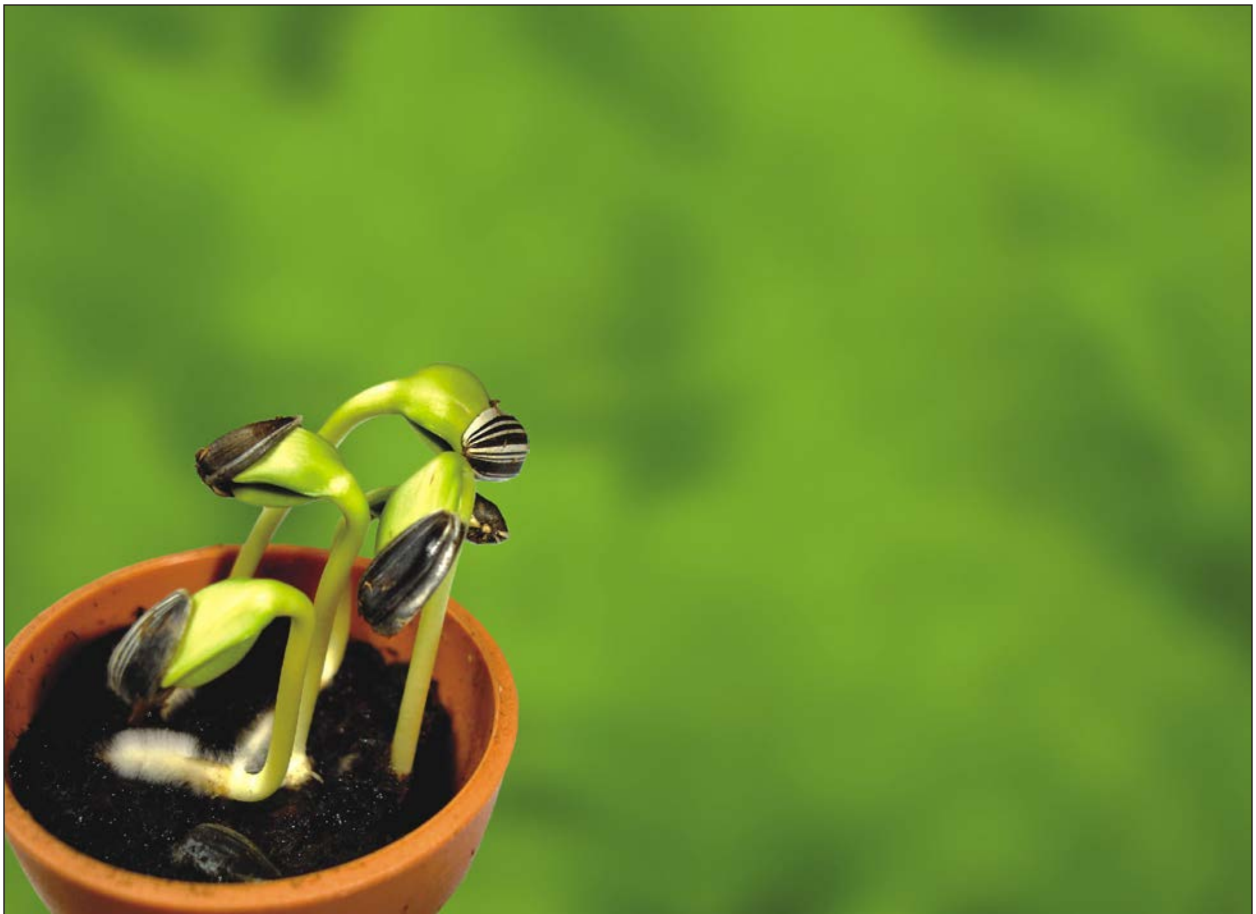

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Assessment of Negative Environmental Impacts of Drainage and Irrigation Canals on *Ipomoea carnea* Jacq. in Egypt

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ABSTRACT

This investigation demonstrates the effect of different environmental pollutants of drainage and irrigation canals on *Ipomoea carnea* Jacq. collected from ten different sites belongs to Dakahlia Governorate. This goal is achieved by using varietal tools as heavy metal analysis such as (Co^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}) of water, soil and plant leaves samples collected from each site, mitotic index, phase index, pollen abortion assay, protein profile using SDS-PAGE technique and molecular study using Random Amplified Polymorphic DNA (RAPD) technique. The effects of heavy metals on *I. carnea* induced a considerable changes in mitotic index and produced number of chromosomal abnormalities such as multinucleated cells at interphase, micronucleus at prophase, stickiness, non-congression, two groups, chromosome ring and disturbed at metaphase, late separation, laggard, bridge and diagonal in anaphase and telophase and reduced the frequency of pollen fertility (low stainability). The damage of DNA was reflected by changes in protein profile and DNA fingerprinting of *I. carnea* through the variation in bands by the appearance or disappearance of these bands.

Key words: *Ipomoea carnea* Jacq., heavy metal, mitotic index, phase index, chromosomal abnormalities, pollen abortion, protein profile, RAPD

INTRODUCTION

Environment pollution is a worldwide problem and its potential to influence the health of human populations is great (Fereidoun *et al*., 2007). Farmers in many parts of the Nile Delta use drainage water for irrigation of their fields because of the agriculture expansions in Egypt depends on the irrigation but the water supply from irrigation canals is not sufficient enough (Khalifa *et al*., 2003). Polluted water consists of industrial discharged effluents, sewage water, rain water pollution (Ashraf *et al*., 2010) and has adverse effects on land, water and its biotic and abiotic component (Al-Dulaimi *et al*., 2012). Plants grown in the contaminated soils when consumed by peoples can result in health problems (Wahid *et al*., 2004) like diarrhea, mental retardation, liver and kidney damage (Uzair *et al*., 2009).

Heavy metals is the generic term for metallic elements having an atomic weight higher than 40.04 (Yu, 2005). Heavy metals are classified as mutagenic, carcinogenic and clastogenic substances (White and Claxton, 2004). It includes Lead (Pb), cadmium (Cd), zinc (Zn), silver (Ag), chromium (Cr), copper (Cu), mercury (Hg), arsenic (As) and platinum group elements. These elements have no beneficial effects in humans and there is no known homeostasis mechanism for them (Vieira *et al*., 2011).

Heavy metals with toxic and/or genotoxic properties released from domestic or industrial sources causing health disorders to human and animals, its effects are not limited to, neurotoxic and carcinogenic actions (Goyer *et al*., 2004; Lu *et al*., 2005). Heavy metals can be toxic to plants, animals and humans, even at very low concentrations (Shahid *et al*., 2012). Heavy-metal-induced damage to DNA may also result in the production of micronuclei which produce chromosome breaks or mitotic anomalies that require passage through mitosis to be recognizable (Soliman and Heikal, 2012).

Ipomoea carnea Jacq., the Pink Morning Glory, is a species of morning glory which is a member of Convolvulaceae that include about 500 tropical and worm temperate species (Mabberley, 1987). *Ipomoea carnea* Jacq. is a shrubby perennial amphibious plant, native to South America, grows in dense population along river beds, river banks, canals and other waterlogged (wetland) areas. It has become naturalized along canals, drains, road sides and field edges in the Nile Delta, Egypt. It was introduced to Egypt for ornamental purpose (Afifi *et al*., 1988). The rapid growth rate, spread and adaptability from xeric to aquatic habitats indicates that this plant may potentially become a disastrous invasive species in Egyptian water bodies (Shaltout *et al*., 2010).

Invasive plant species are often characterized by their abilities to transcend stress conditions that constrain native species and to compete for limited resources to obtain rapid growth (Kercher and Zedler, 2004). This flowering plant has heart-shaped leaves that are a rich green and 6-9 inches long. It can be easily grown from seeds which are toxic and it can be hazardous to cattle. The stem of *I. carnea* can be used for making paper. The plant is also of medicinal value, it contains a component identical to marsilin, a sedative and anti-convulsant. A glycosidic saponins has also purified from it with anti-carcinogenic and oxytoxic properties (Chand and Rohatgi, 1987).

Two basically different approaches can be taken to estimate pollen viability, staining pollen with dyes and *in vitro* germination assay, staining techniques aim to determine pollen enzymatic activity and membrane integrity. *In vitro* germination assays determine the actual germination ability of pollen under suitable conditions (Tuinstra and Wedel, 2000). Additionally, it has been documented that internal, morphological and environmental factors all play a role in determining the duration of pollen viability, with dehydration and/or ultraviolet light producing the most severely damaging effects (Dafni and Firmage, 2000).

Protein electrophoresis is considered a reliable, practical and reproducible method because seed protein is a copy of genomic DNA and largely independent of environmental fluctuations (Javid *et al*., 2004; Iqbal *et al*., 2005). The changes in protein banding patterns are considered to be due to the inherited mutagenic effects of heavy metals. These changes included differences in band intensities or densities and also include changes in bands relative mobilities and appearance of new bands or disappearance of some bands, these changes could be attributed to occurrence of mutational events. The electrophoretic banding pattern of an organism is a mirror image for transcription events occurring due to the expression of genes of that organism.

The random amplified polymorphic DNA approach is used to amplify DNA sequences with single short (9-10) bp primers of arbitrary nucleotide sequence (Williams *et al*., 1990). This technique is used extensively for surveying genomic DNA for evidence of various types of DNA damage and mutation shows that RAPD may potentially from the basis of novel biomarker assays for the detection of DNA damage and mutational events (e.g., rearrangements, point mutation, small insert or deletions of DNA and ploidy changes) in cells of bacteria, plants, invertebrate and vertebrate animals (Savva, 1998; Atienzar *et al*., 2000).

This investigation demonstrates the effect of different environmental variables of drainage and irrigation canals on *I. carnea* collected from Dakahlia Governorate. This goal is achieved by using varietal tools as heavy metal analysis of water, soil and plant leaves, mitotic index, phase index, pollen abortion assay, protein profile using SDS-PAGE technique and Random Amplified Polymorphic DNA assay.

MATERIALS AND METHODS

Seeds, leaves and flower buds of *I. carnea* collected from ten different sites from Dakahlia Governorate. Five of them were collected from drainage canals and the others were collected from irrigation canals as mentioned in Table 1. Water and soil samples where the plant grows also collected. The mutagenic heavy metals (Co^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}) were extracted according to (Allen *et al*., 1974) and its concentrations were measured in water, soil and plant leaf samples using atomic absorption spectrophotometer type PERKIN ELMER, 2380.

Chromosomal aberration assay: Seeds of *I. carnea* from each site were germinated and the radicals of 1-1.5 cm in length were emerged; the root tips were fixed in Carny's solution (glacial acetic acid/ethanol 1:3 ratio) and stored in refrigerator at least for 48 h, washed by distilled water, dried on filter paper then hydrolyzed in 1N HCl at 60°C for 25 min. The root tips were stained using a double staining method combining the modified carbol fuchsin reaction (Koa, 1975a, b) where the root tips were put in carbol fuchsin overnight then in 2% aceto-orcein stain for 2-4 h. One millimeter of mitotic zones were immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover glass.

At least 2000 cells from 20 slides of each site were examined. The cells were recorded as normal or aberrant in the different stages of the mitotic division namely: Interphase, prophase, metaphase, anaphase or telophase. All cells with aberration were counted and photographed using Olympus camera (SC 35 type 12 mode).

Statistical analysis of data: Different phases of mitosis were counted and chromosomal abnormalities were observed to calculate mitotic index, phase indices and total abnormality percentage at different phases of cell division. Site 8 (El-Tawila) is considered as a control and the results were represented as Mean±SE and statistically significant difference between control and samples was determined using T-test. All statistical analyses were based on 0.05 significance level (Snedecor and Cochran, 1976).

Pollen abortion assay: The collected flower buds from *I. carnea* were fixed simultaneously in a fresh mixture of absolute alcohol, chloroform and glacial acetic acid (6:3:1). Flower buds were

Table 1: Sites where *Ipomoea carnea* collected from Dakahlia Governorate

Site No.	Site names	District names	Divisions
1	Meet-Khamis	Mansoura	Drainage canals
2	Belgay	Mansoura	
3	Amaar	Bilqas	
4	Agiena	Bilqas	
5	Gamasa	Gamasa	
6	El-Sallab	Mansoura	Irrigation canals
7	Shawa	Mansoura	
8	El-Tawila	Talkha	
9	Basandila	Talkha	
10	Rezka	Sherbin	

Table 2: List of primers and their nucleotide sequence used in RAPD analysis

No.	Primer code	Sequence 5'to 3'
1	OP-O01	5-GGCACGTAAG-3
2	OP-O05	5-CCCAGTCACT-3
3	OP-O08	5-CCTCCAGTGT-3
4	OP-O12	5-CAGTGCTGTG-3
5	OP-O19	5-GGTGCACGTT-3
6	OP-A01	5-CAGGCCCTTC-3
7	OP-A10	5-GTGATCGCAG-3
8	OP-A15	5-TTCCGAACCC-3

RAPD: Random amplified polymorphism DNA

removed from the fixative then washed. Anthers were excised, stained and squashed in 4% alcoholic hydrochloric acid carmine (Snow, 1963). Either stained or large pollen were counted as fertile where unstained or small pollen were counted as sterile.

Protein analysis: SDS-PAGE technique was carried out according to (Laemmli, 1970) to assessment of environmental variable impacts on *I. carnea* collected from ten different sites along Dakahlia Governorate by their protein profile.

RAPD fingerprinting assay: DNA was extracted according to El-Fiky *et al*. (2002). Eight random primers listed in Table 2 were applied. The PCR reaction was conducted according to Williams *et al*. (1990). The gels of DNA were visualized and photographed by gel documentation system (Gel-Doc Bio-Rad 2000) under UV trans-illuminator. The RAPD bands were scored as present (1) or absent (0), each of which was treated as independent character regardless of its intensity. The levels of polymorphism were calculated by dividing the polymorphic bands by the total number of scored bands.

RESULTS AND DISCUSSION

Environmental pollution is the presence of a pollutant in the environment which may be poisonous or toxic and will cause objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death to living things lay on this environment (Duruibe *et al*., 2007).

Heavy metals are capable of interfering with the spindle apparatus of dividing cells to produce DNA damage (Johnson, 1998).

In present study, heavy metals (Cu, Cd, Pb, Co and Zn) were measured as mg L^{-1} using atomic absorption spectrophotometer type PERKIN ELMER, 2380 in, water, soil and plant leaf samples. The data obtained were tabulated in Table 3.

Table 3 and Fig. 1 showed that, the concentration of mentioned heavy metals in water samples where the highest value of copper ion concentration was 0.0611 mg L^{-1} in site 6 (El-Sallab) whereas, the lowest value was 0.0018 mg L^{-1} at site 1 (Meet-Khamis) with mean value 0.0295. As seen; copper is not detected in site 9 (Basandila). The cadmium ion concentration was slightly varied where the highest value was 0.0185 mg L^{-1} in site 4 (Agiena), the lowest value was 0.0018 mg L^{-1} in site 2 (Belgay) with mean value 0.00563 and there is no recording for cadmium in site 3 (Amaar). The highest value of Pb was 0.4455 mg L^{-1} in site 4 (Agiena), the lowest value was 0.1050 mg L^{-1} in site 8 (El-Tawila) with mean value 0.21141. For cobalt ion, the highest concentration value was 0.073 mg L^{-1} in site 4 (Agiena) while the lowest value was 0.002 mg L^{-1} in site 8 (El-Tawila) with mean value 0.02664. For zinc ion, the highest concentration was 0.2771 mg L^{-1} in site 3 (Amaar) and the lowest value was 0.0931 mg L^{-1} in site 8 (El-Tawila) with mean value 0.15296.

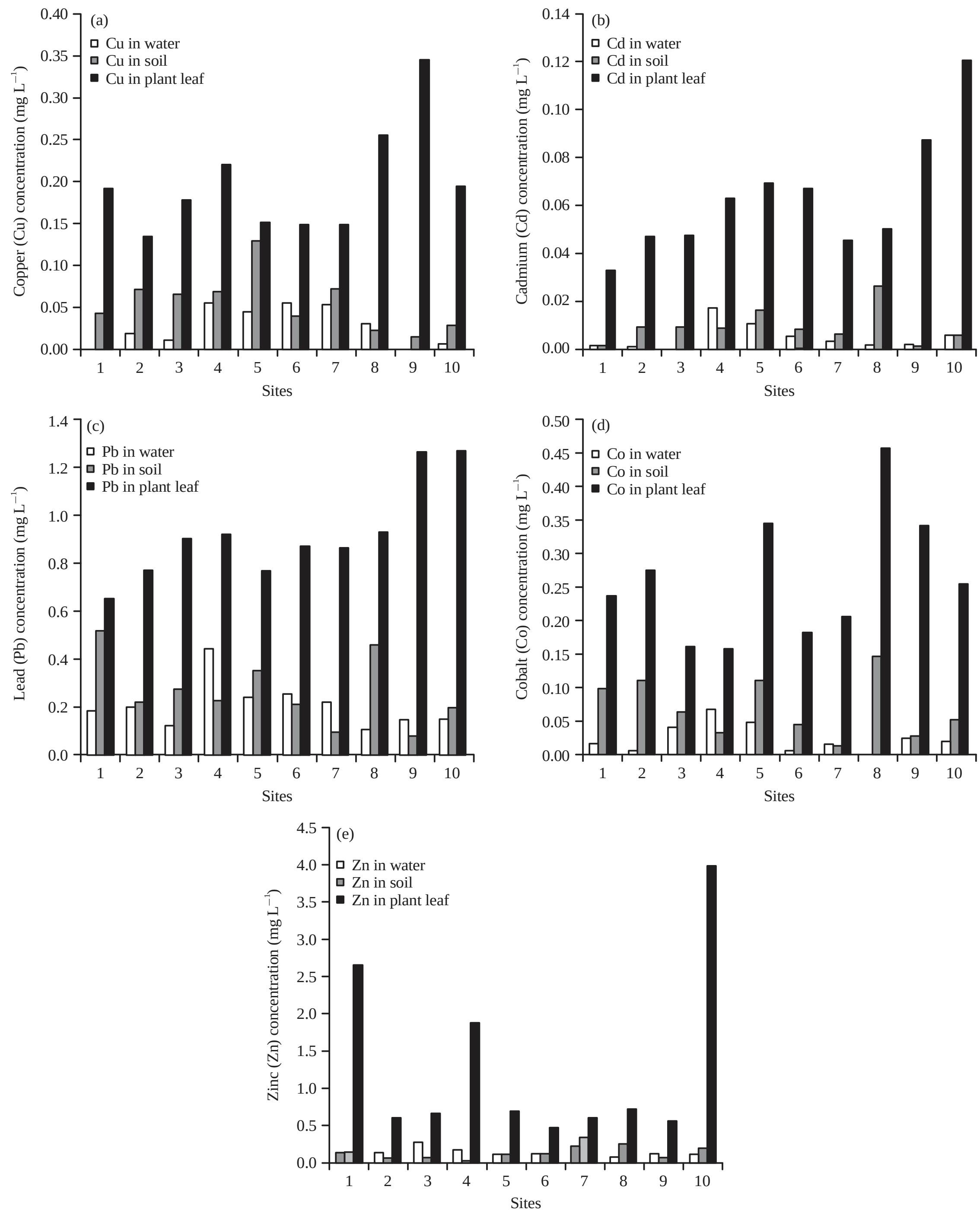

Fig. 1(a-e): Spatial variation in (a) Copper, (b) Cadmium, (c) Lead, (d) Cobalt and (e) Zinc of water, soil and leaf samples collected from the study sites. 1 = Meet-Khamis drainage canal Mansoura district, 2 = Belgay drainage canal, Mansoura district, 3 = Amaar drainage canal, Bilqas district, 4 = Agiena drainage canal, Bilqas district, 5 = Gamasa drainage canal, Gamasa district, 6 = El-Sallab irrigation canal, Mansoura district, 7 = Shawa irrigation canal, Mansoura district, 8 = El-Tawila irrigation canal, Mansoura district, 9 = Basandila irrigation canal, Talkha district, 10 = Rezka, irrigation canal, Sherbin district

Table 3: Heavy metals concentrations (mg L^{-1}) in water, soil and plant leaf samples collected from the study sites

Sites	Cu	Cd	Pb	Co	Zn
Water samples					
1	0.0018	0.0026	0.1891	0.0195	0.1281
2	0.0196	0.0018	0.2026	0.008	0.1357
3	0.0133	0.0000	0.1272	0.0425	0.2771
4	0.0577	0.0185	0.4455	0.073	0.1715
5	0.0463	0.0110	0.2526	0.0514	0.1113
6	0.0611	0.0062	0.2606	0.005	0.1240
7	0.0558	0.0043	0.2282	0.019	0.2312
8	0.0316	0.0021	0.105	0.002	0.0931
9	0.0000	0.0030	0.1508	0.025	0.1416
10	0.0081	0.0068	0.1525	0.021	0.1160
Mean	0.0295±0.0076	0.00563±0.0017	0.21141±0.0308	0.02664±0.0071	0.15296±0.0184
Maximum	0.0611	0.0185	0.4455	0.073	0.2771
Minimum	0.0018	0.0018	0.105	0.002	0.0931
Soil samples					
1	0.0445	0.0031	0.5226	0.103	0.1495
2	0.0735	0.0098	0.2214	0.113	0.0856
3	0.0665	0.0104	0.2832	0.066	0.0752
4	0.0715	0.0096	0.2316	0.036	0.0376
5	0.1316	0.0181	0.3608	0.115	0.1180
6	0.0419	0.0095	0.2157	0.049	0.1250
7	0.0740	0.0070	0.1006	0.015	0.3468
8	0.0244	0.0274	0.4654	0.150	0.2384
9	0.0164	0.0014	0.0825	0.028	0.0816
10	0.0302	0.0069	0.2021	0.055	0.1923
Mean	0.0575±0.0107	0.01032±0.0024	0.2686±0.0454	0.073±0.0141	0.145±0.0292
Maximum	0.1316	0.0274	0.5226	0.15	0.3468
Minimum	0.0164	0.0014	0.0825	0.015	0.0376
Leaf samples					
1	0.1935	0.0345	0.6598	0.240	2.6569
2	0.1361	0.0479	0.7809	0.272	0.6158
3	0.1802	0.0480	0.9135	0.167	0.6839
4	0.2225	0.0636	0.9295	0.163	1.8848
5	0.1530	0.070	0.7812	0.347	0.7201
6	0.1530	0.0684	0.8812	0.186	0.5025
7	0.1487	0.0470	0.8795	0.211	0.5788
8	0.2577	0.0517	0.9411	0.461	0.7269
9	0.3474	0.0895	1.2810	0.345	0.5658
10	0.1967	0.1214	1.2797	0.258	3.9960
Mean	0.1989±0.0203	0.0642±0.0081	0.9327±0.064	0.265±0.0301	1.2932±0.375
Maximum	0.3474	0.1214	1.2810	0.461	3.9960
Minimum	0.1361	0.0345	0.6598	0.163	0.5025

The highest value of copper ion concentration in soil samples was 0.1316 mg L^{-1} in site 5 (Gamasa) whereas, the lowest value was 0.0164 mg L^{-1} in site 9 (Basandila) with mean value 0.0575 Table 3 and Fig. 1. Regarding to cadmium ion, the highest concentration value was 0.0274 mg L^{-1} in site 8 (El-Tawila) while the lowest value 0.0014 mg L^{-1} in site 9 (Basandila) with mean value 0.01032. For lead ion, the highest concentration value was reported to be 0.5226 mg L^{-1} in site 1 (Meet-Khamis) while the lowest value was 0.0825 mg L^{-1} in site 9 (Basandila) with mean value 0.2686. The highest value of cobalt ion concentration was 0.1500 mg L^{-1} in site 8 (El-Tawila) while the lowest value was 0.0150 mg L^{-1} in site 7 (Shawa) with mean value 0.0730. The highest value of zinc ion concentration was 0.3468 mg L^{-1} in case of site 7 (Shawa) while the lowest value was 0.0376 mg L^{-1} in site 4 (Agiena) with mean value 0.1450.

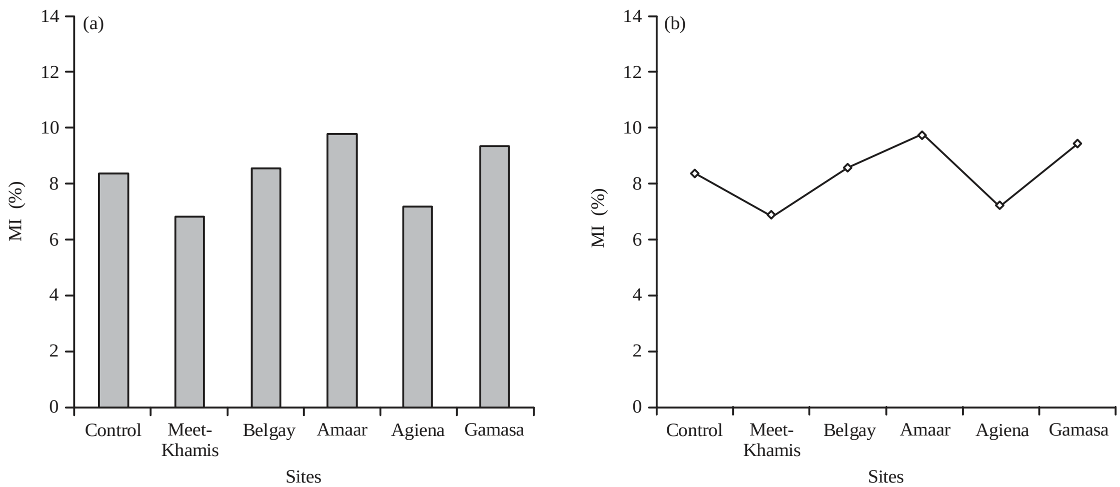

Fig. 2(a-b): (a, b) Percentages of mitotic index in drainage canals, C: Control (El-Tawila)

Heavy metals concentration in plant samples showed in Table 3 and Fig. 1. According to copper ion, the highest value of was 0.3474 mg L^{-1} in site 9 (Basandila), the lowest value was 0.1361 mg L^{-1} in site 2 (Belgay) and the mean value was 0.1989. For cadmium ion, the highest concentration value was 0.1214 mg L^{-1} in site 10 (Rezka) while the lowest value 0.0345 mg L^{-1} noted in site1 (Meet-Khamis) and the mean value was 0.0642. About lead ion, its concentration in plant leaf was slightly high compared to the other elements where the highest value was 1.2810 mg L^{-1} in site 9 (Basandila) while the lowest value was 0.6598 in site 1 (Meet-Khamis) and the mean value was 0.9327. Regarding to cobalt, the highest concentration value was 0.4610 mg L^{-1} in site 8 (El-Tawila) while the lowest value was 0.1630 mg L^{-1} in site 4 (Agiena) with a mean value of 0.2650. Concerning to zinc, the highest concentration value was 3.9960 mg L^{-1} in site 10 (Rezka) while the lowest value was 0.5025 mg L^{-1} in site 6 (El-Sallab) with a mean value of 1.2932.

The genotoxicity of heavy metals in plants influences the synthesis, the duplication of DNA and chromosomes and inducing chromosomal aberration (Soliman and Abdel Migid, 2003). The effects relate positively to heavy metal dosage (Zhang, 1997). Plant chromosomes are highly sensitive to chemical mutagens, giving rise to chromosomal aberrations (Grant *et al.*, 1992). The cytotoxicity levels of agent can be determined by the increase or decrease in the Mitotic Index (MI) (Fernandes *et al.*, 2007). Mitotic index is considered as one parameter used to estimate the frequency of cellular division (Marcano *et al.*, 2004; Leme and Marin-Morales, 2009).

It is an acceptable measure of cytotoxicity for all living organisms that cytotoxicity was defined as a decrease in the mitotic index (Smaka-Kinel *et al.*, 1996). The reduction and the increase in MI are important indicators in monitoring environmental pollution, especially for the assessment of contaminants that present toxic and cytotoxic potential.

The present investigation aims to study the negative impacts of different environmental variables on *I. carnea* collected from five drainage and irrigation canals on Mitotic Index (MI%) and Phase Index (PI%). The results presented in Table 4 and expressed graphically in Fig. 2 and 3. The chromosomal aberrations were observed in Fig. 4 and 5.

According to drainage canals, Table 4 and Fig. 2 showed that drainage canals had a considerable decreasing or increasing effect on mitotic indices than that of control (El-Tawila), whereas, site 3 (Amaar) and site 5 (Gamasa) showed increasing of MI% 9.83 and 9.38%,

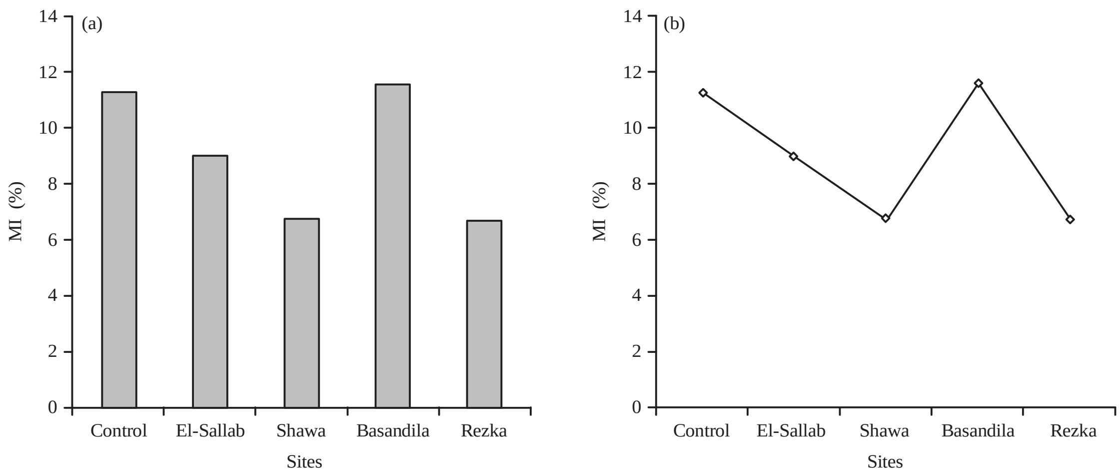

Fig. 3(a-b): (a, b) Percentages of mitotic index in irrigation canals, C: Control (El-Tawila)

Table 4: Mitotic index, normal and abnormal phase indices and total abnormalities in non-dividing and dividing cells of *Ipomoea carnea* in drainage canals and irrigation canals collected from Dakahlia Governorate

		Phase index										
		Prophase (%)		Metaphase (%)		Anaphase (%)		Telophase (%)		Total abnormalities		
Sites	MI (%)	Mitotic	Abn.	Mitotic	Abn.	Mitotic	Abn.	Mitotic	Abn.	Interphase	Mitosis	Division
C	8.40±0.70	7.89	0.04	51.93	0.59	13.24	0.18	26.64	0.04	0.02±0.12	0.85±0.58	Drainage canals
1	6.85±0.55*	2.96	0.00	52.52	0.49	20.49	0.00	21.80	0.10	0.02 ± 0.07^{ns}	0.59±0.20*	
2	8.60 ± 1.27^{ns}	0.96	0.00	54.90	0.41	21.06	0.10	21.54	0.04	0.00±0.00*	0.55±0.59*	
3	9.83 ± 0.94^{ns}	13.08	0.00	60.07	0.64	13.21	0.12	16.47	0.10	0.00±0.00*	0.86 ± 0.58^{ns}	
4	7.20 ± 0.71^{ns}	7.65	0.00	66.67	0.69	11.22	0.20	15.48	0.02	0.00±0.00*	0.91±0.62*	
5	9.38 ± 0.83^{ns}	0.94	0.00	74.69	0.63	15.72	0.06	9.28	0.06	0.00±0.00*	0.75 ± 0.42^{ns}	
6	9.08 ± 0.82^{ns}	3.97	0.02	67.16	0.68	17.09	0.12	11.42	0.06	0.00±0.00*	0.88 ± 0.25^{ns}	Irrigation canals
7	11.63±1.16*	2.92	0.02	61.25	0.65	17.85	0.12	18.82	0.04	0.04 ± 0.74^{ns}	0.83 ± 0.31^{ns}	
9	11.32±0.67*	2.82	0.01	41.92	0.45	31.57	0.30	23.69	0.05	0.04 ± 0.14^{ns}	0.81 ± 0.14^{ns}	
10	6.79±0.51*	7.56	0.05	57.33	0.61	19.86	0.19	15.25	0.05	0.00±0.00*	0.90 ± 0.25^{ns}	

1: Meet-Khamis drainage canal, Mansoura district, 2: Belgay drainage canal, Mansoura district, 3: Amaar drainage canal, Bilqas district, 4: Agiena drainage canal, Bilqas district, 5: Gamasa drainage canal, Gamasa district, 6: El-Sallab irrigation canal, Mansoura district, 7: Shawa irrigation canal, Mansoura district, 8: El-Tawila irrigation canal, Mansoura district, 9: Basandila irrigation canal, Talkha district, 10: Rezka, irrigation canal, Sherbin district. Total number of examined cells = 2000, ns: Not significant at 0.05 level from control, *Two means are significantly different at the 0.05 level., C: Control “site 8 (El-Tawila)

respectively while site 1 (Meet-Khamis) showed decreasing of MI% 6.85% compared with control 8.40%. The frequency of prophase reached a maximum value 13.08% in site 3 (Amaar) and the lowest value 0.94% in site 5 (Gamasa), compared with control which observed to have 7.89%. The frequency of metaphase showed a massive increase in site 5 (Meet-Khamis) 74.69% while the lowest value was 52.52% in site 1 (Meet-Khamis). About anaphase, the frequency reached the maximum value 21.06% in site 2 (Belgay) and the minimum value 11.22% in site 4 (Agiena) compared with 13.24% in control.

The highest value of telophase percentage was 21.80% in site 1 (Meet-Khamis) while the lowest value was 9.28% in site 5 (Gamasa). For interphase, the frequency of anomalies was noticed in site 1 (Meet-Khamis) with a value of 0.02%. The highest percentages of abnormal mitosis were 0.91% in site 4 (Agiena). As seen in Fig. 4, the chromosomal abnormalities found in *I. carnea* collected from drainage canals under the study during mitosis such as micronucleus at interphase, stickiness, non congression, two groups, chromosome ring and disturbed at metaphase, late separation and diagonal in anaphase, disturbed at telophase and bridge and laggard in both anaphase and telophase.

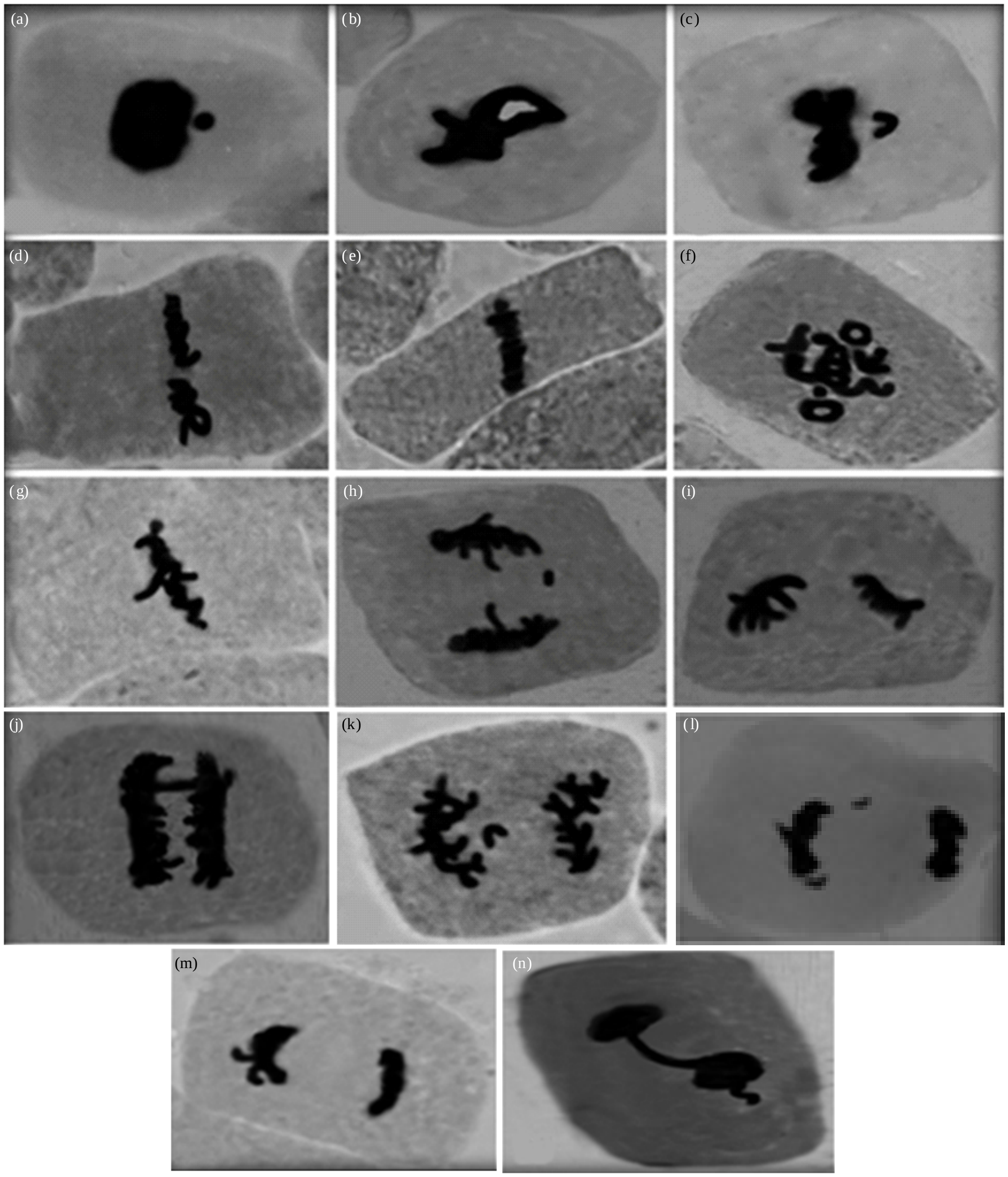

Fig. 4(a-n): Types of mitotic abnormalities were observed in case of drainage canals, (a) Micronucleus at interphase (Site 1), (b) Stickiness at metaphase (Site 1), (c) Non-congression at metaphase (Site 2), (d) Two-groups at metaphase (Site 3), (e) Oblique at metaphase (Site 3), (f) Chromosome ring at metaphase (Site 2), (g) Disturbed metaphase (Site 5), (h) Late separation at anaphase (Site 3), (i) Diagonal at anaphase (Site 4), (j) Bridge at anaphase (Site 3), (k) Laggard at anaphase (Site 3), (l) Laggard at telophase (Site 3), (m) Disturbed telophase (Site 3) and (n) Bridge at telophase (Site 2), (X = 1000)

For irrigation canals, Table 4 and Fig. 3 showed that site 7 (Shawa) and site 9 (Basandila) had increasing of MI% while site 10 (Rezka) showed decreasing of MI% 11.63, 11.32 and 6.79%, respectively compared with control 8.40%. About the percentage of different mitotic phases, it was found that, the frequency of prophase reached a maximum value 7.56% in site 10 (Rezka) and the lowest percentage 2.82% in site 9 (Basandila), The frequency of metaphase showed a great increase in site 6 (El-Sallab) 67.16%% while the lowest percentage was 41.92% in site 9 (Basandila). According to anaphase, the highest frequency was 31.57% in site 9 (Basandila) and the lowest value was 17.09% in site 6 (El-Sallab).

At telophase, the highest value of telophase percentage was 23.69% in site 9 (Basandila) while the lowest value was 11.42% in site 6 (El-Sallab). The highest percentage of abnormal mitosis was 0.90% in site 10 (Rezka). Next to interphase, the frequency of abnormalities was noticed in site 7 (Shawa) and site 9 (Basandila) with a value of 0.04%. As represented in Fig. 5, there were different chromosomal abnormalities were found in *I. carnea* collected from irrigation canals during mitosis such as multinucleated cells at interphase, micronucleus at prophase, stickiness, noncongression, two groups, oblique, ring, disturbed at metaphase, late separation and bridge in anaphase, disturbed at telophase and laggard in anaphase and telophase.

The genotoxicity of the water samples may be attributed to the effluent from the industrial establishment confirming that the source of mutagens comes from the industrial effluent in the study area. Among those mutagens, heavy metals are a potentially mutagenic class of environmental pollutant and some of them induced the tumors in experimental organisms and exposed humans (Minissi and Lombi, 1997).

Heavy metals induce the clastogenic and aneugenic effects including disturbance in mitosis and cytokinesis (Dovgaliuk *et al*., 2001), the obtained results showed that variation in mitotic activity of meristematic cells and causes irregularities in the chromosome and nucleus. Bridges, stickiness and micronuclei were recorded as mitotic irregularities and caused by cadmium (Zhang and Yang, 1994). Jiang *et al.* (2000) reported a rapid decrease in mitotic index caused by copper. Lead has the ability to complex with many biomolecules, so it is likely to be a selective agent which acts on and influences the genetic structure (Johnson, 1998).

Native flora and the crop plant species appear to be capable of serving as a bioindicators of genotoxicity (Micieta, 1990, 1993). Another reliable bioassay for *in situ* monitoring is pollen abortive tests with wild plants as indicators (Murin, 1995; Micieta and Murin, 1997).

Pollen viability tests are used as indicator of plant tolerance against environmental stresses and based on staining techniques that determine pollen enzymatic activity, membrane integrity and the stainability of the cytoplasm and nucleus (Nepi and Franchi, 2000; Vizintin and Bohanec, 2004), they have been widely adopted as a simple indicator of plant tolerance and incorporated into environmental biomonitoring assays. There are many non-genetic causes of decline in pollen viability, including pollen age and exposure to environmental stresses such as temperature and humidity so pollen may be used as a sensitive biological indicator of pollution (Stone *et al*., 1995; Kelly *et al*., 2002).

Pollen abortion assays are highly sensitive since the target cells (microspores) are haploid and detect lethal mutations which affect the development of pollen. Another advantage of this experiment model is that indicator species are directly collected from the environment (Micieta and Murin, 1997). The traditional method is to stain with aceto carmine or cotton blue and this allow a clear difference between pollen grains with and without cytoplasm. Those without cytoplasm are certainly sterile, but those with cytoplasm are not necessarily fully fertile (Ockendon and Gates, 1976).

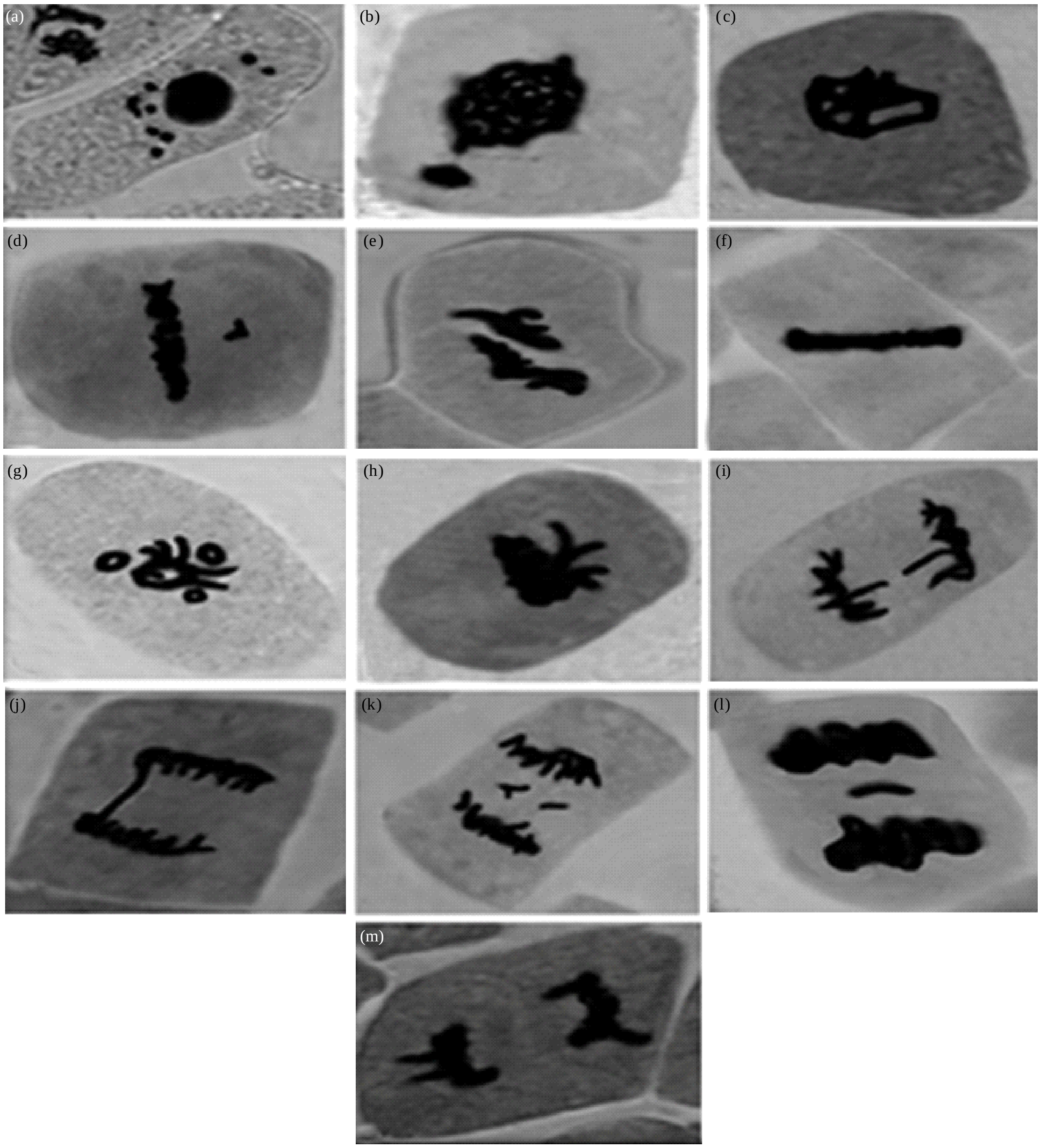

Fig. 5(a-m): Types of mitotic abnormalities were observed in case of irrigation canals, (a) Multinucleated cell at interphase (Site 7), (b) Micronucleus at prophase (Site 6), (c) Stickiness at metaphase (Site 7), (d) Non-Congression at metaphase (Site 6), (e) Two-groups at metaphase (Site 6), (f) Oblique at metaphase (Site 6), (g) Chromosome ring at metaphase (Site 7), (h) Disturbed at metaphase (Site 6), (i) Late separation at anaphase (Site 8), (j) Bridge at anaphase (Site 8), (k) Laggard at anaphase (Site 8), (l) Laggard at telophase (Site 9) and (m) Disturbed telophase (Site 7), (X = 1000)

Pollen abortion assay is used to study the genotoxic effect of environmental variables on the fertility of pollen grains of *I. carnea* collected from ten different sites belongs to Dakahlia Governorate. The obtained percentage of pollen fertility and sterility were tabulated in Table 5 and

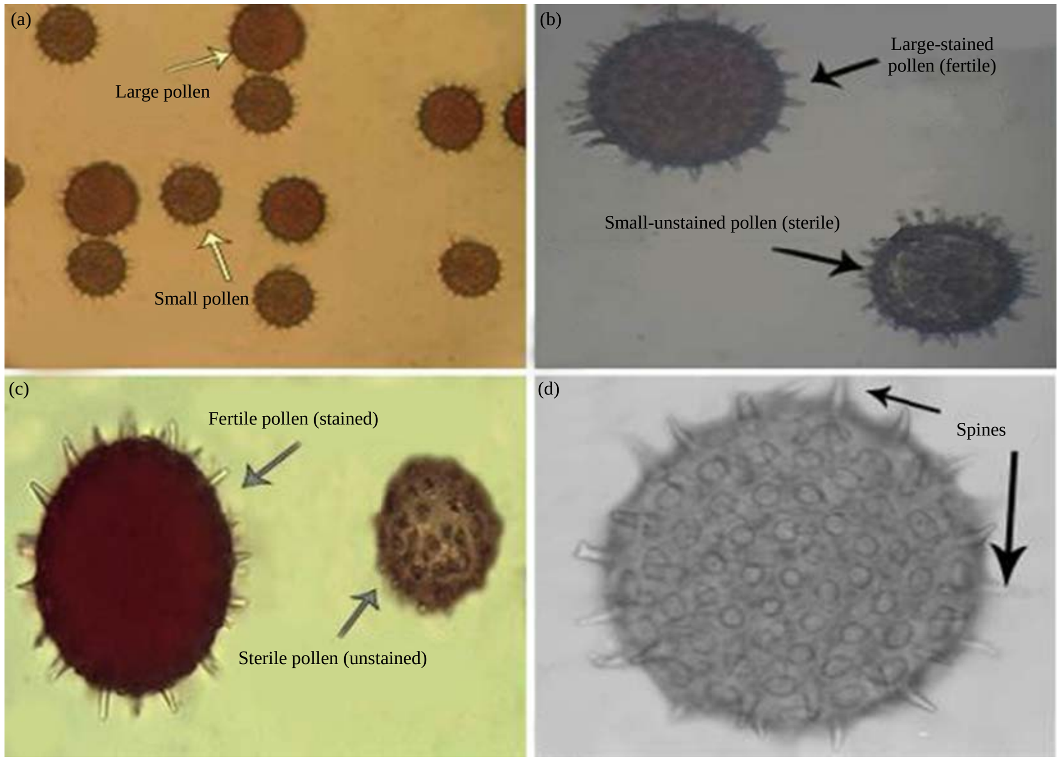

Fig. 6(a-d): Showing the types of pollen grains of *Ipomoea carnea*, (a) Pollen grains at X = 40, (b, c) Pollen grains at X = 100 and (d) Pollen grains at X = 1000

Table 5: Percentage of pollen fertility and abortive pollen grains in *Ipomoea carnea* collected from study sites

Site No.	No. of stained or large pollen	No. of unstained or small size pollen	Total No. of pollen	Fertility (%)	Sterility (%)
1	400	610	1010	39.60	60.40
2	930	420	1350	68.89	31.11
3	692	193	885	78.19	21.81
4	231	78	309	74.76	25.24
5	360	698	1058	34.03	65.97
6	583	118	701	83.17	16.83
7	947	600	1547	61.22	38.78
8	308	50	358	86.03	13.97
9	743	120	863	86.10	13.90
10	1232	104	1336	92.22	7.78

1: Meet-Khamis drainage canal, Mansoura district, 2: Belgay drainage canal, Mansoura district, 3: Amaar drainage canal, Bilqas district, 4: Agiena drainage canal, Bilqas district, 5: Gamasa drainage canal, Gamasa district, 6: El-Sallab irrigation canal, Mansoura district, 7: Shawa irrigation canal, Mansoura district, 8: El-Tawila irrigation canal, Mansoura district, 9: Basandila irrigation canal, Talkha district, 10: Rezka, irrigation canal, Sherbin district

resembled in Fig. 6. It is clear that, the highest percentage of abortive pollen was 65.97% in site 5 (Gamasa) and in turn had the lowest percentage of pollen fertility 34.03% while the highest pollen fertility was 92.22% in site 10 (Rezka) and in turn had the lowest pollen sterility percentage of 7.78%. The reduction of pollen fertility in many plants is often associated with irregular meiosis of pollen mother cells (Brown, 1972; Soliman, 1987), since this leads to the production of non-functional gametes.

The electrophoretic banding patterns of extracted proteins have been studied using a protein marker of molecular weight ranging from 12-97 kDa. As seen in Table 6, the total number of bands

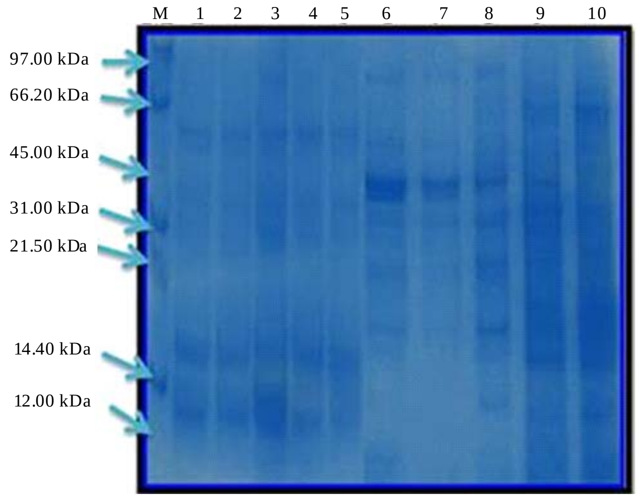

Fig. 7: Scanning of SDS-PAGE gel illustrating protein bands of *Ipomoea carnea* seeds, M: Marker

Table 6: SDS-PAGE of protein banding pattern of *Ipomoea carnea* collected from the study sites

No.	Molecular weight (kDa)	Sites 1	2	3	4	5	6	7	8	9	10
1	91.70	0	0	0	0	0	1	1	1	0	0
2	78.79	0	0	0	1	0	0	0	0	0	0
3	74.60	0	0	0	0	0	0	0	1	0	0
4	71.90	0	0	0	0	0	1	1	1	0	1
5	65.60	0	0	0	0	0	0	1	1	1	1
6	59.30	0	0	0	0	0	0	1	1	1	1
7	56.23	1	1	1	1	1	1	1	1	1	1
8	51.08	0	1	0	0	1	1	1	1	0	0
9	46.50	0	0	0	0	0	0	0	0	0	1
10	40.50	0	0	0	0	0	1	1	1	1	1
11	35.00	1	1	1	1	1	1	1	1	1	1
12	29.66	1	1	1	1	0	0	0	0	1	1
13	25.70	0	0	0	0	0	1	1	1	1	1
14	21.70	0	0	0	0	0	0	0	0	1	1
15	19.30	1	1	1	1	1	1	1	1	1	1
16	13.88	1	0	1	1	0	0	0	1	0	0
17	12.86	1	1	1	1	1	0	0	0	1	1
Total bands		6	6	6	7	5	7	10	12	10	12
Polymorphism (%)		17.65	17.65	17.65	23.53	11.77	23.33	41.18	52.94	41.18	52.94

1: Meet-Khamis drainage canal, Mansoura district, 2: Belgay drainage canal, Mansoura district, 3: Amaar drainage canal, Bilqas district, 4: Agiena drainage canal, Bilqas district, 5: Gamasa drainage canal, Gamasa district, 6: El-Sallab irrigation canal, Mansoura district, 7: Shawa irrigation canal, Mansoura district, 8: El-Tawila irrigation canal, Mansoura district, 9: Basandila irrigation canal, Talkha district, 10: Rezka, irrigation canal, Sherbin district

was 17 with molecular weights ranging between 12.86 and 91.7 kDa. There are 3 monomorphic bands noticed in all samples at molecular weight of 19.30, 35.00 and 56.23 kDa. Three unique bands were observed with molecular weight 43.50, 74.60 and 78.79 kDa, these bands could be used as positive molecular marker for site 10 (Rezka), site 8 (El-Tawila) and site 4 (Agiena), respectively (Fig. 7).

The bands of molecular weight 40.50 and 25.70 kDa noticed to be found in irrigation canals only “site 6 (El-Sallab), site 7 (Shawa), site 8 (El-Tawila), site 9 (Basandila) and site 10 (Rezka)” so that these bands could be used as a positive marker for irrigation canals and differentiate between irrigation and drainage canals.

In Table 6, the highest percentage of polymorphism were expressed in site 8 (El-Tawila) and site 10 (Rezka) by a value of 52.94% while the lowest value was 11.77% and found in site 5 (Gamasa). The changes in protein banding patterns may be correlated to the wide range of cytological abnormalities induced by the heavy metals. This conclusion was in accordance with (El-Abidin Salam *et al*., 1993; Hussam EL-Din, 1996).

Molecular genetics has provided a number of new methods for genotoxicity measurement such as Random Amplified Polymorphism DNA (RAPD). The RAPD has been widely used in genetic analyses to detect polymorphism in genetic mapping, taxonomy and phylogenetic studies, genotoxicity (Soliman and Heikal, 2012) and carcinogenesis studies. It has several advantages, including high polymorphism and independence from effects related to environmental condition and the physiological stage of the plant among these marker techniques (Ovesna *et al*., 2002).

The RAPD and similar techniques have been used to detect not only DNA damage and mutations but also changes in genetic diversity and gene frequencies (Atienzar and Jha, 2006). The polymorphism of the random amplified DNA represents the polymorphism of the genomic DNA corresponding to it. Damage to the genomic DNA will then result in changes of the binding site and PCR product and future more alter the electrophoresis pattern, these results make it possible to use this method to detect the genotoxicity of pollutants (Atienzar *et al*., 2000).

In the present study, as seen in Fig. 8 there were eight primers used to study the effect of different environmental variables of drainage and irrigation canals on *I. carnea* collected from Dakahlia Governorate. The percentages of polymorphisms were recorded. The data matrix of RAPD-PCR for *I. carnea* was recorded in Table 7. Total bands obtained ranged from 6-9 bands where primer A15 produced a total number of 6 bands and primer O12, O19 produced a total number of 9 bands. The number of monomorphic bands ranged from 3 in primer O19 to 5 bands in primer O5, O8, O12 and A10.

The band of molecular size 200 bp in primer O19 could differentiate between *I. carnea* collected from drainage canals and those collected from irrigation canals because this band found only in irrigation canals. The band of molecular size 800 bp in primer A15 observed to be restricted to the first five sites (drainage canals) so that this band could differentiate between *I. carnea* collected from drainage canals and *I. carnea* collected from irrigation canals while the band of molecular size 1100 bp was restricted to the last five sites (irrigation canals) so that this band could be differentiate between *I. carnea* collected from irrigation canals and *I. carnea* collected from drainage canals.

Regarding the polymorphism of all sites, the highest value of polymorphism 66.66% recorded in primer O19. However, the remaining percentages of polymorphism took specific trends where 50.00% in primer A1, 44.44% in primer O12, 42.86% in primer O1, 37.50% in primer O8 and A10, 33.33% in primer A15 and finally 28.57% was the lowest value and recorded in primer O5 as shown in Table 8. Variation in DNA sequences lead to polymorphism (Gajera *et al*., 2010).

Previous studies have shown that changes in DNA fingerprint (i.e., band patterns) observed reflect DNA alterations in genome from single base changes (point mutations) to chromosomal rearrangements (Atienzar *et al*., 1999, 2000) and that DNA fingerprinting offers a useful biomarker assay in ecotoxicology (Savva, 1998).

In this study, DNA damage induced by heavy metals in the drainage and irrigation canals on *I. carnea* was reflected by changes in RAPD profiles: Variation in band intensity, disappearance of bands and appearance of new PCR products occurred on the profiles. These results indicated that genomic template stability was significantly affected by heavy metals stress.

Table 7: Data matrix of RAPD-PCR for *Ipomoea carnea* collected from ten different sites from Dakahlia Governorate

Primer O1 Bp	1	2	3	4	5	6	7	8	9	10
1180	0	1	1	0	0	0	0	0	0	0
1130	1	1	1	1	1	1	1	1	1	1
840	1	1	1	1	1	1	1	1	1	1
730	1	1	1	1	1	1	1	1	1	1
640	1	0	0	1	1	1	1	1	1	1
400	1	1	1	1	1	1	1	1	1	1
200	0	1	1	0	0	0	0	0	0	0
Amplified bands	5	6	6	5	5	5	5	5	5	5

Primer O5 Bp	1	2	3	4	5	6	7	8	9	10
1180	1	1	1	1	1	1	1	1	1	1
1130	1	1	1	1	1	1	1	1	1	1
940	0	0	1	1	0	0	0	0	0	0
840	1	1	1	1	1	1	1	1	1	1
730	1	1	1	1	1	1	1	1	1	1
640	1	1	1	1	1	0	1	1	1	0
400	1	1	1	1	1	1	1	1	1	1
Amplified bands	6	6	7	7	6	5	6	5	6	5

Primer O8 Bp	1	2	3	4	5	6	7	8	9	10
1350	1	1	0	1	1	1	1	0	1	1
1100	1	1	1	1	1	1	1	1	1	1
900	1	1	1	1	1	1	1	1	1	1
800	1	1	1	1	1	1	1	1	1	1
680	0	0	0	0	0	1	0	0	0	0
605	1	1	1	1	1	1	1	1	1	1
445	1	1	1	1	1	1	1	1	0	0
330	1	1	1	1	1	1	1	1	1	1
Amplified bands	7	7	6	7	7	8	7	6	6	6

O12 Bp	1	2	3	4	5	6	7	8	9	10
1350	1	1	1	0	1	1	0	1	1	0
1100	1	1	1	1	1	1	1	1	1	1
900	1	1	1	1	1	1	1	1	1	1
800	1	1	1	1	1	1	1	1	1	1
680	0	0	0	0	0	0	1	0	0	0
605	1	1	1	1	1	1	1	1	1	1
645	0	0	0	0	0	1	0	0	1	0
500	1	0	0	1	0	1	0	0	0	0
330	1	1	1	1	1	1	1	1	1	1
Amplified bands	7	6	6	6	6	8	6	6	7	5

Primer O19 Bp	1	2	3	4	5	6	7	8	9	10
1090	1	1	0	1	1	1	1	1	1	1
920	1	1	1	1	1	1	1	1	1	1
850	1	1	1	1	1	1	1	1	1	1
650	1	1	1	1	1	1	1	1	1	1
625	1	1	1	0	0	0	0	0	1	0
445	0	0	0	1	1	1	1	1	1	0
325	0	0	0	0	0	0	0	1	0	0
290	1	1	1	1	1	1	1	1	1	0
200	0	0	0	0	0	1	1	1	1	1
Amplified bands	5	6	4	6	6	7	7	8	8	4

Primer A1 Bp	1	2	3	4	5	6	7	8	9	10
1500	0	1	1	1	1	1	1	0	0	1
1350	1	1	1	1	1	1	1	1	0	1
1000	1	1	1	1	1	1	1	1	1	1
900	1	1	1	1	1	1	0	0	0	1
800	0	0	0	1	1	1	1	1	1	1
700	1	1	1	1	1	1	1	1	1	1
600	1	1	1	1	1	1	1	1	1	1
450	1	1	1	1	1	1	1	1	1	1
Amplified bands	6	7	7	8	8	8	7	6	5	8

Primer A10 Bp	1	2	3	4	5	6	7	8	9	10
1800	1	0	0	0	0	0	0	1	0	1
1450	1	1	1	1	1	1	1	1	1	1
1600	1	1	1	1	1	1	0	1	0	0
1500	1	1	1	1	1	1	1	1	1	1
750	1	1	1	1	1	1	1	1	1	1
600	1	1	1	1	1	1	1	1	1	1
400	1	1	0	0	0	0	0	0	0	0
Amplified bands	8	7	6	6	6	6	5	7	5	6

Primer A15 Bp	1	2	3	4	5	6	7	8	9	10
1300	1	1	1	1	1	1	1	1	1	1
1100	0	0	0	0	0	1	1	1	1	1
1000	1	1	1	1	1	1	1	1	1	1
850	1	1	1	1	1	1	1	1	1	1
800	1	1	1	1	1	0	0	0	0	0
700	1	1	1	1	1	1	1	1	1	1
Amplified bands	5	5	5	5	5	5	5	5	5	4

Table 8: Polymorphic bands of *Ipomoea carnea* collected from Dakahlia Governorate

	Bands					Polymorphism per primer (%)									
Primers	Range of products (bp)	Monomorphic	Polymorphic	Total	Polymorphism (%)	1	2	3	4	5	6	7	8	9	10
O1	200-1180	4	3	7	42.86	14.29	28.59	28.57	14.29	14.29	14.29	14.29	14.29	14.29	14.29
O5	400-1180	5	2	7	28.57	14.29	14.29	28.57	28.57	14.29	0.00	14.29	0.00	14.29	0.00
O8	330-1350	5	3	8	37.50	25.00	25.00	12.50	25.00	25.00	37.50	25.00	12.50	12.50	12.50
O12	330-1350	5	4	9	44.44	22.22	11.11	11.11	11.11	11.11	33.33	11.11	11.11	22.22	0.00
O19	200-1090	3	6	9	66.60	22.22	33.33	11.11	33.33	33.33	44.44	44.44	55.56	55.56	11.11
A1	450-1500	4	4	8	50.00	25.00	37.50	37.50	50.00	50.00	50.00	37.50	25.00	12.50	50.00
A10	400-1800	5	3	8	37.50	37.50	25.00	12.50	12.50	12.50	12.50	0.00	25.00	0.00	12.50
A15	700-1300	4	2	6	33.33	16.67	16.67	16.67	16.67	16.67	16.67	16.67	16.67	16.67	0.00

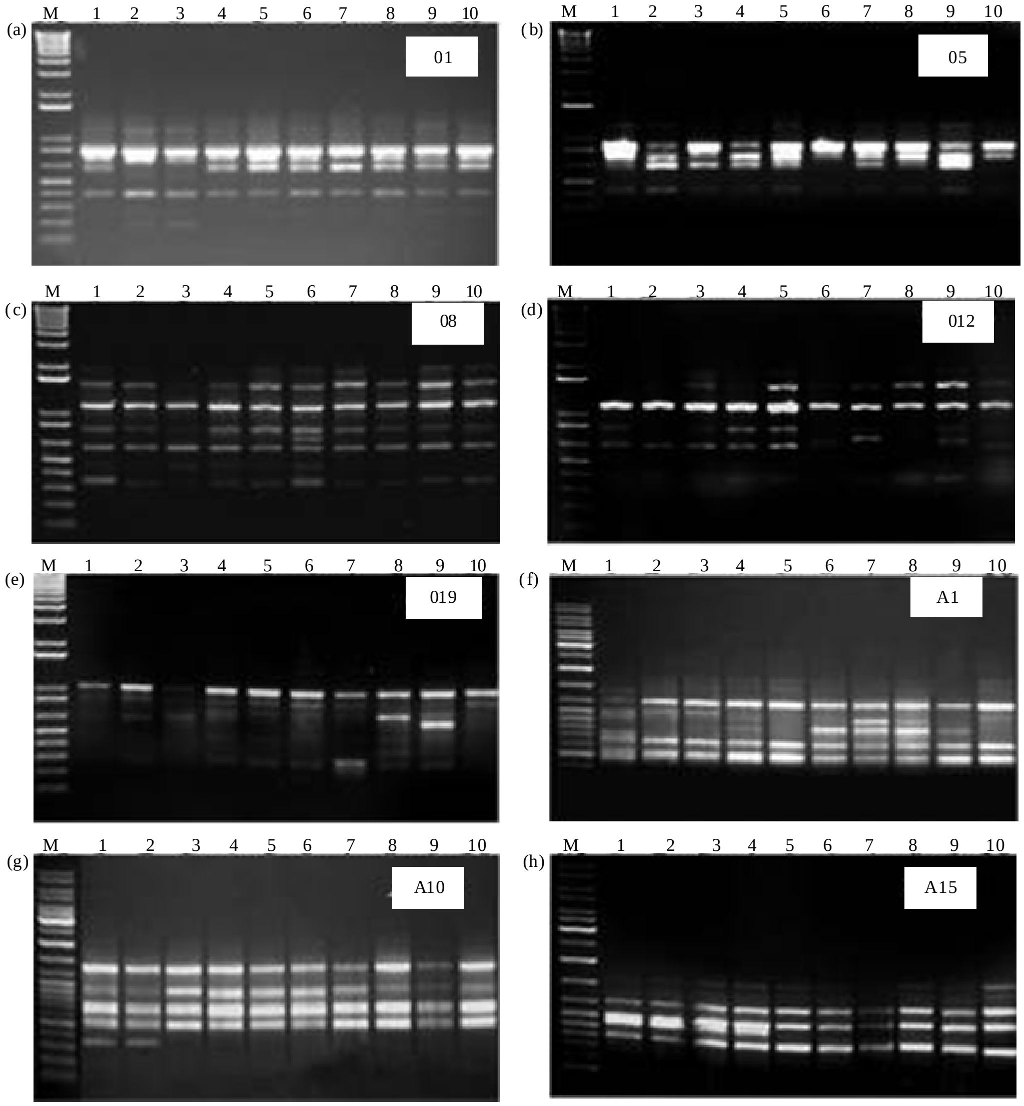

Fig. 8(a-h): Amplification profiles of the ten sites, (a) 01, (b) 05, (c) 08, (d) 012, (e) 019, (f) A1, (g) A10 and (h) A15 of *Ipomoea carnea* generated from eight primers collected from Dakahlia Governorate, M: Marker molecular size. 1: Meet-Khamis drainage canal, Mansoura district, 2: Belgay drainage canal, Mansoura district, 3: Amaar drainage canal, Bilqas district, 4: Agiena drainage canal, Bilqas district, 5: Gamasa drainage canal, Gamasa district, 6: El-Sallab irrigation canal, Mansoura district, 7: Shawa irrigation canal, Mansoura district, 8: El-Tawila irrigation canal, Mansoura district, 9: Basandila irrigation canal, Talkha district, 10: Rezka, irrigation canal, Sherbin district

CONCLUSION

The present study showed that the polluted water of irrigated and drainage canals with heavy metals can cause negative effects on *Ipomoea carnea* plants by causing induction of mitotic abnormalities such as stickiness, lagging, bridges, oblique, non-congression, disturbed and many others were observed in different mitotic stages. Reduce the fertility of pollen grains as well as changes in protein and RAPD profiles. This proved that the cytotoxicity of the heavy metals of the polluted water of drainage and irrigation canals used in this study. This study ensure that heavy metals are genotoxic and cytotoxic agents in plant cells that cause loss in crop yields irrigated by polluted water and planted in polluted soil so it is important to control of water quality and soil must be remediated. We can also conclude that the concentration of heavy metals in the plant leaf samples is higher than the concentration in the water and soil samples so that *Ipomoea carnea* Jacq. act as a hyper accumulator plant to heavy metals which present in the polluted soil and water.

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