Journal of Mediterranean Ecology **vol. 11, 2011: 5-20**

Cytotoxicity and mutagenic effects of soil radionuclides on some black sand plant species

M.S.A. Soliman[1], A.K. Hegazy[2]*, S.K. Goda[3] , M.H. Emam[4] and A.A. Al-Atar[2]

[1] *Botany Department, Faculty of Science, Helwan University, Helwan, Egypt*
[2] *Department of Botany & Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.*
[*] *Corresponding author email: akhegazy@yahoo.com*
[3] *Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt.*
[4] *Botany Department, Faculty of Science, Cairo University, Giza, Egypt.*

Key word: Black sands; Mediterranean; Chromosomal aberrations; Proteins; Amino acids; Uranium; Thorium.

Abstract

Three plant species (*Cakile maritima* Scop., *Senecio glaucus* L. and *Rumex pictus* Forssk) were selected from three black sand habitats along the Mediterranean coast in Egypt for cytogenetic studies and to recognizes the mechanism by which plants withstand high concentration of the absorbed radionuclides through determination of the electrophoratic banding pattern of protein and amino acid profiles. The study showed that exposure of the study plant species to soil radionuclides causes decrease in the percentage of prophase and prophase to metaphase ratio, while the percentage of anaphase and telophase increases with soil radioactivity. The results revealed chromosomal aberrations, e.g., C-metaphase, star metaphase, chromosome stickiness at metaphase stage, C-anaphase and chromosomal breaks at the different mitotic stages with fluctuation in the index of mitotic phases. High radionuclide content of plants causes alterations in the bands relative mobility and intensities, expression of new proteins and suppression of some proteins. Study of amino acid profile of plants indicated that radioactive elements stimulate the biosynthesis of some amino acids e.g. proline, cysteine, serine and thereonine while inhibit some other amino acids such as arginine. Aspartic acid is the most abundant amino acid in the three study species.

Introduction

Black sand accumulations along large stretches of Mediterranean coast in Egypt extend from Abu Qir Bay in the west to Rafaa on the east between longitudes 30 12' and 34 10 E')El-Hadry, 1998, Hegazy & Emam, 2010). This part of the Mediterranean coast reaches about 500 km in length (Fig. 1). These sands originally derived as erosional products of the crystalline igneous rocks from the mountainous ranges in Sudan and Abyssinian and carried down the courses of the River Nile. Rosetta promontory is the area with the highest black sand placers in Egypt (Sheppard et al., 2005). Even though black sand habitats are rich in radioactive minerals several sites are not devoid of vegetation. Tomsett and Thurman (1988) reported that although contaminated soils drastically affect the growth of plant and soil–living microbes, these environments are not totally devoid of flora and fauna as many species are adapted to tolerate the increased metal concentrations. The effect of radionuclide deposits on the vegetation was studied by many authors (Gulati et al., 1980; Murthy et al., 1984; Meyer and McLendon, 1997; Hakonson-Hays et al., 2002; Sheppard et al., 2005).

In our natural environments, living organisms are chronically exposed to low doses and dose rates of ion-

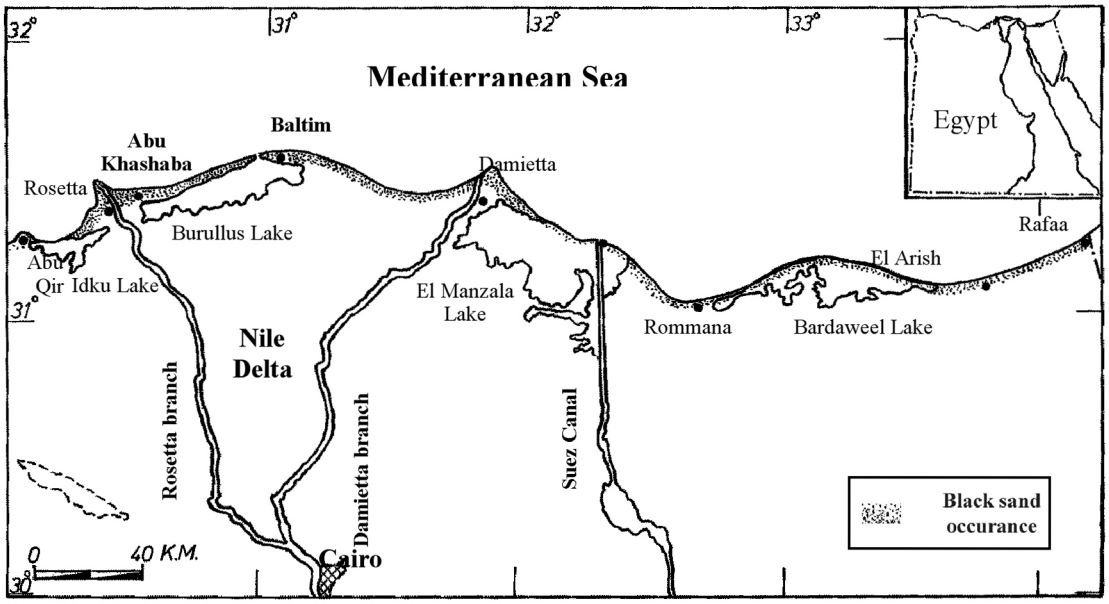


Fig.1. Map of northern part of Egypt showing the black sand deposits (dotted coastal strip) and the study sites location in Abu Khashaba and Baltim.

izing radiation (Zaka et al., 2002). Human beings are exposed to background radiation that stems both from natural and man-made sources. Natural background radiation, which is equivalent to 2.4 mSv per person, makes up approximately 80% of the total radiation dose a person is exposed in a year (Taskin et al., 2009). Soil radionuclide activity concentration is one of the main determinants of the natural background radiation. Radioisotopes that are present in soil significantly affect terrestrial gamma radiation levels. It is critical to evaluate soil radioactivity in order to understand background radiation concentrations. Measuring terrestrial gamma dose rates is also essential since gamma radiation provides information concerning excess lifetime cancer risks (Taskin et al., 2009).

Assessment of the ecological and genetic impact of radiation on plant populations is of great importance as considered the ecosystem producer (Kovalchuk et al., 1998). There's a great need to quantify changes in plant genomes after exposure to radiation as many agricultural areas have been polluted with long-live radioisotopes (Kovalchuk et al., 1998). Scoring the incidence of chromosomal aberrations and micronuclei in root tip cells has provided an easy method to study the effects of different mutagenic agents (Gulati et al., 1994). A number of plant bioassays have been developed for the detection of environmental mutagens, as these assays are relatively easy to perform, inexpensive and they provide a wide range of genetic endpoints. The test is based on assessment of cytotoxic and genotoxic potential by recording mitotic abnormalities and chromosomal aberrations in root tips (Tkalec et al., 2009).

Cytogenetic studies have been conducted on chromosomal aberrations in plants after their exposure to radiation by Kovalchuk et al. (1998) on *Allium cepa* to measure the genotoxicity of soils of inhabited area in the Ukraine contaminated by the Chernobyl accident in 1986 that showed high genotoxicity of radioactively polluted soils. The genetic consequences of radioactive contamination to agricultural crops have been studied by Geraskin et al. (2003) where the analysis of genetic variability in three sequential generations of rye and wheat revealed increased cytogenetic damage in plants exposed to chronic irradiation during second and third years. The percentage of root tip meristem cells displaying chromosome aberrations was estimated immediately after irradiation as shown by Zaka et al. (2002) when he studied the effect of external irradiation on induction of chromosome aberration in *Pisum sativum* root tip meristem.

It is well established that uranium exposure result in both chemical and radiological toxicity (Hartmann et al., 2000). Under some circumstances the chemical toxicity of soluble uranium compounds can even surpass the potential radiotoxic effects (Domingo, 2001). To protect themselves from metal poisoning, plant cells must have developed a mechanism by which the metal ion, entering the cytosol of the cell, is immediately complexed and inactivated (Zenk, 1996), thus preventing the metal from inactivating catalytically active or structural proteins. Physiological and biochemical alterations are common response to environmental stress (Belles et al., 2005). Alteration of SDS-PAGE protein profile may be regarded as an indicator of a mutagenic

potential for the contaminants (Barakat and Hassan, 1997). Amino acids play a central role in plant primary metabolism. These compounds, being early products of photosynthesis and nitrogen assimilation, are known to be particularly affected by environmental factors (Malallah et al., 1998). Environmental pollution, which leads to physiological stress, markedly affects the free amino acid pool considered to be elevated by endogenous protein degradation (Malallah et al., 1998).

The objective of this study is to estimate the accumulation of radionuclides from the black sand deposits by naturally growing wild plants and to quantitively measure changes in the genome of these species to monitor the cyto- and genotoxicity of black sand soils. The mechanism by which these plants can withstand high concentration of absorbed radionuclides will be recognized through determination of the electrophoratic banding pattern of protein and amino acid profiles.

Materials and Methods

Study sites and plant materials

Three sites were selected for this study; two sites in Rosetta represent the coastal sand plain and the sand mounds of Abu Khashaba while the third site was in the coastal sand dunes of Baltim called Al Narreges (Fig. 1). Soil from each site was collected for the radiometric analysis and the greenhouse experiment (cf. Hegazy & emam, 2010). From the plant populations recorded in the study habitats, seeds of the three plant species *Cakile maritima* Scop., *Senecio glaucus* L. and *Rumex pictus* Forssk. were collected from the natueally growing populations.

Cytological study

The radiometric analysis of the soil collected from the study sites was performed using high purity HGPe detector connected to multichannel analyzer of gamma ray spectrometry. Seeds were allowed to germinate in laboratory at 25°C to study the cytological characters of root tip meristematic cells. Cytological study was carried out using aceto-orcein squash method, according to Fukui and Nakayama (1996). Under a light microscope (X10 objective) the preparations were examined. The percentage of the different mitotic, types of chromosomal aberrations and percentage of different types of interphase stage abnormalities were determined. Mitotic index (MI) was determined and prophase to metaphase (P / M) ratio was calculated. Photomicrographs of the different mitotic abnormalities detected in the study plants were taken using oil lens (X100). The obtained data were statistically analyzed by using one way ANOVA and t-test. In case of one way ANOVA the probability level was 0.05. In t-test the significant differences are decided by considering the value of T at 0.05, 0.01 and 0.001 probability levels.

Greenhouse experiment

In an open greenhouse conditions, plants were grown in soil from their natural habitats and in control soil collected from Wadi El Natron. The experiment was conducted during February to June 2005. At the flowering stage, the plants were harvested for determination of uranium and thorium accumulated by the plant. The change in amino acids content and protein banding pattern with the increasing radionuclide content was estimated in the plant tissues.

1. Electrophoratic detection of protein

Cytoplasmic proteins were extracted and purified from the test species for SDS-PAGE analysis based on Nelson et al. (1984). The protein sample was separated by SDS-PAGE on 11% polyacrylamide gel and electrophoresed at 30 milliamper (m.A) at 10oC for 3 hours according to the method of Laemmili (1970). The silver staining method as described by Sammons et al. (1981) was used for protein detection.

2. Amino acid content

Free amino acids were extracted according to Shad et al. (2002). Several amino acids were examined using a HPLC system (model hp1050) with a UV detector at 254 nm.

3. Uranium and thorium content of plants

Uranium and thorium content was determined in the plant material by inductively coupled plasma mass Spectrometer (ICP–MS technique) model JEOL JMS – plasma X2 high resolution ICP–MS, the Central Laboratory for Elemental and Isotopic Analysis, Nuclear Research Center, Atomic Energy Authority, Egypt.

Results

Gamma-radiation

The levels of gamma-radiation in the study black sand habitats are summarized in Table 1. The gamma-radiation of the soil from the coastal sand plain of Abu Khashaba was the highest (39 μSh^{-1}) in comparison to the radiation from the soil of Al Narreges coastal sand dune and the sand mounds of Abu Khashaba where the gamma-radiation was 23 and 34 μSh^{-1} respectively. The black sand habitats under investigation can be arranged

Table 1. Radiological characteristics of the soil from the study black sand habitats

Study Site	Gamma-Radiation (μSh^{-1})
Al Narreges coastal sand dune	23
Sand mounds of Abu Khashaba	34
Coastal sand plain of Abu Khashaba	39

with respect to the level of gamma radiation from the soil in the order of, Abu Khashaba coastal sand plain > Abu Khashaba sand mounds > Al Narreges coastal sand dune.

Mitotic stages

The percentage of prophase in root tip cells of *C. maritima* decreased from 27.27±4.05% to 13.25±1.43% and 13.96±1.57 % with increasing gamma–radiation from 23 $\mu S\ h^{-1}$ in the soil of Al Narreges coastal sand dune to 34 $\mu S\ h^{-1}$ and 39 $\mu S\ h^{-1}$ in the soil from sand mounds and coastal sand plain of Abu Khashaba respectively (Fig. 2). In root tip cells of *S. glaucus* and *R. pictus* there was non significant difference in the prophase percentage with increasing gamma-radiation in the soil of Abu Khashaba sand mounds.

Percentage of metaphase in *C. maritima* from Abu Khashaba sand mounds was 22.04±3.06% and increased to 31.65±2.47% in *C. maritima* from Abu Khashaba coastal sand plain with increasing the level of gamma-radiation while in roots of *S. glaucus* the percentage of metaphase attained non significant increase. There is an inverse correlation between the percentage of prophase and metaphase in root tip cells of *C. maritima* and *S. glaucus* as demonstrated by prophase / metaphase ratio (P/M) where it equal the unity in *C. maritima* from Al Narreges coastal sand dune while it exhibited a value lower than the unity in the plants of *C. maritima* from the coastal sand plain of Abu Khashaba (Fig. 2). Also that was observed in P/M ratio of *S. glaucus*, where P/M ratio was higher than the unity in the plants of Al Narreges coastal sand dune and decreased to be lower than the unity in the plants from the two sites of Abu Khashaba with increasing gamma-radiation.

Percentage of anaphase and telophase in root tip cells of *C. maritima* from Al Narreges coastal sand dune attained the value of 44.25±5.52% and increased to 64.70±2.93% in the plants of this species from Abu Khashaba sand mound by increasing radiation. In root cells of *R. pictus* a significant increase of anaphase and telophase percentage is observed in the plants from Al Narreges coastal sand dune and Abu Khashaba sand mounds (from 33.88±3.61% to 47.28±4.08%). Generally, the analysis of the results of mitotic stage percentage revealed that the percentage of prophase and prophase to metaphase ratio are negatively correlated with gamma radiation in soil while the percentage of anaphase and telophase are positively correlated with the level of radioactivity in the soil.

Mitotic Index

The MI of *C. maritima* and *S. glaucus* root tip cells from Al Narreges coastal sand dune attained non significant difference from the two sites of Abu Khashaba (Fig. 2). The MI of *R. pictus* root tip cells significantly increased in the soil of Al Narreges coastal sand dune compared to Abu Khashaba sand mounds (from 7.74±0.24% to 10.06±0.49%).

Chromosomal aberrations

The fraction of cells with aberrations at interphase stage in root tip cells of *C. maritima* increased from 1.02±0.35% in the plants from Al Narreges coastal sand dune to 3.84±1.09% and 2.38±0.75 in the plants from Abu Khashaba sand mounds and coastal sand plain respectively with increasing soil radioactivity (Fig. 2). The same result was observed in root cells of *S. glaucus* where the percentage of the abnormal cells at the interphase stage in the plants from Al Narreges coastal sand dune increased in the plants from Abu Khashaba coastal sand plain (from 2.90±0.61% to 7.13±2.78%). The percentage of aberrant cells at the interphase stage in *R. pictus* of Al Narreges and the sand mounds of Abu Khashaba are nearly the same which indicates that the fluctuation in the percentage of interphase abnormalities as induced by gamma-radiation is dependant on the plant species.

The percentage of abnormalities at different mitotic stages indicate that exposure of the plant to gamma-radiation induced high percentage of aberrations at prophase and metaphase stages and this aberrations increased with increasing the level of gamma-radiation (Fig. 2). In contrast to prophase and metaphase the percentage of abnormality at anaphase and telophase stage decreased in root tip cells of *C. maritima* from 7.25±2.49% in the seeds from Al Narreges coastal sand dune to 0.53±0.53% in the seeds collected from the sand mounds of Abu Khashaba with increasing gamma-radiation. It was observed that, the percentage of aberrations at metaphase and prophase stages was higher than that at interphase, anaphase & telophase stages.

Chromosomal aberrations in the study species showed that the most conspicuous effect of gamma-radiation on metaphase stage of *C. maritima*, *S. glaucus*

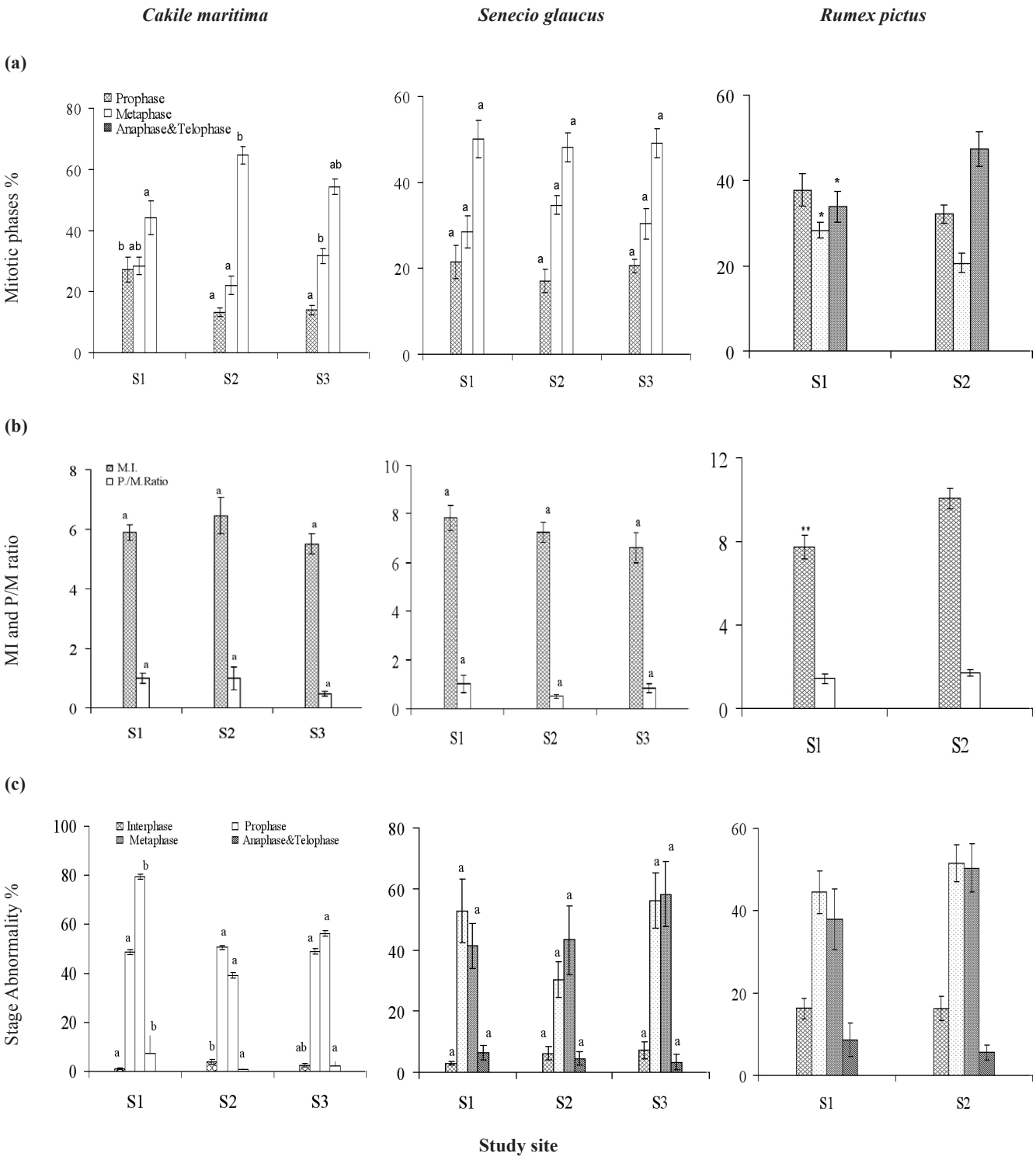


Fig. 2. (a) Percentage of mitotic phases, (b) Mitotic index (MI), prophase to metaphase (P/M) ratio and (c) Percentage of abnormality in interphase and mitotic phases in root tip cells of *Cakile maritima*, *Senecio glaucus* and *Rumex pictus* seeds collected from the study black sand habitats S1=Al Narreges coastal sand dune, S2 = Sand mounds of Abu Khashaba and S3 = Coastal sand plain of Abu Khashaba. Bars represent standard error of the mean and the different letters indicate a significant difference at P = 0.05.

and *R. pictus* in the formation of C-metaphase and stare metaphase (Table 2). In addition to C-metaphase in *S. glaucus* chromosome stickiness was highly common in the metaphase stage. In root tip cells of *C. maritima*, increased gamma-radiation in the soil of Abu Khashaba coastal sand plain more than in the soil of Al Narreges induced increase of the percentage of C-metaphase (2n) from 27.47±4.45% to 39.36±4.13%. A significant increase in the percentage of C-metaphase (2n) is observed in root tip cells of *S. glaucus* from 16.91±5.24% to 39.62±8.02% with increasing soil radioactivity. C-metaphase (4n) was recorded only in the root cells of *C. maritima* and *S. glaucus* seeds collected from the two sites of Abu Khashaba. Percentage

of disturbed chromosomes at metaphase stage of *C. maritima* from Al Narreges coastal sand dune increased from 1.43±1.43% to 4.63±2.45% and 8.06±4.30% by increasing gamma radiation in the soil of sand mounds and the coastal sand plain of Abu Khashaba respectively. Metaphase non-congression was detected in root tip cells of *R. pictus* seeds collected from the soil of Al Narreges coastal sand dune and the soil of Abu Khashaba sand mounds by ratios of 3.17±1.38% and 6.43±3.06% respectively.

The most common type of abnormalities in anaphase and telophase stage of *C. maritima* and *R. pictus* was C-anaphase and in *S. glaucus* was chromosomal breaks (Table 2). At interphase stage, the most conspicuous effect of gamma-radiation in the formation of vacuolated nucleuses, it represents the highest percentage of interphase aberrations in the study species throughout the different level of gamma-radiation. The percentage of vacuolated interphase increased with increasing the level of gamma-radiation. Some types of chromosomal aberrations e.g. C-metaphase, C-anaphase, lag chromosome, free chromosome, chromosomal breaks and sticky metaphase in the study species at different mitotic stages are represented in Plate 1.

Electrophoratic banding pattern of protein

The mutagenic effect of uranium and thorium absorbed by plants on the banding pattern of protein are shown in Fig. 3 and Appendix Table 1. With regard to *C. maritima*, by SDS-PAGE the expression of 14 protein bands was indicated in the plants grown in the soil from the coastal sand dunes of Al Narreges, where the plants accumulated 0.21 ppm uranium (Fig. 3a). The number of expressed proteins in the plants grown in the soils from Abu Khashaba sand mounds was 17 protein bands where the accumulated uranium by the plants was 0.4 ppm. The bands region spanning the molecular weight range 160-65 K.Da. exhibited the highest degree of banding pattern changes as compared with the lower region. By SDS-PAGE the expression of some proteins were detected only in the plants grown in the soil collected from Al Narreges coastal sand dune but these proteins were not expressed in the plants grown in the soil from both sites of Abu Khashaba with increased uranium concentration in the plant tissues. The proteins of molecular weights 57, 51, 40, and 17 K.Da. were detected in the plants of *C. maritima* grown in the soil from Al Narreges coastal sand dune with estimated amounts; 9.34, 7.54, 19.81 and 19.69 % respectively, but these intensities decreased to 6.72, 6.66, 4.34 and 15.01 % respectively with increased uranium content in the plants grown in the soil of Abu Khashaba. The intensities of most proteins are generally lower in the plants grown in the control soil than the plant's natural habitat soils.

The protein banding pattern of *S. glaucus* (Fig. 3b) the expression of the proteins having molecular weights 116, 70, 48, 44, 35 and 24 K.Da. were detected in the plants grown in the soil from the sand mounds of Abu Khashaba from which the plants accumulated 0.27 ppm uranium but new proteins were expressed in the plants grown in the soil from the coastal sand plain of Abu Khashaba and Al Narreges coastal sand dune, where the plants accumulated 1.2 ppm and 3.69 ppm of uranium, respectively. In *S. glaucus*, the amount of proteins of molecular weights 155, 56 and 28 K.Da. were 4.97, 15.05 and 1.78 % respectively in the plants grown in the soil of Abu Khashaba sand mounds. The protein intensities increased to 16.34, 22.76 and 2.63 % respectively, in the plants grown in the soil of Al Narreges coastal sand dune which accumulated more uranium and thorium. The percentage of one protein of molecular weight 17 K.Da. decreased from 19.5% in the plants of Abu Khashaba sand mounds to 6% in the plants of Abu Khashaba coastal sand plain with the increase of radioactive elements content of the plants. The number of detected bands in the plants grown in the control soil was higher than the number in the plants grown in the soil from their natural habitats but, the intensities of most bands decreased when the plants were grown in the control soil.

In SDS-PAGE of *R. pictus* 16 bands are observed in the plants grown in the soil from the sand mounds of Abu Khashaba, where the plants accumulated 0.51 ppm of uranium and 0.59 ppm of thorium (Fig. 3c). The number of bands decreased to 13 in the plants grown in the soil of Al Narreges costal sand dune where uranium and thorium increased in the plant tissues to 0.87 and 1.80 ppm while decreased to 12 protein in the plants grown in the control soil from which the plants accumulated 1.1 and 2.6 ppm of uranium and thorium respectively. Most of bands that appeared in the plants grown in the soil from Abu Khashaba sand mounds were disappeared in the plants grown in the soil of Al Narreges except for the bands of molecular weights 145, 16 and 12 K.Da. Two bands with molecular weights 145 and 16 K.Da. were detected in the plants grown in soil from the sand mounds of Abu Khashaba with amounts 3.55 and 26.03 % respectively, which decreased to 1.84 and 22.87 % respectively in the plants grown in the control soil.

Table 2. Number and percentage of normal and abnormal interphase, prophase and metaphase stages with number and percentage of different types of chromosomal aberrations at these stages in root tips cells of study species seeds collected from the study black sand habitats. Data are expressed as mean ± standard error. Different superscript letters for the same species indicate a significant difference at P = 0.05. C.M. = C-metaphase and C.A. = C-anaphase.

species					*Cakile maritima*			*Senecio glaucus*			*Rumex pictus*	
Study Site					Coastal sand dunes	Abu Khashaba		Coastal sand dunes	Abu Khashaba		Coastal sand dunes	Sand mounds
						Sand mounds	Coastal sand plain		Sand mounds	Coastal sand plain		
Interphase	Total				604.60±56.92a	551.56±50.05a	697.90±45.13a	544.70±94.56a	480.67±65.10a	488.00±56.20a	471.00±61.67	453.80±50.26
	Normal	Number			598.70±57.06a	529.00±46.37a	682.30±46.60a	530.30±93.79a	456.67±68.55a	457.40±55.41a	387.40±46.73	371.50±33.69
		%			98.98±0.35b	96.16±1.09a	97.62±0.75ab	97.10±0.61a	93.86±2.19a	92.87±2.78a	83.75±2.48	83.78±2.90
	Abnormal	Multinuclei			0.30±0.15a	1.44±1.09a	0.10±0.10a	0.40±0.40a	0.11±0.11a	0.10±0.10a	0.80±0.36	0.60±0.22
		Micronucleuse			0.10±0.10a	------	------	0.10±0.10a	------	0.10±0.10a	0.20±0.13	------
		Restitution			0.70±0.30b	------	------	------	------	------	2.30±1.79	------
		Vaculation			4.50±2.05a	16.22±4.28b	14.90±4.53ab	13.80±3.79a	23.89±7.38a	30.40±11.98a	80.30±20.09	81.70±22.16
		Abnormal			0.30±0.15a	4.89±3.23a	0.60±0.50a	0.10±0.10a	------	------	------	------
		Total	Number		5.90±1.96a	22.56±6.94b	15.60±4.69ab	14.40±3.70a	24.00±7.38a	30.60±12.04a	83.60±19.77	82.30±22.07
			%		1.02±0.35a	3.84±1.09b	2.38±0.75ab	2.90±0.61a	6.14±2.19a	7.13±2.78a	16.25±2.48	16.22±2.90
Prophase	Total				10.90±2.33b	5.11±0.92a	5.60±0.75a	10.90±4.08a	6.00±0.94a	6.70±0.94a	15.10±2.75	16.10±1.93
	Normal	Number			7.10±2.55a	2.89±0.90a	3.30±0.93a	6.70±4.07a	4.00±0.58a	3.10±0.81a	8.10±1.44	7.90±1.29
		%			51.52±11.17a	49.44±11.15a	51.07±10.51a	47.20±10.27a	69.74±5.74a	43.91±8.98a	55.61±5.18	48.52±4.53
	Abnormal	Number			3.80±0.92a	2.22±0.57a	2.30±0.47a	4.20±0.85b	2.00±0.50a	3.60±0.67ab	7.00±1.81	8.20±1.23
		%			48.48±11.17a	50.56±11.15a	48.93±10.51a	52.80±10.27a	30.26±5.74a	56.09±8.98a	44.39±5.18	51.48±4.53
Metaphase	Total				10.80±1.55a	8.33±1.44a	12.60±1.17a	10.70±1.16ab	12.67±1.54b	9.00±0.77a	11.20±1.85	10.70±1.87
	Normal	Number			1.70±0.40a	5.78±1.24b	5.50±0.97b	5.90±0.7ab	8.22±2.30b	3.60±0.99a	6.50±1.09	5.60±1.28
		%			20.65±5.95a	60.94±9.83b	43.76±6.77b	58.60±7.23a	56.71±11.15a	41.75±10.64a	62.18±7.35	49.72±5.86
	Abnormal	C.M.(2n)	Number		3.30±0.82b	1.00±0.29a	5.00±0.77b	2.00±0.65ab	1.00±0.41a	3.70±0.75b	1.90±0.38	2.40±0.58
			%		27.47±4.45a	21.13±10.52a	39.36±4.13a	16.91±5.24a	10.80±5.23a	39.62±8.02b	17.40±4.22	21.10±4.75
		C.M.(4n)	Number		------	0.11±0.11a	0.20±0.20a	------	0.22±0.22a	------	------	------
			%		------	0.85±0.85a	1.00±1.00a	------	1.59±1.59a	------	------	------
		Stare	Number		1.10±0.53a	0.44±0.18a	0.50±0.31a	0.90±0.35a	0.78±0.28a	0.80±0.20a	1.20±0.33	1.30±0.30
			%		8.55±3.41a	5.35±2.38a	2.89±1.63a	7.60±3.22a	6.73±2.72a	9.31±2.36a	10.15±2.67	14.56±3.66
		Stick	Number		4.00±1.15b	------	0.30±0.21a	1.40±0.73a	1.00±0.47a	0.30±0.15a	0.60±0.43	0.10±0.10
			%		34.99±5.69b	------	3.21±2.52a	13.11±6.32a	11.22±5.50a	3.06±1.57a	3.25±2.18	1.25±1.25
		Non Congression	Number		0.40±0.22a	0.22±0.15a	0.10±0.10a	0.10±0.10a	0.56±0.44a	0.20±0.13a	0.50±0.22	0.50±0.22
			%		4.48±2.90a	2.86±2.00a	1.00±1.00a	1.00±1.00a	5.03±3.47a	2.36±1.58a	3.17±1.38	6.43±3.06
		Disturbed	Number		0.10±0.10a	0.33±0.17a	0.90±0.46a	0.20±0.20a	0.11±0.11a	0.10±0.10a	0.10±0.10	0.70±0.30
			%		1.43±1.43a	4.63±2.45a	8.06±4.30a	1.25±1.25a	0.79±0.79a	0.83±0.83a	0.50±0.50	6.23±2.69
		Breaks	Number		0.20±0.13a	------	------	0.10±0.10a	0.11±0.11a	0.30±0.15a	0.20±0.13	0.10±0.10
			%		2.43±1.65a	------	------	0.91±0.91a	0.79±0.79a	3.08±1.62a	1.21±0.83	0.71±0.71
		Abnormal	Number		------	0.44±0.34a	0.10±0.10a	0.10±0.10ab	0.67±0.37b	------	0.20±0.13	0.00±0.00
			%		------	4.23±2.83a	0.71±0.71a	0.63±0.63ab	6.35±3.70b	------	2.14±1.52	0.00±0.00
		Total	Number		9.10±1.72b	2.56±0.58a	7.10±1.22b	4.80±0.96a	4.44±1.09a	5.40±1.02a	4.70±1.13	5.10±0.80
			%		79.35±5.95b	39.06±9.83a	56.24±6.77a	41.40±7.23a	43.29±11.15a	58.25±10.64a	37.82±7.35	50.28±5.86

Table 2. Continued.

species				Cakile maritima			Senecio glaucus			Rumex pictus	
Study Site				Coastal sand dunes	Abu Khashaba		Coastal sand dunes	Abu Khashaba		Coastal sand dunes	Sand mounds
					Sand mounds	Coastal sand plain		Sand mounds	Coastal sand plain		
Anaphase & Telophase	Total			16.30±2.30a	24.56±3.28b	22.00±2.04ab	22.50±4.02a	18.78±4.30a	16.70±2.57a	13.60±2.11a	23.40±2.38b
	Normal	Number		15.30±2.39a	24.44±3.30b	21.50±1.93ab	21.30±4.02a	18.11±4.38a	16.30±2.72a	12.20±2.02a	22.40±2.49b
		%		92.75±2.49a	99.47±0.53b	98.04±0.86b	93.52±2.33a	95.52±2.29a	95.42±3.75a	91.34±4.12	94.35±1.82
	Abnormal	C.A.	Number	0.30±0.15a	0.11±0.11a	0.20±0.20a	0.20±0.13a	0.11±0.11a	0.20±0.13a	0.50±0.40	0.40±0.16
			%	1.81±0.97a	0.53±0.53a	0.69±0.69a	1.48±1.13a	0.62±0.62a	2.08±1.42a	3.19±2.41	1.95±0.84
		Chromosome	Number	------	------	------	0.20±0.13a	0.22±0.15a	------	0.30±0.21	0.30±0.21
		Bridge	%	------	------	------	0.63±0.43a	1.62±1.09a	------	2.01±1.36	1.05±0.74
		Lag	Number	0.10±0.10a	------	------	0.20±0.13a	------	0.10±0.10a	0.10±0.10	------
		Chromosome	%	0.56±0.56a	------	------	1.37±1.11a	------	1.25±1.25a	0.53±0.53	------
		Free	Number	0.20±0.13a	------	------	0.20±0.13a	0.11±0.11a	------	0.20±0.20	------
		Chromosome	%	1.39±0.95a	------	------	1.19±0.80a	0.93±0.93a	------	1.18±1.18	------
		Distributed	Number	------	------	------	0.10±0.10a	------	------	------	------
			%	------	------	------	0.37±0.37a	------	------	------	------
		Stare	Number	0.10±0.10a	------	------	------	------	------	------	0.10±0.10
			%	1.11±1.11a	------	------	------	------	------	------	0.67±0.67
		Multipolar	Number	------	------	------	------	------	------	------	0.10±0.10
			%	------	------	------	------	------	------	------	1.43±1.43
		Breaks	Number	0.10±0.00a	------	0.30±0.15a	0.30±0.21a	0.22±0.15a	------	0.30±0.21	0.10±0.10
			%	0.83±0.83a	------	1.28±0.68a	1.44±0.96a	1.31±0.87a	------	1.76±1.26	0.56±0.56
		Abnormal	Number	0.20±0.13a	------	------	------	------	------	------	------
			%	1.56±1.09a	------	------	------	------	------	------	------
		Total	Number	1.00±0.39b	0.11±0.11a	0.50±0.22ab	1.20±0.36a	0.67±0.33a	0.30±0.21a	1.40±0.70	1.00±0.30
			%	7.25±2.49b	0.53±0.53a	1.96±0.86a	6.48±2.33a	4.48±2.29a	3.33±2.55a	8.66±4.12	5.65±1.82

Free amino acid content

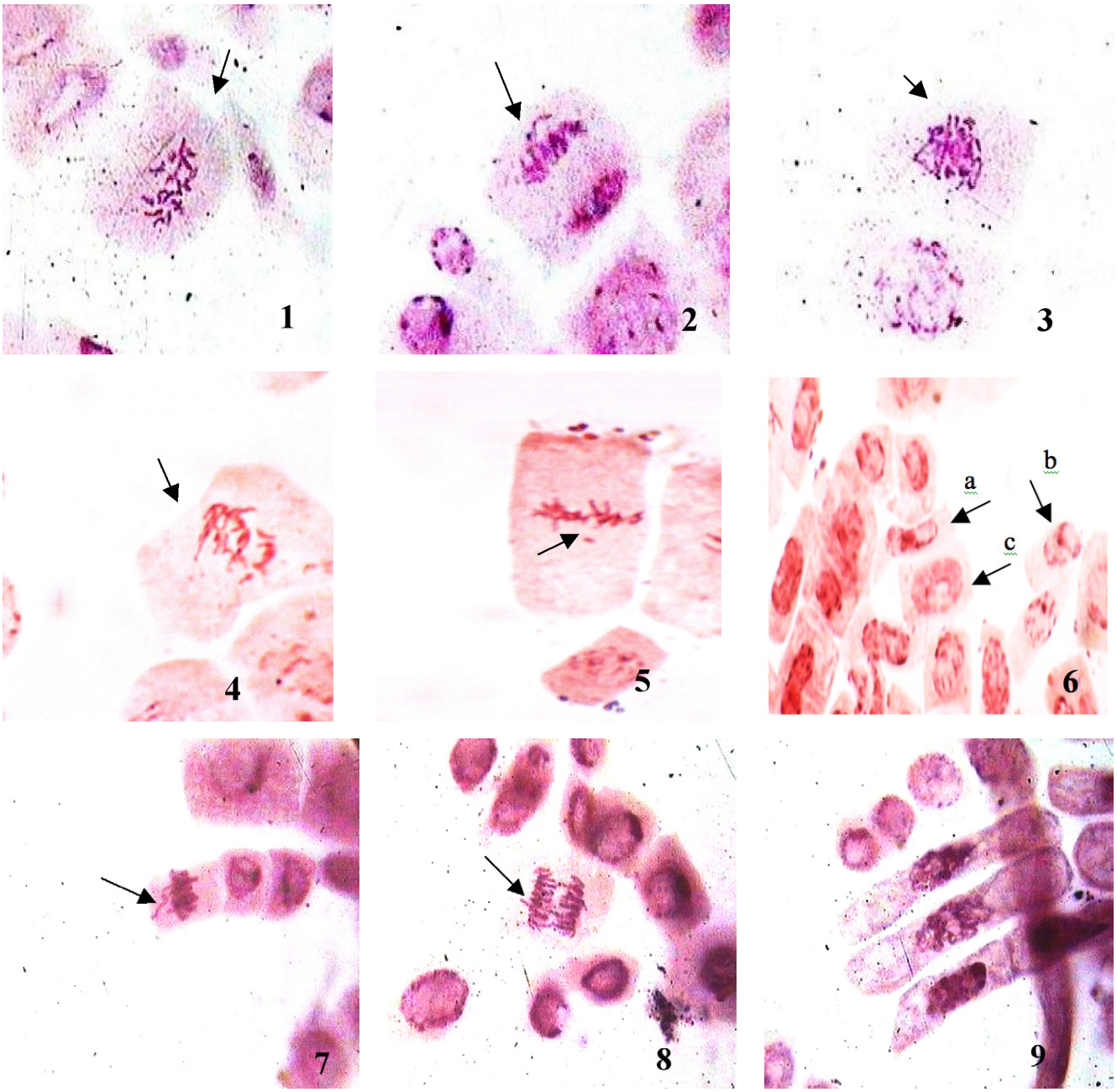
Free amino acid content of the three study species grown in black sand and control soil are shown in Table 3. With regard to *C. maritima*, thereonine and tryptophane detected with concentrations 0.24 and 0.023 mggm^{-1} freeze dry plant tissue respectively in the plants grown in soils of Al Narreges coastal sand dune, and detected with concentrations 0.082 and 0.017 mggm^{-1} freeze dry plant tissue respectively in the plants grown in the soil from the sand mounds of Abu Khashaba. Further decrease of thereonine into 0.017 mggm^{-1} freeze dry plant tissue with complete disappearance of tryptophane was observed after growing the plants in the soil from the coastal sand plain of Abu Khashaba. Alternatively, the concentration of aspartic acid and cysteine increased with the increase of uranium uptake by the plant. Glysine, arginine, valine and histidine were recorded in the plants grown in the soil collected from Al Narreges coastal sand dune but these amino acids were inhibited in the plants grown in the soil from the two sites of Abu Khashaba and new amino acids were synthesized. The free amino acid content in the plants grown in the control soil is higher than that in the plants grown in the soil from the black sand habitats.

In *S. glaucus*, aspartic acid attained the highest content in all plants with serine which considered as codominant amino acid. The content of glutamic acid and arginine were 0.414 and 0.094 mggm^{-1} freeze plant tissue respectively in the plants grown in the soil from the sand mounds of Abu Khashaba, but were not detected in the plants grown in the soil from Al Narreges coastal sand dune. Tryptophane, leucine, histidine and tyrosine were recorded only in the plants grown in the soil from sand mounds of Abu Khashaba. The contents of aspartic acid, serine and cysteine

were increased with increasing the radioactive element content of the plants. Proline, phenylalanine and hydrxyproline were detected only in the plants grown in the soil from Al Narreges coastal sand dune where uranium and thorium content of plants attained the highest values. Concentration of some amino acids decreased while some others increased after growing the plants in a control soil. Moreover, some amino acids such as cysteine and phenylalanine disappeared in the plants grown in the control soil.

Considering *R. pictus*, only four amino acids were recorded out of 19 amino acids analyzed in the plants grown in the soil collected from the sand mounds of Abu Khashaba. This number rose to seven in the plants grown in the soil of Al Narreges coastal sand dune. The content of aspartic acid and valine were 4.69 and 0.05 mggm^{-1} freeze plant tissue respectively in the plants grown in the soil of Abu Khashaba sand mounds and increased to 8.17 and 0.86 mggm^{-1} freeze plant tissue respectively in the plants of Al Narreges coastal sand dune. Thereonine and phenylalanine were detected in the plants grown in the soil of Abu

Plate 1. Photos from 1 to 3 represent chromosomal aberrations observed in root tip cells of Cakile maritima 1- C-metaphase 2- free chromosome at anaphase stage 3- polyploid cell at metaphase stage. Photos from 4 to 6 represent chromosomal aberrations observed in root tip cells of Senecio glaucus 4- Chromosomal breaks at metaphase 5- free chromosome at metaphase stage 6- (a, b, c) Vaculation in telophase. Photos from 7 to 9 represent chromosomal aberrations observed in root tip cells of Rumex pictus 7- non-congression at metaphase stage 8- chromosome bridge 9- fragmentation of chromosome material at prophase stage.



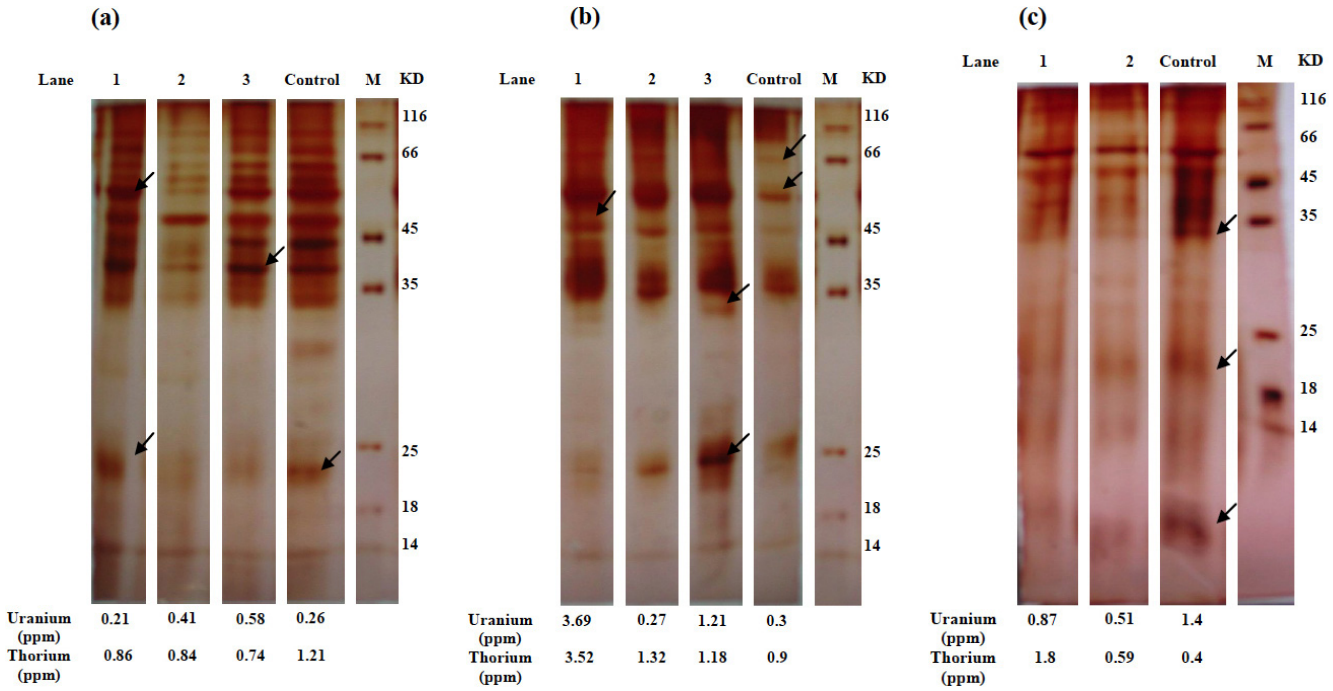


Fig. 3. Change in SDS-PAGE of proteins in the three study plant species grown in the black sand and control soil. (a) Cakile maritima, (b) Senecio glaucus and (c) Rumex pictus. Lane M: molecular weight standards (marker), lane 1: the plants grown in the soil from the coastal sand dunes of Al Narreges, lane 2: the plants grown in the soil from Abu Khashaba sand mounds, lane 3: the plants grown in the soil from Abu Khashaba coastal sand plain. The arrows refer to the protein bands which might be affected by the increased uranium and thorium content in the plant tissues.

Khashaba sand mounds and inhibited after growing the plants in Al Narreges soil. Free amino acid contents detected in the plants grown in the soil from their natural habitats decreased after growing the plants in the control soil where the plants accumulated more uranium. Glutamic acid and cysteine were recorded only in the plants grown in the control soil. Aspartic acid was the most detectable amino acid in *R. pictus* in black sand and control soil, while serine attained the highest values in the control and coastal sand dunes of Al Narreges.

Discussion

Cytological study

Mutagenic environmental effects may be analyzed by macroscopic parameters such as root shape and root growth and cytological parameters (Nielsen and Rank, 1994; Fiskesjo, 1997). The results of these parameters permit an estimation of the cytotoxicity, genotoxicity and mutagenicity of environmental pollutants that have a direct or indirect influence on living organisms. The results of the present study revealed that as the plants exposed to soil radioactivity the percentage of prophase and prophase to metaphase ratio decrease so they are negatively correlated with gamma-radiation of the soil in contrast to the percentage of anaphase and telophase which positively correlated with the radioactivity of the soil. This change in the stages of mitosis indicates that the increase of gamma-radiation affects the relative duration of each stage (Kabarity et al., 1974). The mitotic index is not affected by the increased gamma-radiation in case of *C. maritima* and *S. glaucus*. These results is in agreement with the results obtained by Geraskin et al. (2003) who mentioned that, the mitotic index didn't show correlation with the radiation dose.

The results of chromosomal abnormalities in the present study indicated that gamma-radiation induced high percentage of chromosomal aberrations throughout interphase and mitotic stages. This finding is supported by Kovalchuk et al. (1998) study on Allium cepa where a strong positive correlation between the aberration rates in the root tip cells and the radioactivity in the soil was noticed. In our study gamma-radiation in black sand soil induced additional types of chromosomal aberrations including the increase in the percentage of certain types of abnormalities (e.g. C-metaphase, disturbed chromosomes and metaphase non-congression) or decrease in the percentage of other types (C-anaphase, free and lag chromosome at prophase stage). It was observed that with increasing the level of radiation one or more type of aberrations appeared weren't found at low level of radiation (e.g.

Table 3. Changes in free amino acids (expressed as mggm-1 freeze dry plant tissue) with uranium and thorium content (ppm) of the study species grown in the black sand and control soil. 1 = Plants grown in the soil from the coastal sand dunes of Al Narreges, 2 = Plants grown in the soil from the sand mounds of Abu Khashaba, 3 = Plants grown in the soil from the coastal sand plain of Abu Khashaba.

Free amino acids (mg/gm plant)	Cakile maritima				Senecio glaucus				Rumex pictus		
	Control soil	Black sand soil			Control soil	Black sand soil			Control soil	Black sand soil	
		3	2	1		3	2	1		2	1
Aspartic acid	1.842	0.272	3.346	2.108	0.142	1.073	1.198	1.679	1.162	4.693	8.170
Serine	0.638	0.127	3.345	-	0.328	0.442	0.659	0.674	2.244	-	8.168
Glutamic acid	0.255	0.084	-	-	0.212	0.288	0.414	-	1.318	-	-
Glysine	0.208	-	-	0.244	0.274	0.268	-	0.279	-	-	-
Thereonine	0.051	0.017	0.082	0.243	0.395	-	0.085	0.100	-	0.125	-
Arginine	0.063	-	-	0.136	-	0.153	0.094	-	-	-	-
Proline	-	0.032	-	-	0.332	-	-	0.109	-	-	-
Methionine	0.095	0.016	-	0.136	0.280	0.133	-	0.106	0.122	-	0.073
Valine	-	-	-	0.066	0.052	-	0.075	0.011	-	0.050	0.086
Tryptophane	0.011	-	0.017	0.023	-	-	0.013	-	-	-	0.019
Leucine	0.070	-	0.098	-	-	-	1.376	-	-	-	0.092
Cysteine	-	0.005	-	0.003	-	0.003	0.054	0.210	0.015	-	-
Cystine	-	-	0.008	-	-	-	0.580	0.042	0.026	-	0.051
Phenylalanine	-	-	-	-	-	-	-	0.638	-	0.002	-
Alanine	0.001	-	0.005	-	-	-	0.006	0.003	-	-	-
Lysine	-	0.001	-	-	-	-	-	-	-	-	-
Histidine	-	-	-	0.032	0.001	0.037	0.002	-	-	-	-
Hydroxyproline	0.099	-	-	-	0.003	-	-	0.086	-	-	-
Tyrosine	-	-	-	-	0.001	-	0.051	-	-	-	-
total	**3.333**	**0.554**	**6.901**	**2.991**	**2.02**	**2.397**	**4.607**	**3.937**	**4.887**	**4.87**	**16.659**
Uranium (ppm)	0.26	0.58	0.41	0.21	0.30	1.20	0.27	3.69	1.40	0.51	0.87
Thorium (ppm)	1.21	0.74	0.84	0.86	0.91	1.18	1.32	3.52	0.40	0.59	1.80

multipolar anaphase, star anaphase and C. metaphase 4n). These results are in accordance with those obtained by Kovalchuk et al. (1998) where the proportion of cells with C-mitosis, multipolar anaphase, vagrant chromosomes and sticky chromosomes increased with increasing soil radioactivity.

As reported by Zaka et al. (2002), the chromosomal aberration types are radiation influenced especially the mitotic spindle function. Mitotic spindles are known to be sensitive to radiation, especially in the interphase (Ushida et al., 1975). The most common type of aberrations throughout the mitotic phases at different level of radiation was C-metaphase and C-anaphase and telophase. The C-metaphase was produced as a result of inhibition of spindle fibers formation (Deysson, 1968). The centromeres of the cells already present in C-metaphase divides, and the two chromatids are separated but it doesn't migrate to the poles due to the absence of spindle fibers that will result in C-anaphase (Amer and Farah, 1975). A high level of chromosomal stickiness takes place at the metaphase stage of *S. glaucus* and *R. pictus*. It has been suggested by Patil and Bahat (1992) that, stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material. Chromosomal breaks at metaphase and anaphase stages were recorded in root tip cells of the study species exposed to high level of gamma-radiation. The presence of breaks in both metaphase and anaphase indicates that chromosomal breakage occurs after DNA synthesis (Soliman, 1981).

Free and lag chromosomes during anaphase and telophase stages were observed in root tip cells of the study species especially which were collected from Al Narreges coastal sand dune. The occurrence of laggards at metaphase cells may result from hindrance of the chromosome movement and adhesion of the centromeres to the plasma membrane during prometaphase (Amer and Mikhael, 1987). The induction of laggards could be attributed to the failure of the spindle apparatus to organize and function in a normal way (Patil and Bahat, 1992). The most conspicuous effect of gamma-radiation on interphase stage is the formation of vacuolated nuclei due to a deficiency of DNA in the nucleus (Crockers, 1953). The result of the present study are in agreement with the finding of

Shevchenko and Grinikh (1990) who reported that a high percentage of chromosome aberrations was found in the meristems of wheat and rye plants grown in the 30 km Chernobyl zone, and Kordium and Sidorenko (1997) where increase of chromosome aberrations were correlating with the increase of gamma-radiation level for several wild grass species grown within the exclusion zone around the Chernobyl NPP.

Electrophoratic banding pattern

The study of electrophoratic banding pattern of proteins indicated the change of SDS-PAGE of proteins with the increase of radionuclides content of the plants. The main characteristic changes included alterations in the band relative mobility, the band intensities, and disappearance of some bands and appearance of new bands indicating that protein expression is highly modified in the three study species. High concentrations of radionuclides in the plant tissues caused expression of new proteins, while suppression of some proteins occurred at law concentration of radionuclides with general decrease of the total protein content.

Radionuclides absorbed by the study species emits an internal radiation as reported by Nayar et al. (1970) who pointed out that the radiations from the absorbed radionuclides are much more important than the external radiations in the production of the biological changes. These internal radiations may be the reasons of the changes observed in the protein banding pattern of the study species with the increased radionuclide content of plants as explained by Zhenxing et al. (2007) who reported that, concentration of protein extracts was decreased significantly after radiation exposure, meanwhile new protein bands appeared. Radiation can lead to the formation of new proteins by the bonding of free amino acids to protein and protein molecules to protein aggregation.

A protein banding pattern of an organism represents a biochemical genetic finger print to that organism (Abdelsalam et al., 1996). Changes in the protein banding pattern in the present study may be due to two mutational types i.e. gene mutation and cytological aberrations (Abdelsalam et al., 1993). The correlation between the changes in the protein pattern and the recorded cytological anomalies produced by soil radionuclides can be summarized as changes in bands intensity. This is probably attributed to alteration in the structural genes performance (Hassan, 1995). This may lead to constitutive protein production or to attenuation or complete suppression of the concerned genes and well produced intensive bands, faint bands or complete band disappearance. The increase in band intensity may be interpreted on the basis of gene duplication. It was concluded by Abdelsalam et al. (1993) that the increase in band intensity was due to gene duplication resulted from bridges, breaks and laggards induced after treating Vicia faba with some pesticides. Induction of laggards, breaks, bridges and micronuclei may lead to loss of some of the genetic material. Therefore, band disappearance may be explained on the basis of loss of the genetic material due to the chromosomal aberrations which in turn lead to loss of genes encoding for that band. The protein banding pattern of *C. maritima* revealed that the most pronounced changes due to high concentration of uranium and thorium were in the band regions spanning the molecular weight range 160-65 K.Da, this may reflect the high sensitivity of the genes encoding protein bands of that region to the mutagenic effects of absorbed radionuclides (Abdelsalam et al., 1993; Hassan, 1995).

Free amino acid profile

The results showed that aspartic acid and serine are the most abundant amino acids in the three study species and their contents increased with the increase of plant radionuclides content. The biosynthetic path way of amino acids in *C. maritima* and *S. glaucus* decreased as the plants accumulated more uranium and thorium, meanwhile inhibition of synthesis of some amino acids and stimulation of new amino acids have occurred. The synthesis of proline at high radionuclides content of *C. maritima* and *S. glaucus* and the presence of thereonine, methionine and cysteine at high concentrations of radioactive elements was obvious in the three study species.

A number of plant taxa have been shown to synthesize metal-binding complexes when grown in the presence of cadmium or copper, and when these complexes are purified further, they contained the amino acids, predominantly cysteine, aspartic acid, glutamic acid, glycine, serine, thereonine, alanine and lysine (Tomsett and Thurman, 1988). It might be the same mechanisms by which the plants growing in the black sand habitats can tolerate the increased level of absorbed radionuclides and that explain the increased level of aspartic acid, serine, cysteine and thereonine in the plants having high radionuclide content. The variations which were observed in the free amino acid profile can be discussed on the basis of internal radiations emitted by the absorbed radionuclides. Gamma radiation most probably altered the genetic code for amino acid and protein synthesis which led to the disappearance of some amino acids and the appearance of others (Awny et al., 1988). Accumulation of free proline in response to heavy metal exposure seems

to be wide-spread among plants (Schat et al., 1997). Exposure to heavy metals is known to deteriorate the plant water balance. In addition, proline could be involved in metal chelation in the cytoplasm. This may be an explanation for the biosynthesis of proline at high concentration of radionuclides.

Conclusions

The present study proved that the naturally growing plant populations in the black sand habitats are exposed to gamma-radiation by radionuclides which can result in cytotoxicity and genotoxicity of these plants according to the level of radiation, moreover the results revealed that high radionuclide content of plants causes change of SDS-PAGE of proteins indicated by the alterations in the bands relative mobility and intensities, expression of new proteins and suppression of some proteins. Study of amino acid profile of plants indicated that radioactive elements stimulate the biosynthesis of some amino acids e.g. proline, cysteine, serine and thereonine while inhibit some other amino acids such as arginine. Aspartic acid and serine are the most abundant amino acids in the three study species and their contents increased with the increase of radionuclides content.

Acknowledgements

We thank Prof. A. Galmad, Geology Department, Faculty of Science, Cairo University and Prof. Sofia Afifi, Nuclear Materials Authority, Cairo for their kind help and offering facilities in soil and plant radionuclides analysis. We also acknowledge Center of Excellence for Research in Biodiversity, King Saud University for offering facilities to support this publication.

Molecular weight (K.Da.)	% amount										
	Cakile maritima				Senecio glaucus				Rumex pictus		
	Control soil	Black sand soil			Control soil	Black sand soil			Control soil	Black sand soil	
		3	2	1		3	2	1		2	1
160	-	-	0.13	-	-	-	-	-	-	-	-
157	-	-	-	-	13.07	21.1	-	-	-	-	-
155	3.23	9.43	-	-	-	-	4.97	16.3	-	-	-
153	-	-	10.1	-	-	-	-	-	-	-	-
151	-	-	-	7.32	-	-	-	-	-	-	-
145	-	-	-	-	-	-	-	-	3.04	3.55	4.26
133	-	-	-	-	-	-	-	-	-	-	-
116	-	-	-	-	-	-	12.9	-	-	-	-
111	-	-	-	-	-	-	-	-	-	7.55	-
109	-	-	-	-	-	-	-	-	6.25	-	-
108	-	-	-	-	4.54	-	-	-	-	-	-
106	-	-	-	3.79	-	-	-	-	-	-	-
105	2.82	4.19	-	-	-	-	-	-	-	-	-
104	-	-	1.96	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-	-	-	4.8
97	1.22	-	1.03	-	-	-	-	-	-	-	-
90	-	1.58	-	-	-	-	-	-	-	-	-
83	-	-	-	4.77	-	-	-	-	-	-	-
80	-	3.07	-	-	-	-	-	-	-	-	-
79	4.87	-	3.52	-	-	-	-	-	-	-	-
77	-	-	-	-	6.66	-	-	-	-	-	-
70	-	-	-	-	-	-	7.28	-	-	-	-
68	-	-	-	-	-	-	-	-	-	-	2.84
65	-	-	-	3.67	-	-	-	-	-	-	-
64	4.48	4.23	3.27	-	-	-	-	-	-	-	-
61	-	4.46	3.66	-	2.00	-	-	-	-	-	-
59	-	-	-	-	-	-	-	-	-	-	4.5
58	-	-	4.50	-	7.87	-	-	-	-	-	-
57	-	6.72	-	9.3	-	13.4	-	-	-	-	-
56	10.93	-	-	-	-	-	15.1	22.8	-	5.90	-
55	-	-	-	-	-	-	-	-	10.5	-	-
54	-	-	-	-	-	-	-	-	-	-	6.6

Molecular weight (K.Da.)	% amount										
	Cakile maritima				Senecio glaucus				Rumex pictus		
	Control soil	Black sand soil			Control soil	Black sand soil			Control soil	Black sand soil	
		3	2	1		3	2	1		2	1
51	-	-	6.66	7.54	-	-	-	-	-	-	-
50	-	7.53	-	-	-	-	-	-	-	-	-
49	7.23	-	-	-	4.25	4.72	-	5.30	-	-	-
48	-	-	-	-	-	-	5.35	-	6.91	9.19	-
47	-	-	-	-	-	-	-	-	-	-	8.39
45	-	-	-	6.28	-	-	-	-	-	-	-
44	8.10	5.78	-	-	-	-	3.08	-	-	-	-
42	-	-	7.32	-	-	-	-	-	-	-	-
40	-	-	4.34	19.8	-	-	-	-	20.68	11.09	-
39	8.70	18.3	-	-	-	-	-	-	-	-	-
38	-	-	-	-	-	-	-	-	-	-	19.5
37	-	-	-	-	-	-	-	24.1	-	-	-
36	-	-	-	-	25.72	18.1	-	-	-	-	-
35	-	-	-	-	-	-	18.1	-	-	-	-
34	-	-	10.9	-	-	-	-	-	-	-	-
32	8.66	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	3.81	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	5.45	-
29	-	3.30	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	1.78	2.63	-	-	-
26	-	-	-	-	-	2.16	-	-	-	-	-
25	-	-	-	3.10	-	-	-	1.96	-	-	-
24	8.10	-	-	-	6.76	-	7.78	-	-	1.11	-
23	-	6.79	8.36	5.21	-	-	-	-	-	4.62	-
22	-	-	-	3.53	0.77	-	-	-	4.22	-	2.00
21	3.42	-	2.47	-	-	-	-	-	-	-	-
20	-	-	3.35	-	-	-	-	9.24	-	-	-
18	-	-	-	-	13.48	28.14	-	-	-	-	-
17	14.19	15.4	15.0	19.7	7.63	-	19.5	6.00	-	-	-
16	-	-	-	-	-	-	-	6.95	22.31	26.03	16.36
14	4.80	-	-	1.92	-	5.57	-	-			
12	4.20	-	13.5	4.02	7.25	3.04	4.18	-	8.97	8.60	19.81
11	-	9.15	-	-	-	-	-	4.73	-	-	-
10	-	-	-	-	-	-	-	-	6.24	-	-
9	-	-	-	-	-	-	-	-	-	3.42	-
7	-	-	-	-	-	-	-	-	-	1.01	-
6	-	-	-	-	-	-	-	-	-	-	8.75
5	-	-	-	-	-	-	-	-	10.86	-	-
4	-	-	-	-	-	-	-	-	-	7.57	-
Uranium (ppm)	0.26	0.58	0.41	0.21	0.30	1.20	0.27	3.69	1.40	0.51	0.87
Thorium (ppm)	1.21	0.74	0.84	0.86	0.91	1.18	1.32	3.52	0.40	0.59	1.80

Appendix Table 1. Electrophoratic banding patterns of protein in Cakile maritima, Senecio glaucus and Rumex pictus grown in the black sand and control soil. 1 = Plants grown in the soil from the coastal sand dunes of Al Narreges, 2 = Plants grown in the soil from sand mounds of Abu Khashaba and 3 = Plants grown in the soil from coastal sand plain of Abu Khashaba. The measured values of uranium and thorium in plant shoot are shown.

Reference

Abdelsalam, AZE, Hassan, HZ, Fatma, MI, Waffaa, MA-N. 1993. The mutagenic potentialities of three pesticides on three biological systems. *Egyptian Journal of Genetic and Cytology* 22: 109-128.

Abdelsalam, AZE, Hassan, HZ, Soliman, KhA. 1996. The mutagenicity of two aromatic systemic pesticides using three biological system. *Egyptian Journal of Genetic and Cytology* 23: 185-195.

Amer, SM, Farah, OR. 1975. Cytological effects of pesticides. XII. Mitotic effects of isopropyl-N-phenyl carbamate and Duphar. *Cytologia* 40(1): 21-30.

Amer, SM, Mikhael, E. 1987. Effect of rotenone on the meiosis of *Vicia faba*. *Annals of Agricultural Science, Faculty of Science, Ain Shams University*, Cairo Egypt 32(1): 469-478.

Awny, NM, Salama, AM, El-Zawahry, YA, Abo El-Khair, IA. 1988. Viability and amino acid picture of *Paecilomyces violacea* as affected by gamma radiation. *Egyptian Journal of Radiation Science and Application* 5: 179-191.

Barakat, HM, Hassan, HZ. 1997. Mutagenic effects of pendimethalin herbicide on *Vicia faba* and *Allium cepa* plants. *Egyptian Journal of Botany* 37(1): 13-29.

Bellés, M, Albina, ML, Linares, V, Gómez, M, Sanchez, DJ, Domingo, JL. 2005. Combined action of uranium and stress in the rat. *Toxicology Letters* 158: 176-185.

Crockers, BH. 1953. Effects of 2, 4 dichloro-phenoxy acetic acids on mitosis in *Allium cepa*. *Botanical Gazette* 144: 274.

Deysson, G. 1968. Antimitotic substances. *International Review of Cytology* 24: 99.

Domingo, JL. 2001. Reproductive and developmental toxicity of natural and depleted uranium: A Review. *Reproductive Toxicology* 15: 603-609.

El-Hadry, AFA. 1998. *Geological, Sedimentological and radiometric studies in black sand deposits, west Rosetta beach which emphasis a heavy economic minerals*. Ph.D. thesis, Cairo University.

Fiskesjo, G. 1997. *Allium* test for screening chemicals; evaluation of cytological parameters, in : W. Wang, J.W. Gorsuch, J.S. Hughes, (Eds.), *Plants for Environmental Studies*, CRC, Lewis publishers, New York, 307-333.

Fukui, K, Nakayama, S. 1996. *Plant Chromosomes: Laboratory Methods*. CRC Press–Boka Roton, New York, London, Tokyo.

Geraskin, SA, Dikarev, VG, Zyablitskaya, YeYa, Oudalova, AA, Spirin, YeV, Alexakhin, R.M. 2003. Genetic consequences of radioactive contamination by the Chernobyl fallout to agricultural crops. *Journal of Environmental Radioactivity* 66: 155-169.

Gulati, KL, Oswal, MC, Nagpaul, KK. 1980. Assimilation of uranium by wheat and tomato plants. *Plant and Soil* 55: 55-59.

Gulati, A, Prakash, S, Gupta, SP. 1994. Genotoxicity testing for relative efficiency of selected pesticides on *Allium cepa*. *Journal of Environmental Biology* 15: 89-95.

Hakonson-Hayes, AC, Fresquez, PR, Whicker, FW. 2002. Assessing potential risks from exposure to natural uranium in well water. *Environmental Radioactivity* 59: 29-40.

Hartmann, HM, Monette, FA, Avci, HI. 2000. Overview of toxicity data and risk assessment methods for evaluating the chemical effects of depleted uranium compounds. *Human Ecology and Risk Assessment* 6: 851-874.

Hassan, HZ. 1995. Evaluation of mutagenic effects of two insecticides basudin and decis on *Vicia faba* plants. *Egyptian Journal of Genetic and Cytology* 25: 27-38.

Hegazy, A. K. and Emam, M. H. (2011). Accumulation and soil-to-plant transfer of radionuclides in the Nile Delta coastal black sand habitats. International Journal of Phytoremediation, 13: 140-155.

Kabarity, A, El-Bayoumi, AS, Habid, A. 1974. Effect of morphine sulphate on mitosis of *Allium cepa* L. root tips. *Biology of plant* 16(4): 275-282.

Kovalchuk, O, Kovalchuk, I, Arkhipov, A, Telyuk, P, Hohn, B, Kovalchuk, L. 1998. The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in Ukraine contaminated by the Chernobyl accident. *Mutation Research* 415: 47-57.

Kordium, EL, Sidorenko, PG. 1997. The results of the cytogenetic monitoring of the species of angiosperm plants growing in the area of the radionuclide contamination after the accident at the Chernobyl Atomic Electric power station. *Tsitologiiai Genetika* 31: 39-46.

Laemmili, UK. 1970. Cleavage of structural proteins during the assembly of the head of Bactriophage T4. *Nature* 227: 680-685.

Malallah, G, Afial, M, Kurian, M, Gulshan, S, Dhami, MSI. 1998. Impact of oil pollution on some desert plants. *Environment International* 24 (8): 919-924.

Meyer, MC, McLendon, T. 1997. Phytotoxicity of depleted uranium on three grasses characteristics of different successional stages. *Journal of Environmental Quality* 26: 748-752.

Murthy, TCS, Weinberger, P, Measure, MP. 1984. Uranium effects on the growth of soybean (*Glycine max* (L.) Merr.). *Bulletin of Environmental Contamination Toxicology* 32: 580-586.

Nayar, GG, George, KP, Gopal-Ayengar, AR. 1970. On the biological effects of high background radioactivity: studies on *Tradescantia* grown in radioactive monazite sand. *Radiation Botany* 10: 287-292.

Nelson, T, Harps, MH, Mayfield, SP, Taylor, WC. 1984. Light regulated gene-expression during maize leaf development. *Journal of Cell Biology* 98: 558-564.

Nielsen, MN, Rank, J. 1994. Screening of toxicity and genotoxicity in wastewater by the use of *Allium* test. *Hereditas* 121: 249-254.

Patil, BC, Bahat, GL. 1992. A comparative study of MH and EMS in the induction of chromosomal aberration on lateral root meristem in *Clitoria ternatea* L. *Cytologia* 57: 295.

Sammons, DW, Adams, LD, Nishizawa, EE. 1981. Ultrasensitive silver based color staining of polypeptides in polyacrylamide gels. *Electrophoresis* 2: 135.

Schat, H, Sharma, SS, Vooijs, R. 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. *Physiologia Plantarum* 101: 477-482.

Seddeek, MK, Badran, HM, Sharshar, T, Elnimr, T. 2005. Characteristics, spatial distribution and vertical profile of gamma-ray emitting radionuclides in the coastal environment of North Sinai. *Journal of Environmental Radioactivity* 84: 21-50.

Shad MA, Ansari, H, Pervez, MR, Mahmood, T. 2002. Changes in sugar, amino acid and mineral content of leaves of two mango varieties affected by quick decline disease. *Journal of Biological Sciences* 2 (10): 694-696.

Sheppard, SC, Sheppard, MI, Gallerand, M-O, Sanipelli, B. 2005. Derivation of ecotoxicity thresholds for uranium. *Journal of Environmental Radioactivity* 79: 55-83.

Shevchenko, VV, Grinikh, LI. 1990. Cytogenetic effects in native populations of *Crepis tectorum* exposed to chronic irradiation in the vicinity of the Chernobyl Nuclear Power Station. Induction of chromosome aberrations during the first 2 years following the accident. *Radiologiia* 30: 728-734.

Soliman, M. 1981. *Effect of some pesticides on the sequence of cell division in plants*. M.Sc. thesis, Ain Shams University.

Taskin, H, Karavus, M, Ay, P, Topuzoglu, A, Hidiroglu, S, Karahan, G. 2009. Radionuclide concentrations in soil and lifetime cancer risk due to gamma radioactivity in Kirklareli, Turkey. *Journal of Environmental Radioactivity* 100: 49–53

Tkalec, M, Malarić, K, Pavlica, M, Pevalek-Kozlina, B, Vidakovic-Cifrek, Ž. 2009. Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of *Allium cepa* L. *Mutation Research* 672: 76–81

Tomsett, AB, Thurman, DA. 1988. Molecular biology of metal tolerances of plants. *Plant, Cell and Environment* 11: 383-394.

Ushida, I, Viola Lee, C, Byrnes, E. 1975. Chromosome aberrations induced in vitro by low doses of radiation: nondisjunction in lymphocytes of young adults. *American Journal of Humane Genetics* 27: 419–429.

Zaka, R, Chenal, C, Misset, MT. 2002. Study of external low irradiation dose effects on induction of chromosome aberrations in *Pisum sativum* root tip meristem. *Mutation Research* 517: 87-99.

Zhenxing, L, Hong, L, Limin, C, Jamil, K. 2007. The influence of gamma radiation on the allergenicity of shrimp (*Penaeus vannamei*). *Journal of Food Engineering* 79: 945-949.

Zenk, MH. 1996. Heavy metal detoxification in higher plants: A Review. *Gene* 179: 21-30.