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Effect of Residual and Accumulative Sewage Sludge on Heavy Metals Bioaccumulation: Gene Action and Some Yield Parameters of *Vicia faba*

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Abstract: Field experiments were conducted to evaluate the effect of using sewage sludge as organic fertilizer on different cytological and yield parameters of *Vicia faba*. Sewage sludge treatment rates were 0, 10, 20, 30 and 40 T/F and applied to the soil in three successive additions during 1999-2001. Different rates of residual and repeated application of sewage sludge increased heavy metals concentrations in the soil before sowing and after harvesting of *Vicia faba*. Sludge treatments did not affect some yield parameters, but mature plant height and number of tillers/plant were increased or decreased at different treatments of sludge. The seed index and fresh and dry weights of shoots and roots were increased. The number of seeds/pot recorded the highest value by 10 T/F of the one residual addition, while the lowest value was obtained at 20 T/F two additions. Also, sludge treatments increased mature plant height, seed index, seed density, the number of nodules/plant and fresh and dry weights of nodules except at the highest treatments of two and three additions. Generally, it decreased nodules efficiency percentage. The root tolerance index was increased by sludge treatments. The sludge treatments increased the mean mitotic index and those of three cumulative additions were higher than that of one residual addition treatment. Sludge treatments included a number of abnormalities in all mitotic phases and non dividing cells. The percentage of abnormal cells was increased by three cumulative additions treatments than the one residual addition.

Key words: Sewage sludge, heavy metals, chlorophyll, nodules, seed characters, chromosomes, *Vicia faba*

INTRODUCTION

Wastewater sludge produced by sewage treatment plants are represented as a good source of macro- and microelements and generally contain a high quality of organic matter (Bierman and Rosen, 1994; Logan and Harrison, 1995). Their application in agriculture translates into improved soil fertility. Sludge also maintains soil structure, soil water holding ability, soil cation exchange capacity and soil biology activity (Lindsay and Logan, 1998). Although, the recycling of sludge is an attractive alternative, its use often brings about certain risks to the environment caused by accumulation of heavy metals and organic compounds and potential contamination from the pathogenic organisms (Alloway and Steinnes, 1999).

Heavy metals toxicity may affect growth, morphology and biochemical activities of microorganisms (Ibekwe *et al*., 1996). Variations in effective *Rhizobium* population size were apparent in relation to sludge application rate and type, heavy metal concentrations and soil pH (Obbard, 2001).

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Repeated applications of heavy metal-contaminated sewage sludge can result in an accumulation in toxic metals in the soil which causes injuries to soil microorganisms (McGrath *et al*., 2000).

Mench *et al*. (1993) reported that the highly metal-polluted sludge reduced the yield of leaves of maize. However, Khalil *et al*. (2000) and Mater (2000) found that the addition of different organic biosolid increased significantly the dry matter yield of plants.

Plants have been the material of choice to study the cytotoxic and mutagenic effects of metals and can provide a good system for studies related to environmental monitoring (Fiskesjo, 1988). Because of the similarity of effects and target materials in all organisms, the effects of metals on plants can be usefully applied to other systems as well (Minissi *et al*., 1998; Rank and Nielsen, 1998; Zhang and Xiao, 1998; Amin, 2001; Steinkellner *et al*., 2002; Ivanova *et al*., 2005), found that the frequencies of chromosomal aberrations increased significantly in plants exposed to different concentrations of heavy metals.

In order to increase accuracy and reduce environmental risks, an evaluation of the bioavailability of metals to the plants and the heavy metal content in soil solution and ground water is necessary. Therefore, assessment of the degree of solubility in water is important. The goal of this study is to compare the efficiency of different rates of sludge as residual or cumulative treatments on the chemical nature of soil, the genome of *Vicia faba* (as a short plant bioassay to be excellent for mutagenicity studies) and some of its yield parameters (e.g., plant height, pod length, seeds pod^{-1}, seed index, seed density, nodules number and activity and fresh and dry weight).

Plant bioassay have many advantages among them are being less expensive, similar in chromosome morphology and response to mutagens to mammals and suitability to in situ experiments.

MATERIALS AND METHODS

Physical and Chemical Properties of Sludge

This investigation was carried out at the field station of the Faculty of Agriculture, Alexandria University at Abis area during the period (1999-2001). Composted sewage sludge was collected from Alexandria General Organization of Sanitary Drainage (AGOSD) of Alexandria city in February, 1999. The chemical composition of sludge and physical and chemical characteristics of the soil were determined according to Page *et al*. (1982) and are presented in Amin and Sherif (2001).

On March 1999 (before the experimental study started), sewage sludge was amended to the soil at the rates of 0, 10, 20, 30 and 40 T/F and was planted with *Zea mays*. After harvesting, the plot was divided into two subplots; one received a second sludge addition at the previous rates while the other was left without further addition to compare the effect of the residual and accumulation treatments. *Vicia faba* Giza 461 was sown in the two subplots (following *Zea mays*) on November, 1999. On July, 2000 another study using *Zea mays* was grown following *Vicia faba* Giza 461 on the same soil without any additions. In the following year (2000-2001) a third experiment was conducted by using *Vicia faba* on the same soil but the subplot which have the second sludge addition received a third sludge addition at the same rate.

Soils Sampling and Analysis

Surface soil samples were collected from each plot, before cultivation and after harvesting at soil depth 20 cm. The collected samples were air-dried, ground, passed through 2 mm sieve and stored in polyethylene bags for analyses. Heavy metals analysis of AB-DTPA (Ammonium Bicarbonate-diethylene triamine-pentacetic acid) extracts were made for heavy metals analysis using Atomic Absorption Spectrophotometer (Parken Elmer model 3300).

Plant Analysis

Thirty, 45 and 75 days of sowing and mature plant samples of *Vicia faba* were collected . Also, mature seeds from each plot were sampled, washed with distilled water, oven dried at 70°C, then stored in paper bags for analysis. Leaves and mature seeds from each treatment were homogenized and wet digested using concentrated sulfuric acid and hydrogen peroxide (FAO, 1980) and analyzed for extractable heavy metals.

Chlorophyll Estimation

Seventh leaf stage samples were collected, washed with tap water, then with distilled water and dried between paper towels. Known weight (about 0.1 g fresh weight) of leaves were immersed in 10 mL N, N-dimethyl formamide (DMF) and kept overnight at 4°C. After incubation, Chlorophyll a and b was determined in the extract according to Inskeep and Bloom (1985). Absorption spectra of the extracts were measured at 645 and 647 nm using spectrophotometer (uk) using the following formula:

$$\text{Chlorophyll a} = 10.4\ E_{645}\text{-}E_{647}$$
$$\text{Chlorophyll b} = 19.6\ E_{647}\text{-}E_{645}$$

Morphological Measurements

After 4 weeks of planting, seedling height and number of tillers per plant were determined. At maturity ten characters were recorded: plant height, seed index (100 seeds weight), number of seeds pod^{-1}, number of sterile and fertile seeds, seeds pod^{-1}, seed color, hilum color and nodules number and activity. The nodule activity (efficiency) was measured according to the density of red colored of the nodules; dark red is highly efficiency, light red is low and colorless in not active. Seed density was calculated by dividing the weight of 10 seeds by its volume (Kharkwal and Chaudhary, 1997).

The rate of root growth day^{-1} was determined by sowing five replica each of fifteen homogenous seeds in the different treated soil samples in pots. Root tolerance index was calculated after 2 and 3 days of planting (root growth increase expressed as a percentage of the control, Wierzbicka, 1999).

Cytological Studies

Homogenous seeds of *Vicia faba* were germinated in sewage sludge treatments after soaking and incubation. Three replica root meristems were stained with the conventional Feulgen technique (Sharma and DPhil, 1980); squashed and permanent slides were examined to determine the mitotic index and mitotic distribution, in addition to the rate and types of abnormal dividing (ADCs) and non dividing cells (ANDCs).

Statistical Analysis

Statistical analysis for data were evaluated by applying the analysis of variance and least significant differences test, using COSTAT program.

RESULTS

Effect of Sludge Treatments on Heavy Metals Content in Soil

Using one residual addition, soil heavy metals increased significantly with increasing sludge application rates before cultivation (21 months from 1st addition) (Table 1). After harvesting (25 months from 1st addition), the heavy metals content decreased but still higher than the control. In the meantime, cumulative effect of three additions showed that extractable Zn, Cd and Pb increased significantly before cultivation (20 months from 1st addition+12 months from 2nd addition+8 days from 3rd addition) and after harvesting (25 months from 1st addition +17 months from 2nd addition +5 months from 3rd addition).

Table 1: Heavy metal concentrations in the sludge amended soil before *Vicia faba* sowing and after harvesting using one addition and three cumulative additions

	Treatments (T/F)				
Variables	Control	10	20	30	40
Before sowing (mg kg^{-1})					
One residual addition					
Cu	10.17a	10.80a	11.62a	10.80a	11.88a
Zn	3.09b	2.71b	3.59b	4.70ab	5.81a
Cd	0.16b	0.00c	0.00c	0.36a	0.18b
Pb	4.63a	4.33a	4.94a	4.58a	5.92a
Three cumulative additions					
Cu	10.17a	11.55a	11.06a	11.23a	12.07a
Zn	3.09c	5.02 b	6.78b	6.17b	11.66a
Cd	0.16c	0.32 c	0.84b	0.54c	1.14a
Pb	4.63c	7.31 b	7.24b	6.99b	10.81a
After harvest (mg kg^{-1})					
One residual addition					
Cu	9.70a	9.21a	10.01a	10.11a	10.23a
Zn	2.47b	3.41b	3.83a	5.25a	5.49a
Cd	0.10c	0.30b	0.30b	0.00c	0.48a
Pb	3.98a	5.22a	5.47a	5.62a	5.44a
Three cumulative additions					
Cu	9.70a	7.11b	11.19a	11.13a	10.38a
Zn	2.47b	2.74b	5.25a	6.59a	5.08a
Cd	0.10b	0.00c	0.70a	0.74a	0.18b
Pb	3.98b	3.46b	7.15a	6.98a	5.86a

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test

Table 2: Heavy metals concentrations in leaves and seeds of *Vicia faba* plants using one addition and three cumulative additions

	Treatments (T/F)				
Variables	Control	10	20	30	40
Leaves (mg kg^{-1})					
One residual addition					
Cu	12.31a	11.88a	9.55b	10.98a	7.93b
Zn	41.78b	38.48c	51.07a	43.54b	41.67b
Cd	nd	nd	nd	nd	nd
Pb	1.00b	1.00b	1.00b	9.00a	1.00b
Three cumulative additions					
Cu	12.31b	15.41a	11.70b	9.49c	12.87b
Zn	41.78c	47.53b	41.87c	47.98b	63.16a
Cd	nd	nd	nd	nd	nd
Pb	1.00c	6.00a	2.00c	4.00b	2.00c
Seeds (mg kg^{-1})					
One residual addition					
Cu	12.31b	15.41a	11.70b	9.49c	12.87b
Cu	10.79a	10.01a	9.04a	-	-
Zn	34.00b	31.39b	39.39a	-	-
Cd	nd	nd	nd	-	-
Pb	nd	nd	nd	-	-
Three cumulative additions					
Cu	10.79a	0.00c	11.05a	8.78a	6.50b
Zn	34.64a	25.53b	34.56a	32.04a	33.43a
Cd	nd	nd	nd	nd	nd
Pb	0.00d	2.20b	1.00c	3.00a	0.00d

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test, -: Missed data, nd: Not detected

Effect of Sludge Treatments on Heavy Metals Content in Plant

One residual addition significantly reduced the concentration of Cu in leaves in all sludge treatments (Table 2). However, the three cumulative additions reduced Cu content in seeds.

Table 3: Effect of different rates of sludge treatments on yield components of *Vicia faba*

Treat-ments (T/F)	30 days plant height (cm)	Mature plant height (cm)	Tillers $plant^{-1}$ (No.)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	Pod length (cm)	Seeds pod^{-1} (No.)	Seed index (g)	Seed density ($g\ cm^{-1}$)
Control	6.46a	136.63ab	8.75abc	157.3h	80.00e	20.0g	7.8c	6.32a	2.92b	75.00 bc	1.08a
One residual addition											
10	14.8a	141.75ab	9.00ab	510.0b	145.0bc	23.3f	6.4e	6.56a	4.02a	83.07ab	1.15a
20	13.8a	136.00ab	7.75bc	310.0e	155.0ab	30.3b	7.8c	6.95a	2.74b	77.07abc	1.10a
30	14.8a	138.50ab	8.75abc	210.0g	100.0d	25.3d	6.5e	6.18a	2.71b	83.30 ab	1.12a
40	15.2a	130.50b	5.75c	475.0c	135.0c	24.4e	8.6b	6.20a	2.65b	80.00abc	1.08a
Two cumulative additions											
10	15.8a	142.50ab	8.50abc	220.0g	80.0e	28.90c	26.0a	7.14a	2.8b	84.60a	1.1a
20	13.5a	147.25a	11.00a	700.0a	165.0a	36.30a	26.0a	6.09a	1.6c	82.33ab	1.0a
30	15.8a	133.75b	7.25bc	250.0f	145.0bc	23.20f	6.8d	6.09a	2.5b	73.20c	1.0a
40	15.6a	134.75ab	8.25abc	325.0d	95.0d	24.70de	6.2e	6.09 a	2.6b	77.13abc	1.1a

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test

Table 4: M_2 seed characters of *Vicia faba* grown on soil amended with sludge

Treatments (T/F)	Seed pod (No.)	Total seeds (No.)	Sterility (%)	White hilum (%)	Coat color (%) Mottled Black	Mottled Brown	Mottled Red	Green	Red	Σ Abnormal seeds (%)
Control	2.92	343.0	10.23	0.39	4.14	4.30	0.84	-	-	10.58
One residual addition										
10	2.75	539.0	5.94	0.56	3.34	0.74	-	1.11	-	5.75
20	2.74	312.0	9.94	-	2.88	14.74	-			24.68
30	2.35	342.0	5.85	-	-	0.88	-	1.46	-	2.34
40	2.68	175.0	9.71	-	1.14	4.00	-	-	-	5.14
Two cumulative additions										
10	3.12	431.00	17.87	-	6.26	0.93	-	-	-	7.20
20	2.52	201.00	22.39	1.49	16.42	35.82	2.99	-	-	56.72
30	2.53	197.00	11.68	-	3.05	2.54	-	-	-	10.66
40	2.68	206.00	16.02	0.97	6.80	10.68	0.49	3.40	1.0	23.30

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test

Effect of Sludge Addition on Plant Yield and Nodule Formation

One Residual Addition and Two Cumulative Additions Effect

Some sludge residual treatments increased the mature plant height. In the meantime, the number of tillers per plant for the two additions treatments was significantly higher than that of the residual treatment (mean treatments were 8.75 and 7.81, respectively). The number of seeds per pod recorded the highest value by 10 T/F of the one residual addition, while the lowest value recorded by 20 T/F of the two cumulative additions. On the contrary of that, seed index increased significantly. Generally, increasing of sludge rates increased significantly the fresh and dry weights of shoot and root (Table 3).

One residual addition treatments showed low sterility percentage compared to the two cumulative additions (Table 4). Seeds with white hilum were recorded in three treatments only. M_2 Seed coat color was found to be uniform green or red and blotched black, beige or red instead of brown after sludge treatments (Table 4). Mottled, green and red colors were also found in 17 and 52% of seeds after residual and cumulative treatments, respectively.

The number of mature nodules $plant^{-1}$ exhibited significant increase in all residual treatments except at 10T/F (Table 5). Nevertheless, the lowest treatment of two cumulative additions recorded the highest significant increase. In the same time, while the fresh and dry weights of nodules $plant^{-1}$ increased significantly by all sludge treatments, the levels of nodules efficiency percentage decreased (Table 5).

Table 5: Effect of different rates of sludge treatments on nodules of *Vicia faba*

	Nodules/Plant				
		Efficiency (%)		Weight (g)	
Treatments (T/F)	No.	High (++)	Low (+)	FW	DW
Control	86.00d	31.00abc	30.00ab	1.20d	0.40abc
One residual addition					
10	78.00e	32.00ab	19.00c	1.80bc	0.55ab
20	99.00c	23.00cd	33.00ab	1.40cd	0.49abc
30	123.00a	30.10abc	19.50c	2.10ab	0.67a
40	100.00c	33.00a	26.00bc	2.60a	0.60a
Three cumulative additions					
10	110.00b	36.60a	31.40ab	2.50a	0.66a
20	50.00g	18.00de	30.30ab	0.80d	0.34bc
30	58.00f	24.13bcd	37.93a	0.80d	0.25c
40	55.00fg	14.50e	30.90ab	1.30cd	0.29c

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test

Table 6: Effect of sludge treatments on mature plant productivity of *Vicia faba*

Treatments (T/F)	Plant height (cm)	Pod length (cm)	Seeds pod^{-1} (No.)	Seed index (g)	Seed density (g cm^{-3})
Control	60.68e	7.14a	2.53a	83.00b	1.15b
One residual addition					
10	64.10cd	8.70a	3.00a	100.00a	1.53ab
20	54.40e	6.30a	2.60a	55.00d	1.57a
30	48.70f	-	-	-	-
40	64.00cd	-	-	-	-
Three cumulative additions					
10	67.10c	6.01a	2.22a	70.00c	1.35ab
20	72.60b	6.97a	2.63a	83.00b	1.26b
30	76.10a	6.57a	1.77a	101.00a	1.33b
40	61.70d	5.94a	2.53a	74.00c	1.19b

Mean values within a column followed by the same letter are non-significantly different at 5% level according to Duncan´s multiple range test

Table 7: Effect of different rates of sludge treatments on root and shoot fresh and dry weights of *Vicia faba*

	No. of days after sowing											
	30				45				75			
Treat-ments	Shoot		Root		Shoot		Root		Shoot		Root	
(T/F)	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
Control	8.20d	0.76b	1.94de	0.15d	22.8e	2.85d	1.35f	0.24d	65.58d	7.6ef	3.87c	1.00b
One residual addition												
10	13.8ab	1.50a	2.90b	00.21abd	60.1a	7.17a	3.88a	0.64a	63.12d	10.5ce	4.88bc	0.97b
20	11.1bc	1.22ab	2.75bc	0.25abd	33.7d	3.54c	3.07b	0.48ab	73.95b	8.8def	6.4b	1.30b
30	13.3ab	1.33ab	2.60bc	0.20abd	22.1e	2.61d	1.82e	0.27d	59.66d	11.5bcd	5.9b	1.20b
40	13.5ab	1.51a	1.70e	0.16cd	31.7d	3.68c	2.25d	0.41bc	42.08e	6.6f	4.9bc	1.00b
Three cumulative additions												
10	11.3bc	1.23ab	2.26cd	0.18bcd	25.5e	2.67d	1.01g	0.19d	87.50b	12.4bc	6.02b	1.10b
20	11.7bc	1.08ab	3.77a	0.28a	26.5e	3.00d	2.00e	0.32cd	90.27a	15.4a	8.10a	1.70a
30	15.0a	1.68a	3.80a	0.26ab	38.5c	4.60b	2.63c	0.42bc	43.04e	11.0cd	5.0bc	1.00b
40	9.7cd	0.93b	2.40bcd	0.17bcd	42.6b	4.86b	3.17b	0.48ab	77.90c	14.4cb	8.76a	1.80a

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test. FW: Fresh weight, DW: Dry weight

Cumulative Effect of Three Additions

The different yield parameters increased significantly by all sludge treatments (Table 6, 7). This was accompanied by the decrease of the fresh weight of shoots after 75 days.

The number of nodules per plant was significantly increased in most sludge treatments (Table 8). However, the mean percent of efficiency (high and low) was higher at one residual addition than at repeated applications of sewage sludge.

Effect of Sewage Sludge on Chlorophyll Mutation and Photosynthetic Pigments

Table 9 indicated that chlorophyll b content increased significantly by 10 and 40 T/F one residual addition treatments and 20 and 30 T/F three cumulative additions treatments.

Effect of Sewage Sludge on Cell Division and Chromosomes

Root Growth, Mitotic Activity, Phase Distribution and Cell Aberrations

Root tolerance index (Fig. 1) increased by sludge treatments and by increasing the addition rates, which indicated that stimulation of root growth occurred.

The effect of sludge residual treatments and three cumulative additions on mitosis and chromosomes is presented in Table 10. An increase in mitotic activity (MI) was found in all treatments. The increase in cell division by sludge treatment was accompanied by an increase in the

Table 8: Effect of different rates of sludge treatments on nodules number, efficiency [low (+), high (++)] and fresh (FW) and dry weight (DW) of *Vicia faba*

		Efficiency (%)		Weight (g)	
Treatments (T/F)	No. of nodules	High (++)	Low (+)	FW	DW
Control	34.00f	47.10b	44.10e	0.44a	nd
30 days					
One residual addition					
10	70.00b	50.00a	42.80e	0.35a	nd
20	56.00d	41.10c	44.60e	0.38a	nd
30	80.00a	22.50f	72.50a	0.26ab	nd
40	46.00e	36.90d	54.30d	0.15b	nd
Three cumulative additions					
10	70.00b	25.70e	64.30b	0.12b	nd
20	64.00c	26.60e	57.80c	0.05b	nd
30	25.00g	28.00e	32.00g	0.07b	nd
40	22.00g	13.60g	36.40f	0.07b	nd
Control	74.00g	6.80c	92.90b	0.28f	0.07bc
45 days					
One residual addition					
10	125.00c	3.20d	96.00a	0.59cd	0.13abc
20	157.00a	1.50e	99.10a	1.12a	0.19a
30	102.00e	1.00ef	96.00a	0.98b	0.15ab
40	122.00c	0.40f	97.50a	0.70c	0.16ab
Three cumulative additions					
10	108.00d	9.00b	90.00b	0.52de	0.11abc
20	130.00b	1.50e	98.50a	0.46e	0.12abc
30	82.00f	1.20ef	91.20b	0.69e	0.11abc
40	70.00g	10.00a	84.80c	0.44e	0.06c
Control	94.00d	5.25f	82.40b	2.18bcd	0.20a
75 days					
One residual addition					
10	83.00e	6.00e	92.80a	2.01cde	0.30a
20	45.00f	8.90d	88.90ab	1.74de	0.20a
30	95.00d	8.40d	85.30ab	2.57b	0.40a
40	86.00e	9.30d	69.70c	1.60e	0.20a
Three cumulative additions					
10	152.00a	13.20c	85.20ab	2.85a	0.40a
20	103.00c	15.50b	72.80c	2.30bc	0.30a
30	90.00d	32.00a	68.00c	0.93f	0.10a
40	128.00b	7.80d	90.60a	1.81de	0.20a

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test. nd: Not detected

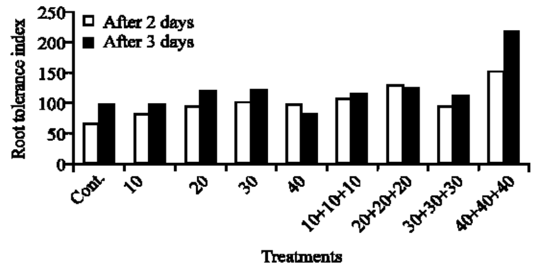

Fig. 1: Root tolerance index of root growth after two and three days germinated seeds grown on sludge amended soil

Table 9: Chlorophyll a and b in *Vicia faba*

Treatments (T/F)	Chlorophyll a	Chlorophyll b	Chlorophyll a (%)	Ratio a/b
	---------------- (mg g^{-1} plant) -------------			
Control	10.14a	6.94ab	59.16	1.45
One residual addition				
10	10.00a	5.66c	64.06	1.78
20	10.28a	7.97a	56.33	1.29
30	10.29a	7.95a	56.57	1.30
40	9.96a	6.46bc	60.88	1.56
Three cumulative additions				
10	10.31a	7.37ab	57.15	1.40
20	9.67a	4.79d	66.87	2.02
30	10.18a	5.88c	63.38	1.73
40	10.27a	7.22ab	58.72	1.42

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test

Table 10: Root growth, mitotic activity, phase distribution and abnormal dividing (ADCs) and non dividing cells (ANDCs) of *Vicia faba* primary roots grown on sludge amended soil

Treatments (T/F)	Primary root length (cm)	Examined cells (No.)	M/P ratio	(A+T)/M ratio	MI	ADCs	ANDCs
					------------------	(%)	------------------
Control	2.72 ab	4264	0.22	1.52	5.19a	9.15c	1.59b
One residual addition							
10	3.38a	3154	0.19	1.66	6.00a	12.28b	1.11b
20	2.68ab	3662	0.35	1.34	5.82a	10.90c	1.35b
30	2.17ab	3412	0.26	1.23	6.47a	12.07b	3.25a
40	2.43ab	2275	0.53	1.21	5.23a	17.37ab	2.35ab
Three cumulative additions							
10	2.07ab	2915	0.45	1.56	5.68a	14.49b	1.67b
20	3.45a	2839	0.46	1.08	7.40a	19.38ab	3.43a
30	2.32ab	2726	0.62	1.42	6.10a	19.35ab	3.86a
40	2.00b	2762	0.46	1.23	7.25a	21.01a	3.73a

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range, M/P: Metaphase Prophase ratio, A: Anaphase, T: Telophase, M: Metaphase, MI: Mitotic index, ADCs: Abnormal dividing cells, ANDCs: Abnormal nondividing cells

frequency of metaphase. As indicated from Table 10, metaphase-anaphase transition was increased generally by sludge treatments.

Aberration Spectrum

The percentage of abnormal dividing cells was increased by the three cumulative additions treatments (Table 11, Fig. 2a-f). Chromosome stickiness was the most dominant abnormality apparent in all phases.

Drastic changes in spindle apparatus and centromeres leading to impairment of chromosome alignment (scattered chromosomes) on metaphase plate, abnormal spindle orientation, abnormal chromosome movement and c-mitosis also occurred (Table 11, Fig. 2).

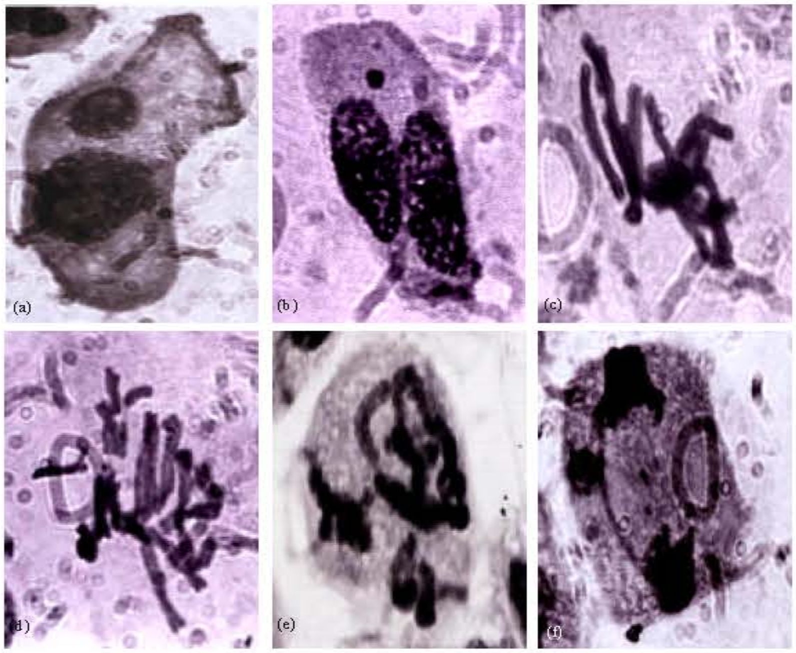

Fig. 2: Non- dividing and dividing cells of *Vicia faba* root meristems affected with different rates of sewage sludge. (a) Interphase with large micronucleus, (b) Binucleate with two small micronuclei, (c) Sticky metaphase, (d) Fragmented metaphase (e) Sticky multigroups metaphase and (f) Telophase with bridge and with sticky and ring laggards

Table 11: Effect of different rates of sewage sludge treatments on the chromosome behavior of root meristems of *Vicia faba* plants

		One additions (T/F)				Three additions (T/F)			
Treatments	Control	10	20	30	40	10	20	30	40
Total dividing cells	204.00	188.30	206.83	218.57	118.67	166.00	203.16	165.83	186.6
Abn. D.Cs (%)	9.15c	12.28b	10.90c	12.07b	17.36ab	14.49b	19.38ab	19.35ab	21.0a
Aberration/cell	1.09	1.12	1.18	1.18	1.03	1.09	1.11	1.09	1.15
Abn. P (Pi) (%)	2.45	1.49	0.64	3.05	1.40	0.60	1.48	2.72	1.16
Abn. M (%)	2.94	2.65	3.33	2.59	3.94	4.31	7.78	6.53	8.84
Sticky M	1.39	1.22	2.56	0.60	1.96	3.31	4.34	5.02	6.60
Abn. Chrom.move	0.08	0.53	0.55	0.30	1.10	0.19	0.57	0.00	0.44
Fragmented M	0.56	0.53	0.00	0.76	0.00	0.38	1.55	1.30	1.41
Micronucleus	0.81	0.44	0.73	0.76	1.12	0.50	1.48	1.00	1.07
C-metaphase	0.08	0.53	0.42	0.15	0.00	0.39	0.65	0.00	0.18
Mmultigroup	0.00	0.00	0.40	0.15	0.00	0.00	0.32	0.00	0.00
Abn. A+T (%)	3.59	6.53	6.91	7.13	10.11	9.34	10.01	9.95	12.05
Sticky	0.49	2.92	1.85	0.00	1.12	2.10	0.57	3.82	5.59
Bridge	0.65	1.76	1.36	1.83	1.39	2.50	3.36	3.41	3.03
Abn.Chrom. move	1.22	0.62	1.45	3.50	1.68	1.00	1.14	1.10	0.80
Abn. Group Ori.	0.00	0.25	1.45	2.13	2.52	2.80	1.64	0.90	2.84
Fragment	0.40	0.53	0.48	0.30	1.12	0.30	2.05	0.90	0.79
Micronucleus	0.32	0.27	0.96	1.21	1.39	0.80	0.82	0.70	1.33
Disturbed	0.00	0.79	0.00	0.15	0.56	0.50	1.31	1.00	0.00
Multigroup	0.00	0.00	0.00	0.15	0.56	0.19	0.16	0.00	0.17
Total NDCs (No.)	4060.00	2966.00	3456.00	3194.00	2156.70	2749.83	2636.66	2560.66	257.66
Abn.NDCs (%)	1.59b	1.11b	1.35b	3.25a	2.35ab	1.67b	3.43a	3.86a	3.73a
Micronucleate	1.34	0.88	1.26	2.90	2.33	1.56	3.20	3.87	3.60
Multinucleate	0.04	0.01	0.14	0.24	0.09	0.20	0.18	0.20	0.12

Mean values within a column followed by the same letter(s) are non-significantly different at 5% level according to Duncan´s multiple range test. Abn: Abnormal, DC: Dividing cells, NDC: Non dividing cells, Chrom. Mov: Chromosome movement, Pi: Prophase with micronucleus, Ori: Orientation

Multigroups in metaphase and anaphase-telophase cells and abnormal group orientation represented other forms of spindle disturbance were also scored and recorded. Micronuclei were found to increase by additive sludge treatments (Table 11, Fig. 2).

Generally, chromosome fragments are frequently increased after sludge treatments. In the present study, a considerable number of micronuclei were also recorded in all treatments.

Abnormal interphase cells increased by sludge treatments and gave the highest values by the cumulative treatments (Table 11). The highest percentage of multinucleate and binucleate cells was recorded for the 40 T/F of one residual treatment. Dissolution of the chromatin material was also observed in some cells. Most of the aberrant dividing cells contained one type of aberrations. However, 1.2 aberrations per cell was found after 20 and 30 T/F (Table 11).

DISCUSSION

Effect of Sludge Treatments on Heavy Metals Content in Soil and Plant Parts

Soil heavy metals increased significantly with increasing sludge application rates before cultivation. However, it decreased after harvesting but was still higher than the control. These results were in agreement with McBride *et al.* (2000), Sherif *et al.* (2000), Granto *et al.* (2004) and Ashworth and Alloway (2004).

The concentration of Cu, Pb and Zn in leaves in all one residual addition sludge treatments were significantly reduced, this is similar to that reported by Tiffany *et al.* (2000), Antoinadis and Alloway (2002) and Martinez *et al.* (2003). However, the three cumulative additions reduced Cu content only in the seeds. This may be due to its accumulation in roots or its chelation by the high organic matter content of soil (Shorrocks, 1984; Amin *et al.*, 2007; Bose *et al.*, 2008). Also, Kim *et al.* (2007) stated that significant Cu loss in soybean seeds was related with the degree of Cu mobilization.

Effect of Sludge Addition on Plant Yield and Nodule Formation

Seed yield is a complex trait and quantitatively inherited with low heritable value (Saravanan *et al.*, 2000; El-Shakhess and Sommer, 2003). So, high yield ability must be associated with its components; number of pods/plant, plant height, length of fruiting structure and number of tillers plant^{-1} (Zhang and Yishow, 2002).

The number of tillers plant^{-1}, increased significantly in the two additions treatments than that of the one residual treatment. While, seeds per pod and seed index increased in one residual addition than that of two addition treatment. Generally, increasing of sludge rates increased significantly the fresh and dry weights of shoot and root. This may be due to increasing the concentration of macronutrients (N and P) of plants (Khalil *et al.*, 2000; Meyer *et al.*, 2004).

Seeds with white hilum were recorded in three treatments only. Higgins *et al.* (1988) and Bould and Crofton (1987) found that hilum color of most recent varieties is controlled by two alleles (N and n) with the black allele being completely dominant to white. Also, M_2 seed coat color was found to be green, red and blotched black (mottled), beige or red instead of brown after residual and cumulative sludge treatments. Ricciardi *et al.* (1985) reported that the mottled seed coat color is dominant over the uniform one.

Therefore, it can be concluded that the sludge treatments may mutate the N allele to n revealing that the genotype of black hilum was either homozygous for N (NN) or heterozygous (Nn). Also, all two cumulative additions and 20 T/F of one residual addition were more effective on gene mutations of seed coat color.

Toxicity threshold of sewage sludge application on plants and soil microorganisms was estimated herein by counting the number and efficiency of root nodules. The inhibition of nodulation may be due to several sludge additions. This is disagree with Iqbal *et al.* (2007), who stated that soil with residual sludge provide better nodule formation of *L. leucocephala.* In the same time, while the fresh and dry weighs of nodules/plant increased significantly by all sludge treatments, the levels of nodules efficiency percentage decreased. This can be interpreted as the plant got its all nitrogen requirement from sludge (Chaudrie *et al*., 2000) and in the meantime the heavy metals content of sludge caused toxicity to soil microorganisms. This would lead to the decrease of nodule efficiency percentage as well as affecting host signal transduction genes decreasing host-microsymbiont interaction (Werner, 1992; Ibekwe *et al*., 1996; Siddique and Loss, 1999). Accordingly, nodulation of plants can be used as sensitive bioindicator towards toxic chemicals in soil by nodule genes (nod genes, Werner, 1992).

The significant increase in different yield parameters by all sludge treatments of three additions may indicate that the crop fulfilled its requirements of N, P and essential nutrients from sewage sludge (Korcak and Fanning, 1985). However, the decrease of the fresh weight of shoots after 75 days may be due to the increase of soil salinity (induced by cumulative sludge addition) leading to the decrease of yield as reported by Sherif *et al.* (2001). Anyhow, Kirkham (1976) attributed the drop of yields at high rates of sludge additions to the production of phytotoxic substances during sludge decomposition.

Effect of Sewage Sludge on Chlorophyll Mutation and Photosynthetic Pigments

Many gene mutations are known to affect the main pigments associated with photosynthesis which form chlorophyll a and b protein complexes (King, 1991). Chlorophyll b content and chlorophyll a/b ratios increased significantly by one residual addition and three cumulative additions. The increase in total chlorophyll content of the leaves could be attributed to the increase in nitrogen content of the sludge amended soil. Similar results were obtained by Amin and Sherif (2001). However, mutations also occurred in chlorophyll b genes (chlorina f2; King, 1991) more than those of chlorophyll a giving high a/b ratio and revealing that the conversion of chlorophyll a to chlorophyll b was affected (Westhoff and Kloppstech, 1998).

Effect of Sewage Sludge on Cell Division and Chromosomes

Parameters such as root growth, frequency of mitosis and abnormal cell divisions were analyzed to estimate the cytotoxicity, genotoxicity and mutagenecity of environmental mutants.

Stimulation of root growth (root tolerance index) occurred by sludge treatments and by increasing the addition rates, which indicated that this stimulation may affect certain metabolic processes (Vygas *et al*., 1985) leading to the increase of endogenous growth regulators (Grover and Tejpaul, 1981; El-Antably *et al*., 1994). Since root tolerance index is a way to asses the phytotoxicity (Wierzbicka, 1999), herein, sewage sludge treatments enhanced root tolerance especially after the repeated applications. An increase in mitotic activity (MI) was also found in all treatments, which proved that the addition of sludge induced an increase in both cell division and cell elongation. However, herein, there is no relation between high MI and root elongation. This means that sludge treatments disturb the growth hormones responsible for cell cycle and cell elongation (Chen, 2001; Mouchel *et al*., 2004).

Reversible protein phosphorylation is an important regulatory mechanism in the control of cell cycle progression. Central to this regulation are the Cyclin-Dependent Kinases (CDK) and their activating proteins-cyclins. The Mitotic Cyclin-Dependent Kinase (MCDK) complex consists of a Cdc2 catalytic subunit and a cyclin B regulatory subunit. A critical event in the regulation of Cdc2/cyclin B involves its dephosphorylation by Cdc25C (Xiaoqi Liu and Erikson, 2002). The increase in cell division by sludge treatments was accompanied by an increase in the frequency of metaphases, in the present study, might be due to the activation of spindle checkpoint genes through cyclin B leading to a general increase in M/P ratio (Tsukaya and Beemster, 2006). In the meantime, the increase of rate of biosynthesis of nucleic acids and Mitosis Promoting Factors (MPF) would lead also to the acceleration of the rate of cell division (Kornberg and Baker, 1992). Recently it has been shown that when the tension associated with proper attachment is absent the kinetochore becomes phosphorylated and anaphase is delayed. It has been proposed that the kinetochore protein dephosphorylation caused by tension is the all-clear signal to the checkpoint. The involvement of Cyclin B/cdc2 in the events surrounding this mitotic checkpoint have yet to be documented, but the fact that Cyclin B/cdc2 is degraded at the metaphase-anaphase transition, suggests that MPF is the direct target of this checkpoint (Xiaoqi Liu and Erikson, 2002).

Herein, metaphase-anaphase transition was increased generally by sludge treatments, probably due to stimulation of the synthesis of Anaphase Promoting Complex (APC) which is a check point protein required to reduce B-cyclin levels as cells pass through anaphase and telophase regulates the transition, during mitosis (Yu *et al*., 2000). Moreover, the APC/C is required to coordinate chromosome separation at the metaphase-to-anaphase transition in a process that requires Cdk activity (Harper *et al*., 2002).

In other word, Abnormal spindle (Asp) is affected by Microtubule-Associated Protein (MAP), found at the poles of mitotic spindles. Abnormal spindle mutants exhibit a mitotic metaphase checkpoint arrest with abnormal spindle poles; that reflects a requirement for Asp for the integrity of microtubule organizing centers (MTOCs). The absence of a strong spindle integrity checkpoint enables Asp mutant cells to proceed through anaphase and telophase (Mendes-Bonato1 *et al*., 2006). However, the central spindle region is not correctly organized and cells frequently fail to complete cytokinesis.

The increase in the percentage of abnormal dividing cells by the three cumulative additions treatments could be due to the cumulative effect of genotoxic compounds found in sewage sludge, high heavy metals content, household detergents and chlorophenols (Ivanova *et al*., 2005). Chromosome stickiness which were recorded in appreciable percentage in this study and increased by all sludge treatments is probably caused through immediate reactions with DNA causing DNA-DNA or DNA-protein cross linking (El-Kodary *et al*., 1990; El-Ghamery *et al*., 2000; Kovaleva, 2008). consequently, sticky chromosome bridges were also recorded.

Spindle disturbances caused by drastic changes in spindle apparatus and centromeres leading to impairment of chromosome alignment (scattered chromosomes) on metaphase plate, abnormal spindle orientation (and split spindle and then Multigroups), abnormal chromosome movement (laggards and then micronuclei) and c-mitosis also occurred. The mechanistic background to spindle disturbances with compounds might be partially based on the partitioning (sharing) of some household chemicals and metals into hydrophobic compounds of the cell (Onfelt, 1987). In addition, the presence of Ca^{+2} (El-Bagouri, 1999) as well as chlorophenols (UNESCO, 1996) in sewage water and consequently sewage sludge, could be the cause of the occurrence of c-mitosis. Dipanker and Crothers (1986) suggested that Ca^{+2} neutralizes the negative charges on chromatin fibrils, which would increase chromosome repulsion and chromatin condensation causing c-mitosis. Also, scattered chromosomes, herein, might be a consequence of the increase of Ca^{+2} concentration of the soil (Amin and Sherif, 2001) and afterwards, at the cellular level during cell division affecting glutathione and ATP contents causing the disruption of spindle mechanism (Onfelt, 1987). Heavy metals like Pb was found to be associated with the spindle protein tubulin (Jonson, 1998) leading to inhibition of polymerization and/or microtubule formation (Aardema *et al*., 1998) disrupting chromosome movement (Oshimura and Barrett, 1986). Moreover, Pb could block the combination of spindle microtubules with the associated proteins essential for the sliding function of microtubules during anaphase and thus disrupt the movement of the chromosomes (Oshimura and Barrett, 1986). However, in addition to all previous causes for spindle abnormalities, the possibility of mutation of one or more of the genes responsible for the assembly and function of microtubules (Onfelt, 1986) could not be neglected. The abnormal orientation of some chromosomes was found to be due to altered quantity and quality of kinetochore heterochromatin (Graves and Zelesco,1988) or mutation of one or more of the genes responsible for pole determination (Rajendra and Bates, 1981). Micronuclei are true mutagenic indicators of the exposure to genotoxic agent (Minissi *et al*., 1998; Grover and Kaur, 1999; Kovaleva, 2008) were found herein to increase by additive sludge treatments. The increase of multinucleate and binucleate interphase cells by sludge treatments is an indication of the inhibition of cell wall formation at the end of mitosis.

In conclusion, this study suggests that sewage sludge is good source of nitrogen content and macro- and micronutrients leading to high plant productivity. This was reflected as increased mean mitotic index. However, it also contains heavy metals and its application lead to harmful accumulation in soil and plants but no phytotoxic effects were detected. However, chromosome aberrations and gene mutations occurred and were reflected as: (A) the disturbance of balance between chlorophyll type a and b, (B) affecting nodulation genes and (C) occurrence of gene mutations of genes that regulate the seed hilum and coat color. Taking into consideration, the long-term applications of sewage sludge would carry a risk of progressive heavy metal toxic levels. This might be considered as a yellow but rather a red light of warning to the hazardous effect of using sewage sludge as a recycle of wastes.

Thus regular sludge, soil and plant analysis are needed to check for low levels of sewage sludge-borne metals used as soil amendments. Further studies should be encouraged towards more investigations on the different methods for secondary treatments of sewage sludge reducing hazardous compounds (e.g., heavy metals and house hold detergents) to benefit from high nitrogen content and micro- and macro nutrients found in sewage sludge. This type of study might be useful to urge decision makers to design guide lines to the producers and users of sewage sludge in agriculture.

REFERENCES

Aardema, M.J., P. Abertin, S. Arni, L.M. Meidersdon, M. Kirsch-Volders, J.M. Mackay, A.M. Asarrif, D.A. Stringer and R.D.F. Taalman, 1998. Aneuploidy: A report of an ectoc task force. Mut. Res., 410: 3-97.

Alloway, B.J. and E. Steinnes, 1999. Anthropogenic Additions of Cadmium to Soils. In: Cadmium in Soils and Plants, McLaughlin, M.J. and B.R. Singh (Eds.). Kluwer Academic Publishers, Dordrecht, Netherlands, pp: 97-123.

Amin, A.W., 2001. Heavy metals content in maize as affected by sewage sludge application. ii-cytological effects. Egypt. J. Genet. Cytol., 29: 131-140.

Amin, A.W. and F.K. Sherif, 2001. Heavy metal contents in maize as affected by sewage sludge application morphological characters and yield. Pak. J. Biol. Sci., 4: 1451-1455.

Amin, A.W., F.M. Ashour, N.M. El-Nahas and L.M. El-Sadek, 2007. Evaluation of sewage sludge use in agriculture: 1-Genetic changes of M1 yield parameters and meiocytes of wheat. Assiut. Univ. J. Bot., 36: 23-40.

Antoniadis, V. and B.J. Alloway, 2002. The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge-amended soils. Environ. Pollut., 117: 515-521.

Ashworth, D.J. and B.J Alloway, 2004. Soil mobility of sewage sludge-derived dissolved organic matter, copper, nickel and zinc. Environ. Pollut., 127: 137-144.

Bierman, M.P. and C.J. Rosen, 1994. Phosphate and trace metal availability from sewage sludge incinerator ash. J. Environ. Qual., 23: 822-830.

Bose, S., A. Jain, V. Rai and A.L. Ramanathan, 2008. Chemical fractionation and translocation of heavy metals in *Canna indica* L. grown on industrial waste amended soil. J. Hazardous Mater., 160: 187-193.

Bould, A. and G.R.A. Croften, 1987. Variation in the expression of hilum color in field varieties in relation to seed certification standers. Seed Sci. Technol., 15: 651-662.

Chaudrie, A.M., C.M.G. Allain, V.L.B. Jefferson, F.A. Nicholson, P. Chambers and M. Baud Grath, 2000. A study of the impact of Zn and Cu on two rhizobial species in soils of along-term field experiment. Plant Soil, 221: 167-179.

Chen, J.G., 2001. Dual auxin signaling pathways control cell elongation and division. J. Plant Growth Regul., 20: 255-264.

Dipankar, S. and D.M. Crothers, 1986. Influence of DNA binding drugs on chromatin condensation. Biochemistry, 25: 1503-1509.

El-Anatably, H., M.A. Amer, I. EI-Shamey and A. Raafat, 1994. Endogenous growth substances of tomato under salinization. I. Effect of salinity stress in relation to ontogeny. Egypt. J. Bot., 34: 199-208.

El-Bagouri, I.H., 1999. Potentials and constraints of the reuse of wastewater in desert agriculture. Final Report of Land and Water Management Programmed, Center for Environmental and Developmental for the Arab Region and Europe, pp: 1-16.

El-Ghamery, A.A., A.I. EI-Nahas and M.M. Mansour, 2000. Effect of the herbicide goal oxyfluorfen on cell division and nucleic acids content in root tips of *Allium cepa* L. and *Vicia faba* L. Egypt. J. Bot., 402: 173-190.

El-Kodary, S.A., A. Habib and A. Halim, 1990. Effects of the herbicide tribunal on root mitosis of *Allium cepa*. Cytologia, 55: 209-215.

EL-Shakhess and A.M. Sommer, 2003. Heterosis and inbreeding depression in sesame. Egypt. J. Plant Breed., 7: 7-5.

FAO., 1980. Soil and plant testing as a basis of fertility recommendation. Soil Bull., 38: 49-100.

Fiskesjo, G., 1988. The *Allium cepa*-test an alternative in environmental studies, the relative toxicity of metal ions. Mut. Res., 197: 243-260.

Granto, T.C., R.I. Pietz, G.J. Knafl, C.R. Carlson, Jr. P. Tata and C. Lue-Hing, 2004. Trace element concentrations in soil, corn leaves and grain after cessation of biosolids applications. J. Environ. Qual., 33: 2078-2089.

Graves, J.A. and P.A. Zelesco, 1988. Chromosome segregation from cell hybrids. V. Does segregation result from asynchronous centromere separation?. Genome, 30: 124-128.

Grover, I.S. and S.K. Tejpaul, 1981. Antagonistic effect of gamma radiation on the dormant seed of *Avena tatua*. Radiat. Bot., 15: 439-450.

Grover, I.S. and S. Kaur, 1999. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the allium root anaphase aberration and micronucleus assay. Mut. Res., 426: 183-188.

Haque, I., E.A. Aduayi and S. Sibanda, 1993. Copper in soil, plants and ruminant animal nutrition in special references to sub-saharan. Afr. J. Plant Nutr., 16: 2149-2212.

Harper, J.W., J.L. Burton and M.K.J. Solomon, 2002. The anaphase-promoting complex: It's not just for mitosis any more. Genes Dev., 16: 2179-2206.

Higgins, J., J.L. Evans and J.R. Law, 1988. A revised classification and description of faba bean cultivars (*Vicia faba* L.). Plant Varieties Seeds, 1: 27-35.

Ibekwe, A.M., J.S. Angle, R.L. Chaney and P. Berkum, 1996. Zinc and cadmium toxicity to alfalfa and its microsymbiont. J. Environ. Qual., 25: 1032-1040.

Inskeep, W.P. and P.R. Bloom, 1985. Extinction coefficients of chlorophyll a and b in N, N-dimethylformamaide and 80% acetone. Plant Physiol., 77: 483-485.

Iqbal, G.M.A., S.M.S. Huda, M. Sujauddin and M.K. Hossain, 2007. Effects of sludge on germination and initial growth performance of Leucaena leucocephala seedlings in the nursery. J. For. Res., 18: 226-230.

Ivanova, E., T.A. Staikova and I. Velcheva, 2005. Cytogenetic testing of heavy metal and cyanide contaminated river waters in a mining region of Southwest Bulgaria. J. Cell Mol. Biol., 4: 99-106.

Johnson, F.M., 1998. The Genetic effect of environmental lead. Mut. Res., 410: 123-140.

Khalil, M.E.A., N.M. Badran and M.A.A. El-Emam, 2000. Effect of organic manures on growth and nutritional status of corn. Egypt. J. Soil Sci., 40: 245-263.

Kharkwal, M.C. and H.B. Chaudhary, 1997. Grain density as selection criterion in chickpea and wheat. Indian J. Genet., 57: 415-423.

Kim, B., M.B. McBride, B.K. Richards and T.S. Steenhuis, 2007. The long-term effect of sludge application on Cu, Zn and Mo behaviour in soils and accumulation in soybean seeds. Plant Soil, 299: 227-236.

King, J., 1991. Mutants Affecting the Chloroplast and Photoset: The Genetic Basis of Plant Physiological Processes. Oxford Univ. Press, New York, Oxford.

Kirkham, M.B., 1976. Trace elements in sludge on land effect on plant, soil and ground water. Proceedings of Cornell. Agric. Waste Management Conference, 1976, USA., pp: 209-247.

Kornberg, A. and T.A. Baker, 1992. DAN Replication. 2nd Edn., Free Man and Co., New York, pp: 759-769.

Kovaleva, O.A., 2008. Cytogenetic anomalies and causes for their occurrence in somatic cells. Cytol. Genet., 42: 48-59.

Lindsay, B.J. and T.J. Logan, 1998. Field response of soil physical properties to sewage sludge. J. Environ. Qual., 27: 534-542.

Logan, T.J. and B.J. Harrison, 1995. Physical characteristics of alkaline stabilized sewage sludge in vivo soil physical properties. J. Environ. Qual., 24: 153-164.

Martínez, F., G. Cuevas, R. Calvo and I. Walter, 2003. Biowaste effects on soil and native plants in a semi arid ecosystem. J. Environ. Qual., 32: 472-479.

Mater, J.E., 2000. Role of organic acids on detoxification of aluminum in higher plants. Plant Cell Physiol., 41: 383-390.

McBride, M.B., B.K. Richards, T. Steenhuis and G. Spiers, 2000. Molybdenum uptake by forage crops grown on sewage sludge amended soils in the field and green house. J. Environ. Qual., 29: 848-854.

McGrath, S.P., F.J. Zhao, S.J. Dunnam, A.R. Crosland and K. Coleman, 2000. Long term changes in the extractability and bioavailability of zinc and cadmium after sludge application. J. Environ. Qual., 29: 875-883.

Mench, M.J., E. Martin and P. Solda, 1993. After effects of metals derived from a highly metasl-polluted sludge on maize (*Zea mays* L.). Water Air Soil Pollut., 75: 277-291.

Mendes-Bonato1, A.B., M.S. Pagliarini1 and C. Borges do Valle, 2006. Abnormal spindle orientation during microsporogenesis in an interspecific Brachiaria (Gramineae) hybrid. Genet. Mol. Biol., 29: 122-125.

Meyer, V.F., E.F. Redente, K.A. Barbarick, R.B. Brobst, M.W. Paschke and A.L Miller, 2004. Plant and soil responses to biosolids application following forest fire. J. Environ. Qual., 33: 873-881.

Minissi, C., C. Passqlium Grella and Rizzoni, 1998. Mutagenecity (micronucleus test on *Vicia faba* root tips), polycyclic aromatic hydrocarbons and heavily metal content of sediments collected Tiber River and its tributaries within the urban area of Rome. Mut. Res., 420: 77-84.

Mouchel, C.F., G.C. Briggs and C.S. Hardtke, 2004. Natural Genetic Variation in *Arabidopsis* Identifies BREVIS RADIX, a Novel Regulator of Cell Proliferation and Elongation in the Root. Cold Spring Harbor Laboratory Press, New York.

Obbard, J.P., 2001. Toxicological assessment of heavy metals in sewage sludge amended soils. Applied Geochem., 16: 1405-1411.

Onfelt, A., 1986. Mechanistic aspects on chemical induction of spindle disturbances and abnormal chromosome numbers. Mut. Res., 168: 249-300.

Onfelt, A., 1987. Spindle disturbances in mammalian cells. III: Toxicity, c-mitosis and aneuploidy with 22 different compounds. Specific and unspecific mechanisms. Mut. Res., 182: 135-154.

Oshimura, M. and J.C. Barret, 1986. Chemically-induced aneuploidy in mammalian cell: Mechanisms of biological significant in cancer. Environ. Mol. Mutagen., 8: 129-159.

Page, A.L., R.H. Miller and D.R. Keeney, 1982. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd Edn., SSSA Publisher, Madison, Wisconsin, USA.

Rajendra, B.R. and L.S. Bates, 1981. Genetic male and chemically induced sterility in *Triticum* aestivum. J. Heredity, 72: 52-56.

Rank, J. and M.H. Nielsen, 1998. Genotoxicity testing of wastewater sludge using the *Allium cepa* anaphase-telophase chromosome aberration assay. Mut. Res., 418: 113-119.

Ricciardi, L., A. Filippetti, C. De Pace and C.F. MarZano, 1985. In Heritance of seed coat in broad bean (*Vicia faba*). Euphytica, 34: 43-51.

Saravanan, T., S. Thirugnana Kumar and J. Gahessand, 2000. Genetics of earliness characters in sesame (*sesamum indicum* L.). Sesame Safflower, 15: 14-18.

Sharma, A.K. and A.S. DPhil, 1980. Chromosome Techniques. Fakenham Press Ltd., Fakenham, Norfolk, pp: 95-105.

Sherif, F.K., S.M. El-Arabla, M.N. Felafel and I.M Ghoneim, 2000. Effect of bio-fertilization under varying sludge rates on growth, yield potentials and elemental composition of potato. Adv. Agric. Res., 5: 1631-1647.

Sherif, F.K., A.M. Awad, A.F. Aswad and A.T. Farag, 2001. Heavy metals and phosphorus availability for potato yield as affected by sludge-amended soil. Alex. J. Agric. Res., 46: 181-203.

Shorrocks, R.M., 1984. Micronutrient deficiencies and their correction. Span, 27: 119-121.

Siddique, K.H. and S.P. Loss, 1999. Studies on sowing depth for Chick pea (*Cicer Arietinum* L.), Faba bean (*Vicia faba.*) and Lentil (*Lens culinaris*) in mediterranean type environment of South Western Australia. J. Agron. Crop Sci., 182: 105-112.

Steinkellner, H., K. Mun-Sik, C. Helma, S. Ecker, Th. Ma, O. Horak, M. Kunid and S. Knsumullar, 2002. Genotoxic effects of heavy metals: Comparative investigation with plant bioassays. Radiates Biol. Radioecol., 42: 369-383.

Tiffany, M.E., I.R. Mcdowell, G.A. O'Connor. H. Negyen, F.G. Martin, N.S. Wikinosn and E.C. Cardoso, 2000. Effect of pasture applied biosolids of forage and soil concentration over a grazing season in north Florida. ii microminerals. Commun. Soil Sci. Plant Anal., 31: 215-227.

Tsukaya, H. and G.T.S. Beemster, 2006. Genetics, cell cycle expansion in organogenesis in plants. J. Plant Res., 119: 1-4.

UNESCO., 1996. Ecological management of municipal wastewater. A case study of slow-rate land treatment in Shynyang, China. Final Report of the Co-operative Ecological Research Project (CERP).

Vygas, S.P., S. Kothju, B.K. Garg and A.N. Lahiri, 1985. Performance and metabolic alteration in *Seasaum indicum* L. under different intensities of water stress. Ann. Bot., 56: 323-329.

Werner, D., 1992. Physiology of Nitrogen Fixing Legume Nodules: Compartments and Function. In: Biological Nitrogen Fixation, Stacey, G., R. Burris and H.J. Evans (Eds.). Chapman Hall, New York, London, pp: 359-431.

Westhoff, P. and K. Kloppstech, 1998. Light, Phytohormones and the Biological Clock as Inducers and Modulators of Development From Molecular Plant Development. From Gene to Plant, Oxford, New York, Tokyo, pp: 110-115.

Wierzbicka, M., 1999. Comparison of seed tolerance in *Allium cepa* with other plant species. Environ. Pollut., 104: 41-52.

Xiaoqi, Liu and Erikson, 2002. Activation of Cdc2/cyclin B and inhibition of centrosome amplification in cells depleted of Plk1 by siRNA. Proc. Natl. Acad. Sci. USA., 99: 8672-8676.

Yu, H., E.N. Hiat and R. K. Dae, 2000. The plant kinetochore. Trends Plant Sci., 5: 543-547.

Zhang, Y. and H. Xiao, 1998. Antagonistic effect of calcium, zinc and selenium against cadmium induced chromosomal aberrations and micronuclei in root cells of *Hordeum vulgare*. Mut. Res., 420: 1-6.

Zhang, Y. and W. Yishow, 2002. Genotypic correlations and path coefficient analysis in sesame. Sesame Safflower Newslett., 17: 10-16.