J. Agric. Sci. Mansoura Univ., 32 (5): 3971 - 3995, 2007

EFFECT OF RESIDUAL AND ACCUMULATIVE SEWAGE SLUDGE APPLICATION ON HEAVY METALS BIOACCUMULATION, GENE ACTION AND SOME YIELD PARAMETERS OF *Vicia faba*

Sherif, Fatma.K.[*]; Amal, W. Amin[**]; H. A. El-Atar [*]; Heba Ez-Eldin[*]
[*] Soil & water Science Dept, Fac. of Agric. Alex. Univ.
[**] Faculty of Science, Botany Dept.

ABSTRACT

Field experiments were conducted to evaluate the effect of using sewage sludge as organic fertilizer on different yield parameters of Vicia faba. Sewage sludge treatments rate were 0, 10, 20, 30, and 40 ton/Fed were 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 of Vicia faba (Giza461), but, affected mature plant height and number of tillers/plant which 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 ton/Fed of the residual one addition. The lowest value was obtained at 20 ton/Fed 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 of Vicia faba (Giza 461 and 77) except at the highest treatment of two addition and three additions. Generally, it decreased nodules efficiency percentage. In the same time, the percentage of ineffective nodules was increased in case of Vicia faba (Giza 461). However, in case of Vicia faba (Giza 77) in the percentage of effective nodules after 30, 45, and 75 days from sowing was increased except at the high sludge rates of cumulative three additions after 30 and 45 days. In the same time, the percentage ineffective nodules were increased after 30 and 40 days at the highest sludge rates. In addition the percent of nodules which have low efficiency was higher than the percent of high ineffective. The rate of root growth of Vicia faba (Giza 77), after all sludge treatments, recorded lower values than the control except those of 10 and 30 ton/Fed residual one addition. The root tolerance index increased by sludge treatments. The sludge treatments increased the mean mitotic index and those of cumulative three additions treatments were higher than that of residual one addition treatment. Sludge treatments included a number of abnormalities in all mitotic phases and non dividing cells of Vicia faba (Giza 77). The percentage of abnormal cells increased by cumulative three additions treatments than the residual one addition treatment.

Keywords:-sewage sludge, heavy metals, chloropyll, nodules, seed characters, chromosomes, *vicia faba*

INTRODUCTION

Wastewater sludge produced by sewage treatment plants are represented as a good source of macro elements and microelements and generally contain a high quantity of organic matter. Their application in agriculture translates into improved soil fertility, Maguire et al. (2001). 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 toxic organic compounds and potential contamination from

pathogenic organisms. Heavy metal such as Cd, Cu, Ni, Zn, and Pb may also be among the common contaminants found in sludge, and significant accumulation of these metals in food crops may result in potential health problems for the consumers. Also, the release of these metals in biologically available form as a result of human activity may damage or alter both natural or man made ecosystems (Show and Chadwick, 1999).

Antoniadis and Alloway (2002) studied the effect of application of sewage sludge at rates 0, 10 and 50 t/ha on the uptake of Cd, Ni, and Zn and reported that sludge applications significantly increased the extractability of metals and also their uptake by ryegrass.

Also,Heavy metals toxicity may affect growth, morphology, and biochemical activities of microorganisms. Ibekwe et al. (1996) reported that white clover grown in metal contaminated, biosoilds-amended soil exhibited reduced yeilds and N content. Nodule isolates from the plants were demonstrated to be ineffective in N_2 fixation. Variations in effective-*Rhizobium* population size were apparent in relation to sludge application rate and type, heavy metal concentrations, and soil pH. Obbard, (2001) reported that in soils which had received metal-spiked sludge at the lower application rate and high pH soil, their population was reduced by 42% compared to its equivalent non-spiked sludge treatment. In the low pH soil this effect was even more sever.

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). The effects of Cd at different concentrations (0.5-20 PPm) on root growth, cell division and chromosomal morphology of *Hordeum vulgar* were studied by Zhang and Yang, (1994) and found that the rate of root growth and mitotic index decreased progressively with increasing cadmium andcaused mitotic irregularities comprising c-mitosis, anaphase bridges, breaks stickiness, lagging and vagrant chromosomes and micronuclei. The intensity of the toxic effects is basically dependent on the cadmium concentration and duration of treatment. Amin. (2001) found that increasing sludge rates increased total aberrant pollen mother cells in immature young male tassels at the booting stage in maize causing drastic changes in both spindle apparatus and centromeres. Therefore, the reduction of heavy metals from sewage sludge, before using it as biofertilizer is required to decrease the rate of gene mutation and chromosomal abnormalities.

According to the general consensus about the importance of mutaginicity testing of environment sample, the determination of the genotoxic potential of wastewater sludge could provide important information about sludge quality and thus contribute to proper decision – making process for the proper treatment and use of sludge (Show and Chadwick, 1999).

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 its solubility in water is important.

The goal of this work was to compare the efficiency of different doses of sludge as residual or cumulative treatments on heavy metal concentrations

in soil and *Vicia faba* plant, and their effects on DNA and some yield parameters of *Vicia faba* plant.

MATERIALS AND METHODS

Field experiments were carried out at Agriculture Research Station of the Faculty of Agriculture, Alexandria University at Abis area during the period (1999 - 2001). The soil of the station is calcareous lacustrine.

Soil Physical and Chemical Properties

Soil samples (0-20cm) were collected, air dried, ground, passed through 2-mm sieve and stored in polyethylene bags for analysis. The physical and chemical characteristics of the soil were determined according to Page *et al.* (1982) and are presented in Table (1).

Table 1: Some chemical characteristics of the experimental soil

Soil charact.			**AB-DTPA**	
***EC**	**dS m^{-1}**	**0.68**	**K mg kg^{-1}**	**114.0**
*pH		7.60	Fe mg kg^{-1}	3.47
Clay	gkg^{-1}	455.00	Zn mg kg^{-1}	2.31
Sand	gkg^{-1}	418.00	Mn mg kg^{-1}	6.20
Silt	gkg^{-1}	137.0	Cu mg kg^{-1}	1.70
$CaCO_3$	gkg^{-1}	152.00	Pb mg kg^{-1}	1.75
O.M	gkg^{-1}	10.70	Cd mg kg^{-1}	0.07
TKN	gkg^{-1}	1.36	Ni mg kg^{-1}	0.39
AB-DTPA-P	mg kg^{-1}	33.60	Cr mg kg^{-1}	0.15

*** EC and pH in water extract (1: 2.5).** **[3]TKN= total Kjeldahl nitrogen**

Composted sewage sludge was collected from Site 9N Alexandria General Organization of Sanitary Drainage (AGOSD) in February, 1999. The chemical composition of the used sewage sludge is summarized in Table (2).

Table 2. Some chemical characteristics of the sludge used.

Sludge charact.		**AB-DTPA**	**mg kg^{-1}**
***EC, dSm^{-1}**	**5.05**	**Zn**	**812**
*pH	7.20	Mn	160
$CaCO_3$, %	10.20	Cu	475
OM., g kg^{-1}	435.00	Pb	170.30
TKN-N, g kg^{-1}	21.60	Cd	8.05
TP, g kg^{-1}	5.40	Ni	108.04
TK, g kg^{-1}	1.60	Cr	118.06
TFe, g kg^{-1}	160.80		

***EC and pH in water extract (1: 2.5).**

Experimental Layout

Before starting the experimental study, the sewage sludge was incorporated within the upper 20 cm of soil at the rates: 0, 10, 20, 30, and 40 ton/Fed on March 99 and planted with *Zea mays*. After *Zea mays* harvest,

each plot was divided into two subplots, the first subplot received second sludge addition at the rate of 0, 10, 20, 30, 40 ton/Fed while, the second subplot did not receive any application to compare between the residual and accumulation effect on *Vicia faba. Vicia faba* Giza 461 was sown in the same soil following the *Zea mays* on November, 1999-2000. 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 using *Vicia faba,* (Giza 77) on the same soil but the subplot which have the second sludge addition were received the third sludge addition at the same rates.

Split plots design was used and each plot had six rows. The experimental plot unit had area of 20 m^2. All the other cultivation practices commonly used in the region have been carried out.

Soil Sampling and Analysis

Surface soil samples were collected (0-20cm) from each plot, just before cultivation and after harvesting. These soils were air-dried, ground, passed through 2 mm sieve and stored in polyethylene bags for analyses. The concentrations of AB-DTPA (Ammoniun Bicarbonate-diethylene triamine- pentacetic acid) extractable heavy metals were determined using Atomic Absorption Spectrophotometer (Parken Elmer model 3300) as described by Soltanpour and Schwab (1977).

Sampling and Plant Analysis

Mature plant samples of *Vicia faba* (Giza 461) were collected. In case of *Vicia faba* (Giza 77) plant samples were collected after 30, 45, and 75 days of sowing from each plot. Also, seeds from each plot were sampled, washed with distilled water, oven dried at 70° C, then stored for analysis. The oven-dried plant materials were homogenized and wet digested (FAO, 1980).

Chlorophyll Estimation in *Vicia faba* (Giza 77)

The seventh leaves were collected, washed, and air-dried. The concentration of Chlorophyll a and b was determined according to Inskeep and Bloom (1985).

Morphological Measurements

***a- Vicia faba* (Giza 461)**

After 4 weeks of planting, seedling height and number of tillers per plant were determined. At maturity seven characters were recorded: plant height, seed index (100 seeds weight), number of seeds/pod, number of sterile and fertile seeds / pod, seed color and hilum color. Seed density was calculated by dividing the weight of 10 seeds by its volume (Kharkwal and Chaudhary, 1997).

***b- Vicia faba* (Giza 77)**

Fifteen seeds were sown in treated soil samples. Root length of five replicates per treatment, rate of root growth per day, and root tolerance index, (Wierzbicka, 1999) were calculated. At maturity plant length and productivity (plant height, pod length, seeds/pod, seed index, and seed density, nodules number and activity) were recorded.

Cytological Studies Using *Vicia faba* (Giza 77) Test

Seeds of *Vicia faba,* (Giza 77) were germinated in sewage sludge treatments in order to determine the mitotic index in addition to mitotic

distribution using three replicates of carbol fuschsin stained root tips. Also, abnormal dividing cells (ADCs) and non dividing cells (ANDCs) were calculated. The different kinds of aberrant nondividing cells were restricted as micronuclei (one or more). While the kinds of aberrant dividing cells were classified as: chromosome stickiness, laggards, c-metaphase, disturbed chromosomes, bridges, fragments and micronuclei.

Statistical Analysis

The analytical and morphological variations were evaluated by applying the analysis of variance and least significant differences test, using COSTAT program.

RESULTS AND DISCUSSION

I-Effect of Sludge Treatments on Heavy Metals Content in Soil

1. Residual one addition effect

Before cultivation of *Vicia faba* (Giza 77), the results in Table 3 indicated that the AB-DTPA extractable Zn, and Cd increased significantly with increasing sludge application rates. However, the amounts of ab-DTPA exractable Cu and pb increased markedly but not significantly. Similar results were obtained by Darmody *et al.* (1983); Cambreco *et al.*, 1996: Tsadilas *et al.,* (1995) and Ashworth and Alloway (2004).

After *Vicia faba* harvesting, Table (3) represented that the increasing of sludge rates increased AB-DTPA extractable Zn and Cd. However, the concentration declined at 10 ton/Fed and 30 ton/Fed for Cu and Cd respectively. This may be due to mobility of these metals with dissolved organic matter (DOM).

Table 3: The concentrations of AB-DTPA extractable heavy metals ($mgkg^{-1}$)in the sludge amended soil planted with *Vicia faba* (Giza 77) before sowing (21 months from 1st add.) and after harvesting (25 months from 1st add.).

Treatment (ton/Fed)		Cont.	10	20	30	40
Before sowing	**Cu**	**10.17 a**	**10.80 a**	**11.62 a**	**10.80 a**	**11.88 a**
	Zn	3.09 b	2.71 b	3.59 b	4.70 ab	5.81 a
	Cd	0.16 b	0.00 c	0.00 c	0.36 a	0.18 b
	Pb	4.63 a	4.33 a	4.94 a	4.58 a	5.92 a
After harvest	Cu	9.70 a	9.21 a	10.01 a	10.11 a	10.23 a
	Zn	2.47 b	3.41 b	3.83 a	5.25 a	5.49 a
	Cd	0.10 c	0.30 b	0.30 b	0.00 c	0.48 a
	Pb	3.98 a	5.22 a	5.47 a	5.62 a	5.44 a

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.
1st add.: the first addition of sewage sludge.

2. Cumulative effect of three additions

Before sowing and after harvesting of *Vicia faba* (Giza 77), Table (4) indicated that AB-DTPA extractable Cu did not respond significantly to sludge applications, whereas, extractable Zn, Cd and Pb increased significantly.

These results were in agreement with Andersson and Nilsson (1976); McBride *et al.*, (2000); and Sherif *et al.*, (2000) and Granto *et al.* (2004).

Table 4: The concentrations of AB-DTPA extractable heavy metals (mgkg^{-1})in the sludge amended soil planted with *Vicia faba* (Giza 77) before sowing (20 months from 1st add.+12 months from 2nd add.+8 days from 3rd add.) and after harvesting (25 months from 1st add.+17 months from 2nd add.+5 months from 3rd add.).

Treat. (ton/Fed)		Cont	10	20	30	40
Before sowing	Cu	10.17 a	11.55 a	11.06 a	11.23 a	12.07 a
	Zn	3.09 c	5.02 b	6.78 b	6.17 b	11.66 a
	Cd	0.16 c	0.32 c	0.84 b	0.54 c	1.14 a
	Pb	4.63 c	7.31 b	7.24 b	6.99 b	10.81 a
After harvest	Cu	9.70 a	7.11 b	11.19 a	11.13 a	10.38 a
	Zn	2.47 b	2.74 b	5.25 a	6.59 a	5.08 a
	Cd	0.10 b	0.00 c	0.70 a	0.74 a	0.18 b
	Pb	3.98 b	3.46 b	7.15 a	6.98 a	5.86 a

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.
1st add.: the first addition of sewage sludge. 2nd add.: the second addition of sewage sludge.
3rd add.: the third addition of sewage sludge

II-Effect of Sludge on Heavy Metals Content in Plant

1. Residual one addition effect

Table (5) showed that the concentration of Cu was decreased significantly in leaves of *Vicia faba* in all sludge treatments but the decrease was insignificant in seeds. This result is similar to that reported by Tiffany *et al.* (2000) for bahiagrass. Zn concentrations in leaves were increased significantly by the application of sludge rates up to 20 ton/Fed. However, the concentration of Zn in seeds reduced significantly with sludge rate of 10 ton/Fed, then increased significantly at 20 ton/Fed. Similar results were obtained by Antoinadis and Alloway 2002; and Martinez *et al.* 2003.

Table 5: Heavy metals concentrations (mgkg^{-1}) in leaves and seeds of *Vicia faba* (Giza 77)

Treat.(ton/Fed)		Cont.	10	20	30	40
Leaves	Cu	12.31 a	11.88 a	9.55 b	10.98 a	7.93 b
	Zn	41.78 b	38.48 c	51.07 a	43.54 b	41.67 b
	Cd	nd	nd	Nd	nd	nd
	Pb	1.00 b	1.00 b	1.00 b	9.00 a	1.00 b
Seeds	Cu	10.79 a	10.01 a	9.04 a	-	-
	Zn	34.00 b	31.39 b	39.39 a	-	-
	Cd	nd	nd	nd	-	-
	Pb	nd	nd	nd	-	-

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.
- missed data. nd.: Not detected

The Cd concentration in leaves and seeds were not detected with all sludge application rates, while, Pb concentrations in leaves were increased by increasing sludge rates. Chaney (1988) found that the concentration of Pb from sludge does not raise plant concentration unless it is very high. The concentrations of Pb in seeds did not respond significantly to sludge addition rate and were not detected

2.Cumulative effect of three additions

Table (6) showed that Cu concentration in leaves was significantly influenced by sludge application rate, while it was not influenced significantly in seeds. The reduction of Cu in leaves and seeds of *Vicia faba* may be due to chelating of Cu by the high content of organic matter derived from cumulative sludge application **(**Shorrocks, 1984) or to accumulation of Cu in roots than in shoots.

Concentration of Zn in leaves influenced significantly by sludge application rates. Similar results were obtained by Baham and Sposito, (1994), and Gaskin *et al.* (2003) However, the concentration of Zn in the seeds declined significantly at 10 ton/Fed sludge rate compared with control.

Table 6: Heavy metals concentrations in leaves (mgkg^{-1}) (22 months from 1st add.+14 months from 2nd add.+2months from 3rd add.) and seeds of *Vicia faba* (Giza 77) plants

Treat.(ton/Fed)		Cont.	10	20	30	40
Leaves	Cu	12.31b	15.41 a	11.70 b	9.49 c	12.87 b
	Zn	41.78 c	47.53 b	41.87 c	47.98 b	63.16 a
	Cd	nd	nd	nd	nd	nd
	Pb	1.00 c	6.00 a	2.00 c	4.00 b	2.00 c
Seeds	Cu	10.79 a	0.00 c	11.05 a	8.78 a	6.50 b
	Zn	34.64a	25.53 b	34.56 a	32.04 a	33.43 a
	Cd	nd	nd	nd	nd	nd
	Pb	0.00 d	2.20 b	1.00c	3.00 a	0.00 d

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

1st add.: the first addition of sludge. 2nd add.: the second addition of sludge.

3rd add.: the third addition of sludge. nd.: Not detected

Concentrations of Cd in leaves and seeds of were not influenced significantly by sludge rates. However, Pb concentration in leaves increased significantly with increasing sludge rates. The 10 ton/Fed treatment recorded the highest concentration content in grain compared with the other treatments. Also, the concentration of Pb increased significantly in seeds with increasing sludge rate.

III. Effect of Sludge on Plant Yield and Nodule Formation

a- *Vicia faba* (Giza 461)

All sludge treatments decreased the height of 30 days old plant, while the residual treatments of 10 and 30 ton/Fed and of two additions of 10 and 20 ton/Fed increased the mature plant height (Table 7). Generally, the number of tillers per plant decreased significantly at 20 and 40 ton/Fed

residual one addition and at 30 ton/Fed cumulative two additions giving the mean 7.81 for residual treatment and 8.75 for the two applications treatments.

Values of main pod length and seed density showed no significant difference among the different treatments and the control. However, the number of seeds per pod recorded the highest value by 10 ton/Fed of the residual one addition while the lowest value recorded by 20 ton/Fed of the cumulative two additions. Contrarely, the seed index increased significantly with sludge treatments.

The increasing sludge rates increased significantly the fresh and dry weight of shoot and root at the residual and cumulative two sludge treatments except at 10 and 30 ton/Fed residual one addition and 30 and 40 ton/Fed cumulative two additions for dry weight of root.. This may be due to increasing the concentration of macronutrients (N and P) in plants (Epstein *et al.*, 1976; Khalil *et al.* 2000 and Meyer *et al.* 2004).

Table7: The yield components of *Vicia faba* (Giza 461) as affected by sludge treatments.

Treat. ton/Fed	30days Plant height (cm)	Mature plant height (cm)	Tillers/ plant (no.)	Shoot		Root		Pod Length cm)	Seeds / pod (no.)	Seed index (gm)	Seed density (gm/cm^{3})
				F.W (gm)	D.W (gm)	F.W (gm)	D.W (gm)				
Residual one addition											
Cont	6.46a	136.63 ab	8.75abc	157.3 h	80.00e	2.00 g	7.8c	6.32a	2.92b	75.00 bc	1.08 a
10	14.8a	141.75 ab	9.00 ab	5100 b	145.0bc	23.3f	6.4e	6.56a	4.02a	83.07 ab	1.15 a
20	13.8a	136.00 ab	7.75 bc	3100 e	155.0ab	30.3 b	7.8c	6.95a	2.74b	77.07 ab	1.10 a
30	14.8a	138.50 ab	8.75 abc	2100 g	100.0 d	25.3 d	6.5e	6.18a	2.71b	83.30 ab	1.12 a
40	15.2a	130.50 b	5.75 c	4750 c	135.0 c	24.4 e	8.6b	6.20a	2.65b	80.00 ab	1.08 a
Cumulative two additions											
10	15.8a	142.50ab	8.50abc	220.g	80.0 e	28.90 c	26.0 a	7.14 a	2.8b	84.60 a	1.1a
20	13.5a	147.25 a	11.00 a	700.0 a	165.0 a	36.30 a	26.0 a	6.09 a	1.6c	82.33 ab	1.0a
30	15.8a	133.75 b	7.25 bc	250.0 f	145.0 bc	23.20 f	6.8 d	6.09 a	2.5b	73.20 c	1.0a
40	15.6a	134.75ab	8.25 abc	325.0 d	95.0 d	24.70de	6.2 e	6.09 a	2.6b	77.13ab	1.1a

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

Different characters of seeds are presented in Table (8). It was observed that the sludge treatments of residual one addition decreased the sterility percentage, while the treatments of the cumulative two additions increased that percentage. It is clear, therefore, that the percentage of total abnormal seeds was increased by all treatments of cumulative two additions. While, 20 and 40 ton/Fed residual one addition treatment induced high values.

The percentage of small sized seeds' number increased by 20 ton/Fed, residual one addition, while by 30 ton/Fed of these treatments recorded the least number. On the average, cumulative two additions of 10, 20, and 30 ton/Fed recorded higher small size number and by 40 ton/Fed recorded the least number relative to control.

Table 8: M_2 Seed Characters of *Vicia faba* (Giza 461) plants

Treat. (ton/Fe)	Seed pod (no)	Total Seeds (no)	Ster. %	Small size (no)	White hilum %	% Coat color					
						Bloched			Green	Red	%∑ab seeds
						Black	Brown	Red			
Residual one addition											
Cont.	2.92	343.0	10.23	3.31	0.39	4.14	4.30	0.84	-	-	13.89
10	2.75	539.0	5.94	-	0.56	3.34	0.74	-	1.11	-	5.75
20	2.74	312.0	9.94	6.73	-	2.88	14.74	-			31.41
30	2.35	342.0	5.85	2.92	-	-	0.88	-	1.46	-	5.26
40	2.68	175.0	9.71	-	-	1.14	4.00	-	-	-	5.14
Cumulative two additions											
10	3.12	431.00	17.87	3.94	-	6.26	0.93	-	-	-	11.14
20	2.52	201.00	22.39	3.98	1.49	16.42	35.82	2.99	-	-	60.70
30	2.53	197.00	11.68	3.63	-	3.05	2.54	-	-	-	14.29
40	2.68	206.00	16.02	2.91	0.97	6.80	10.68	0.49	3.40	1.0	26.21

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

Seeds having white hilum color were recorded in three treatments only. In the majority of recent varieties hilum color has been controlled by two alleles (N and n) with black being completely dominant to white (Bould and Crofton, 1987). Therefore, it can be concluded that the sludge treatments may mutate the N allele responsible for black hilum, to n allele for white hilum.

The M_1 seed coat was brown. In case of M_2 seed, the color modified into uniform green or red and blotched black, beige or red (Table 6). Ricciardi *et al.* (1985) reported that the mottled seed coat color is dominant over the uniform one. Accordingly, it can be concluded that all cumulative two additions treatments were more effective on gene mutations of seed coat color in addition to 20 ton/Fed residual one addition.

McBride (1995) concluded that metals entering soil may be potentially harmful to various soil plant systems. His concerns were based on the organic matter - time bomb hypothesis, where metal availability and hence toxicity, were postulated to increase as sludge organic matter in soil oxidizes and/or mineralizes over time.

In the present study, the number of mature nodules/plant exhibited significant increase in all treatments of residual one sludge addition (Table 9). However, in case of cumulative two additions, 10 ton/Fed recorded the highest significant value. This result indicated that the highest treatment of sludge for several times caused inhibition of nodulation. This may be due to enough content of nitrogen for the plant requirements at these treatments (Ferreira and Castro, 1995). Contrarily, this may be due to high concentrations of heavy metals at these rates. Also, Chaudri *et al.* (2000) found a relation between lack of nodules in Pea and white clover plants and suffering metal phytotoxicity. In the same time, generally the fresh and dry weight of nodules/plant increased significantly at all sludge treatments (Table9).

Table 9: Effect of different rates of sludge treatments on nodules of *Vicia faba* (Giza 461).

Treat. (ton/Fed)	Nodules/Plant					
	Number	% Efficiency			Weight	
		High (++)	Low (+)	Negative (-)	F.W (gm)	D.W (gm)
Residual one addition						
Cont.	86.00d	31.0 abc	30.00 ab	39.0 bcd	1.20 d	0.40 abc
10	78.00 e	32.0 ab	19.00 c	45.0 bc	1.80 bc	0.55 ab
20	99.00 c	23.0 cd	33.00 ab	44.0 bc	1.40 cd	0.49 abc
30	123.00 a	30.1 abc	19.50 c	43.0 bc	2.10 ab	0.67 a
40	100.00 c	33.0 a	26.00 bc	34.0 d	2.60 a	0.60 a
Cumulative three additions						
10	110.00 b	36.60 a	31.40 ab	32.00 d	2.50 a	0.66 a
20	50.00 g	18.00 de	30.30 ab	50.00 ab	0.80 d	0.34 bc
30	58.00 f	24.13 bcd	37.93 a	37.93 d	0.80 d	0.25 c
40	55.00 fg	14.50 e	30.90 ab	54.50 a	1.30 cd	0.29 c

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

b. *Vicia faba* (Giza 77)

All sludge treatments increased significantly the mature plant height, except with20 and 30 ton/Fed of the residual treatment, and the maximum height was obtained with 30 ton/ Fed cumulative three additions Table (10). Generally, the pod length showed no significant differences among the different treatments and the control. On the other side, seed density increased at all sludge treatments. The highest value of seed number per pod was induced by 10 ton/Fed of the residual treatment; however the lowest value occurred by 30 ton/Fed of the cumulative two additions treatment. In case of seed index it was observed that only the 10 ton/Fed of the residual treatment and 30 ton/Fed of the two additions treatment giving higher values than the control.

Table (10): The mature plant length and productivity of *Vicia faba* (Giza 77) as affected by sludge treatments.

Treat. (ton/Fed)	Plant height	Pod length (cm)	Seeds/pod (no.)	Seed Index (gm)	Seed density (gm/cm^3)
Residual one addition					
Cont.	60.68 e	7.14a	2.53 a	83.0 b	1.15 b
10	64.10 cd	8.70a	3.00 a	100.0 a	1.53 ab
20	54.40 e	6.30a	2.60 a	55.0d	1.57 a
30	48.70 f	-	-	-	-
40	64.00 cd	-	-	-	-
Cumulative three additions					
10	67.10 c	6.01a	2.22 a	70.00 c	1.35 ab
20	72.60 b	6.97a	2.63 a	83.00b	1.26 b
30	76.10 a	6.57a	1.77 a	101.0a	1.33 b
40	61.70 d	5.94a	2.53 a	74.00 c	1.19 b

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

- Missed data

Data in Table (11) showed that the fresh and dry weights of *Vicia faba* (Giza 77) increased significantly with increasing sludge rates. This may be due to sewage sludge used as a source of N and P in the production of

crops (Korcak and Fanning, 1985). However, the highest value after 45 days was recorded at 10 ton/Fed. This may be due to rapid mineralization of organic matter at this rate which release nitrogen in available form (Aguilar *et al.* 1994), the highest value of fresh and dry weight of shoot and root were recorded at 20 ton/Fed and 40 ton/Fed cumulative three additions after 75 days. This may be due to that the cumulative sludge applications were high and increased macronutrients NPK with time (Martinez *et al.*, 2003). In contrary, at 40 ton/Fed the fresh weight of shoot declined below the control. This may be due to that increasing sludge rate which caused decrease in the plant yield Sherif *et al.* (2001). The same trend was obtained by Sherif (1996) who found that salinity induced by sludge application affects wheat and soyabean yields.

Table11: Effect of of sludge rates on *Vicia faba* (Giza 77).

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

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

Table **(**12) showed that the number of nodules per plant, after 30 days of sowing, exhibited significant increase in all sludge treatments of residual one sludge addition. However, in case of cumulative three additions, the lowest treatment 10 ton/Fed recorded the highest significant value. Also, after 45 day of sowing, the maximum number of nodules/plant was recorded with the residual one addition of sludge, while, cumulative three additions treatments showed the lower number in comparison with residual treatments except at 20 ton/Fed. In the comparison of the number of nodules after 30 and 45 days of sowing it was found that number of nodules was increased to 180.35% at 20 ton/Fed residual one addition and increased to 228% at 30 ton/Fed cumulative three additions relative to control.

However, number of nodules per plant after 75 days was low by, residual one addition treatment. The cumulative three additions of sludge recorded the highest number except at sludge rate of 30 ton/Fed. The highest increase in the number of nodules at repeated three additions of sludge may be due to increasing the content of organic matter in soil which improved all properties of soil. Similar results were obtained by Heckman *et al.* (1988).

Concerning the nodules efficiency's percentage, **1-** After 30 days, low efficiency (+) of nodules was increased significantly with increasing sludge rates except at 30 and 40 t/.f cumulative three additions, which might indicates that the crop is not in need for more nutrients. However, high efficiency (++) was below the control value except at 10 ton/Fed residual one addition. In case of negative efficiency the percent was below the control value except at 20 ton/Fed residual one addition and at all treatments of cumulative three additions. **2-** After 45 days of sowing, all sludge treatments (residual one addition and cumulative three additions) caused increased in low efficiency (+) except at high sludge rate 40 ton/Fed three addition. However, the percent of nodules which have high efficiency (++) declined below the control value at all sludge applications except at 10 and 40 ton/Fed three additions, where the percentage increased to 9% and 10% respectively, relative to the control. In case of negative efficiency the percent was below the control value at all sludge treatments except at 30 ton/Fed of cumulative three additions. Accordingly, it is clear that sewage sludge treatments after 30 and 45 days of sowing did not inhibit nodules efficiency because the percent of efficiency (high and low) was higher than the percent of negative efficiency. However, the mean percent of efficiency (high and low) was higher at residual one addition than at repeated applications of sewage sludge. **3-** after 75 days, high efficiency (++) percentages were increased significantly with sludge applications in comparison with the control. The high percentage was 32% at 30ton/Fed cumulative three additions. Also, the low efficiency (+) percentage increased with increasing sludge rates relative to control except at 40 ton/Fed residual one addition and 20 ton/Fed cumulative three additions. However, the percent of high efficiency was lesser than the percent of low efficiency.

In case of negative efficiency the percent was declined below the control value at all sludge treatments except at 40 ton/Fed residual one addition. Obbard (2001) reported that the population was reduced by 42% in high pH soil received sludge at lower rate.

The fresh weight of nodules/plant after 30 days decreased significantly below the control at all sludge treatments. However, after 45 days it was increased and the highest value was recorded at 20 ton/Fed residual one addition in comparison with three additions.

The fresh weight of nodules/plant after 75 days at residual one addition increased significantly with increasing sludge rates except at 40 ton/Fed. Also, fresh weight of nodules at cumulative third addition of sludge significantly with increasing sludge rates except at 30 and 40 ton/Fed.

IV. Effect of Sludge on Chlorophyll Mutation and Photosynthetic Pigments

Data in Table (13) indicated that chlorophyll b was increased significantly by 20 and 30 ton/Fed residual one addition treatments and by 10 and 40 ton/Fed cumulative three additions treatments. However, there was no significant effect on chlorophyll a. The chlorophyll a/b ratios gave the same trend of chlorophyll b content. The increase in total chlorophyll content of the leaves could be attributed to the increase in nitrogen applied to the soil with the sludge. Similar results were obtained by Amin and Sherif (2001).

Also no visible symptoms of chlorosis or necrosis were found on the above ground parts of the plants, indicating no sludge toxicity, but their leaves were greener than those of the control plants.

Table 12: The number of nodules, efficiency, and weight after 30, 45, and 75 days of sowing of *Vicia faba* (Giza 77) as affected by sludge treatments

Treatment (ton/Fed)	No. of nodules	% Efficiency			Weight	
		++	+	-	F.W (gm)	D.W (gm)
After 30 days						
Residual one addition						
Cont.	34.00 f	47.10 b	44.10 e	8.80 de	0.44 a	nd
10	70.00 b	50.00 a	42.80 e	7.10 ef	0.35 a	nd
20	56.00d	41.10 c	44.60 e	14.30 c	0.38 a	nd
30	80.00 a	22.50 f	72.50 a	5.00 fg	0.26 ab	nd
40	46.00 e	36.90 d	54.30 d	4.30 g	0.15 b	nd
Cumulative three additions						
10	70.00 b	25.70 e	64.30 b	10.00 d	0.12 b	nd
20	64.00 c	26.60 e	57.80 c	15.60 c	0.05 b	nd
30	25.00 g	28.00 e	32.00 g	40.00 b	0.07 b	nd
40	22.00 g	13.60 g	36.40 f	50.00 a	0.07 b	nd
After 45 days						
Residual one addition						
Cont.	74.00 g	6.80 c	92.90 b	1.30 c	0.28 f	0.07 bc
10	125.00 c	3.20 d	96.00 a	0.00 d	0.59 cd	0.13 abc
20	157.00 a	1.50 e	99.10 a	0.40 d	1.12 a	0.19 a
30	102.00 e	1.00 ef	96.00 a	2.00 b	0.98 b	0.15 ab
40	122.00 c	0.40 f	97.50 a	0.00 d	0.70 c	0.16 ab
Cumulative three additions						
10	108.00 d	9.00 b	90.00 b	0.00 d	0.52 de	0.11 abc
20	130.00 b	1.50 e	98.50 a	0.00 d	0.46 e	0.12 abc
30	82.00 f	1.20 ef	91.20 b	8.50 a	0.69 e	0.11 abc
40	70.00 g	10.00 a	84.80 c	4.20 b	0.44 e	0.06 c
After 75 days						
Residual one addition						
Cont.	94.00 d	5.25 f	82.40 b	10.50 b	2.18 bcd	0.20 a
10	83.00 e	6.00 e	92.80 a	1.20 f	2.01 cde	0.30 a
20	45.00 f	8.90 d	88.90 ab	2.20 de	1.74 de	0.20 a
30	95.00 d	8.40 d	85.30 ab	6.30 c	2.57 b	0.40 a
40	86.00 e	9.30 d	69.70 c	18.00a	1.60 e	0.20 a
Cumulative three additions						
10	152.00 a	13.20 c	85.20 ab	2.60 d	2.85 a	0.40 a
20	103.00 c	15.50 b	72.80 c	9.70 b	2.30 bc	0.30 a
30	90.00 d	32.00 a	68.00 c	0.00 g	0.93 f	0.10 a
40	128.00 b	7.80 d	90.60 a	1.60 ef	1.81 de	0.20 a

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

++ % of high efficiency + % of low efficiency - % negative efficiency

One type of mutation, in higher plants which has been widely studied, results in the reduced expression of chlorophyll b. This pigment comprises about one-fourth of the chlorophyll in higher plants and is bound in pigment-protein complexes associated with both photosystem I and II. The affected

plants appear with no detectable chlorophyll b or with reduced amount of the pigment. According to Markwell *et al.* (1986)**,** chlorina mutants are responsible for these variations. One of the many such mutants expressed little or no chlorophyll b and known as chlorina f_2 (King, 1991), since the expression of the chlorina f2 mutation eliminated chlorophyll b from the plant. However, according to Westhoff and Kloppstech (1998)**,** the mutations in chlorophyll b genes occurred more than those of chlorophyll a, revealing that the conversion of chlorophyll a to chlorophyll b was affected.

Table13: Chlorophyll contents in leaves of *Vicia faba* (Giza 77) plants

Treatment (ton/Fed)		Chlorophyll a	Chlorophyll b	Chlorophyll a	Ratio a/b
		mg/g plant		%	
Cont.		10.14 a	6.94 ab	59.16	1.45
Residual one addition	10	10.00 a	5.66 c	64.06	1.78
	20	10.28 a	7.97 a	56.33	1.29
	30	10.29 a	7.95 a	56.57	1.30
	40	9.96 a	6.46 bc	60.88	1.56
Cumulative three additions	10	10.31 a	7.37 ab	57.15	1.40
	20	9.67 a	4.79 d	66.87	2.02
	30	10.18 a	5.88 c	63.38	1.73
	40	10.27 a	7.22 ab	58.72	1.42

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

IIV. Cytological Studies *Vicia faba* (Giza 77)

a. Mitotic index and phases spectrum

The lengths of primary roots were measured after one, two and three days (Table 14) at the beginning of germination, 10 ton/Fed residual one addition and 20 ton/Fed cumulative three additions treatments were recorded significantly the highest root length. However, 40 ton/Fed cumulative three additions treatment induced the lowest root length after the three different duration times. The rate of root growth after all sludge treatment recorded lower values than the control except that of 10 and 30 ton/Fed residual one addition after two days.

Values of root tolerance index (Fig.1) increased by sludge treatments and by increasing the addition rate. After two days, 10 ton/Fed of the residual one addition and 30 ton/Fed of the cumulative three additions treatment recorded the same index, while, after three days 40 ton/Fed of the former treatment attained the minimum index, and the same rate of the later treatments attained the maximum index. Therefore, it could be concluded that sewage sludge treatment enhances the tolerance especially after the repeated application.

Parameters such as root growth, frequency of mitosis and abnormal cell divisions were analyzed to estimate the cytotoxicity, genotoxicity and mutagenecity of environmental mutants (Kovalchuk *et al.*, 1998). The present study provides evidence that sewage sludge treatment caused growth retardation or stimulation after one day of germination. Generally all treatments caused growth root stimulation, in spite of that more days of germination had retarded the growth. The inhibition or stimulation of growth may be due to that sewage sludge affected certain metabolic processes

(Vygas *et al.* 1985) leading to the disturbance of endogenous growth regulators (Grover and Tejpaul, 1981), such as endogenous auxins, gibbrellins and cytokinins (El-Antably *et al.*, 1994).

The effect of sludge residual treatments and cumulative three sludge additions on mitotic index (MI) and frequency of mitotic phase of primary roots are represented in Table (15). It is clear that all sludge treatments increased the mean mitotic index. In addition, the mean mitotic index of cumulative three additions treatments was higher than that of residual one-addition treatments. The increase in mitotic activity can be explained as that addition of sludge had induced the acceleration rate of cell division by increasing the rate of biosynthesis of nucleic acids and other factors required for cell division (El-Kerady *et al.* 1975). Kornberg and Baker (1992) stated that the entry in mitosis is controlled by the cytoplasmic mitosis promoting factor (MPF) which triggers mitosis by its protein kinase activity. The protein synthesis requirement for entry into mitosis allows the production of the critical level of cyclin needed to activate MPF. (Murray and Kirschner, 1989).

Table 14: The length of primary roots and the growth rate of *Vicia faba* (Giza 77) as affected by sludge.

Treatment (ton/Fed)		Length of primary root (cm)			Growth rate (cm/day)	
		After 1day	After 2days	After 3 days	After2days	After3days
	Cont.	2.41 a	4.08 bc	5.08 a	2.04	2.54
Residual one addition	10	3.48 a	5.10 ab	5.16 a	2.55	2.58
	20	2.95 a	4.38 bc	4.25 a	2.19	2.13
	30	2.17 a	5.04 a	4.10 a	2.52	2.05
	40	2.37 a	4.20 ab	6.00 a	2.11	2.50
Cumulative Three additions	10	2.25 a	3.83 bc	4.43 a	1.92	2.22
	20	3.13 a	3.23 bc	4.13 a	1.62	2.07
	30	2.25 a	4.38 ab	4.55 a	2.19	2.28
	40	2.15 a	2.73 c	2.33 b	1.37	1.17

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

It is obvious that the primary root length is attributed to cell division or elongation. Therefore, in the present study, there was no relation between the root length and mitotic index. In the same time, Metaphase Prophase ratios (M/P) increased than those of the control (Table 15).

Ratio reaches 2.26 times than that of the control after cumulative three additions treatments and 1.5 times after residual one addition treatment. The passage of a cell through the cell cycle is controlled by several proteins. Therefore, there are several systems for interrupting the cell cycle if something goes wrong. Some of these are spindle checkpoints, which detect any failure of spindle fibers to attach to kinetochores and arrest the cell in metaphase.

F1

Table 15: Values of mitotic index (M1) and phases spectrum of *Vicia faba* (Giza 77)

Treatment (ton/Fed)		Primary root length (cm)	Total Cells	MI	% ADCs	%ANDCs	%P	%M	%(A+T)	M/P ratio
Cont.		2.72 ab	4264.35	5.19 a	9.15 c	1.59 b	64.40	14.10	21.50	0.22
Residual one addition	10	3.38 a	3154.47	6.00 a	12.28 b	1.11 b	67.10	12.50	20.73	0.19
	20	2.68 ab	3662.83	5.82 a	10.90 c	1.35 b	54.70	19.40	25.90	0.35
	30	2.17 ab	3412.95	6.47 a	12.07 b	3.25 a	61.65	16.30	20.00	0.26
	40	2.43 ab	2275.37	5.23 a	17.37 ab	2.35 ab	46.17	24.30	29.52	0.53
Cumulative Three additions	10	2.07 ab	2915.83	5.68 a	14.49 b	1.67 b	46.18	21.00	32.85	0.45
	20	3.45 a	2839.82	7.40 a	19.38 ab	3.43 a	50.90	23.60	25.48	0.46
	30	2.32 ab	2726.49	6.10 a	19.35 ab	3.86 a	43.20	23.44	33.40	0.62
	40	2.00 b	2762.32	7.25 a	21.01 a	3.73 a	49.30	22.70	27.90	0.46

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.

b. Frequency of aberrant mitotic cells

Chromosomal aberrations occur due to lesions in both DNA and chromosomal and spindle proteins causing genetic damage. In the present study, sludge treatments induced a number of abnormalities in all mitotic phases of *Vicia faba* (Giza77) root meristem including chromosome stickiness, laggards, c-metaphase, disturbed chromosomes, bridges, fragments and micronucleus ect. (Table 16 and Plate1). The percentage of abnormal dividing cells (ADCs) increased by cumulative three additions treatments than the residual one addition treatment and the control. It increased twice than the control after cumulative three additions treatments and 1.5 times after residual one addition treatment. This could be due to the increased content of heavy metals of the cumulative treatments

Aberrant prophase cells formed the lowest percentage while aberrant anaphase-telophase (A-T) cells formed the highest percentage of the total dividing cells in all treatments. The aberrant prophase cells recorded one type of aberration, one or more micronuclei (Plate 1). Generally, the percentage of aberrant metaphase cells increased by using sludge treatment and by

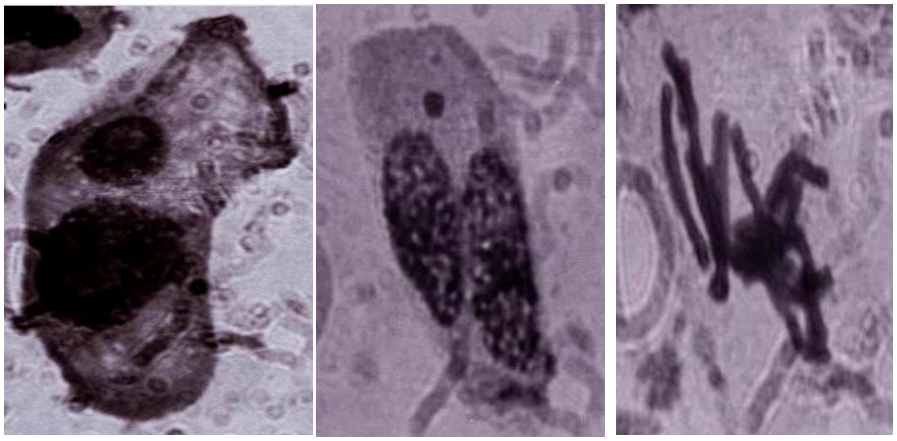
increasing the concentrations. Also, it reached 2.3 times of the control after the cumulative treatments.

Chromosome stickiness was the most dominant abnormality induced in all phases. The percentage of sticky figures induced by cumulative treatments increased by increasing the sludge concentration with a mean of 4.82% as compared with a mean of 1.59% for the residual treatment and 1.39% for the control. Chromosome stickiness is caused probably through immediate reactions with DNA during its inhibition period causing DNA-DNA or DNA-protein cross linking (Amin, 1991), or through reactions with the lysosomal system altering the physiochemical properties of nucleic acid and/or nucleoproteins.The liquefaction of the chromatin material (Adam and Farah, 1989), which consequently lead to the hindrance of daughter chromatids separation at anaphase giving sticky chromosome bridges which were recorded in appreciable percentage in this study. This agrees with the data obtained by El-Khodary *et al.* (1990). Chromosome bridge were recorded in anaphase-telophase in root meristems and increased by all sludge treatments. The three additions treatments had the highest mean value which reached 4.7 times of the control, while the one addition had value of 2.5 times.

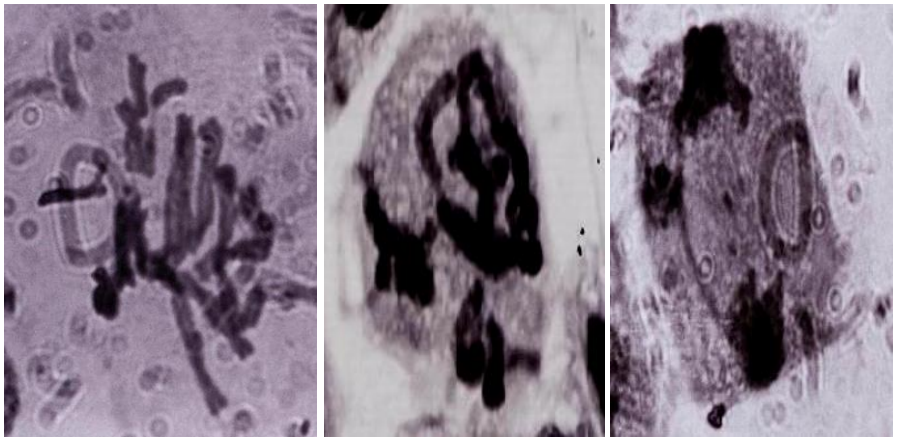
Table 16: The chromosome behavior of *Vicia faba* (Giza 77) plants as affected by sludge treatments.

Treatment	Cont.	One Addition (ton/Fed)				Three Additions (ton/Fed)			
		10	20	30	40	10	20	30	40
Total D.Cs	204.00	188.30	206.83	218.57	118.67	166.00	203.16	165.83	186.66
%Abn. D.Cs	9.15 c	12.28 b	10.90 c	12.07 b	17.36 ab	14.49 b	19.38 ab	19.35 ab	21.01 a
Aberration/cell 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-meta.	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
Σ NDCs (no.)	4060	2966	3456	3194	2156.70	2749.83	2636.66	2560.66	2575.66
%Abn.NDCs	1.59 b	1.11 b	1.35 b	3.25 a	2.35 ab	1.67 b	3.43 a	3.86 a	3.73 a
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

Means within a column with same letter are non-significantly different at 5% level according to Duncan´s test.



A B C



D E F

Plate 1: Non dividing and dividing cells of *Vicia faba* (Giza 77) root merstimes 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 F. Telophase with bridge, sticky and ring laggered chromosomes

The genetic damage which occurs in the present study was probably due to the effect of genotoxic compounds found in sewage sludge. This caused drastic changes in chromatin, spindle apparatus and centromere leading to impairment of chromosome alignment on metaphase plate, abnormal spindle orientation, abnormal chromosome movement and c-mitosis. The mechanistic background to spindle disturbances with compounds might be partially based on the partitioning of some household chemicals or 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 could be the cause of the occurrence of c-mitosis. In the mean time, the increase in Ca^{+2} neutralizes the negative charges on chromatin fibrilis, which would increase chromosome repulsion and chromatin condensation causing c-mitosis (Dipanker and Crothers, 1986).

Heavy metals like Pb was found to be associated with the spindle protein "tubulin" (Johnson, 1998) leading to inhibition of polymerization and/or microtubule formation (Aardema *et al.*, 1998) disrupting chromosome movement (Oshimura and Barret, 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 Barret, 1986). In addition, calcium ion concentration is a major functional component of spindle apparatus (Dellarco *et al.*, 1985). The impairment of chromosome movement and the occurrence of scattered chromosomes in the present study might also be due to the increase of calcium ion concentration of the soil (Amin and Sherif, 2001) and most probably at the cellular level during cell division as found by Onfelt (1987). In the mean time, the presence of $calcium^{++}$ neutralize the negative charges on chromatin fibrils causing a decrease of repulsion force and increase in chromatin condensation forming scattered chromosomes (Dipanker and Crothers, 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.

Disturbed chromosomes at metaphase (M) and anaphase-telophase (A-T) were scored after sludge treatments. However, c-metaphase figures as a form of disturbed chromosomes recorded zero value after 40 ton/Fed residual one addition and 30 ton/Fed three additions treatments. Also, disturbed A-T gave zero value after 20 ton/Fed one addition and 40 ton/Fed three additions treatments. This phenomenon may be induced as a result of disruption of spindle mechanism which is affected by glutathione and ATP contents (Onfelt, 1987).

Multigroups in metaphase and anaphase-telophase cells was another figure of disturbed chromosomes. They were induced by sludge treatments and only recorded a mean of 0.16 for residual one addition treatments and 0.11 for three cumulative treatments. In addition, sludge treatments induced the appearance of abnormal group orientation in anaphase – telophase cells. Abnormal chromosome movement, presented as laggards and precocious chromosomes, increased at metaphase and anaphase - telophase. The abnormal orientation of some chromosomes was found to be due to altered quantity and quality of kinetochore heterochromatin (Jennifer *et al.,* 1988). This may lead to formation of laggards and micronuclei. However, the possibility of mutation of one or more of the genes responsible for pole determination (Rajendra and Bates, 1981) can not be excluded.

Generally, chromosome fragments are frequently increased after sludge treatments giving the highest mean value by cumulative three additions (1.09). The induction of abnormal chromosome movement and

chromosome fragments may lead to formation of micronuclei. Micronuclei are true mutagenic aspects that may lead to loss of the genetic material. In the present study, a considerable number of micronuclei were recorded in all treatments showing the highest percent in prophase cells of 30 ton/Fed of both treatments, metaphase-cells of 40 ton/Fed one residual addition and 20 ton/Fed cumulative three additions and A-T cells of 40 ton/Fed of both treatments.

The effect of sewage sludge was also detected in interphase as it showed micronuclei and binucleate. The percentages of abnormal interphase cells increased by sludge treatments and gave the highest values by the cumulative treatments (Table 22). One type of aberrant nondividing cells (ANDCs) was distinct as one or more micronuclei. The occurrence of micronuclei in a tissue indicates its exposure to a genotoxic agent (Ma *et al.*, 1995; Dakhly and Abdel-Rahem, 1997; Dimitrov and Gadeva, 1997; Minissi *et al.*, 1998 and Grover and Kaur, 1999). This means that sludge components are genotoxic to *Vicia faba* plants. Also, multinucleate cells induced by sludge treatments recording the highest value for the 40 ton/Fed of one residual treatment. The presence of binucleate cells reveals the inhibition of cell wall formation between cells. Dissolution of the chromatin material was observed in some cells (El-Abidin Salam *et al.*, 1993).

The percentage of abnormal nondividing cells (interphase) followed the same trend of abnormal-dividing cells. The mean percent after residual one addition treatments was 2.02 and that after cumulative three additions treatments was 3.17.

Most of the aberrant dividing cells contained one type of aberrations. The highest ratio of aberrations/dividing cells was 1.18 found after 20 and 30 ton/Fed, residual one addition (Table 16).

Finally; the addition of sewage sludge seems to benefit the soil fertility and plant nutrition. Hazardous effects occurred in the nutrition contents and its balance in plant tissues. Mutants also occurred and are reflected on chlorophyll contents and balance between chlorophyll a and b. The sludge treatments increased the mean mitotic index which increased by the cumulative three additions, and induced the number of abnormalities in all mitotic phases which increased by cumulative three additions.

These abnormalities are due to sludge's heavy metals and other chemicals. This study might be considered as yellow but rather red light of warning to the hazardous effect of using sewage sludge as a recycle of wastes. Finally, further studies should be encouraged towards more clear and wide investigations that might include all or some of the followings: (1) The effect of chemical phytotoxic materials that might be present in the sewage sludge, (2) the cumulative effects of sewage sludge additions on both the mitotic and meiosis cell division. This might lead to (i) bread for beneficial properties of crops productions. (ii) guide the sewage sludge pre-treatments methods which might reduce the hazardous effects of such practices, and be useful to design guide lines to the producers and users of sewage sludge in agriculture.

REFRENCES

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. Cited in: 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.

Adam, Z.M. and O.R. Farah. 1989. Cytological effect of water extracts of cymbogon proxmus (half albarr) on *Vicia faba*. Cytologia, 54: 489- 492. Cited in: 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.

Aguilar R., S.R. Loftin, and P.R. Fresquez. 1994. Microbial populations and enzyme activity in soil treated with heavy metals. Water, Air, and Soil Pollution. 96 (11): 61- 71.

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 K.F. Sherif. 2001. Heavy metal contents in maize as affected by sewage sludge application. 1-morphological characters and yield. J. of Biological Sci. 4(12): 1451-1455.

Andersson, A., and K.O. Nilsson. 1976. Enrichment of trace element from sewage sludge fertilizer in soil and plant. A M Bio. 1: 176- 178.

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. Environmental Pollution. 117: 515-521.

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

Baham, J. and G. Sposito 1994. Adsorption of dissolved organic carbon extracted from sewage sludge on montmorilonite and kaolinite in the presence of metal ions. J. Environ. Qual. 23: 147-153. Cited in: 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. Pollution. 117: 515-521.

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 Science and Technology. 15: 651-662.

Chaney, R.L. 1988. Effective utilization of sewage sludge on cropland in the united state and toxicological consideration for land application. In: Proc. second int. symp. land application of sewage, Tokyo, 1988. Association for the utilization of sludge. Tokyo. 77-105. Cited in : Alloway B. J., and A. P. Jackson. 1991. The behaviour of heavy metals in sewage sludge amended soils. Science of Total Environ. 100: 151-176.

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

Dakhly, O.F., and A.T. Abdel- Rahem.1997. Cytological features, growth and yield of maize as affected by chemical and bio-fertilizers. Egypt. J. Genet. 26: 183-193.

Darmody, R.G., J.E. Foss, M. Mcintosh, and D.C. Wolf. 1983. Municipal sludge compost - amended soils: some spatiotemporal treatment effects. J. Environ. Qual. 12: 231-236.

Dellaroc, V.L., K.H. Mavournin, and RR. Tice. 1985. Aneuploidy and health risk assessment: current ststus and future directions. Environ. Mutagen., 7: 405- 424.

Dimitrov, B. and P. Gadeva. 1997. Genotoxicity studies on the insecticide dursban in root meristem cells of *Crepis capillaris* L. Environ. Exp. Bot. 372-3. 199-209.

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

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

El- Kerady, M.S., A. Abd- Alla, and S. El- Aishy . 1975. Effect of gibberelic acid on mitotic activity, root and shoot growth of wheat grown from seeds irradiated with game rays- Egypt. J. Gent. Cytol. 4: 317- 323.

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-Abidin Salam, A.Z., E.H.A. Hussein, A.A. El-Itriby, W.A. Anwar, and S.A. Mansour. 1993. The mutaginicity of gramoxone (Parquat) on different eukaryotics system. Mutation Res. 319: 89-101.

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 (2):199-208.

Esptin, E., J.M. Taylor, and R.L. Chaney. 1976. Effect of sewage sludge compostt applied to soil on some soil physical and chemical properties J. Enviro. Qual. 5: 422- 426.

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

Ferreira, E.M., and I V. Castro. 1995. Nodulation and growth of subterranean clover (trifolium subterranean l.) in soils previously treated with sewage sludge. Soil Biol. Biochem. 27 (9): 1177-1183.

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

Gaskin, J.W., R. B. Brobst, W. P. Miller, and E.W. Tollner. 2003. Long - term biosoilid application effect on metal concentrations in soil and bermudagrass forage. J. Environ. Qual. 32 : 146-152.

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.

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.

Heckman, J.R., J.S. Angle, and R.L Chaney. 1988. Soybean nodulation and nitrogen fixation on soil previously a mended with sewage sludge. Biol. Fertil. Soils. 2: 181- 185. Cited in : Alloway B. J., and A. P. Jackson. 1991. The behaviour of heavy metals in sewage sludge amended soils. Science Total Environ. 100: 151-176.

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-di methyl formamide and 80% acetone plant physiol. 77: 483- 485.

Jannifer, A.M., Graves and P.A .Zelesco. 1988. Chromosome Segregation from Cell Hybrids. V. Does Segregation Result from a synchronous centromere separation? Genome. 30: 124-128.

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 (1-2): 245-263.

Kharkwal M.C. and H.b. Chaudhary. 1997. Grain density as selection criterion in chechpea and wheat. Indiah. J. Genet. 57: 415- 423.

King, J. 1991. Mutants affecting the chloroplast and photoset. Cited in: The Genetic Basis of Plant Physiological Processes.150-223. New York, Oxford. Oxford Univ. press.

Korcak, R.F., and D.S. Fanning 1985. Availability of applied heavy metals as a function of type of soil material and metal source. Soil Science. 140(1)

Kornberg, A., and T.A. Baker. 1992. DAN Replication (second edition), Free Man and Company, New York. 759- 769.

Kovalchuk, O., I. Kovalchuk, A. Arkhipov, P. Telyuk, B. Hohn, and Kovalchuk 1998. The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the ukaine contaminated by the chernobyl accident. Mut. Res., 415: 47-57.

Ma, T., Xu Zbidong, Xu. Chengen, H. Mcconnell, E.V. Rabago, G.A. Arreola, H. Andzhang. 1995. The improved *Allium* / *Vicia* root tip micronucleus assay for clastogenicity of environmental pollution. Mut. Res. 334: 185-195.

Markwell, J.P., S.J. Danko, H. Bauwe, J. Osterman, H.J. Gorz, and F.A. Haskins. (1986). A termperature- sensitive chlorophyll b- deficient mutant of sweet clover (*Maliolotu alba*). Plant Physiol. 81: 329- 334.

Martenz, F., G.R. CuevasCalvo, and I. Walter. 2003. Biowaste effects on soil and native plants in a semi arid ecosystem. J. Environ. Qual. 23: 822-830.

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.

McBride, M.B.1995. Toxic metal accumulation from agricultural use of sludge: are the USEPA regulations projectile. J.Envir. Qual.24:5-18.

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, P. Grella, and C. 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.

Murray, A.W., and M.W. Kirschner. 1989. Cylcin synthesis derives the early embryonic cell cycle. Nature. 339: 275- 280.

Obbard, J.P. 2001. Toxicological assessment of heavy metals in sewage sludge amended soils. Applied geochemistry. 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 DR keency 1982. Chemical and microbiological properties. Part 2 Madison, Wisconsin U.S.A.

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

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.

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 (1): 181-203.

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 (1): 181-203.

Sherif, F.K. 1996. Phytoavailability of zinc and copper by soybean and wheat grown in sand culture and sewage sludge amended soil in relation to plant growth and metal mobility in soil. PH. D. Thesis. Soil and Water Sci. dept. Faculty of Agriculture Alex. University. Alexandria. Egypt.

Shorrocks, R.M. 1984. Micronutrient deficiencies and their correction. Span. 27 (3): 119- 121. Cited in: Haque, I., E. A. Aduayi, and S. Sibanda. 1993. Copper in soil, plants, aqnd ruminant animal nutrition ith special references to sub-saharan. Africa. J. of Plant Nutrition. 16(11): 2149-2212.

Show I. and J. Chadwick. 1999. Principles of Environmental Toxicology. Taylor and Frances (Ltd).

Soltanpour, P.N., and A.P. Schwab. 1997. A new soil test for simultaneous extraction of macro and micro- nutrients in alkaline soils. Commun Soil Sci. Plant Anal. 8: 195- 207.

Tasdilas, C.D., T.N. Barbayiannis, and D. Dimoyiannis. 1995. Influence of sewage sludge application on soil properties and the distribution and availability of of heavy metals fractions. Commun. Soil. Sci. Plant Anal. 26 (15 & 16): 2603-2619.

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 inShynyang, 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 S*easaum indicum* L. under different intensities of water stress. Ann. Bot. 56: 323.

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, 110- 115. Oxford-New York, Tokyo.

Wierzbicka M. and J. Obidzniska. 1998. The effects of lead on seed imbibitions and germination in different plant species. Plant Science. 137: 155- 171.

Zhang Y., and X. Yang. 1994. The toxic effects of cadmium on cell division and chromosomal morphology of *Hordeum vulgare*. Mut .Res. 312: 121-126.

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

التأثير التراكمى والمتبقى من إضافات الحمأه على محتوى الأرض من العناصر الثقيلة وعلى الصفات الجينية لنبات الفول

***فاطمة كمال شريف. **أمال أمين, * حاتم العطار, * هبه عز الدين**

***كلية الزراعة- قسم الأراضى والمياه**

**** كلية العلوم- قسم النبات**

تم إجراء دراسة حقلية فى مزرعة كلية الزراعة جامعة الإسكندرية أثناء الفترة من (1999 – 2001) لكى نقيم تأثير استخدام الحمأة كسماد عضوى على مختلف عناصر الإنتاج لنبات الفول صنف (جيزة 461 و 77) ولتأثيرها أيضا على سلوك الكروموسومات لنبات الفول صنف (جيزة 77). قبل بداية التجربة تحت الدراسة. تم إضافة الحمأة وخلطت بالتربة بمعدلات 0 ,10 ,20 ,30 ,40طن/فدان. بصفة عامة فإن المعدلات المختلفة من الإضافة الواحدة المتبقية والإضافات المتكررة من المعاملة بالحمأة أدت إلى زيادة تركيز العناصر الثقيلة فى الأرض قبل الزراعة وبعد الحصاد لنبات الفول (جيزة 77). أيضا محتوى الأرض من العناصر الثقيلة ازداد نتيجة المعاملات المتكررة من الحمأة وذلك عند مقارنتها بالإضافة الواحدة المتبقية قبل الزراعة. المعاملة بالحمأة أدت إلى زيادة نسبة الإنبات والعدد الكلى للكيزان لكل معاملة أيضا أدت إلى زيادة الوزن الجاف للأوراق ما عدا المعاملات 10، 40 طن/فدان للإضافة الواحدة و 20، 30 طن/فدان للإضافتان. أيضا لم تؤثر المعاملة بالحمأة على بعض عوامل الإنتاج لنبات الفول (جيزة 461) ولكنها أثرت على طول النبات الناضج وأدت إلى زيادة الوزن الرطب والجاف للمجموع الخضرى والجذور ومتوسط وزن 100 حبة وبالنسبة لعدد البذور لكل قرن فقد سجلت أعلى قيمة عند 10 طن/فدان للإضافة الواحدة بينما أقل قيمة عند 20 طن/فدان للإضافتان. أيضا المعاملة بالحمأة لم تؤثر على بعض عوامل الإنتاج لنبات الفول (جيزة 77) ولكنها أدت إلى زيادة طول النبات الناضج ومتوسط وزن 100 حبة وكثافة البذور. أيضا المعاملة بالحمأة أثرت على خصائص بذور الجيل الثانى لنبات الفول (جيزة 461) وتشمل لون قصرة وسرة البذور وحجم البذور حيث نسبة مجموع البذور المشوهة زاد عند جميع معدلات الحمأة للإضافتان التراكميتان ومعدل20 طن/فدان للإضافة الواحدة المتبقية. فى حالة الفول (جيزة 77) حدث زيادة غى نسبة العقد ذات الكفاءة بعد 30، 45، 75 يوم من الزراعة ما عدا عند المعدلات العالية من الإضافة الثالثة من الحمأة بعد 30و45 يوم وفى نفس الوقت ازدادت نسبة العقد عديمة الكفاءة بعد 30و45 يوم عند المعدلات العالية من الإضافة التراكمية. ونسبة العقد ذات الكفاءة المنخفضة كانت أعلى من نسبة العقد ذات الكفاءة العالية. معدل نمو الجذور لنبات الفول (جيزة 77) عند جميع معاملات الحمأة سجلت قيم منخفضة عن نباتات الأرض الغير معاملة ما عدا المعاملات 10و30 طن/فدان للإضافة الواحدة المتبقية بينما زاد مؤشر تحمل الجذور بمعاملات الحمأة. معاملات الحمأة أدت إلى زيادة النشاط الميتوزى للخلايا ومتوسط النشاط الميتوزى للثلاث إضافات تراكمية أكبر من الإضافة الواحدة المتبقية. وأدت إلى تواجد عدد من التشوهات فى جميع أطوار الانقسام الميتوزى والخلايا الغير منقسمة لنبات الفول (جيزة 77). ونسبة الخلايا المشوهة زادت بالثلاث إضافات التراكمية عن الإضافة الواحدة المتبقية.