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CYTOGENETIC EFFECT AND HEAVY METALS CONTENT IN MAIZE AS AFFECTED BY SEWAGE SLUDGE APPLICATION

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ABSTRACT

Although the application of sewage sludge on agricultural land is beneficial to crops as a source of nutrients such as N and P, there is considerable concern for the amounts of heavy metals added as constituents of sludge. An aerobically digested sewage sludge-obtained from Alexandria General Organization of Sanitary Drainage (AGOSD) was applied to corn grown on calcareous lacustrine soil at different rates (0, 10, 20, 30, and 40 ton fed.$^{-1}$). Treatment with NH_4NO_3 supplying the recommended dose was included for comparison. Immature young male tassels at the booting stage were collected to examine various chromosomal irregularities. Plant height and chlorophyll content in leaves were determined at different growth stages. At maturity ten quantitative characters were recorded: number of ears per plant, ear length, ear weight, cob weight, number and weight of kernels per ear, kernel index, kernel yield per plant, volume of 100 kernels, and kernel density. Soil was analyzed before cultivation and after harvesting for macro, micronutrients and heavy metals. Corn grain was analyzed for Pb, Cd, Ni, and Cr. Increasing sludge rates increased significantly initial growth, cob weight, total chlorophyll, chlorophyll a and b in leaves, lead and nickel in kernels and total aberrant mother cells, recording many kinds of aberrations, while no significant differences were observed for the other characters. The concentrations of Pb, Cd, Ni, and Cr in corn grain due to sludge application were generally higher than the normal concentration values.

INTRODUCTION

Sewage sludge is the final residue produced from the treatment of domestic and industrial effluents. Utilization of sewage wastes on land for enhancement of crop production is an age-old practice. In the last few decades concern for environmental contamination prompted a rethinking of the concept of utilization of sludge on land. Agricultural utilization of sewage sludge has the potential benefit of utilization of the nutrients and organic matter for crop production and soil management. In addition to plant macronutrients, sewage sludge contains heavy metals and other trace elements such as cadmium, copper, nickel, zinc, mercury and lead (Show and Chadwick, 1999; Rank and Nielsen, 1998; Rasmy, 1996; Alloway, 1995,). The levels of toxic metals found in sewage sludge are considerably higher than those found in typical agricultural land with more than 300 times as much as zinc and 100 times as much boron and copper (Rasmy, 1996). In Egypt, El-Keiy, 1983 found that the application of sewage sludge at the rate of 50 g kg^{-1} soil, increased the amounts of N, P, K, Fe, Mn, Co, Cu, Ni, Pb, and Pb in the leaves of the five plant species. Significant accumulation of these metals in food crops may result in potential health problems for the consumers. According to the general consensus about the importance of mutaginicity testing of environmental samples, the determination of the genotoxic potential of sludge could provide important information about sludge quality and thus contribute to proper decision-making process for the proper treatment and use of sludge.

Various genotoxic short-term tests have been carried out on industrial wastewater as reviewed by Houk (1991). But, relatively few genotoxicity studies on wastewater sludge have been published. Hopke *et al* (1982) reported the induction of point mutations in germ cells of *Zea mays,* and clastoginic effect in *Tradescantia paludosa.*

The present study is conducted to justify the use of treated sewage sludge as a biofertilizer on the chromosomes and yields of maize.

MATERIALS AND METHODS

This investigation was carried out at the field station of the faculty of Agriculture, Alexandria University at Abis area. The soil of the station is calcareous lacustrine. The main physical and chemical characteristics of the soil was determined according to Page *et al* (1982) and presented in (Table 1).

Composted sewage sludge used in the present study as a biofertilizer was obtained from Alexandria General Organization for Sanitary Drainage (AGOSD) of Alexandria City. The raw sewage sludge was air-dried, aerobically composted at site 9N (45 Km southwest Alexandria city, Amriya). The chemical composition of used sewage sludge is summarized in (Table 2). In March, 1999, the sludge was applied and mixed evenly with the 15 cm top soil three months before cultivation using five rates of sludge applications: 0, 10, 20, 30 and 40 Ton/Fadden (T/F). For comparison ammonium nitrate (33.5% N), was added as a mineral fertilizer after one month of sowing to a control plot in a rate of 0.04 T/F, which is normally used.

Table 1: Some chemical and physical characteristics of the experimental soil.

Soil characteristics	Value	Soil characteristics	Value
[1]EC, dSm^{-1}	0.34	NH_4-OAC-K, mg kg^{-1}	114.0
[2]PH	7.60	DTPA-Fe, mg kg^{-1}	3.47
$CaCO_3$, %	15.20	DTPA-Zn, mg kg^{-1}	2.31
OM. G kg^{-1}	10.7	DTPA-Mn, mg kg^{-1}	6.20
Sand, g kg^{-1}	418.0	DTPA-Cu, mg kg^{-1}	1.70
Silt, g kg^{-1}	13	DTPA-Pb, mg kg^{-}	1.7

	7.0		5
Clay, g kg^{-1}	455.0	DTPA-Cd, mg kg^{-1}	0.07
Soil texture class	Clay	DTPA-Ni, mg kg^{-1}	0.39
[3]TKN. g kg^{-1}	1.36	DTPA-Cr, mg kg^{-1}	0.15
$NaHCO_3$-P, mg kg^{-1}	33.6		

[1]EC in soil water extract (1:5) **[3]TKN= total Kjeldahl nitrogen**
[2]pH in soil suspension (1:2.5)

Grains of *Zea mays,* var. Alexandria 11, were used to test and compare the efficiency of the used fertilizers. A split plot design with four replicates for each treatment was used and each plot had six rows.

Soil sampling and analysis:

Surface soil samples were collected from each plot, just before cultivation and after harvesting. The collected samples were air-dried, and ground to 2-mm and prepared for organic matter content, total nitrogen, available phosphorous and potassium and DTPA (diethylenetriamine-pentaacetic acid) -extractable heavy metals (Fe, Zn, Mn, Cu, Pb, Cd, Ni and Cr) analysis. Spectrophotometer model 21 D) was used for phosphorous determination and atomic absorption spectrophotometer (Perken Elmer model 3300) for heavy metals determination. These analyses were made according to the standard methods used by Page *et al* (1982).

Table 2: Some chemical characteristics, nutrient contents and total concentration of heavy metals (T-) of used sludge.

Sludge characteristics	Value	Sludge characteristics	Value
EC, dSm^{-1}	5.05	TFe, g kg^{-1}	160.80
PH	7.20	TZn, mg kg^{-1}	812.00
$CaCO_3$, %	10.20	TMn, mg kg^{-1}	160.00
OM., g kg^{-1}	435.00	TCu, mg kg^{-1}	475.00
TKN-N, g kg^{-1}	21.60	TPb, mg kg^{-1}	170.30
TP, g kg^{-1}	5.40	TCd, mg kg^{-1}	8.05
TK, g kg^{-1}	1.60	TNi, mg kg^{-1}	108.04
		TCr, mg kg^{-1}	118.06

EC and pH in water extract (1: 2.5). **T-metal = Total heavy metals**
TKN= total Kjeldahl nitrogen.

Chlorophyll determination and grain analysis for heavy metals:

At the seventh leaf stage, leaf samples were collected, washed with tap water, then with redistilled water, and dried between paper towels. Chlorophyll a, b and the total chlorophyll content were determined according to (Mackinney, 1941).

Grains of four mature ears from each plot were sampled, washed with distilled water, oven dried at 70°C then homogenized and wet digested using concentrated sulfuric acid and hydrogen peroxide (FAO, 1980) and analyzed for extractable Pb, Cd, Ni and Cr. To characterize the transfer and the accumulation of metals from the soil to corn grains, the transfer coefficient (TC) was used according to Labrecque *et al* (1995).

$$\mathbf{TC = (Mf - Me) / Ms}$$

Where Mf and Me are metals accumulated (μg) in grains of fertilized and control plants respectively, while Ms is the metal brought into the soil (μg) by the dose of sludge used.

Morphological Measurements:

Plant heights of sixty-four plants per treatment were determined after three and five weeks. At maturity (after three months of sowing) ten quantitative characters were recorded (twelve plants from each treatments): number of ears per plant, ear length, ear weight, cob weight, kernel's number and weight per ear, 100 kernel's weight (index), kernel's yield per plant, volume of 100 kernel and Kernel's density. Kernel's volume was determined by using distilled water displacement. Kernel density was calculated by dividing the weight of the kernel by its volume (Kharkwal and Chaudhary, 1997).

Cytological Parameters:

Immature male tassels at the booting stage were collected from five different plants from each replicate and fixed immediately in fresh solution of ethanol: glacial acetic acid (3:1, V/V). Semiperminant acetocarmine meiocyte preparations were examined. Hundreds of pollen mother cells (PMCs) at different meiotic stages were screened and various chromosomal and cellular irregularities were recorded.

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

1-Effect of sludge application on soil

After three months of sludge application and before cultivation, the increasing of sludge rates increased the organic matter content, available phosphorous and potassium significantly (Table 3). The extractable trace elements (Fe, Zn) increased significantly with increasing sludge rates from 3.47 to 13.79 and 2.31 to 6.75 mg/ kg for Fe and Zn respectively. Extractable (Cd, Ni, and Pb) also, increased significantly due to the sludge addition.

While, Cr decreased with increasing sludge rates. This increase was reduced after harvesting to over 50% in case of Fe, Zn, Mn and Cr, and lower than that in the case of the other metals in the following order: Ni<Cu<Pb<Cd. This decrease did not necessarily be translated into an increase in the concentration of these metals in the same order within the plant.

Table 3: Some chemical analysis of soil before cultivation and after harvesting

Treat T/F	E.C	pH	OM %	TKN %	P	K	Fe	Zn	Mn	Cu	Pb	Cd	Ni	Cr
					$\mu g\ g^{-1}$									
Before Cultivation														
0	0.34	7.60	1.07	0.13	33.5	114	3.47	2.31	6.20	1.70	1.75	0.07	0.39	0.15
10	0.90	7.50	1.13	0.16	56.2	180	9.42	3.95	8.95	6.52	1.82	0.08	0.48	0.16
20	1.12	7.42	1.21	0.14	45.3	69.5	8.71	4.25	7.80	4.78	3.17	0.06	0.60	0.15
30	1.18	7.38	1.33	0.20	61.2	14	11.3	3.21	12.4	8.22	3.14	0.14	0.82	0.12
40	1.22	7.40	1.56	0.25	47.0	125	13.7	6.75	15.6	11.5	3.5	0.12	0.79	0.09
L.S.D	0.02	0.02	0.02	0.01	0.13	1.56	0.55	0.07	0.01	0.01	0.21	0.01	0.01	0.01
After Harvesting														
Mineral	0.42	7.6	0.75	0.06	3.9	65.2	4.86	1.34	17.8	1.63	1.09	0.03	0.11	0.12
0	0.34	7.59	0.72	.004	0.0	0.0	3.06	0.77	10.8	1.34	0.88	0.04	0.02	0.12
10	0.86	7.65	0.83	0.05	34.7	142	6.05	3.43	8.58	1.42	0.38	0.04	0.07	0.10
20	0.99	7.63	0.97	0.08	34.0	31.2	10.7	3.67	7.61	2.17	0.88	0.02	0.10	0.07
30	0.71	7.7	1.18	0.09	59.7	97.5	9.13	2.76	7.75	2.82	0.97	0.04	0.16	0.08
40	0.75	7.57	1.21	0.08	49.5	93.7	7.49	7.73	9.35	2.42	0.76	0.05	0.09	0.18
L.S.D	0.02	0.02	0.02	0.01	0.15	0.32	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01

2. Effect of sludge application on chlorophyll content:

Increasing sludge rate increased chlorophyll a (C_a), chlorophyll b (C_b), and total chlorophyll (C_T) content, significantly than the control (Table 4). The 30 T/F recorded the highest chlorophyll content. This could be attributed to the increase in the nitrogen content of the soil due to the increase in sludge concentration. Labrecque *et al.* (1995) reported that the increase in nitrogen concentration in the soil to a certain extent induced a proportional increase in total leaf nitrogen, subsequently an increase in total chlorophyll content occurred. Sherif *et al.* (2000) found an increase in both N and Fe accompanying the increase in chlorophyll content of potato leaves. In addition, Narwal *et al.* (1990) suggested that the increase in chlorophyll content could result from high Cd concentration in the soil. This suggestion confirmed the results obtained in Table 4 except the 40 T/F sludge treatment, which resulted in lowering chlorophyll content in leaves.

Table 4: Chlorophyll content (mg L^{-1}) in Corn Leaves

TreatmentT/F	Ca	Cb	C total
mineral	0.017615	0.013468	0.031083
0	0.006559	0.004156	0.010715
10	0.007078	0.0046	0.011678
20	0.00876	0.005689	0.014449
30	0.011545	0.007949	0.019494
40	0.008589	0.005599	0.014187
$L.S.D_{0.05}$	0.00046	0.002	0.00048

3. Effects of sludge application on heavy metal contents in grains:

Table 5 showed that increasing the rate of sludge application increased Pb, Ni, Cd and Cr significantly in corn grain (about 4, 2, 1.5 and 4 times respectively). The 10T/F treatment recorded the highest concentration content of Pb and Ni in corn grain compared with other treatments.

While the 30T/F treatment recorded the highest concentration of Cd and the 40T/F treatment recorded the highest concentration of Cr. It was noticed that corn grains accumulated the four analyzed metals in a different order as follows: Ni>Pb>Cr>Cd. This accumulation pattern suggested that there was a selective uptake of these metals probably due to both their different solubility in the soil solution and different transfer coefficients, and thus made it immediately available to the plants. Bagouri (1999) also found a similar case

Table 5: Heavy metals concentrations ($\mu g\ g^{-1}$) in corn grains

Treatment	Pb	Ni	Cd	Cr
Mineral	3.75	8.21	0.05	1.45
0	4.01	9.62	0.00	1.32
10	12.03	15.9	1.23	4.69
20	6.5	7.01	0.01	1.85
30	4.25	3.24	1.56	1.72
40	11.3	13.02	0.45	5.61
$L.S.D_{0.05}$	0.11	0.01	0.01	0.02

The transfer coefficient (TC) of metals from soil to plants differs from one metal to the other. In the present study, it was observed that Cd and Cr were the most transferable with the highest TC values after 10 T/F treatment (15.4 and 21.1, respectively) and 47.7 by Cr after 40 T/F treatment, while Ni and Pb were the lowest transferable elements (Table 6). However, although the transfer coefficient of Cr is the highest, corn grains had the lowest concentration of it. This might indicate that Ni which showed lower transfer coefficient value than Cr was accumulated in grains in a higher concentration, thus depending on its higher rate of solubility in soil solution as was suggested by Labrecque *et. al.* (1995) and Petruzzelli (1989). In the meantime, the lowest and highest sludge treatments (10 and 40 T/F) caused the highest level of accumulation of the four metals in corn grains, while the intermediate concentrations caused lower accumulation. The high accumulation of metals in grains after the application of the lowest concentration may be due to the dilution effect, which enhanced the rate of translocation (transfer) of these metals (Rank and Nielsen, 1998).

Table 6: Transfer coefficient ($\mu g\ g^{-1}$) of heavy metals in corn grains of treated plants using different rates of sludge

Treatment T/F	Pb	Ni	Cd	Cr
10	4.41	13.08	15.38	21.06
20	0.79	-4.35*	0.17	3.53
30	0.08	-7.78*	11.14	3.33
40	2.08	4.3	3.75	47.66

***negative values means that the heavy metals content of control corn grains was higher than the control one.**

While, the low accumulation of metals in the latter case could be interpreted as the organic matter in the sludge acted as chelating agent (Das *et at, 1997).* However, the recorded concentrations were found to be higher than safe limits recorded by Risser and Barker (1990).

4. Morphological Parameters:

No visible symptoms of chlorosis or necrosis developed on the above ground parts of the plants indicating no sludge toxicity, but their leaves were slightly greener than those of the control plants. The results of the morphological and yield parameters are represented in Table 7. Sludge treatment increased the initial plant height (three weeks old) significantly than either the control or ammonium nitrate treatment. However, after five weeks, the mineral fertilizer induced the best growth, while the sludge treatments gave lower values than the control except that treatment using 40 T/F which was not significantly different. Ear length showed a similar trend. A significant variation in mean ear and cob weight were found between the control and the treatment using sewage sludge at 40 T/F. This was accompanied by no significant variation in the mean grain index between all treatments. The mineral fertilizer induced the highest percent of sterility (4.1%) and pitted kernels (28%). The increase in pitted kernels amounted to 2.5 times higher than that of sludge treatments and 14.0 times higher than that of the control. However, all the other means of morphological characters were not significantly different from each other. The morphological characters of corn plants generally increased by sludge treatments in spite of that, most parameters were insignificantly different from each other and the control. The highest values of the morphological parameters were recorded by using the 40T/F treatment, accompanied by the highest heavy metals content in the corn grains. This increase in the yield and growth were attributed to the increase in the available nitrogen content of the soil (Labrecque *et al,* 1995 and Rappaport *et al,* 1987).

Table 7: Effect of mineral fertilizer and different rates of sludge on the yield and yield components of Zea mays.

Treatment	Control		Sludge Treatment			
	(-M)	(+M)	10	20	30	40
Pl. Length (3Weeks)	16.16	16.76	17.12	17.46	19.16	19.68
Pl. Length (5Weeks)	126.08	145.23	112.9	114.9	120.3	127.6
Ear's number/plant	1.00	1.37	1.33	1.37	1.46	1.37
Ear Diameter	17.39	18.32	18.14	16.72	18.46	20.15
Ear weight	13.02	13.84	13.39	13.45	13.72	13.88
Rows number	117.38	133.74	125.4	123.2	133.4	151.8
Kernel's number/ear	12.89	13.18	12.54	13.33	13.0	13.72
Kernel's weight/ear	96.91	101.50	364.5	363.4	374.3	415.1
Cob weight	20.47	32.12	99.49	99.52	108.77	120.23
Grain index	26.26	28.87	26.21	26.76	27.15	28.89
Grain density	1.19	1.23	1.20	1.24	1.15	1.23
Pitted Kernel's percent	1.82	28.01	9.38	8.82	12.72	13.18
Sterility percent	0.36	4.07	0.56	1.22	0.14	0.36

However, the insignificant increase in these parameters could be due to the high content of heavy metals in plant tissues leading to its accumulation in grains

5. Meiotic analysis:

An appreciable amount of meiotic aberrations were recorded in pollen mother cells (PMC's) of plants grown on soil supplemented with different amounts of sewage sludge or ammonium nitrate. Table 8, 9, 10 and Figure 1.

a. Frequency of aberrant meiocytes and meiotic phases:

The total percentage of aberrant pollen mother cells increased with increasing sewage sludge concentration. The increase was about two folds that induced by the mineral fertilizer (Table 8). The percentages of aberrant PMC's at meiotic division two (D1I) were ~~35~~ times higher than those at meiotic division one (D I). Aberrations in D II generally increased by increasing sewage sludge concentration. Diakinesis stage was severely affected, where all cells had fragmented bivalents, most of them with more or less than ten bivalents. Metaphase one and two aberrations caused by sewage sludge treatments were about three folds that caused by ammonium nitrate treatment. But, the latter treatment caused 6.6% aberrant tetrads, which amounted to 76% of total aberrant DII. While sewage sludge treatments caused a range of 3.3 to 11.7% aberrant tetrads, which amounted to 5.6% to 18.3% only from D II. When the tetrad aberrations were calculated from total tetrads only (Table 10), it was found that the low doses of sludge treatments (10 and 20 T/F) caused lower values than the ammonium nitrate treatment, while the 40 T/F gave the highest value of 37.5%.

Table 8: Percentage of Aberrant stages of meiocytes of plants grown on soil supplemented with either mineral or biofertilizer (calculated from total aberrant meiocytes

Treatment	Control		Sludge Treatment			
	(-M)	(+M)	10	20	30	40
Total PMC'S (no)	566	1396	1638	2069	1988	2032
Aber. PMC'S (%)	12.72	10.24	19.61	18.75	23.79	29.08
Aber. DI (%)		1.58	6.18	6.57	5.58	0.79
MI		1.50	5.41	5.41	3.82	0.69
Ana-Tel.I		0.07	0.77	1.16	1.76	0.10
Aber. DII (%)		8.67	13.43	12.18	18.21	28.3
Pro.II		0.14	1.78	0.29	2.06	0.84
MII		1.00	6.24	6.67	2.26	10.33
Ana-Tel.II		0.43	1.13	0.77	1.16	3.15
Asynchrony		0.50	0.95	0.82	0.96	2.31
Tetrad	12.95	6.59	3.33	3.67	11.77	11.22

b.Meiotic aberration spectrum:

Most aberrant meiocytes contained one kind of aberrations, Table 9. The highest kind of aberrations in division one was abnormal chromosome distribution, which appeared in metaphase one as absence of spindle microtubules or abnormal spindle orientation, and in anaphase one as precocious, laggards and unequal or star shape polar groups. These kinds were more frequent in the 10 and 20 T/F than the other treatments. Sticky figures (metaphase and anaphase) were induced by 20 T/F treatment only. In addition, sludge treatments induced abnormal cell contour, which appeared as protruded or elongated cell walls. Division two meiocytes contained high percent of overlapping cells found in all treatments. The rate increased by increasing the sludge concentration. Sludge treatments increased the rate of asynchronized meiocyte nuclei; abnormal cell and chromosome orientation. The highest percentage of abnormal chromosome movement and spindle inhibition occurred after 10 and 20 T/F treatments respectively. In the same time, abnormal cell contour, inhibition of cytokinesis and the occurrence of sticky figures were found in small rates in sludge treatments only. The percentage of aberrant end products of pollen mother cells (tetrads) increased more than seven times by sludge treatments than the nitrate treatment (Table 10). The different kinds of aberrations were divided into three categories according to: 1- the number of microspores resulting from one meiocytes (diad, triad and pentad), 2- arrangement of microspores within meiocyte wall (linear, T- or L-shape, decussate and apple shape), and 3- structural malformations, i.e. abnormal contour (protruded or elongated cell wall and meiocyte having appendages).

Chromosomal aberrations occur due to lesions in DNA molecule causing genetic damage. In the present study, the gradual increase in the total percentage of aberrant meiocytes of *Zea mays* by increasing sewage sludge concentration was probably due to the effect of genotoxic compounds found in sewage sludge (household chemicals, trace elements, as well as heavy metals beside those analyzed in corn grains). This caused drastic changes in both spindle apparatus and centromeres, leading to impairment of chromosome alignment onto metaphase plate and anaphase separation (scattered chromosomes, abnormal spindle orientation, laggards and precocious chromosome, unequal and star shape groups). Heavy metals like Pb was found to associate 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 Barrett, 1986).

Also, 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). In addition, calcium ion concentration is a major functional component of spindle apparatus (Dellarco *et al.,* 1985 a). The impairment of chromosome movement and the occurrence of scattered chromosomes in the present study might also be due to the increase in calcium ion concentration of the soil (calcareous soil) and most probably, at the cellular level. The increase of calcium ion concentration during cell division, which was found by (Onfelt, 1987) caused partial or complete disruption of chromosomes. In the mean time, the presence of Ca 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.

Abnormal cell contour was another abnormality found in the present study. It was expressed as protruded or elongated cell wall, which was recorded only after sludge treatment. The drastic changes in microtubular cytoskeleton could be the cause of this kind of abnormality causing deformation of the cell shape (Vig and Paweletz, 1988). The occurrence of protruded cytoplasm might be due to non-uniform deposition of cell wall leaving week areas where protrusions occurred (Meijer *et al.,* 1988).

Table 9: Meiotic aberration spectrum of Zea mays M1 meiocytes after treatment with sludge (calculated from total aberrations)

Treatment	**Control**		**Sludge treatment**			
	(-M)	**(+M)**	**10**	**20**	**30**	**40**
Total PMC's	566	1396	1683	2069	1988	2032
Aber./ PMC	1.18	1.13	1.01	1.12	1.13	1.25
Kinds:						
Meiosis I:						
Sp. Inhibition		0.07	4.10	2.27	0.86	0.15
Abn. Chrom. Movement		0.93	1.84	2.71	2.77	0.34
Sticky Chrom.		0.00	0.00	1.21	0.00	0.00
Abn. Cell contour		0.00	0.12	0.05	0.05	0.05
Abn. Sp. Orientation		0.50	0.00	0.48	1.56	0.44
Bridge		0.07	0.12	0.10	0.00	2.10
Meiosis II:						
Overlaping		1.36	5.59	5.27	6.89	13.44
Asynchronised cell		0.50	0.95	0.87	1.01	2.36
Abn. Cell om.		0.14	5.30	1.26	1.76	4.13
Abn. Chrom. Om.		0.00	2.08	0.24	0.20	0.39
Abn. Chrom. Movement		0.00	0.06	2.71	0.15	0.00
Sp. Inhibition		0.00	1.84	1.35	0.55	0.00
Cytokinesis inhibition		0.00	0.00	0.00	0.10	0.00
Sticky chrom.		0.00	0.24	0.87	0.00	0.00
Abn. Cell contour		0.00	0.24	0.10	0.30	0.25
Abn. Tet.	12.72	6.59	3.33	3.67	11.77	12.35

Chromosome stickiness was recorded in meiocytes the 20 T/F treatment. This might be due to a recessive gene mutation resembling that discovered in maize by Beedle (McGill et al., 1974), causing inter and intra-chromosome cross links (Ashour, 1988 and Amin, 1991).

During the present study, it was noticed that the control treatment contained only the tetrad stage, while all other treatments included all meiotic stages in spite of collecting and fixing flower buds of all treatments at the same time. This indicated that the control flowers matured faster than the treated ones. However, tetrad aberrations were frequent even in the control. Overlapping tetrads were the major kind found all over the experiment.

Sludge treatments affected both cell wall elasticity and consequently cell mobility. This caused overlapping of the two daughter cells and/or abnormal alignment in several configurations leading to the abnormal orientation of daughter cells. This might be due to the increase of the Ca concentration in the cell (Aardema *et al.,* 1998).

Dyads, triads and pentads occurred due to the second meiotic division restitution. Veilleux *et al.* (1982); Gill *et al.* (1985) and Smith and Murphy (1986) attributed these irregularities to be under genetic control.

Table 10: The percentage of aberrant tetrads and the different kinds of aberrant microspores (Calculated from total tetrads)

Treatment	Control			Sludge treatment		
	(-M)	(+M)	10	20	30	40
Total tetrads (no.)	566		445	837	920	670
Aber./ tetrads	1.17		1.29	1.43	1.20	1.20
Kinds						
No of microspores/ meiocyte						
Two (diad)	0.00	0.60	0.00	0.00	7.39	0.45
Three (triad)	0.00	0.00	0.45	2.03	1.09	0.90
Five (pentad)	0.00	0.00	0.00	0.12	0.10	0.00
Arrangement of microspores						
Overlaping	12.72	17.71	7.87	7.17	12.72	32.24
T-shape	0.00	0.00	0.45	0.00	1.52	0.15
L-Shape	0.00	0.00	0.67	0.00	0.00	0.00
V-Shape	0.00	0.00	0.00	0.60	0.00	0.00
Apple Shape	0.00	0.00	0.00	0.24	0.00	0.00
Linear	0.00	6.64	6.07	2.75	7.17	9.70
Decussate	0.00	0.00	0.45	0.00	0.22	1.19
Structural malformation						
Appendage	0.00	0.00	0.00	0.00	0.10	0.00
Protusion	0.00	0.00	0.00	0.00	0.22	0.15
Elongation	0.00	0.00	0.22	0.12	0.00	0.00

Accordingly, the abnormalities and irregularities of meiotic division, which was induced by sludge treatments, could be attributed to the mutation of several genes governing the division.

Point mutations that are expressed in kernel characters have been used successfully to evaluate the mutagenic properties of various compounds (Brigges, 1966). Generally, both the mineral fertilizer and the biofertilizer increased the percentage of pitted kernels (the used kernels were not pitted). This increase indicated that the two used fertilizers caused either

point mutation or deletions of the dominant gene "Pt" in meiocytes giving a homozygous recessive grain as suggested by Amano and Smith (1965). The presence of minute fragments in the diakinesis and laggards in other stages of meiotic division supports the occurrence of deletions as a cause for pitted kernels.

The present study suggests the reduction of heavy metals from sewage sludge that will be used as biofertilizer, to decrease the rate of gene mutation and chromosomal abnormalities. Since, the availability of sludge nutrients was found to increase by incubation time (Baldoni *et ai,* 1996 & Berti and Jacobs, 1996) it will be advisable to carry further investigations including second- generation productivity.

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التأثيرات السيتوجينية ومحتوى العناصر الثقيلة فى نبات الذرة نتيجة إضافة الحمأة للأرض

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على الرغم من أن إضافة الحمأه الى الأراضى الزراعية لها أهمية بالغة للمحاصيل كمصدر للعناصر الغذائية مثل النيتروجين والفوسفور إلا أنه يجب أن نضع فى إعتبارنا محتواها من العناصر الثقيلة .وقد أجريت تجربة حقلية فى مزرعة كلية الزراعه بأبيس لدراسة تأثير إضافة معدلات مختلفة من الحمأه (صفر، ١٠/٢٠،٣٠،٤٠ طن / فدان) على محصول الذره.

وقد أخذت عينات من نبات الذره لإختبار التغيرات الغير طبيعية كذلك تم تقدير طول النبات والمحتوى الكلوروفيلى للأوراق فى مراحل النمو المختلفة. وعند النضج سجلت بعض الصفات المورفولوجية مثل عدد الكيزان لكل نبات، طول الكوز، وزنه، وزن القولحه ، عدد الحبوب ، حجم ١٠٠ حبه وكثافتها. كذلك تم تحليل الأرض قبل الزراعة وبعد الحصاد لتقدير محتواها من العناصر الكبرى والصغرى والثقيلة. كذلك تم تقدير العناصر الثقيلة فى حبوب الذره. وقد وجد من النتائج أن زيادة معدلات الحماه تؤدى الى زيادة النمو ووزن القولحه والمحتوى الكلوروفيلى الكلى وكلوروفيل أ. ب فى الأوراق وكذلك الرصاص والنيكل فى حبوب الذره وكذلك سجلت كثير من التغيرات الغير طبيعية فى الخلايا. وبوجه عام فإن تركيزات الرصاص ، الكادميوم، والنيكل والكروميوم فى حبوب الذرة زادت نتيجة زيادة الإضافات المختلفة من الحمأه.