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Residual Effect of Sewage Sludge on Soil and Several Yield Parameters of *Zea mays*

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Abstract: A field experiment was conducted to evaluate the effect of using sewage sludge as an organic fertilizer on different yield parameters of maize. Generally, different rates of residual applications of sewage sludge increased heavy metal concentrations in the soil before sowing and after harvesting of *Zea mays*. Leaf and grain contents of heavy metals were affected by sludge addition except for Cd. In general, the residual one addition of sludge decreased heavy metals contents except Pb. While, the residual two additions increased the concentrations of heavy metals except Cu. Sludge treatments did not affect some plant yield parameters. Increased germination percentage and number of ears per treatment were recorded. The dry weight of leaves increased except at 10 and 40 T/F for one residual addition and 20 and 30 T/F for two residual additions. Mature plant height, number of tillers/plant and dry weights of leaves either increased or decreased for different treatments of sludge. The kernel index decreased in all sludge treatments. Sludge treatments affected the M_2 kernel characters of maize, such as inducing yellow kernels, different colored patches in aleurone layer and non-pitted and shrunken kernels. Therefore, the use of sewage sludge as biofertilizer must be applied after pre-treatment to reduce heavy metals in order to decrease the rate of point mutations affecting kernel germination, color and shape and some yield products.

Key words: Sewage sludge, heavy metals, M_2 kernel characters

INTRODUCTION

Applying municipal sewage sludge to agricultural land has been practiced worldwide for several decades. Most communities regularly use land application as a sludge disposal method, benefiting both the wastewater utility and the agricultural producers. However, because of new regulations governing the application of sludge to land, many questions are being asked by farmers, utility managers, government officials and consultants about this alternative.

While plant nutrients and organic matter are essential to plants, application of excessive rate of nitrogen and phosphorus could contaminate surface and groundwater or interfere with good crop growth when applied above the agronomic rate. Sludge also may contain metals and some other constituents that can be harmful to humans if total accumulated application is not managed properly (William Eberle *et al*., 1994).

Application of sewage sludge to agricultural land presents an opportunity for recovery of essential plant nutrients (Liu *et al*., 2008). Many waste products contain concentrations of plant

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nutrients elements sufficient to produce an agriculturally significant growth response (Bierman and Rosen, 1994), but recycling waste materials through agriculture systems require evaluation of both the agronomic benefits and causes broader environmental consequences.

The reuse of nutrients and organic matter in wastewater sludge via., land application is a desirable goal. However, excessive concentrations of non-essential metals derived from sewage sludge result in phytotoxicity (Berti and Jacobs, 1996). In addition repeated applications of heavy metal-contaminated sewage sludge can result in an accumulation of toxic metals in the soil and can cause potential problems such as phytotoxicity (Richards *et al*., 1998).

Exposure to excess Cu can damage cells and organs (Koppenol, 1994). Other than that, excessive amounts can result in acute damage to the cell membrane and leakage of internal enzymes leading to loss of cell integrity and thereby, cell death (Linder, 2001). Accumulation will occur in the mitochondrial matrix and this is accompanied by dramatic morphological changes (Goldfischer *et al*., 1980). Plants have been the primary choice when it comes to studying cytotoxic and mutagenic effects of metals and can provide a good database for studies related to environmental monitoring (Grant and Owens, 2001). Zhang and Yang (1994) found that the frequencies of chromosomal aberrations increased significantly in plants exposed to different concentrations of Cd. They reported Cd penetrated into the cells inducing physiological and genetically damages when they were exposed to Cd for 24 h. They also mentioned that Cd inhibited cell division and altered the chromosomes.

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 processes for proper treatment and usage of sludge (Show and Chadwick, 1999).

The aims of the present study are to asses the phytotoxic and genetic effects of the treated soil with sewage sludge as organic-fertilizer for *Zea mays*. This evaluation is based on: (1) the assessment of the effects of residual one and two sludge additions on the heavy metal content in the soil and (2) the assessment of the effect of one and two residual sludge additions on the heavy metal content in leaves and M_2 grains and several plant yield parameters.

MATERIALS AND METHODS

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

In March 1999, sewage sludge was amended to the soil at the rates of 0, 10, 20, 30 and 40 T/F and was planted with *Zea*. After harvesting, the plot was divided into two subplots; one received a second sludge addition at the previous rates while the other was left without further addition and was planted with beans. In July 2000, these plots were planted with *Zea mays* variety H320, characterized by white kernels, to determine the effects of residual one and two additions.

Surface soil samples were collected and analyzed for heavy metals using Atomic Absorption Spectrophotometer Soltanpour and Schwab (1977). Seven week leaves and mature grains of *Zea mays* were collected and oven dried at 70°C, homogenized, wet digested using concentrated sulfuric acid and hydrogen peroxide (FAO, 1980) and finally analyzed for heavy metals using spectrophotometry. After 4 weeks of planting, percentage of germinated plants and mean of plant height per treatment were determined. At maturity, seven morphological characters were recorded; height, ear length, total grain number, kernel color (white or yellow), kernel shape (pitted or non pitted) and kernel index (weight of 100 kernel). Seed density was calculated by dividing the weight of 10 seeds by its volume (Kharkwal and Chaudhary, 1997). Statistical analysis of data was carried out by applying the analysis of variance and least significant differences test, using COSTAT program.

RESULTS

Effect of Sludge Treatments on Heavy Metal Content in Soil

Soil treated with one and two residual additions showed a significant increase in heavy metals with increasing sludge application before cultivation and after harvesting (Table 1). However, Cd could not be detected at 10 and 20 T/F for one residual addition and Pb did not respond significantly to sludge additions in several applications.

Effect of Sludge Treatments on Heavy Metal Content in Plant

Table 2 showed that the Cu concentrations in leaves and grains were reduced by sludge additions. However, significant increase of Zn concentrations in leaves and grains occurred in several sludge applications. Pb concentration in leaves and grains fluctuated with increased residual sewage sludge additions in comparison with the control.

The Effect of Residual Sludge Additions on Germination, Morphological Characters and M_1 Productivity

Table 3 shows the effects of residual sludge treatments on germination after 30 days compared to the control. The mean percentage of one residual treatment (55%) was higher than that of two additions (51.87%). The mean number of ears per treatment increased in all sludge treatments except 10 T/F, while it's significant decreased in 30 T/F, while the kernel index decreased. The dry weight of leaves was increased or decreased.

M_2 kernel characters were found to be yellow color instead of white after sludge treatments (Table 4). Sludge treatments induced a significant decrease in the percentage of yellow colored kernels except that of 20 and 30 T/F application treatments, which recorded about 2 and 1.5 times, respectively higher or lower to that of the control. By longitudinal sectioning, it was found that the

Table 1: Heavy metal concentrations in the sludge amended soil before sowing and after harvesting using one and two residual additions

	Treatments (T/F)				
Heavy metals	Control	10	20	30	40
Before sowing (mg kg^{-1})					
(One residual addition)					
Cu	9.62a	10.41a	10.77a	10.19a	10.53a
Zn	3.47b	5.09ab	4.22b	5.33ab	7.01a
Cd	0.17c	0.00c	0.36a	0.01c	0.20b
Pb	4.12a	4.24a	4.40a	4.34a	5.03a
Two residual additions					
Cu	9.62ab	9.32ab	11.35a	7.57b	10.58a
Zn	3.47b	4.02b	9.61a	11.39a	10.74a
Cd	0.17c	0.04c	0.32b	0.52a	0.42ab
Pb	4.12a	4.26a	5.60a	4.80a	4.23a
After harvesting (mg kg^{-1})					
(One residual addition)					
Cu	9.27a	9.58a	10.49a	10.38a	10.59a
Zn	2.82c	3.79b	3.07c	4.87a	5.78a
Cd	0.35b	0.00c	0.00c	0.38b	0.54a
Pb	3.18a	3.56a	4.09a	4.63a	4.09a
Two residual additions					
Cu	9.27a	9.21a	10.04a	10.11a	10.23a
Zn	2.82c	2.02c	3.07c	4.11b	6.35a
Cd	0.35b	0.40b	0.56a	0.62a	0.64a
Pb	3.18bc	3.68bc	5.01ab	5.43a	4.37ab

Mean values within a row followed by the same letter(s) are not significantly different at 5% level according to Duncan´s multiple range test

Table 2: Heavy metal concentrations in leaves and grains of untreated and treated *Zea mays* plants using one and two residual additions

	Treatments (T/F)				
Heavy metals	Control	10	20	30	40
Leaves (mg kg^{-1})					
(One residual addition)					
Cu	6.18a	3.87b	6.34a	2.90b	3.22b
Zn	21.72ab	21.08ab	23.10a	20.28ab	17.18b
Cd	0.00b	0.00b	0.00b	3.10a	0.00b
Pb	2.50b	0.00c	5.00a	6.00a	3.00b
Two residual additions					
Cu	7.10a	5.07a	4.99a	3.59b	5.07a
Zn	21.72c	22.13c	20.91c	25.00b	31.05a
Cd	nd	nd	nd	nd	nd
Pb	2.50b	3.00b	5.00a	4.00a	1.00c
Grains (mg kg^{-1})					
(One residual addition)					
Cu	1.20a	1.00b	0.60c	0.00d	0.00d
Zn	17.54a	13.92b	17.48a	9.24c	13.60b
Cd	nd	nd	nd	nd	nd
Pb	4.00d	11.00a	9.00b	2.00e	6.00c
Two residual additions					
Cu	1.20a	0.00c	1.40a	0.40b	0.00c
Zn	17.54b	21.14a	10.26c	17.03b	18.09b
Cd	nd	nd	nd	nd	nd
Pb	4.00a	2.00b	1.00b	5.00a	4.00a

Mean values within a row followed by the same letter(s) are not significantly different at 5% level according to Duncan´s multiple range test, nd: Not differentiate

Table 3: Effect of different rates of sludge treatments on *Zea mays* germination and plant height after 30 days and plant productivity at maturity

	After 30 days		At maturity							
Treatments (T/F)	Germination (%)	Plant height (cm)	Plant height (cm)	Ears/ treat. (No.)	Ear length (cm)	DW of leaves (g)	Rows/ ear (No.)	Kernel/ ear (No.)	Kernel index (g)	Kernel density (g cm^{-3})
Control	43.9ab	34.0a	162.a	33.3c	17.8a	10.7c	12.7a	448.0a	26.8ab	1.03a
One residual addition										
10	68.9a	35.7 a	137.4a	33.3c	16.0a	7.8d	12.3a	469.7a	22.3c	1.02a
20	56.7a	40.3a	162.7a	38.9bc	16.3a	11.9c	12.8a	467.0a	21.8c	0.93a
30	58.9ab	36.3a	153.2a	43.3ab	16.7a	14.3a	11.5a	407.5a	10.9d	1.10a
40	35.5b	37.1a	147.7a	44.4ab	16.8a	10.4c	12.3a	430.7a	20.8c	0.97a
Two residual additions										
10	55.6ab	33.2a	153.8a	33.8c	15.9a	13.0ab	11.3a	354a	29.3a	1.00a
20	51.1ab	33.9a	146.2a	50.0a	16.4a	5.8e	12.3a	451.0a	22.8bc	1.00a
30	-	-	135.a	22.2d	17.5a	7.4d	12.7a	485.0a	24.8bc	0.92a
40	48.9ab	32.6a	144.a	47.8a	17.6a	13.8a	12.0a	453.0a	21.5c	0.93a

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

endosperm color displayed yellow kernel coloration, while the aleurone layer demonstrated purple, red or brown colors individually or in combination in the same kernel. Residual treatment of 10 T/F resulted in 7.33% of blotched kernels while at 40 T/F it was increased three fold (23%). However, the different rates of the two residual addition treatments induced appearance of one type of blotched kernels.

In addition, kernel shape differed according to presence of (pitted and non-pitted kernels) (Table 4). Non-pitted kernels increased significantly when applied with both 10 and 30 T/F of one residual and 10 and 20 T/F of two residual treatments.

Table 4: Effect of different rates of sludge treatments on M_2 kernel characters of *Zea mays* grown in sludge amended soil

	Kernel color (%)								
	Endosperm		Blotches Aleurone					Kernel shape (%)	
Treatments (T/F)	White	Yellow	Purple	Brown	Red	Red and brown	Purple and brown	Non-pitted	Shrunken
Control	95.15a	4.50c	0.00	0.00	0.00	0.00	0.00	24.10ab	0.35
One residual addition									
10	98.47a	1.20de	3.60	2.48	0.16	1.09	0.00	39.30ab	0.00
20	98.10a	1.90d	0.00	0.00	0.00	0.00	0.00	22.30ab	0.00
30	96.40a	3.60c	0.00	0.00	0.00	0.00	0.00	26.40ab	0.00
40	99.30a	0.60de	21.43	0.24	0.18	0.00	0.00	13.70b	1.56
Two residual additions									
10	91.04a	1.30de	7.66	0.00	0.00	0.00	0.00	33.30ab	2.51
20	84.88a	9.60a	5.52	0.00	0.00	0.00	0.00	48.70a	0.00
30	93.00a	6.60b	0.00	0.00	0.40	0.00	0.00	22.10ab	0.00
40	99.69a	0.04e	0.00	0.27	0.00	0.00	0.00	23.10ab	0.27

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

M_2 shrunken kernels increased by 40 T/F of the one and 10 T/F of the two residual additions. The effect of the latter treatments was higher than the former (31.80 and 25.38, respectively).

The control was found to have 4.5% yellow kernels (Table 4). The percentage of yellow colored kernels was significantly decreased after one residual sludge treatments. However, 20 and 30 T/F two residual applications treatments recorded an increase of about 2 and 1.5 times, respectively to that of the control. By longitudinal sectioning, it was found that the yellow kernel coloration were demonstrated in the endosperm; while the purple, red and brown colors were demonstrated in the aleurone layer of kernel. Sludge residual treatments caused the appearance of different colored patches in aleurone layer represented as individual purple, brown and red or combined in the same kernel. In case of the one residual treatment, 10 T/F caused 7.33% of blotched kernels while 40 T/F increased this percentage to about three times (23%). However, the two lowest rates of both residual additions treatments induced the presence of only the purple or the brown type of blotched kernels.

DISCUSSION

The results of the present study demonstrated that residual sewage sludge had a significant effect on the phenotype and genetics of M_1 morphological characters and productivity. M_2 kernel characters, on the other hand, represent the direct effect on the genetic material.

Effect of Residual Sludge Treatments on Heavy Metal Content of Soil

Before sowing the decrease of Cu after one residual addition may be due to downward movement of residual Cu with time (Darwish and Ahmed, 1997). The increase of Zn may be due to its high concentration in sewage sludge releasing it in the available form (McGrath *et al*., 2000). Also, the significant increase of Cd at sludge rates 20 and 40 T/F using residual one addition may be due to soluble complexes of organic matter (Dunnivant *et al*., 1992). At the lowest sludge concentration of the second addition, the reduction of Cd concentration may be due to the downward movement or its original low concentrations. Pb concentrations did not respond significantly to sludge additions in some applications.

Effect of Residual Sludge Treatments on Heavy Metal Content in Plant

The reduced Cu concentrations in leaves and grains after sludge treatments indicate that the increase of Cu concentration in soil in the presence of organic matter is not translated to higher uptake by plants (Buridge and Berrow, 1984; Labrecque *et al.*, 1995; Tiffany *et al.*, 2000). This may be due to presence of organic matter in the soil and the high affinity of Cu to form Cu-organo complex (Aduayi, 1973). However, the significant increase of Zn concentrations in leaves and grains by applications of sludge rates was in agreement with Martinez *et al.* (2003). This may be due to cumulative sludge load of Zn which complexes with dissolved organic matter releasing it in the available form (McGrath *et al.*, 2000; Antoinadis and Alloway, 2002). Herein, Pb concentration in leaves and grains was influenced by sewage sludge concentration. This agrees with Martinez *et al.* (2003) but Chaney (1988) found that sludge concentration of Pb did not raise Pb plant concentration unless it is very high.

The Effect of Residual Sludge Addition on Germination, Morphological Characters and M_1 Productivity

The germination percentage after 30 days increased through sludge treatment. Iqbal *et al.* (2007) observed that soil amended with residential sludge (1:1) provided better field germination in degraded soil. Howell (1998) recorded that Abscisic acid (ABA) appears to play a major role in controlling kernel germination through vp5 and 7 genes. Therefore, sewage sludge treatments may affect the action of vp5 and 7 genes, followed by the stimulation of ABA synthesis. However, this is not the case using higher sludge rates where reduced germination percentage occurred.

In the present study, the total number of ears per treatment, the dry weight of leaves, kernels per ear, kernel volume and kernel index were affected in all residual treatments. Yield products are known to be controlled by Gibberilic Acid (GA) genes which are expressed from germination to maturity. Howell (1998) found that gibberilic-deficient mutants would affect a subset of gene products within the plant (transcription rate, mRNA stability, or increased efficiency of translation of certain mRNAs). Thus, in the present study, it can be suggested that residual heavy metal in sludge amended soil induced gibberilic-deficient mutants affecting some yield parameters.

Point mutations that are expressed in the kernel characters have been used successfully to evaluate the mutagenic properties of various components. Brigges (1966) stated that there are several mutants which appear after the development of carotenoid pigment in *Zea* endosperm. Prasanna and Sarkar (1995) listed 13 independent recessive mutants that were responsible for yellow (Y gene) or white (y gene) endosperm. If one mutation event of the recessive y gene is reverted to the dominant Y gene, the triploid endosperm will be yellow (Yyy). Thus, in the present study, the significant decrease in the percentage of yellow colored M_2 kernels by residual sludge treatments may be due to the reversion of the y to Y gene.

Many genes are involved in the production of pigment in the kernel aleurone layer in maize: Pr gene gives purple while its allele pr gives red color, Bn_1 or bn_2 control formation of brown color in genotypes capable of pigmentation. Four basic pigment genes, A_1, A_2, C and R are necessary to develop the pigment. A and R genes, C control aleurone color. Different alleles of R are responsible for color patterns (Brigges, 1966). The genotype AcR develops patches of color in the presence of the dominant blotched gene Bh. This may be due to Bh-gene induced mutations of c to C. According to Coe (1962), the C locus alleles in the triploid aleurone tissue of maize Ccc cause colorless kernel to have very infrequent small patches of color. Therefore, it can be concluded that the sludge treatments, which induced colored patched kernels, caused the mutation of dominant C allele to the recessive one c in the presence of the dominant Bh gene. In addition, these treatments caused mutations to produce the Pr gene to give purple color; pr gene to red and Bn_1 or bn_2 for brown color.

Kernel shape, in the present study, was either pitted or non-pitted, (pitted genotype is pt pt pt, Sheridan and Neuffer, 1982). The increase or decrease of non-pitted kernels from that of the spontaneous rate observed in the control means that sludge treatments enhanced the reversion of the pt gene to its dominant Pt state and vice versa. The increase in percentage of shrunken endosperm after some sludge treatment indicates the occurrence of gene mutation form Sh to its recessive state sh causing endosperm collapse during the drying stage at maturity, giving a smooth indentation at the crown (Neuffer and Sheridan, 1980; Sheridan and Neuffer, 1982). This mutation inhibits ADP-glucose phosphorylate required for starch biosynthesis (Bhave *et al*., 1990).

In conclusion, residual sewage sludge treatments may affect the genes responsible for some yield parameters and kernel characters, probably due to the presence of residual (accumulated) heavy metals which were not leached out from the growing zone of *Zea* roots. Thus, there is it is absolutely necessary to lower heavy metals content from the used sewage sludge as biofertilizer to keep soils clean.

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