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RESIDUAL EFFECT OF SEWAGE SLUDGE APPLICATION ON SOIL AND GENES OF SOMEYIELD PARAMETERS OF *ZEA MAYS*

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ABSTRACT

Disposal of sewage sludge by soil application has been practiced as resource utilization. However, such practice may be benefit to agricultural land, growing concern has been expressed about the potential of specific hazard may be associated with sewage sludge application to agricultural land.

A field experiment was conducted to evaluate the effect of using sewage sludge as organic fertilizer on different yield parameters of maize. Generally, different rates of residual applications of sewage sludge increased heavy metals concentrations in the soil before sowing and after harvesting of *Zea mays*. Leaves and grains 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 for Pb. While, the residual two additions increased the concentrations of heavy metals except for Cu. Sludge treatments did not affect some plant yield parameters. However, increasing the germination percentage and number of ears per treatment were recorded. Also, the dry weight of leaves increased except at 10 and 40 T/F for residual one addition and 20 and 30 T/F for residual two additions. Indeed, affected mature plant height and number of tillers/plant increased or decreased at different treatments of sludge. The seed index and fresh and dry weights of shoots and roots were increased.

Sludge treatments affected the M_2 kernel characters of maize, such as inducing yellow kernels, different colored patches in aleurone layer, non pitted and shrunken kernels.

INTRODUCTION

Application of sewage sludge to agricultural land presents an opportunity for recovery of essential plant nutrients. Many waste products contain concentrations of plant nutrients elements sufficient to produce an agriculturally significant growth response, but recycling waste materials through agriculture systems require evaluation of both the agronomic benefits and 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. Also, repeated applications of heavy metal-contaminated sewage sludge can result in an accumulation in 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. In addition, 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 is at least partly in the mitochondrial matrix and is accompanied by dramatic morphological changes [**Goldfischer** ***et al.*****, (1980)**]. 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) and Zhang & Yang (1994)**] found that the frequencies of chromosomal aberrations increased significantly in plants exposed to different concentrations of Cd. They reported that in plants exposed to cadmium for 24 h the Cd penetrated into the cells inducing physiological and genetically damages. 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 process for the proper treatment and use of sludge [**Show & Chadwick (1999)**].

The aims of the present study are to qualify the phytotoxic and genetic effects of the treated soil with sewage sludge as organic-fertilizer for *Zea mays*. This evaluation is based on: I- The assessment of the effect of residual one and two sludge additions on the heavy metals content in the soil. II- The assessment of the effect of residual one and two sludge additions on the heavy metals content in leaves and M_2 grains, and some plant yield parameters.

MATERIALS AND METHODS

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

On 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 corn. 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 were planted with beans. On July 2000, *Zea mays* (var. H320), of the present study, was planted after harvesting beans without any further addition of sludge to compare the effect of one and two residual additions.

Surface soil samples were collected and analyzed for heavy metals using Atomic Absorption Spectrophotometer **[Soltanpour & Schwab (1977)]**. Seven weeks leaves and mature grains of *Zea mays* were collected and analyzed for heavy metals. After 4 weeks of planting, percentage of germinated plants and mean of plant height per treatment were determined. At maturity, seven 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 & Chaudhary (1997)]**.

RESULTS AND DISCUSSIONS

1-Effect of sludge treatments on heavy metals content in soil

Using residual one and two additions, soil heavy metals increased significantly with increasing sludge application rates before cultivation and after harvesting (Table 1). Before sowing the increase of Cu after residual one addition may be due to downward movement of residual Cu with time **[Darwish & Ahmed (1997)]**. While, the second sludge addition raised the percent of organic matter in the soil beside the high affinity of Cu to form Cu-organo complex. The increase of Zn may be due to its high concentration in sewage sludge amended soil which degraded slowly with time and releasing it in available form **[McGrath *et al.*, (2000)]**. Also, the significantly increase of Cd to high percent at sludge rates 20 T/F and 40T/F using residual one addition may be due to composing soluble complexes with organic matter **[Dunnivant *et al.*, (1992)]**. At sludge rate 10 T/F of the second addition, the concentrations of Cd was reduced in comparison with control because of rising the percent of $CaCO_3$ in the soil and consequently precipitate the Cd **[McGrath *et al.*, (2000)]**.

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

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

Means within a row followed by the same letter are non-significantly different at 5% level according to Duncan's multiple range test.

After harvesting heavy metals increased significantly with increasing sludge rates the concentration of Cd could not be detected at 10 and 20 T/F using residual one addition. This could be due to that residual Cd precipitated with $CaCO_3$ or complexed with organic matter forming organic and carbonate residual phases [**McGrath** ***et al.*****, (2000)]**. Pb did not respond significantly to sludge additions in some applications. This may be due to the high content of organic matter in sewage sludge amended soil which chelated the metals and reduced the available form [**Brown** ***et al.*****, (1999)]**.

3-Effect of sludge treatments on heavy metals content in plant

Table (2) showed that Cu concentrations in leaves and grains were reduced by sludge treatments either before sowing or after harvesting. This confirms that the increase of Cu concentration in soil in the presence of organic matter does not translate to higher Cu uptake by plants [**Labrecque** ***et al.*****, (1995) and Tiffany** ***et al.*****, (2000)]**. However, the significant increase of Zn concentrations in leaves and grains by many applications of sludge rates was agree with [**Martinez** ***et al.*** **(2003)]**. This may be due to cumulative sludge load of Zn which complexes with dissolved organic matter [**Antoinadis & Alloway (2002)]**. Pb concentration in leaves and grains was influenced by increasing sewage sludge additions rates. [**Chaney (1988)]** found that concentration of Pb from sludge does not raise plant concentration unless it is very high. However, the concentration of Pb was declined to 100% at 10 T/F in leaves in comparison with control value. This may be due to translocation of Pb from leaves to grains

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

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

Means within a row followed by the same letter are non-significantly different at 5i % level according to Duncan's multiple range test .nd : not detected

4- The Effect of sludge addition on germination, morphological characters and M_1 productivity.

Table (3) represented that germination percentage after 30 days old increased by sludge treatment. However, the mean effect of residual one treatments (55.09%) was more than the two additions treatments (51.59%). The total number of ears per treatment, the dry weight of leaves and kernel index were affected by the increase or decrease either by one or two residual treatments.

Howell (1998) recorded that abscisic acid (ABA) appears to play a major role in controlling kernel germination. Therefore, sewage sludge treatments may affect the action of vp5 and vp7 genes, followed by the stimulation of ABA synthesis except the residual treatment of 40 T/F which inhibit its synthesis and so, reduced germination percentage. However, yield products are controlled by gibberellic acid (GA). *Gibberellic- deficient mutants* are expressed from germination to maturity, by inducing changes in the expression of a subset of gene products within the plants. This may be due to changes in transcription rate, mRNA stability, or increased efficiency of translation of certain mRNAs. Thus, it seems likely that increased and decreased transcription of certain genes will be one of the mechanisms involved in GA action during stem elongation. So, it was concluded that *gibberellic-deficient mutants* were expressed only by genes responsible for number of ears/ plant, dry weight of leaves, number of kernels per ear, kernel volume and kernel index.

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

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

Means within a column followed by the same letter are non-significantly different at 5% level according to Duncan's multiple range test. – missed date

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 two applications treatments, which recorded about 2 and 1.5 times respectively that of the control. The kernel- color genes includes aleurone, pericarp, scutellum and endosperm colors. By longitudinal sectioning, it was found that the endosperm color leads to yellow kernel coloration, while the purple, red, brown colors over demonstrated in aleurone layer. Sludge treatments caused appearance of different colored patches in aleurone layer represented as purple, brown, and red unique or combined in the same kernel. In case of the residual treatment, 10 T/F caused 7.33% of blotches kernels while 40 T/F increased this percentage to about three times (23%). However, the different rates of two residual additions treatment induced presence of one type of blotched kernels, with the highest values occurred by the two lowest rates.

In addition, kernel shape differs according to presence of pitted and non pitted kernels Table (4). Non pitted kernels were increased significantly by sludge treatments of both 10 and 30 T/F of one residual treatments and 10 and 20 T/F of the other treatment.

Table (4): Effect of different rates of sludge treatments on M_2 Kernel characters of *Zea mays* grown in sludge amended soil.

Treatment (T/F)	Kernel color (%)							Kernel shape (%)	
	Endosperm		Blotches Aleurone						
	White	Yellow	Purple	Brown	Red	Red & Brown	Purple & Brown	Non Pitted	Shrunken
Control	95.15a	4.50 c	0.00	0.00	0.00	0.00	0.00	24.10ab	0.35
Residual one addition									
10	98.47a	1.20de	3.60	2.48	0.16	1.09	0.00	39,30ab	0.00
20	98.10a	1.90 d	0.00	0.00	0.00	0.00	0.00	22.30ab	0.00
30	96.40a	3.60 c	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	13.70 b	1.56
Cumulative two additions									
10	91.04a	1.30de	7.66	0.00	0.00	0.00	0.00	33.30ab	2.51
20	84.88a	9.60 a	5.52	0.00	0.00	0.00	0.00	48.70 a	0.00
30	93.00a	6.60 b	0.00	0.00	0.40	0.00	0.00	22.10ab	0.00
40	99.69a	0.04 e	0.00	0.27	0.00	0.00	0.00	23.10ab	0.27

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

M_2 shrunken kernels occurred as endosperm collapses during drying stage at maturity, giving a smooth indentation at the crown. They increased by 40 T/F of the residual one addition and 10 T/F of residual two additions. The effect of residual two additions treatments was higher than the other (31.80 and 25.38, respectively).

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 caretenoid pigment in *Zea* endosperm. **[Prasanna & Sarkar (1995)]** listed 13 independent recessive mutants that were responsible for yellow (Y gene) or white (y gene) endosperm,

In the present study, if one mutation event of the recessive "y" gene is reverted to the dominant "Y" gene either spontaneously- incase of the control- or induced by sludge treatment, the triploid endosperm will be yellow (Yyy). Accordingly, the rate of reversion of the "y" gene was lower or higher than the control after sludge treatment.

Many genes in maize are involved in the production of pigment in the aleurone, Pr gene gives purple and pr gives red color in genotypes capable of pigmentation. Either Bn_1 or bn_2 control formation of brown color. Four basic pigment genes A_1, A_2, C and R are necessary to develop the pigment with A and R genes, C produces aleurone color. C' allele inhibits expression different alleles of R are responsible for alurone color, inhibition is not always complete. Different alleles of R are responsible for color patterns **[Brigges (1966)]**. In the genotype A, c, R, pigment develops patches of color in the presence of dominate blotched (Bh). This may be due to Bh- induced mutations of c to C. According to **[Coe (1962)]**, the C locus alleles in the triploid aleurone tissue of maize Ccc cause color less kernel very infrequent small patches of color. Therefore, it can be concluded that the sludge treatments, which induced colored patched kernels, may mutate the dominant C allele to the recessive one (c) by occurring the Bh gene. In addition, these treatments caused mutation to produce the Pr gene to give purple color, pr gene to red and Bn_1 or bn_2 for brown.

Owing to kernel shape, the kernel of the present study was pitted with genotype pt **[Sheridan & Neuffer (1982)]**. The increase or decrease rates of non-pitted kernels from the spontaneous rate found in the control means that sludge treatments enhanced the reversion of the "pt" gene to the dominant Pt gene with different rates. In the meantime, the increase of the percentage of shrunken endosperm after some sludge treatments indicates the occurrence of gene mutation form Sh to sh **[Neuffer & Sheridan (1980) and Sheridan & Neuffer (1982)]**, which has an effect on endosperm development, leading to starch deficiency mutation in kernels inhibiting ADP- glucose phosphorylase required for starch biosynthesis **[Bhave *et al.*, (1990)]**.

In conclusion, sewage sludge treatments may affect the genes responsible for some yield products (germination, plant stature, ear length, number of ears/ plant, number of kernels per ear, kernel volume and kernel index and dry weight of leaves). In addition, point mutations appeared that are expressed in the kernel characters (color, endosperm, aleurone, and shape either pitted and non pitted; shrunken or full).

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الملخص العربي

معاملة الأرض بالحمأة فى الوقت الحالى يعتبر من طرق تصريف هذه المخلفــات وذلــك للإستفادة منها. حيث أنها مفيدة للأراضى الزراعية لغناها بالعناصر والمادة العضوية. ولكنها تحتوى على العناصر الثقيلة والتى تسبب تأثير سام على النبات.

تم إجراء دراسة حقلية فى مزرعة كلية الزراعة – جامعة الإسكندرية أثنــاء الفتــرة مــن (١٩٩٩ – ٢٠٠١) وذلك لتقويم تأثير استخدام الحمأة كسماد عضوى على مختلف عناصــر الإنتــاج لنبات الذرة. قبل بداية التجربة تحت الدراسة تم خلط الحمأة بالتربة بمعــدلات ٠ ، ١٠ ، ٢٠ ، ٣٠ ، ٤٠ طن/فدان وزرعت بنبات الذرة . وبعد الحصاد تم تقسيم قطعة الأرض إلى شريحتان الشريحة الأولى تم إضافة ثانية لها من الحمأة بنفس المعدلات السابقة بينما الشريحة الأخرى تركت بدون أى إضافات ثم زرعت بنبات الفول. وفى شهر يوليو (٢٠٠٠) تم زراعة الذرة (Hybrid 320) تحت الدراسة بعد حصاد الفول بدون أى إضافات أخرى وذلك لمقارنة التأثير المتبقى لإضافة واحدة أوإضــافتان مــن الحمأة.

بصفة عامة فإن المعدلات المختلفة من الإضافة الواحدة والإضافات المتكررة المتبقية مــن المعاملة بالحمأة أدت إلى زيادة تركيز العناصر الثقيلة فى الأرض قبل الزراعة وبعد الحصاد لنبــات الذرة. أيضا محتوى الأرض من العناصر الثقيلة ازداد نتيجة المعاملات المتكررة من الحمأة وذلك عند مقارنتها بالإضافة الواحدة المتبقية قبل زراعة الذرة.

محتوى أوراق وحبوب نبات الذرة من العناصر الثقيلة تأثر بإضافة الحمأة ما عدا عنــصر الكادميوم. وبصفة عامة فإن الإضافة الواحدة المتبقية من الحمأة أدت إلى انخفاض محتوى العناصــر الثقيلة ما عدا عنصر الرصاص بينما المعاملات المتكررة من الحمأة أدت إلى زيادة محتوى العناصر الثقيلة ما عدا عنصر النحاس.

المعاملة بالحمأة لم تؤثر على بعض عوامل الإنتاج لنبات الذرة ولكنها أدت إلى زيادة نسبة الإنبات والعدد الكلى للكيزان لكل معاملة. أيضا أدت إلى زيادة الوزن الجــاف لــلأوراق مــا عــدا المعاملات ١٠، ٤٠ طن/فدان للإضافة الواحدة و ٢٠، ٣٠ طن/فدان للإضافتان.

أثرت المعاملة بالحمأة على خصائص حبوب الجيل الثانى لنبات الذرة حيث المعاملة بالحمأة أدت إلى ظهور حبوب ذات لون أصفر، وحبوب ذات تبقعات مختلفة الألوان فــى طبقــة الأليــرون، وحبوب غير منقورة وحبوب منكمشة فى بعض المعاملات.