# RESIDUAL AND ACCUMULATION EFFECT OF SEWAGE SLUDGE APPLICATIONS ON METAL CONTENT IN SOIL AND GENETIC CHARACTERS OF *Zea mays.*

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***Received on: 16/4/2007*** ***Accepted on: 13/5/2007***


## 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 that may be associated with sewage sludge application to agricultural land.*

*Field experiments were conducted to evaluate the effect of using sewage sludge as organic fertilizer on different yield characters of Zea mays.*

*Different rates of residual and repeated application 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 excepted for Cd. The residual one addition of sludge decreased heavy metals contents except for Pb. While, the repeated applications of sewage sludge increased the concentrations of heavy metals except for Cu.*

*Sludge treatments did not affect some plant yield parameters of maize. 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 ton/Fed for residual and 20 and 30 ton/Fed for cumulative 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 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 requires 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 occurred 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). Zhang and 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 and Chadwick, 1999).

The aims of the present study were 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 residual and cumulative effect of sludge additions on the heavy metals content in the soil. II- The assessment of residual and cumulative effect of sludge additions on the heavy metals content in leaves and $M_2$ grains, and plant yield.

## MATERIALS AND METHODS

### 1. Site Description and Cultural Practices

Field experiments were conducted at site No. (4) in the Agricultural College Farm, University of Alexandria at Abis sector, Alexandria Egypt during the period 1999 - 2001. Soil samples were collected (0- 20) cm, air dried, ground, passed through 2-mm sieve and stored in polyethylene bags for analysis. The main physical and chemical characteristics of the soil were determined according to Page et al. (1982) and are presented in Table (1).

Composted sewage sludge was collected from Site 9N, Alexandria General Organization of Sanitary Drainage (AGOSD) of Alexandria city in February,

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1999. A composite sample of sewage sludge was air dried, ground, passed through 2-mm sieve for chemical analysis according to the methods outlined in Page *et al.* (1982). The chemical properties of sludge are listed in Table (2).

A Split plot design was used and each plot had six rows. The experimental plot unit had an area of 20 $m^2$. On March, 1999 sewage sludge was applied and mixed within the upper 20 cm soil layer using five rates: 0, 10, 20, 30, and 40 ton/Fed. *Zea mays* was cultivated in this season.

After Zea mays harvest, the 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 in order to compare between the residual and accumulation effect on *Zea mays* Var. Hybrid 320 under study which grown on July, 2000.

**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 $mgkg^{-1}$ | 33.60 | Cr mg $kg^{-1}$ | 0.15 |

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

**Table 2. Some chemical characteristics of the sludge used.**

| Sludge charact. | Value | AB-DTPA | |
|---|---|---|---|
| EC, $dSm^{-1}$ | 5.05 | Zn mg $kg^{-1}$ | 812.00 |
| pH | 7.20 | Mn mg $kg^{-1}$ | 160.00 |
| $CaCO_3$, % | 10.20 | Cu mg $kg^{-1}$ | 475.00 |
| OM., g $kg^{-1}$ | 435.00 | Pb mg $kg^{-1}$ | 170.30 |
| TKN-N, g $kg^{-1}$ | 21.60 | Cd mg $kg^{-1}$ | 8.05 |
| TP, g $kg^{-1}$ | 5.40 | Ni mg $kg^{-1}$ | 108.04 |
| TK, g $kg^{-1}$ | 1.60 | Cr mg $kg^{-1}$ | 118.06 |
| TFe, g $kg^{-1}$ | 160.80 | | |

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

**2-Sampling and analysis**

**A- Soils**

Samples from the upper soil layer (0-20cm)were collected from each plot, just before cultivation and after harvesting. The collected samples were air-dried, ground, passed through 2 mm sieve and stored in polyethylene bags for analyses.

Heavy metals were extracted from soil according to Soltanpour and Schwab (1977) with AB-DTPA (Ammoniun Bicarbonate-diethylene triamine-pentacetic acid) and the concentrations of heavy metals were measured using Atomic Absorption Spectrophotometer ( Parken Elmer 3300).

**B- Plants**

Seven weeks old leaves and mature grains of *Zea mays* were collected, washed by tap water, then distilled water and oven dried at 70°C for 48 hrs and ground finely for analysis. The oven dried plant was digested using concentrated sulfuric acid and hydrogen peroxide (FAO, 1980) and the concentrations of heavy metals were measured using Atomic Absorption Spectrophotometer (Parken Elmer 3300).

**Morphological Measurements**

After 4 weeks of planting, percentage of germinated plants from 30 grains and mean height of 9 plants per treatment were determined. At maturity of sowing, seven characters were recorded using 6 replicates for each treatment: Plant height, ear length, total grain number, kernel color (white or yellow), kernel shape (pitted or non pitted), kernel index (weight of 100 kernel), and kernels density. Kernel density was calculated according to (Kharkwal and Chaudhary, 1997).

## RESULTS AND DISCUSSION

**1-Effect of Sludge Treatments on Heavy Metals Content in Soil**

**A- Residual effect of one addition**

Data in Table 1 represented the concentrations of residual heavy metals after one addition of sewage sludge. The results indicated that extractable Cu increased insignificantly with increasing sludge rates. This may be due to downward movement of residual Cu with time as reported by Darmody et al. (1983), and Darwish and Ahmed, (1997). On the other side, the amounts of extractable-Zn (Table 3) was increased significantly with increasing sludge rates to 53.6 and 102 % at sludge rates 30 and 40 ton/Fed, respectively relative to the control. This may be due to high concentration of Zn in sewage sludge amended soil and slowly degraded bound Zn which is released in available form as concluded by McGrath et al. (2000).

Extractable-Cd (Table 3) increased with sludge application significantly to 52.9% and 17.6 % at sludge rates 20 ton/Fed and 40ton/Fed, respectively relative to the control. This increase may be due to the decomposition of organic matter, derived from sewage sludge performed dissolved organic matter (DOM), which are combined with Cd and form soluble complexes. Study carried out by Dunnivant et al. (1992) showed that DOM has the ability to form stable-soluble complexes with heavy metals (e.g Cd). However, the concentration of extractable-Cd was higher at sludge rate 20 ton/Fed than at 40 ton/Fed and this may be due to downward mobility mobility of Cd at high sludge rate (40 ton/Fed). After harvesting of maize, the concentration of Cd could not be detected at 10 and 20 ton/Fed. This could be due to that residual Cd precipitated with $CaCO_3$ or complexed with organic matter forming organic and carbonate residual phases as reported by McGrath *et al*. (2000).

Extractable Pb (Table 3) did not respond significantly to sludge additions. This may be due to the high content of organic matter in sewage sludge – amended soil which strongly chelated Pb and reduced the extractable fraction. Recent studies indicate that application of biosolids or biosolids compost can reduce the bioavailability of soil Pb (Brown et al., 1999).

Comparing the concentrations of the AB-DTPA extractable metals before sowing and after harvesting of maize, It is clear that extractable-Cu concentration decreased after harvesting at 0, 10, and 20 ton/Fed sewage sludge application rates. This decrease may be due to plant uptake. While at 30 & 40 ton/Fed application rates, Cu concentration increased. This may be due to decomposition of sludge and the release of Cu. While, the amount of the extractable Zn and Pb decreased, at all treatments after harvesting compared by before cultivation which may be due to plant uptake.

Concerning Cd concentration after harvesting, it is clear that Cd increased at control. This may be due to P-fertilizers which originally contain relatively high concentration of Cd. While, at higher sludge rates (30 and 40 ton/Fed) Cd concentration had increased. This may be due to high content of organic matter at this rates which is degraded slowly with time and released Cd and also to decline soil pH (Pales et al., 1996).

**Table3: The concentration of AB-DTPA extractable heavy metal (mgkg$^{-1}$) in the sludge amended soil before sowing (16 months from 1$^{st}$ add.) and after harvesting (20 months from 1$^{st}$ addition).**

| Treat. (ton/Fed) | | Cont. | 10 | 20 | 30 | 40 |
|---|---|---|---|---|---|---|
| Before sowing | 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 | n.d 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 |
| After harvesting | 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 | n.d c | n.d c | 0.38 b | 0.54 a |
| | Pb | 3.18 a | 3.56 a | 4.09 a | 4.63 a | 4.09 a |

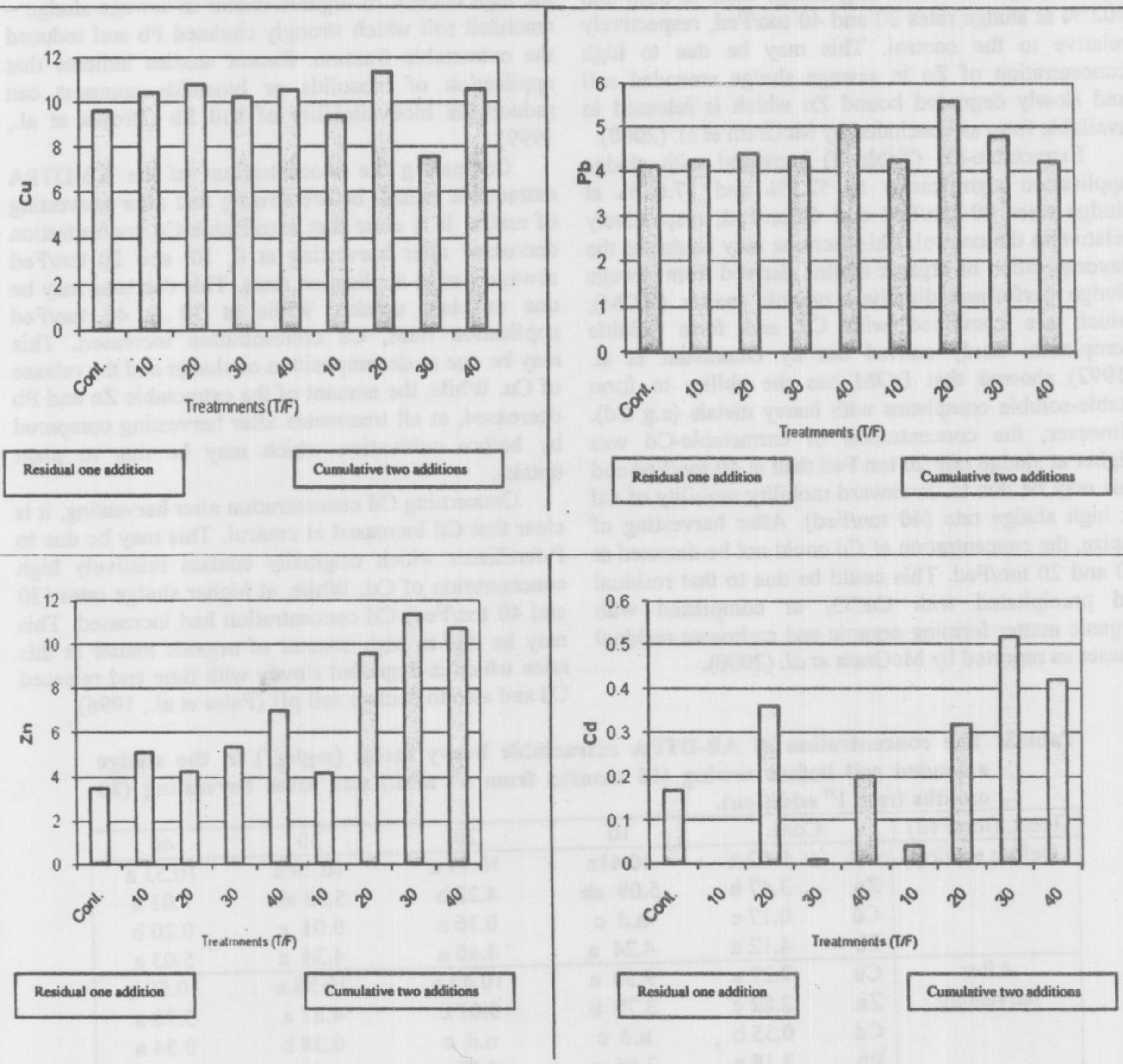
Means within a row followed by the same letter are non-significantly different at 5% level according to Duncan's multiple range 1$^{st}$ add.: the first addition of sewage sludge. n.d : not detected

**B- Cumulative effect of two additions**

The results in Table 4 indicated that sewage sludge applications increased significantly the concentration of AB-DTPA extractable Cu at all sludge rates except that of 10 ton/Fed where the concentration was decreased from 9.62 to 9.32 fot the control and10 ton/Fed sludge treated soil. This decrease may be due to both the occurrence of high level of organic matter in soil as a result of the second sludge addition, the high affinity of Cu to form high stable Cu-organo complex (Aduayi, 1973).

It is also clear that extractable Zn increased significantly with increasing sludge application, this may be due to cumulative sludge load of heavy metals from sewage sludge. Barbarick *et al*. (1998) observed significant accumulations of the trace elements in the plow layer of the biosolids–amended soils.

**Figure (1): Effect of different rates of sludge treatments, residual one addition and comulative two addtions on the concentrations of heavy metals (mg/kg) in the soil before sowing of maize**




The concentrations of extractable Cd increased significantly with increasing sludge addition rates. The highest increase of Cd was 205.8% recorded at 30 ton/Fed. However, at sludge rate 10 ton/Fed the AB-DTPA extractable Cd was markedly reduced to 76.47% as compared with control. This may be due Cd precipitation.

After maize harvesting, Table 4 indicated that the amount of AB-DTPA extractable heavy metals increased significantly with increasing sludge rates except for Cu. The concentration of extractable Zn increased significantly and represented 1 125.2% relative to the control at the highest sludge rate (40 ton/Fed). Also, extractable-Cd and Pb increased significantly with increasing sludge application and represented 82% and 70% relative to control at high sludge rates 40 ton/Fed and 30 ton/Fed respectively.

To compare between the amounts of extractable metals in soils before sowing and after harvesting maize, the results indicated that the concentrations of Cu, Zn, and Pb were decreased after maize harvest except at sludge rate 30 ton/Fed for Cu where the concentration increased by 33%. On the other side, the amounts of Cd were increased in all treatments which could be due to the oxidation of organic material by microorganisms (Emmerich et al., 1982).

Figure1 represents a comparison between residual and cumulative effects of the two additions of sewage sludge before sowing of maize. It is clear that the extractable Zn increased to high percent 127 % at

20ton/Fed cumulative compared with 20 ton/Fed residual, then the percent declined to 21% at 10 ton/Fed cumulative compared with 10 ton/Fed residual. This may be due to mobility of excess Zn in the soil profile with DOM. Also, it is clear that extractable Cd increased from 0.01 mg/kg at 30 ton/Fed residual to 0.52 mg/kg at 30 ton/Fed cumulative. Also, extractable Pb increased at all sludge rates except at 40 ton/Fed cumulative where the percent declined to 15% in comparison with 40 ton/Fed residual. This may be due to the second sludge addition with high sludge rate had increased the content of O.M that chelated Pb and consequently decreased the available form. The same trend was also found with Cu where its concentration percentdeclined at 10 and 30 ton/Fed cumulative to 10.47 and 25% respectively, in comparison with 10 and 30 ton/Fed residual one addition. However, the concentration increased to low level 7 at 20 ton/Fed cumulative compared by 20 ton/Fed residual. These results were in accordance with Granto et al. (2004).

**Table 4: The concentration of AB-DTPA extractable heavy metal (mgkg$^{-1}$) in the sludge amended soil before sowing (16 months from 1st add.+8 months from 2nd add.) and after harvesting (20 months from 1st add.+ 12 months from 2nd ).**

| Treat. (ton/Fed) | | Cont. | 10 | 20 | 30 | 40 |
|---|---|---|---|---|---|---|
| Before sowing | 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 | 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
1st add.: the first addition of sludge. 2nd add.: the second addition of sludge.

**2-Effect of sludge treatments on heavy metals content in plant**

**A-Residual effect**

Data in Table (5) showed that Cu concentrations in leaves and grains of maize were decreased with all sludge treatments compared with the control. This confirms that Cu in soil, in the presence of sewage sludge did not take up in high amounts by plants (Aduyia, 1973, and Tiffany *et al*., 2000).

**Table (5): The concentration of extractable heavy metal (mgkg$^{-1}$) (19 months from 1st add.) and grains (20 months from 1st add.) of untreated and treated *Zea mays* plants .**

| Treat. (ton/Fed) | | Cont. | 10 | 20 | 30 | 40 |
|---|---|---|---|---|---|---|
| Leaves | 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 | nd b | nd b | nd b | 3.10 a | nd b |
| | Pb | 2.50 b | nd c | 5.00 a | 6.00 a | 3.00 b |
| Grains | 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 |

Means within a row followed by the same letter are non-significantly different at 5% level according to Duncan´s multiple range
1st add.: The first addition of sewage sludge. nd.: Not detected

Zn concentrations in leaves of maize were increased significantly by the application of sludge. The highest Zn content was recorded in leaves of plants grown in 20 ton/Fed sludge treated soils. This level represented 6.3 % increase relative to the control. This may be due to complexation of Zn with dissolved organic matter, and consequently increased mobility and uptake by plants (Antoinadis and Alloway, 2002).

Cd concentration in leaves and grains of maize were not influenced significantly by all sludge application rates exceptat 30 ton/Fed, where the concentration of Cd in leaves was increased significantly to 3.1 mg/ kg in comparison with control value. This may be due to complexation of Cd by dissolved organic matter, as reported by Hyun et al. (1998).

Pb concentrations in leaves and grains of maize were significantly influenced by increasing sewage sludge additions rates. The greatest increase in Pb concentrations in leaves was 2.5 fold higher relative to control at 30 ton/Fed. Chaney, (1988) found that concentration of Pb from sludge does not rise plant concentration unless it is very high. However, the concentration of Pb was undetected at 10 ton/Fed in leaves in comparison with control. This may be due to translocation of Pb from leaves to grains which contained the highest concentration of Pb.

B- Cumulative effect of two additions

Copper concentrations in leaves and grains of maize were affected significantly by sludge application rates. The concentrations of Cu in leaves decreased in all sludge treatments in comparison with control except at 20 ton/Fed cumulative two additions where the concentration of Cu in the grains was increased by16 % relative to the control but this increase was insignificant. The decrease in the concentration of Cu in leaves and grains of maize may be due to high percent of organic matter derived from the second addition of sludge which held Cu and consequently reduced the available form. Labrecque et al. (1995) reported that Cu concentration in the biomass was less dependent on their concentration in the soil.

**Table (6): Heavy metals concentrations (mg/kg) in leaves (19 months from 1st add. +11 months from 2nd add.) and grains (20 months from 1st+12 months from 2nd add.) of *Zea* mays plants.**

| Treat. ( ton/Fed) | | Cont. | 10 | 20 | 30 | 40 |
|---|---|---|---|---|---|---|
| Leaves | 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 | Cu | 1.20 a | n.d c | 1.40 a | 0.40 b | n.d 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 5% level according to Duncan's multiple range 1st add.: the first addition of sludge. 2nd add.: the second addition of sludge.
nd : not detected

The concentration of Zn in leaves increased significantly (15.1% and 42.96%) relative to the controlat rates 30ton/Fed and 40 ton/Fed, of sludge respectively compared with control. This may be due to cumulative sludge load of Zn with the second addition. Antoniadis and Alloway (2002) found that Zn concentration was greater in plants at the higher rate of sewage sludge application. The concentration of Zn in grains of maize was increased to 20% at rate of 10 ton/Fed of sludge relative to the control. Whereas the concentration decline to 41% at sludge rate 20 ton/Fed in comparison with control. Meyer *et al.*, (2004) found that Zn concentrations of seeded grasses generally increased with increasing biosolids applications.

As shown in Table 4, Cd concentration was not detected in both leaves and grains with all sludge treatments. Martinez et al. (2003) found that tissue Cd levels did not increase with biowaste application.

The highest increase in Pb concentration in the leaves and grains was 100% and 25% at sludge rate 20 ton/Fed and30 ton/Fed respectively, relative to control. However, the concentration of Pb declined to 60% in leaves at sludge rate 40 ton/Fed and declined to 50 and 75% in the grains at sludge rate 10 and 20 ton/Fed in comparison with control. This may be due to high O.M content which decreased the availability and uptake of Pb by plant (Darwish and Ahmed, 1997).

**3-Effect of sludge on germination, morphological characters and $M_1$ productivity.**

Data in Table (7)showed that Germination percentage after 30 days was increased by sludge treatment. However, the mean effect of residual treatments was more than the two additions treatments. Plant height was not significantly affected by sludge treatment after 30 days and at maturity. Also, the ear length and number of rows per ear among all treatments and the control did not differ significantly. The total number of ears per treatment recorded the highest values with 40 ton/Fed for residual and 20 ton/Fed for cumulative two additions treatments, while the lowest value was recorded with 30 ton/Fed two additions. The mean value of both treatments was approximately 40 ears.

The dry weight of leaves was increased with 20 and 30 ton/Fed of residual treatments besides 10 and 40 ton/Fed by two additions treatments. While 20 ton/Fed of two additions treatments reduced the value to 50% of control and 10 and 30 ton/Fed of residual and two applications treatments respectively reduced the value to 0.71% of its control.

The number of kernels per ear and Kernel density (Table 7) did not affected by sludge rate. Contrarily, the mean kernel index decreased significantly by sludge treatments. The mean kernel index, induced by residual treatment, was lower than that of the two applications treatments (18.95 and 24.59,respectively).

Howell (1998) reported that Abscisic acid (ABA) appears to play a major role in controlling kernel germination. This indicates that 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 ton/Fed which inhibited its synthesis and so, reduced germination percentage. Contrarely, yield products are controlled by Gibberilic acid (GA). *Gibberilic- 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 *gibberilic- 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.

In case of $M_2$ kernel characters, during the determination of *Zea* maize plant productivity, it was found that kernel characters were different from those of control kernels (Table 8). The majority of $M_2$ color kernels of the control were white, resembling the parental kernel color, while the yellow color produced formed 4.50% from the total scored kernels. Sludge treatments induced a significant decrease in the percentage of yellow colored kernels except that of 20 and 30 ton/Fed 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 ton/Fed caused 7.33% of blotches kernels while 40 ton/Fed increased this percentage to about three times (23%). However, the different rates of the two addition treatment induced the presence of one type of blotched kernels, with the highest values occurred by the two lowest rates.

In addition, kernel shape differed according to presence of pitted and non pitted kernels (Table8). Non pitted kernels appeared in the control treatment and increased significantly by sludge treatments of both 10 and 30 ton/Fed of residual treatments and 10 and 20 ton/Fed of two applications treatments. Also, it was noticed that the values of percentage of yellow and non pitted kernels with 20 ton/Fed of two additions were twice than that of the control.

$M_2$ shrunken kernels occurred as endosperm collapses during drying stage at maturity, giving a smooth indentation at the crown. They appeared with low percent in the control treatment and increased by the residual treatment of 40 ton/Fed and by the cumulative two additions treatments of 10 ton/Fed. The effect of two additions treatments was higher than that of the residual treatments (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 and Sarkar (1995) listed 13 independent recessive mutants that were responsible for yellow or white endosperm, that is recessive gene "y", found on chromosome number 6, produced white endosperm; while its dominant allele "Y" produced yellow 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, the outer most layer of the endosperm. 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 which are responsible for alurone color. The 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 that Bh-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 with 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 the $Bn_1$ or $bn_2$ for brown.

Owing to kernel shape, the kernel of the present study was pitted with genotype pt pt pt (pitted gene–pt is located on chromosome number 4, Brigges, 1966; and Sheridan and Neuffer, 1982). The increase or decrease rates of non-pitted kernels from the

**Table 7: Effect of different rates of sludge treatments on *Zea mays* germination and on plant height after 30 days and plant productivity at maturity**

| Treat. (ton/Fed) | 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$^3$ |
| **Residual one addition** | | | | | | | | | | |
| Cont. | 43.90 ab | 34.00 a | 161.95 a | 33.33 c | 17.83 a | 10.67 c | 12.66 a | 448.00 a | 26.80 ab | 1.03 a |
| 10 | 68.90 a | 35.66 a | 137.35 a | 33.33 c | 16.00 a | 7.83 d | 12.33 a | 469.67 a | 22.30 c | 1.02 a |
| 20 | 56.67 a | 40.33 a | 162.72 a | 38.88 bc | 16.25 a | 11.90 c | 12.83 a | 467.00 a | 21.77 c | 0.93 a |
| 30 | 58.90 ab | 36.33 a | 153.19 a | 43.33 ab | 16.66 a | 14.34 a | 11.50 a | 407.50 a | 10.93 d | 1.10 a |
| 40 | 35.53 b | 37.11 a | 147.66 a | 44.44 ab | 16.75 a | 10.43 c | 12.33 a | 430.70 a | 20.78 c | 0.97 a |
| **Cumulative two additions** | | | | | | | | | | |
| 10 | 55.55 ab | 33.22 a | 153.78 a | 33.77 c | 15.92 a | 12.92 ab | 11.30 a | 354.30 a | 29.30 a | 1.00 a |
| 20 | 51.11 ab | 33.88 a | 146.22 a | 50.00 a | 16.42 a | 5.81 e | 12.33 a | 451.00 a | 22.80 bc | 1.00 a |
| 30 | - | - | 135.72 a | 22.22 d | 17.46 a | 7.35 d | 12.66 a | 485.00 a | 24.80 bc | 0.92 a |
| 40 | 48.89 ab | 32.55 a | 144.5 a | 47.77 a | 17.58 a | 13.83 a | 12.00 a | 453.00 a | 21.46 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.

**Table 8: Effect of different rates of sludge treatments on $M_2$ Kernel characters of *Zea mays* grown in sludge amended soil.**

| Treatment (ton/Fed) | Kernel color (%) | | | | | | | Kernel shape (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Endosperm | | Blotches Aleurone | | | | | | | | |
| | White | Yellow | Purple | Brown | Red | Red & Brown | Purple & Brown | Pited | Non Pited | Full | Shrunken |
| **Residual one addition** | | | | | | | | | | | |
| Cont. | 95.15 a | 4.50 c | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 74.60 a | 24.10 ab | 99.65 | 0.35 |
| 10 | 98.47 a | 1.20 de | 3.60 | 2.48 | 0.16 | 1.09 | 0.00 | 60.60 a | 39.30 ab | 100.00 | 0.00 |
| 20 | 98.10 a | 1.90 d | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 77.70 a | 22.30 ab | 100.00 | 0.00 |
| 30 | 96.40 a | 3.60 c | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 73.30 a | 26.40 ab | 100.00 | 0.00 |
| 40 | 99.30 a | 0.60 de | 21.43 | 0.24 | 0.18 | 0.00 | 0.00 | 86.50 a | 13.70 b | 98.35 | 1.56 |
| **Cumulative two additions** | | | | | | | | | | | |
| 10 | 91.04 a | 1.30 de | 7.66 | 0.00 | 0.00 | 0.00 | 0.00 | 77.10 a | 33.30 ab | 7.49 | 2.51 |
| 20 | 84.88 a | 9.60 a | 5.52 | 0.00 | 0.00 | 0.00 | 0.00 | 51.30 a | 48.70 a | 100.00 | 0.00 |
| 30 | 93.00 a | 6.60 b | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 79.60 a | 22.10 ab | 100.00 | 0.00 |
| 40 | 99.69 a | 0.04 e | 0.00 | 0.27 | 0.00 | 0.00 | 0.00 | 75.80 a | 23.10 ab | 99.73 | 0.27 |

Means within a column followed by the same letter are non-significantly different at 5% level according to Duncan's multiple rang 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 (located on chromosome number 3 and 9, Brigges, 1966; and Sheridan and Neuffer, 1982), which has an effect on endosperm development, leading to starch deficiency mutation in kernels and inhibiting ADP-glucose phosphorylate required for starch biosynthesis (Bhave et al., 1990).

Finally, it could be concluded that, 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 and aleurone, and shape either pitted and no pitted; shrunken or full).

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## الملخص العربى

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

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**كلية العلوم– قسم النبات

تعتبر معاملة الأرض بالحمأة احدىطرق تصريف هذه المخلفات وذلك للاستفادة منها فى الأراضى. حيث أنها مفيدة للأراضى الزراعية لغناها بالعناصر الغذائية والمادة العضوية. ولكنها تحتوى على العناصر الثقيلة والتى تسبب تأثيرا ساما على النبات.

تم إجراء دراسة حقلية فى مزرعة كلية الزراعة (قطعة رقم ٤) جامعة الإسكندرية فى قطاع أبيس أثناء الفترة من (١٩٩٩ – ٢٠٠١) وذلك لتقويم تأثير استخدام الحمأة كسماد عضوى على مختلف عناصر الإنتاج لنبات الذرة (Hybrid 320) . تم إضافة الحمأة وخلطت بالتربة بمعدلات ٠، ١٠، ٢٠، ٣٠، ٤٠ طن/فدان فى شهر مارس ١٩٩٩ وزرعت بنبات الذرة . وبعد حصاد الذرة تم تقسيم قطعة الأرض إلى شريحتان الشريحة الأولى تم إضافةالحمأة لها فى إضافة ثانية من الحمأة بنفس المعدلات السابقة بينما الشريحة الأخرى لم يضاف لها أى إضافات أخرى من الحمأة وذلك لكن نقارن بين التأثير المتبقى والتراكمى من الحمأة على نبات الذرة. وفى شهر يوليو (٢٠٠٠) تم زراعة الذرة المعنى بالدراسة.

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 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 عنصر الكادميوم. وبصفة عامة فإن الإضافة الواحدة المتبقية من الحمأة أدت إلى انخفاض محتوى العناصر الثقيلة ما عدا عنصر الرصاص فى نبات الذرة. 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 الجاف للأوراق ما عدا 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 فى طبقة الأليرون، حبوب غير منقورة وحبوب منكمشة فى بعض المعاملات.