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Heavy Metal Contents in Maize as Affected by Sewage Sludge Application I-Morphological Characters and Yield

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Abstract: Aerobically digested sewage sludge was applied to corn at different rates (0, 10, 20, 30, and 40 ton/fed.). At maturity, 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 were recorded. Corn grains were analyzed for Pb, Cd, Ni, and Cr content. Increasing sludge rates increased significantly the initial growth, cob weight, total chlorophyll, chlorophyll-a in leaves, lead and nickel in kernels. No significant differences were observed for 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.

Key words: Transfer coefficient, antagonism, point mutation, chelating agent

Introduction

The provision of sewage treatment in Alexandria has resulted in the production of substantial quantities of effluents and sludge (Hall and El-Hakeh, 1999). In an arid country such as Egypt, these materials should be regarded as valuable resources for agricultural irrigation and soil fertilization (FAO, 2000). However, effluent and sludge need to be treated and managed appropriately to avoid the potentially adverse impacts on the environment and human health (Rappaporte *et al.*, 1988 and Koppe, 1999).

Sewage sludge is the final residue produced from the treatment of domestic and industrial effluents (Rappaporte *et al.*, 1988). Sewage sludge can be used as a valuable source of plant nutrients to substitute the chemical fertilizer (FAO, 2000; Labrecque *et al.*, 1995 and Richards *et al.*, 1998). It contains high percentage of organic matter (Labrecque *et al.*, 1995 and Berti and Jacobs, 1998). However, the levels of toxic metals found in sewage sludge are considerably higher than those found in typical agricultural land (William *et al.*, 1987; Rasmy, 1996 and Sloan *et al.*, 1998). Significant accumulation of these metals in food crops may result in potential health problems for the consumers (Rappaporte *et al.*, 1988; Dowdy *et al.*, 1991; Labrecque *et al.*, 1995 and Richard *et al.*, 1998).

The present study is conducted to justify the use of treated sewage water sludge (from Alexandria east station) as a biofertilizer and its effects on the yield of maize.

Materials and Methods

Site description and cultural practices: This investigation was carried out at the field station of the Faculty of Agriculture, Alexandria University at Abis area from February to September, 1999 and the laboratory studies were carried out at Faculty of Science and Faculty of Agriculture. The soil of the station is calcareous lacustrine. The main physical and chemical characteristics of the soil were determined according to Page *et al.* (1982) and are presented in Table 1a.

Composted sewage sludge used as a biofertilizer, was obtained from Alexandria General Organization for Sanitary Drainage of Alexandria city in February 1999. This was previously air-dried, aerobically composted at site 9N (45 Km southwest Alexandria city, Amriya). The chemical composition of the used sewage sludge is summarized in Table 1b. In March, 1999, the sludge was applied and mixed evenly with 15 cm top soil three months before cultivation using five rates of sludge applications: 0, 10, 20, 30 and 40 ton/fad. For comparison, ammonium nitrate (33.5% N) was also added as a mineral fertilizer after one month of sowing to a control plot at a rate of 0.04 t/f, which is normally used.

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 replica for each treatment combination 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, ground, passed through 2-mm sieve and prepared for the analysis to determine organic carbon content, total nitrogen, sodium bicarbonate, available potassium and DTPA (diethylenetriamine-pentaacetic acid)-extractable heavy metals Fe, Zn, Mn, Cu, Pb, Cd, Ni and Cr. Spectrophotometer (model 21 D) was used for Phosphorous determination and atomic absorption spectrophotometer (Perken Elmer model 3300) for heavy metal determination. These analyses were made according to the standard methods of Page *et al.* (1982).

Chlorophyll estimation and grain analysis for heavy metals: At the seventh leaf stage, leaf samples were collected, washed with tap water, then with bidistilled 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. The transfer coefficient (TC) of metals from the soil to corn grains was estimated according to Labrecque *et al.* (1995).

$$TC = (Mf - Mc) / Ms$$

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

M_1 morphological measurements: Plant heights of sixty-four plants per treatment were determined after three and five weeks of planting. At maturity (after three months of sowing)ten quantitative characters were recorded (twelve plants from each treatment): number of ears per plant, ear length, ear weight, cob weight, kernel's number and weight

Table 1: Some chemical and physical characteristics of
a) experimental soil

Soil characteristics		Values	Soil Characteristics		Values
Soil texture class		Clay	K	mg/kg	114.00
Clay,	g/Kg	455.00	Fe	mg/kg	3.47
Sand,	g/Kg	418.00	Zn	mg/kg	2.31
Silt	g/Kg	137.00	Mn	mg/kg	6.20
Ec,	ds/m^{-1}	0.68	Cu	mg/kg	1.70
pH		7.60	Pb	mg/kg	1.75
$CaCO_3$,	g/Kg	152.00	Cd	mg/kg	0.07
OM.	g/Kg	10.70	Ni	mg/kg	0.39
TKN.	g/Kg	1.36	Cr	mg/kg	0.15
P	mg/Kg	33.60			

b. experimental sludge

Sludge characteristics		Values
EC	ds/m	5.05
pH		7.20
$CaCO_3$	g/kg	102.0
OM	g/kg	435.00
TKN	g/kg	21.60
TP	g/kg	5.40
TK	g/kg	1.60
Fe	g/kg	160.80
Zn	mg/kg	812.80
Mn	mg/kg	160.00
Cu	mg/kg	475.00
Pb	mg/kg	170.30
Cd	mg/kg	8.05
Ni	mg/kg	108.04
Cr	mg/kg	118.06

EC and pH in water extract (1: 2.5)
OM: Organic matter TKN: Total Kjeldahl nitrogen

per ear, 100 kernels weight (index), kernel's yield per plant (number and weight), volume of 100 kernels and kernel's density. Kernel's volume was determined using distilled water displacement. Kernel density was calculated by dividing the weight of the kernels by its volume (Kharkwal and Chaudhary, 1997).

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

Results

Soil and sludge constituents:

Soil: Table 1a shows that soil has heavy texture of clay (40%), with 15.2% total $CaCO_3$, low amounts of organic matter and total nitrogen, and high level of available P. The level of DTPA- extractable trace elements (Fe, Zn and Mn) and heavy metals (Cu, Pb, Cd, Ni and Cr) were normal. Correspondingly, soil fertility and physical properties may be enhanced by addition of organic fertilizer to ensure the high crop production.

Sludge: The analysis of the used sludge showed appreciable amounts of organic matter and total N and low levels of P and K (Table 1b). The chemical composition of this sludge agrees with those previously reported by Alloway (1995). It also contained appreciable amounts of micronutrients (Fe, Zn, Mn and Cu) and a potential source of toxic heavy metals (Pb, Ni, Cd and Cr).

Effects of sludge application on soil: After three months of sludge application and before cultivation, the increase in sludge rates increased the organic matter content, available phosphorous and potassium significantly in soil (Table 2). The trace elements (Fe, Zn) extractability increased significantly with increasing sludge rates from 3.47 to 13.79 and 2.31-to 6.2-mg/ kg for Fe and Zn, respectively. Extractability of Cd, Ni, Cr and Pb increased significantly with sludge application. However, after harvesting, the data showed a decrease in macro and micronutrients and heavy metal concentrations compared with the data before cultivation.

Effect of sludge application on chlorophyll content: Increasing sludge rate increased chlorophyll-a (C_a), and total chlorophyll (C_t) content, significantly than the control (Table 3). However, there was no significant effect of sludge application on chlorophyll-b (C_b). The 30 T/F treatment recorded the highest chlorophyll content. In the mean time, the control with minerals gave the highest values for all chlorophyll contents.

Effects of sludge application on heavy metal contents in grains: Table 4 shows that increasing the rate of sludge application increased grain content of Pb, Ni, Cd and Cr significantly. The 10 T/F treatment recorded the highest concentration content of Pb and Ni in corn grains compared with the other treatments. While the 30 T/F treatment recorded the highest concentration of Cd and the 40 T/F treatment recorded the highest concentration of Cr.
The transfer coefficient (TC) of metals from soil to grains differs from one metal to the other (Table 5). Ni, Cd and Cr were the most transferable with the highest TC values after 10 T/F treatment (13.1, 15.4 and 21.1, respectively). The highest sludge concentration (40 T/F) induced the highest TC value of Cr (47.7). Ni and Pb were the lowest transferable elements.

M_1 Morphological Parameters: No visible symptoms of chlorosis or necrosis were found on the above ground parts of the plants, indicating no sludge toxicity, but their leaves were slightly greener than those of the control plants. The results of the morphology and yield parameters are presented in Table 6. 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 for the treatment using 40 T/F, which was non-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 insignificant variation in the mean grain index between all treatments. The mineral fertilizer induced the highest percentage of aborted kernels (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 non-significantly different from each other.

Discussion

Municipal sewage sludge has been used as biofertilizer in many countries in forest rehabilitation and tree plantation (Hall, 1999). The urgent need for cheap biofertilizers in crop cultivations called for the use of sewage sludge. Sewage sludge application, increased the metal concentration in soil proportional to the concentration of the added sludge. This increase was reduced after harvesting to over 50% in case of Fe, Zn, Mn and Cr, and lower than that in case of the other

Table 2: Effect of sewage sludge application on chemical characteristics, nutrient content and total concentration of heavy metals in soil before cultivation and after harvesting of maize

Treatment (T/F)	EC ds/m	pH	O.M. %	TKN %	Soil characteristics									
					P	K	Fe	Zn	Mn	Cu	Pb	Cd	Ni	Cr
					$\mu g\ g^{-1}$									
Before cultivation														
Cont. ⁻M*	0.34e	7.60a	1.07e	0.136d	33.5e	114.0c	3.47e	2.31e	6.20e	1.70e	1.75cd	0.07cd	0.39e	0.15b
10	0.90d	7.50b	1.13d	0.165c	56.28b	180.0a	9.42c	3.95c	8.95d	6.52c	1.82c	0.08c	0.48d	0.16a
20	1.12c	7.42c	1.21c	0.14d	45.34d	69.5d	8.71d	4.25b	7.80c	4.78d	3.17cd	0.06cd	0.60c	0.15b
30	1.18b	7.38e	1.33b	0.20b	61.25a	14.00e	11.30b	3.21d	12.40a	8.22b	3.14a	0.14a	0.82a	0.12c
40	1.22a	7.40d	1.56a	0.25a	47.00c	125.0b	13.79a	6.75a	15.61b	11.56a	3.50b	0.12b	0.79b	0.09d
L. S. D. $_{0.05}$	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														
Cont. +M**	0.42e	7.60c	0.75e	0.06c	3.90e	65.23e	4.86e	1.34e	17.87a	1.63d	1.09a	0.03c	0.11c	0.12b
Cont. -M*	0.34f	7.59c	0.72f	0.004d	0.00f	0.00f	3.06f	0.77f	10.87b	1.34f	0.88c	0.04b	0.20a	0.12b
10	0.86b	7.65b	0.83d	0.05c	34.77c	142.25a	6.05d	3.43c	8.58d	1.42e	0.38e	0.04b	0.07f	0.10c
20	0.99a	7.63b	0.97c	0.084b	34.05d	31.25d	10.7a	3.67b	7.61f	2.17c	0.88c	0.02d	0.10d	0.07d
30	0.71d	7.70a	1.18b	0.098a	59.71a	97.25b	9.13b	2.76d	7.75e	2.82a	0.97b	0.04b	0.16b	0.08e
40	0.75c	7.57d	1.21a	0.08b	49.51b	93.75c	7.49c	7.73a	9.35c	2.42b	0.76d	0.05	0.09e	0.18a
L.S.D.$_{0.05}$	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

*Control without mineral fertilizer ** Control with mineral fertilizer

Table 3: Chlorophyll content in corn leaves (mg/g)

Treatment (T/F)	Chl.a	Chl.b	Chl.Total
Cont. +M*	17.6	13.5	31.1
Cont. -M**	6.6	4.2	10.7
10	7.1	4.6	11.7
20	8.8	5.7	14.4
30	11.5	7.9	19.5
40	8.6	5.6	14.2
L.S.D.$_{0.05}$	0.46	0.002	0.15

* Control with mineral fertilizer.

** Control without mineral fertilizer

Table 4: Heavy metals concentration in corn grain ($\mu g\ g^{-1}$)

Treatment (T/F)	Pb	Ni	Cd	Cr
Cont. +M*	3.75	8.21	0.05	1.45
Cont. -M**	4.01	9.62	0.00	1.32
10	12.03	15.9	1.23	4.69
20	6.50	7.01	0.01	1.85
30	4.25	3.24	1.56	1.72
40	11.30	13.02	0.45	5.61
L.S.D.$_{0.05}$	0.11	0.01	0.01	0.02

* Control with mineral fertilizer

** Control without mineral fertilizer

Table 5: Transfer coefficient of heavy metals in corn grains of treated plants, using different rates of sludge application

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	4.30	3.75	47.66

*Negative values mean that the heavy metal content of control corn grains was higher than the treated ones.

metals in 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. It was noticed that M_1 corn grains accumulated the four analyzed metals in the order as follows: Ni>Pb>Cd>Cr. 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. This was in agreement with the findings of El-Bagouri (1999). 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 the 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 might be the result of dilution effect, which enhanced the rate of translocation (transfer) of these metals (Rank and Nielsen, 1998). While, the low accumulation of metals in the intermediate concentrations could be interpreted as: the organic matter in sludge acted as chelating agent (Das *et al.*, 1997). This property was at its full effect when the metal concentration was equivalent to organic matter concentration. When this balance was disturbed on either side, the rate of soluble and available metals in soil solution would increase, and the increase in accumulation in plant tissues depended to a great extent on its transfer coefficient value. However, the recorded concentrations in grains were found to be higher than safe limits recorded by Risser and Barker (1990).

Total chlorophyll content and chlorophyll-a of the leaves increased with the increase in sewage sludge concentration (up to 30 T/F). This could be attributed to the increase in nitrogen content of the soil due to 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. However, the antagonism between very high nitrogen content and high Cd content, of the 40 T/F sludge treatment, reduced their availability and probably resulted in lowering the chlorophyll content in leaves.

Table 6: Effect of mineral and different rates of sludge on the yield components of *Zea mays*

Treatment	Control		Sludge treatments (T/F)			
	-M	+M	10	20	30	40
Plant height (2 weeks) (cm)	16.16c	16.76bc	17.12abc	17.46bc	19.16ab	19.68a
Plant height (5 weeks) (cm)	126.08ab	145.23a	112.92b	114.95b	120.38ab	127.67ab
Ear's number/plant	1.00a	1.37a	1.33a	1.37a	1.46a	1.37a
Ear length (cm.)	17.39ab	18.32	18.14ab	16.72b	18.46ab	20.15
Ear diameter (cm.)	13.02a	13.84a	13.39a	13.45a	13.72a	13.88a
Ear weight (gm)	117.38b	133.74ab	125.44ab	123.29ab	13.72	151.89a
Rows number	12.89a	13.18a	12.54a	13.33a	33.40ab	13.72a
Kernel's number/ear	369.44a	390.47a	364.52a	383.47a	13.00ab	415.03a
Kernel's weight/ear (gm.)	96.91a	101.50a	99.49a	99.52a	374.34a	120.23a
Cob weight (gm.)	20.47b	32.12a	26.21ab	26.76ab	108.77a	28.89a
Grain index (%)	26.26a	28.87a	27.56a	25.87a	27.15ab	29.70a
Grain density (%)	1.19a	1.23a	1.20a	1.24a	29.92a	1.23a
Pitted kernels (%)	1.82a	28.01a	9.38a	8.82a	1.15a	13.18a
Aborted kernels (%)	0.36b	4.07a	0.56b	1.22ab	12.72a	0.36b

Values in same row with different letters are significantly different at 0.05 level.

The morphological characters of M_1 corn plants generally increased by sludge treatments although, most parameters were insignificantly different from each other and the control. The highest values of M_1 parameters were recorded using 40T/F treatment, accompanied by the highest heavy metals content in corn grains. This increase in the yield and growth were attributed to the increase in available nitrogen content of the soil (Labrecque *et al.*, 1995 and Rappaport *et al.*, 1988). However, the insignificant increase in these M_1 parameters could be due to the high content of heavy metals in plant tissues leading to its accumulation in grains.

Generally, both the mineral fertilizers and the biofertilizers 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 characters as suggested by Amano and Smith (1965). The presence of minute fragments in the diakinesis and laggards in other stages of meiotic division in PMC's of maize (Amin, 2001) support the occurrence of deletions as a cause of pitted kernels. The application of the 20 T/F treatment caused endosperm failure, 3.4 times higher than the control, leading to formation of aborted kernels. This may be due to defective chromosome number and microtubule organization in meiosis I and II as found by Amin (2001). This also agrees with the findings of Alleman and Doctor (2000). The present study suggests the importance of the reduction of heavy metals from sewage sludge before using it as biofertilizer in order to decrease their rates of accumulation in grains. Since, the availability of sludge nutrients was found to increase with incubation time (Baldoni *et al.*, 1996 & Berti and Jacobs, 1996). It will be advisable to carry further investigations including second-generation productivity and reduce the mutation rates of kernels.

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