Effect of Sewage Sludge Amended Soil on Maize Crop

II. Influence on metal accumulation

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The aim of the present study was to evaluate the influence of sewage sludge application on metal accumulation (Cu, Mn, Zn, Cd, Ni, Pb) at maize (Zea Mays L.). It was applied different doses varying from 0-600 kg/ha of sewage sludge associated with mineral fertilization. The results indicated that copper and manganese contents increase with fertilization and rates equivalent to 200 kg N/ha resulted in statistically significant copper content in the maize leaves. The highest accumulation of manganese in the leaves was recorded at the sludge rate equivalent to 300 kg N/ha, meanwhile in the maize kernels after the increasing sludge rate, the manganese content recorded a decreasing tendency. The zinc content in the maize leaves significantly increased with the increasing rate of sludge, starting with a rate equivalent to 200 kg N/ha, sewage sludge application being a promoter of zinc accumulation but without becoming excessive. As concerns the zinc content in the maize kernels, sewage sludge rates higher than 300 kg N/ha resulted in statistically assured increases. The cadmium level in the maize leaves increased directly proportional with the rate applied and the values were statistically assured at rates higher than 200 kg N/ha. The cadmium content in the maize kernels was not affected by the treatments applied, as the very low values obtained were below the detection limit of the analytical method. The lead content in the maize leaves increased under the influence of sludge fertilization, but the accumulation was generally low, all values in leaves being under 10 mg/kg and in kernels under 1 mg/kg Pb.

Keywords: accumulation, fertilization, leaves, heavy metals, kernels, maize, sewage sludge.

Agricultural land application appears to be a logical and reasonable use of sewage sludge, since it may improve many soil properties, such as *p*H and contents of organic matter and nutrients, can also improve soil physical properties such as porosity, aggregate stability, bulk density, and water retention and movement [1-5]. Sewage sludge application it has been proven to increase soil fertility, being an innovative solution for arable land resources and solid waste disposal, as well [6]. Even if sewage sludge from the treatment of munici-pal wastewater is characterized by high content of organic matter, elements that present importance from agrochemical point of view (N, P, K, Ca, Mg), the presence of some potentially toxic elements (heavy metals) [7-13], organic pollutants [14-16] and pathogenic organisms [16,17] may create some inconvenient related to their undesirable effects.

Contrariwise, due to beneficial effects and from environmental and ecologic reasons, the sewage sludge application to agricultural soils has been investigated in many countries [6, 12, 18-20].

Application of increasing doses of sewage sludge (0, 40, 80, 120 and 240 t/ha) at rice produced a significant increase of Fe, Cu, Zn, Pb in grains, while the Mn content decreased significantly. Also, Cr, Cd and Ni contents were below detection limit of the used method [21].

In the case of *Beta vulgaris* plants, applications of sewage sludge led to increases in heavy metal uptake and shoot and root concentrations of Ni, Cd, Cu, Cr, Pb and Zn in plants as compared to those grown in unamended soil. It was found that accumulation was more in roots than in shoots for most of the heavy metals. Furthermore, accumulation of heavy metals in plants led to reduced photosynthetic rate and chlorophyll pigments, stunted growth and lowering of yield [11].

Other study investigated the effect of municipal solid waste compost on Co and Ni contents of submerged rice paddies. The results evidenced that metal content in rice straw was higher than in rice grain [22].

Mihalache et al. [12] investigated the translocation of metals (Cu, Zn, Pb, Co, Ni, Mn, Cr, Cd) in different organs of various vegetal species (tomato, lettuce, sugar beet, soy, oats, maize) grown on soil amended with 30 t/ha sewage sludge. The results show an important variability of the metals' concentrations in different plant organs. According to tolerance index, the most tolerant specie is lettuce (1.70), followed by maize (1.47), soy (1.39), oats (1.09), tomatoes (1.07), sugar beet (0.94).

A study concerning the influence of sewage sludge on maize yield and metal accumulation [6] indicated that sewage sludge favored dry matter accumulation of the aerial part of the maize and increased grain yield. Cd and Ni levels in maize grains were positively correlated with sewage sludge amendment rates. Mn and Cr levels at 300 t/ha sewage sludge application rate and Zn concentrations at more than 75 t/ha sewage sludge application rate were significantly higher than unamended mudflat soil.

The present study is a continuation of our previously reported researches [12, 23,24]. Therefore, the purpose of the present study was to evaluate the suitability use of sewage sludge for maize crop (*Zea mays* L.) by evaluating the metals accumulation (Cu, Mn, Zn, Cd, Ni, Pb) in leaves and kernels. It was used different sewage sludge rates associated or not with mineral fertilization.

The experimental developed model was a bifactorial one (7x2) and the studied factors were: **A** factor- sewage sludge fertilization with seven degrees representing equivalent doses varying between 0-600 kg/ha and **B** factor – mineral fertilization (NPK) with two degrees: $\mathbf{b_1}$- $N_0P_0K_0$ and $\mathbf{b_2}$ -$N_{100}P_{100}K_{100}$.

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Experimental part

Material and methods

Experimental design

In order to study the influence of sewage sludge application on metal accumulation (Cu, Mn, Zn, Cd, Ni, Pb) in experimental pots were used as test plant maize (*Zea mays* L.).

The experimental developed model was a bifactorial one (7x2) and the studied factors were: **A factor** - sewage sludge fertilization with seven degrees representing equivalent doses varying between 0-600 kg N/ha ($\mathbf{a_1}$ - unfertilized with sewage sludge; $\mathbf{a_2}$ - fertilizations with sewage sludge corresponding to 100 kg N/ha; $\mathbf{a_3}$ - fertilizations with sewage sludge corresponding to 200 kg N/ha; $\mathbf{a_4}$ - fertilizations with sewage sludge corresponding to 300 kg N/ha; $\mathbf{a_5}$ - fertilizations with sewage sludge corresponding to 400 kg N/ha; $\mathbf{a_6}$ - fertilizations with sewage sludge corresponding to 500 kg N/ha; $\mathbf{a_7}$ - fertilizations with sewage sludge corresponding to 600 kg N/ha) and **B factor** - mineral fertilization (NPK) with two degrees: $\mathbf{b_1}$ - $N_0P_0K_0$ and $\mathbf{b_2}$ - $N_{100}P_{100}K_{100}$.

For experimental model there were used 56 vegetation pots (20 L capacity), each of 14 treatments in four repetitions. The same experimental scheme was adopted previously to investigate the suitability use of sewage sludge for sunflower (*Helianthus annuus* L.) [24] and to evaluate the yield and macroelements accumulation for maize [25].

For experiments was used soil collected from A_0 horizon of luvisol meanwhile sewage sludge that was used in experiment was collected from the Wastewater Treatment Plant (WWTP) from Pite^{o}ti. Soil, sewage sludge and soil after treatments were fully characterized and the results were reported in a previous paper [23].

Plant sampling and chemical analysis

Plants were taken to the laboratory and it was measured the height of each sample. Then it was partitioned for analysis into leaves and kernels. All these fractions were washed with distilled water to remove soil particles and oven dried at 70^{0}C until constant weight was achieved.

For heavy metals analysis, 1 g of grounded sample passed through a sieve of 2 mm mesh size was digested with 10 mL from a mixture of acids (HNO_3:H_2SO_4:$HClO_4$, 5:1:1) till a transparent color appeared [11].

Contents of metals (Cu, Mn, Zn, Cd, Ni, Pb) in maize leaves and kernels were determined after filtering the digested samples through atomic absorption spectrometry (*Thermo Scientific AA Spectrometer*). The calibrations curves were plotted using working standards obtained from a multi-element stock solution provided by Merck.

Results and discussions

Characterization of soil and sewage sludge

The soil used in this experiment, which was taken from Ao horizon of Luvisol [26]. It had a loam texture, was moderately acidic (*p*H 5.2), had a low carbon organic content (1.19%) and a low content of macronutrients (N_{total} - 0.140%, P_{AL} - 19 mg/kg and K_{AL} - 40 mg/kg). Sewage sludge contain various amounts of metals: **Cu** (114-122 mg/kg; 117 mg/kg, as average), **Mn** (320-350 mg/kg; 338 mg/kg, as average), **Zn** (1211-1252 mg/kg; 1237 mg/kg, as average), **Cd** (3.04-3.40 mg/kg; 3.21 mg/kg, as average), **Ni** (32-36 mg/kg; 34 mg/kg, as average), **Pb** (91-105 mg/kg; 97 mg/kg, as average).

Heavy metal concentration of sewage sludge used in this research is very low-well below the limits established by the EU for sludges produced by urban sewage farms EU Directive 86/278 CEE [27] and this one it has been transposed through the Order of the Minister of Agriculture, Forests, Waters and Environment no. 344/2004 [28].

Influence of sewage sludge application on copper content in maize leaves and kernels

The evolution of the copper content in the maize leaves under the influence of sewage sludge fertilization, with or without mineral fertilizers, showed that the rates equivalent to 200 kg N/ha resulted in statistically significant copper content in the maize leaves (table 1). The level of accumulation remained within normal limits and did not affect production quality and its usability as roughage.

Mineral fertilization led to no significant change in the copper content of the maize leaves. Although the copper levels in the maize leaves increased nearly three times after fertilization with the maximum rate of sludge, the values we are not statistically when applying sludge mixed with mineral fertilizers. The copper concentration in the maize kernels showed no significant changes after sludge fertilization, with or without mineral fertilizers.

Chang et al. [29] found that total Cu concentra-tion in maize does not exceed the limit of 25 mg/kg, but that this limit could be exceeded when Cu con-centrations greater than 1500 kg/ha are applied to topsoil.

Influence of sewage sludge application on manganese content in maize leaves and kernels

The manganese content in the maize leaves resulted in significant increases after the application of sludge fertilization at a rate equivalent to 200 kg N/ha (table 2).

The $N_{100}P_{100}K_{100}$ mineral fertilization had an acidifying effect on the soil and led to a significant increase of the manganese content in the maize leaves. The application

Table 1

INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON Cu CONTENTS IN LEAVES AND KERNELS

Sewage sludge		Leaves			Kernels		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kg N/ha	3.7	4.0	3.9a*	1.8	1.9	1.9a*
a_2	100 kg N/ha	5.4	6.0	5.7ab	1.9	2.0	2.0a
a_3	200 kg N/ha	6.1	7.5	6.8bc	2.1	2.0	2.1ab
a_4	300 kg N/ha	8.7	9.3	9.0cd	2.3	2.3	2.3abc
a_5	400 kg N/ha	9.0	9.4	9.2cd	2.6	2.7	2.7bc
a_6	500 kg N/ha	9.7	10.3	10.0d	2.7	2.7	2.7bc
a_7	600 kg N/ha	10.7	9.6	10.2d	2.7	2.9	2.8c
Av.(a)		7.6a*	8.0a	-	2.3a*	2.4a	-

b_1 - corresponds to $N_0P_0K_0$ (without mineral fertilization)

b_2 - corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)

Av. - average

*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Table 2

INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON Mn CONTENTS IN LEAVES AND KERNELS

Sewage sludge		Leaves			Kernels		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0kg N/ha	186	180	183a*	9.2	9.3	9.3a*
a_2	100 kg N/ha	189	222	206ab	9.1	8.3	8.7ab
a_3	200 kg N/ha	201	232	217b	8.4	8.4	8.4ab
a_4	300 kg N/ha	242	244	243c	8.3	8.0	8.2ab
a_5	400 kg N/ha	197	232	215b	8.0	8.3	8.2ab
a_6	500 kg N/ha	211	221	216b	7.7	7.4	7.6b
a_7	600 kg N/ha	193	212	203b	7.6	7.9	7.8b
Av.(a)		203a*	220b	-	8.3a*	8.2a	-

b_1 - corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 - corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

of sewage sludge mixed with mineral fertilizers had no significant change in the manganese content of the maize leaves. The highest accumulation of manganese in the leaves was recorded at the sludge rate equivalent to 300 kg N/ha. When this rate was exceeded, the values started to decline due to the amendment effect of sewage sludge; however, the values remained significantly higher than those obtained in the unfertilized variants. The manganese values in the maize leaves were maintained within the normal range.

The data recorded showed that sewage sludge fertilization, with or without mineral fertilizers, led to no statistically significant changes of the manganese content in the maize kernels. Only the manganese content recorded a decreasing tendency in the maize kernels after the increasing sludge rate, which was explained by the amendment effect of sewage sludge.

Influence of sewage sludge application on zinc content in maize leaves and kernels

Regarding the influence of sewage sludge fertilization, with or without mineral fertilizers, on the zinc content in the maize leaves, the results showed the following:

- the zinc content in the maize leaves significantly increased with the increasing rate of sludge, starting with a rate equivalent to 200 kg N/ha (table 3);
- the lowest zinc values in the maize leaves were recorded in the unfertilized variants while the highest (over 6 times higher) were identified in the variants fertilized with the highest sludge rates, i.e. equivalent to 600 kg N/ha.

Sewage sludge promoted an increase in total Zn concentration without becoming excessive for human consumption [8].

Mineral fertilization led to a significant increase of the zinc content in the maize leaves. The application of sewage sludge mixed with mineral fertilizers led to no statistically zinc changes in the maize leaves, as the values were very close to those obtained after sludge-only fertilization. Zinc accumulation in the maize leaves was not likely to impose restrictions on its use as fodder.

As concerns the zinc content in the maize kernels, sewage sludge rates higher than 300 kg N/ha resulted in statistically assured increases. When this rate was exceeded, the level of zinc accumulation was not statistically assured.

The application of mineral fertilizers, with or without sewage sludge, resulted in no statistically significant changes of the zinc concentration in maize kernels.

Influence of sewage sludge application on cadmium content in maize leaves and kernels

The cadmium level in the maize leaves increased directly proportional with the rate applied. The values were statistically assured at rates higher than 200 kg N/ha. At a sludge rate equivalent to 600 kg N/ha, the cadmium content in the maize leaves was three times higher than in the control (table 4).

Mineral fertilization, with or without sludge, resulted in no statistically significant changes of the cadmium content in leaves, as its level was the same as the one achieved through organic fertilization. Even at the highest rate, corresponding to 600 kg N/ha, the cadmium content in leaves was below the zootoxic level.

The cadmium content in the maize kernels was not affected by the treatments applied, as the very low values obtained were below the detection limit of the analytical method.

Table 3

INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON Zn CONTENTS IN LEAVES AND KERNELS

Sewage sludge		Leaves			Kernels		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kg N/ha	39	40	40a*	22	22	22a*
a_2	100 kg N/ha	58	77	68ab	23	24	24ab
a_3	200 kg N/ha	81	110	96bc	25	25	25ab
a_4	300 kg N/ha	102	124	113c	27	28	28bc
a_5	400 kg N/ha	162	192	177d	27	29	28bc
a_6	500 kg N/ha	233	237	235e	29	30	30c
a_7	600 kg N/ha	249	248	249e	28	31	30c
Av.(a)		132a*	147b	-	26a*	27a	-

b_1 - corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 - corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Table 4

INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON Cd CONTENTS IN LEAVES AND KERNELS

Sewage sludge		Leaves			Kernels		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kg N/ha	0.30	0.34	0.32a*	nd	nd	-
a_2	100 kg N/ha	0.35	0.43	0.39ab	nd	nd	-
a_3	200 kg N/ha	0.42	0.49	0.46b	nd	nd	-
a_4	300 kg N/ha	0.47	0.55	0.51b	nd	nd	-
a_5	400 kg N/ha	0.79	0.76	0.78c	nd	nd	-
a_6	500 kg N/ha	0.89	0.80	0.85cd	nd	nd	-
a_7	600 kg N/ha	0.91	0.92	0.92d	nd	nd	-
Av.(a)		0.59a*	0.61a	-	-	-	-

b_1 - corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 - corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
nd - not detected
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Influence of sewage sludge application on nickel content in maize leaves and kernels

Statistically assured changes in the nickel content of the maize leaves were recorded in sludge treatment at a rate equivalent to 200 kg N/ha. However, when this rate was exceeded, accumulation was not statistically assured (table 5). Mineral fertilization, with or without sewage sludge, did not significantly alter the nickel level in the maize leaves.

Sewage sludge fertilization (with or without mineral fertilizers) produced no statistically changes in the nickel level of the maize kernels.

Melo et al. [30] conducted a study on maize uptake of Ni and found that the addition of sewage sludge to the soil increases the content of this metal in the shoots, but not in the grain. This shows that Ni translocation from the leaves and stem to the grain is not significant. According to this study, the nickel content in the maize leaves was low and did not affect plant quality.

Influence of sewage sludge application on lead content in maize leaves and kernels

The lead content in the maize leaves increased under the influence of sludge fertilization. The values became statistically assured after the application of a rate equivalent to 200 kg Na/ha. Yet, accumulation was generally low and resulted in no negative changes in plant quality as animal fodder. All values were below 10 mg/kg Pb (table 6).

Also, mineral fertilization, with or without organic fertilization, resulted in no statistically assured changes of the lead concentration in the leaves.

The lead content in the maize kernels recorded no statistically assured change, regardless of the fertilization system (sewage sludge, with or without fertilizers).

Table 5

INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON Ni CONTENTS IN LEAVES AND KERNELS

Sewage sludge		Leaves			Kernels		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kg N/ha	2.0	2.3	2.2a*	1.48	1.53	1.51a*
a_2	100 kg N/ha	2.4	2.3	2.4a	1.60	1.55	1.58a
a_3	200 kg N/ha	2.5	2.4	2.5ab	1.48	1.48	1.48a
a_4	300 kg N/ha	2.7	2.8	2.8ab	1.50	1.43	1.47a
a_5	400 kg N/ha	2.8	2.8	2.8ab	1.40	1.35	1.38a
a_6	500 kg N/ha	2.9	2.8	2.9ab	1.33	1.33	1.33a
a_7	600 kg N/ha	3.1	3.3	3.2b	1.30	1.36	1.33a
Av.(a)		2.6a*	2.7a	-	1.44a*	1.43a	-

b_1 - corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 - corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Table 6

INFLUENCE OF SEWAGE SLUDGE APPLICATION (WITHOUT/WITH MINERAL FERTILIZATION) ON Pb CONTENTS IN LEAVES AND KERNELS

Sewage sludge		Leaves			Kernels		
		b_1	b_2	Av.(b)	b_1	b_2	Av.(b)
a_1	0 kg N/ha	3.7	3.5	3.6a*	0.63	0.58	0.61a*
a_2	100 kg N/ha	5.1	5.8	5.5ab	0.68	0.58	0.63a
a_3	200 kg N/ha	6.2	7.1	6.7bc	0.70	0.68	0.69a
a_4	300 kg N/ha	7.8	8.1	8.0c	0.68	0.69	0.69a
a_5	400 kg N/ha	7.8	8.0	7.9c	0.68	0.70	0.69a
a_6	500 kg N/ha	7.9	7.9	7.9c	0.73	0.82	0.78a
a_7	600 kg N/ha	7.9	7.6	7.8c	0.80	0.83	0.82a
Av.(a)		6.6a*	6.9a	-	0.70a*	0.70a	-

b_1 - corresponds to $N_0P_0K_0$ (without mineral fertilization)
b_2 - corresponds to $N_{100}P_{100}K_{100}$ (mineral fertilization)
Av. - average
*Mean values accompanied by same letter (a or b) does not present significant differences (Tukey multiple comparison test - significance level 0.05)

Correlation between different equivalents nitrogen doses from sludge applied and Cu, Mn, Zn, Cd, Ni and Pb uptake by plant

The content of Cu and Zn in maize leaves and kernels was very significantly correlated with N doses determined by applying sludge in increasing doses with or without mineral fertilizer.

Sewage sludge different doses applied has the effect on releasing nitrogen in the soil and on Mn accumulation in maize leaves and kernels evidenced by very significant and significant correlations at mineral fertilized variants and not significantly for Mn content in leaves in variants without mineral fertilizer.

The correlations between content of Cd, Ni and Pb from maize (leaves / kernels) and increasing doses of N corresponding to sewage sludge applied without/with mineral fertilizer indicates coefficients of correlation very significant in leaf (Cd, Pb, Ni, Pb) and distinctly significant in kernels (Ni in variants without/ with mineral fertilization, Pb in variants with mineral fertilization) (figs. 1-6).

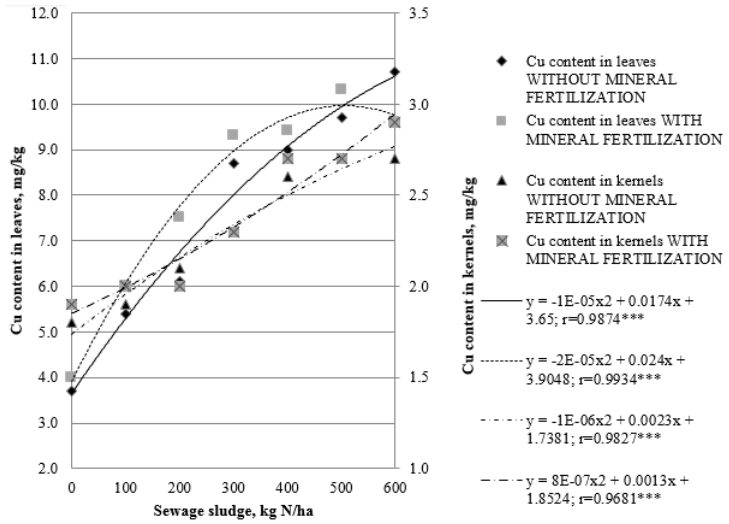

Fig.1. Correlation between Cu content in leaves and kernels with fertilization

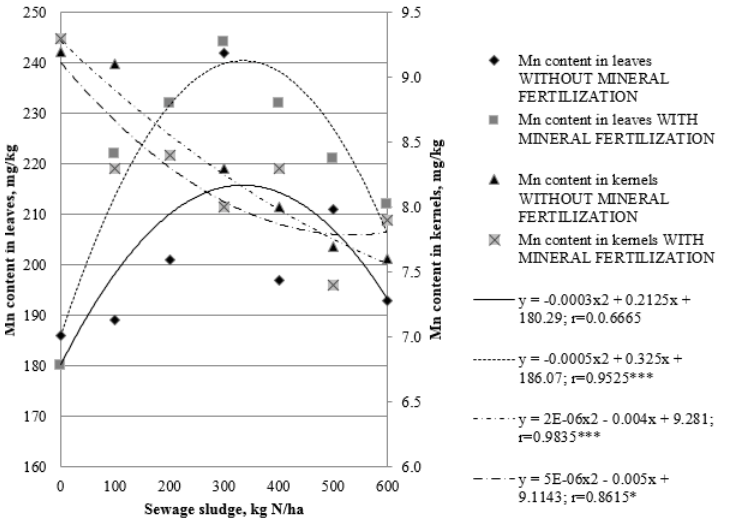

Fig.2. Correlation between Mn content in leaves and kernels with fertilization

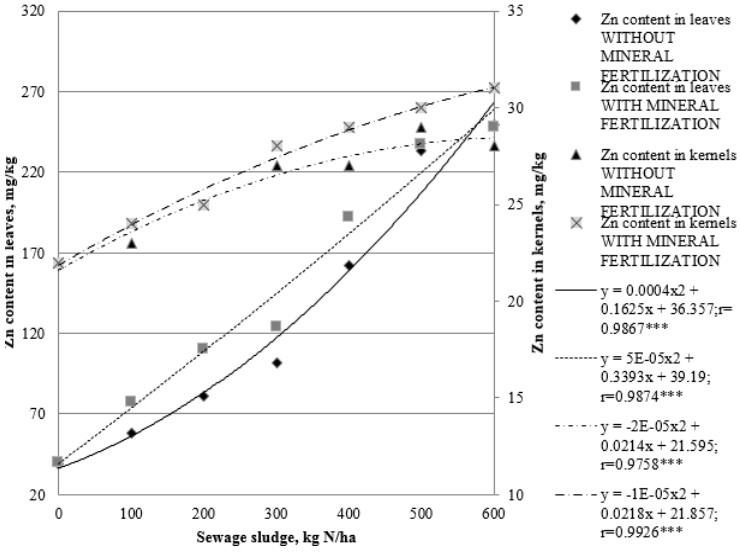

Fig.3. Correlation between Zn content in leaves and kernels with fertilization

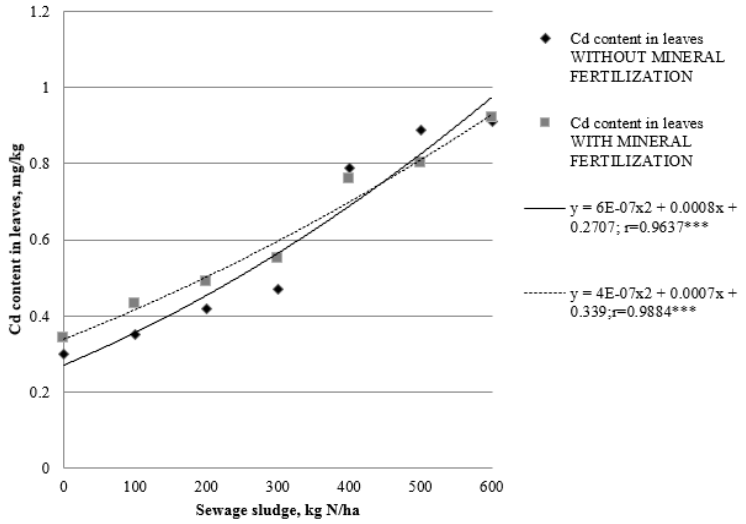

Fig.4. Correlation between Cd content in leaves and kernels with fertilization

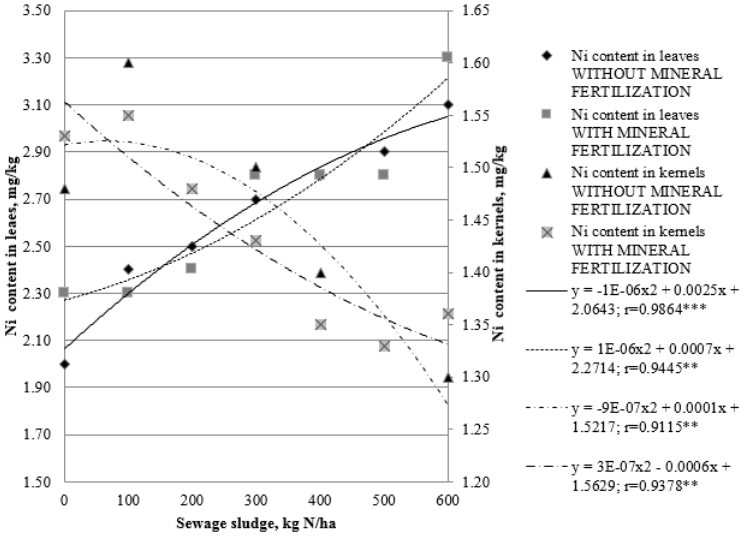

Fig.5. Correlation between Ni content in leaves and kernels with fertilization

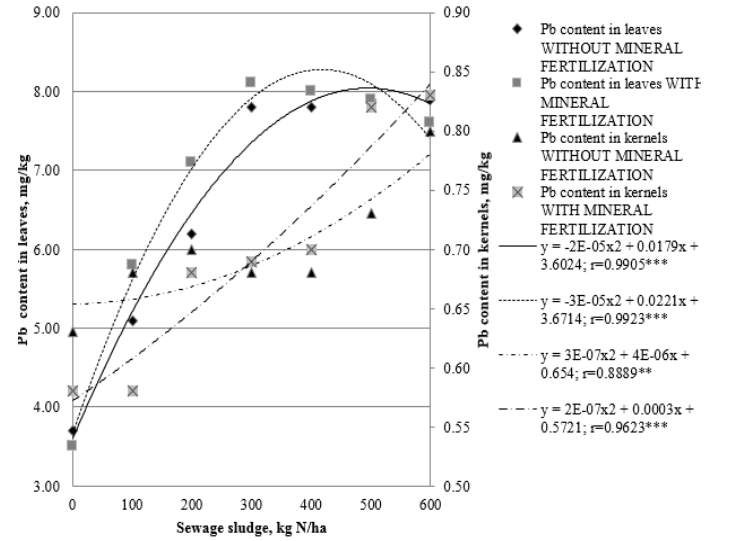

Fig. 6. Correlation between Pb content in leaves and kernels with fertilization

Conclusions

The main objective of the present study was to evaluate the effect of sewage sludge application in various doses (associated or not with mineral fertilizers) on metal accumulation (Cu, Mn, Zn, Cd, Ni, Pb) in the case of maize (*Zea Mays* L.). Based upon the findings of this study, the main conclusions are presented below.

Copper and manganese contents increase with fertilization and rates equivalent to 200 kg N/ha resulted in statistically significant copper content in the maize leaves.

The cadmium content in the maize kernels was not affected by the treatments applied, as the very low values obtained were below the detection limit of the analytical method.

The highest accumulation of manganese in the leaves was recorded at the sludge rate equivalent to 300 kg N/ha, meanwhile in the maize kernels after the increasing sludge rate, the manganese content recorded a decreasing tendency.

The zinc content in the maize leaves significantly increased with the increasing rate of sludge, starting with a rate equivalent to 200 kg N/ha, sewage sludge application being a promoter of zinc accumulation but without becoming excessive. As concerns the zinc content in the

maize kernels, sewage sludge rates higher than 300 kg N/ha resulted in statistically assured increases.

The cadmium level in the maize leaves increased directly proportional with the rate applied and the values were statistically assured at rates higher than 200 kg N/ha. The cadmium content in the maize kernels was not affected by the treatments applied, as the very low values obtained were below the detection limit of the analytical method.

The lead content in the maize leaves increased under the influence of sludge fertilization, but the accumulation was generally low, all values in leaves being under 10 mg/kg and in kernels under 1 mg/kg Pb.

As a general conclusion, it can be appreciated that sewage sludge rates equivalent to 600 kg N/ha did not affect the quality of agricultural production reflected in the content of investigated heavy metals.

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