Plants Growth on Different Fertilized Agricultural Land and Metal Bioavailability

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Abstract
The study compares the amounts of Zea mays, grains, cobs and stalks, harvested on fertilized / unfertilized variant with different fertilizers: M-control, V_1-fertilized with sewage sludge 50 t/ha + indigenous volcanic tuff, 5 t/ha, V_2-fertilized with sewage sludge 50 t/ha, V_3-fertilized with cattle manure, 50 t/ha. The amount of grains and cobs harvested from variant fertilized with cattle manure or sewage sludge mixed with indigenous volcanic tuff were similar. The amount of cobs and grains harvests from variant fertilized with sewage sludge mixed with indigenous volcanic tuff were higher between 15.1-17.5%, respectively 15.4-19.1% than those harvested from variant fertilized with sewage sludge. The indigenous volcanic tuff used as addition at sewage sludge was an important factor for the reduction of bioavailability of metals in the aerial tissues of the plant. The amount of toxic metals, Cd, Cr, Ni, Pb and Zn from the tissues of Zea mays plants was below the maximum limits allowed by the UE rules.

Keywords: cattle manure, metal bioaccumulation, sewage sludge, volcanic tuff, Zea mays.

1. Introduction

High quantities of sewage sludge result from the treatment of city wastewater. This organic material has a huge potential of providing nitrogen and phosphorus based nutrients. The organic substances that contain nitrogen have an important property regarding soil fertilizing namely the nitrogen is released in much slower rate than the nitrogen found in other fertilizing materials. This property of the sewage sludge reduces the nutrient loss from the soil and maintains the necessary reserve throughout the entire growth cycle of the plant. In addition to this, the use of sewage sludge ensures an improvement of the agricultural properties of the soil: water retention, ventilation of the soil, positive modification of the aggregation of soil particles, etc. [1-3]. However, due to its content in potentially toxic traces of biological load and the presence of metals in sewage sludge, it can affect the quality of the crops. Due to these inconveniences, the spreading of sewage sludge on farming lands is presently restricted. On the other hand, the application of treatments through which fresh sewage sludge is brought to forms with a low toxic potential is not out of the question. Results from research fields [4-6] show that the bioavailable metals from fertilizers can be bound to organic radicals, which diminish their release into the soil. The availability and bioavailability of metals from fertilizers has been determined through aerobic and anaerobic treatments to which the sewage

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sludge has been subjected. On the other hand, the effect of some amendments brought to sewage sludge has been reported and it regards the release mode of metals in the soil and their bioavailability [7]. The addition of compost sewage sludge has caused bioaccumulation of Ca in feed plants and Zn in corn. The use of unstabilized sewage sludge has led to the reduction of metal bioavailability for plants, similar to the reduction of metal bioaccumulation from harvests obtained from soil amended with whitewash [8].

2. Materials and methods

The following aspects were taken into study in the present research:

1. Sewage sludge fertilizing options of soil cultivated with Zea mais in the presence/absence of indigenous volcanic tuff;
2. Soil variants cultivated with Zea mais, with or without stable manure fertilizing;
3. Comparative quantitative analysis of crops, expressed in cobs, ears of maze and kernels;
4. The Fe, Mn, Cd, Cr, Cu, Ni, Pb and Zn quantity found in the aerial parts of the Zea mais plant.

The experimental lot was based in the Banat`s University of Agricultural Sciences and Veterinary Medicine „King Mihai I of Romania" from Timisoara, at the experimental unit of the Ecology and Environment Protection discipline, Biotechnology Department, Bioengineering Faculty of Animal Resources (45°47'03.2"N 21°12'54.6"E). The lot was separated into 4 experimental variants: M-control, V_1-sewage sludge fertilizing 50t/ha + volcanic tuff 5t/ha, V_2-sewage sludge fertilizing 50t/ha, V_3-cattle manure fertilizing 50t/ha. Every variant has an 18.2 m^2 surface. Every experimental variant has been reproduced into 3 identical replications. Sewage sludge characteristics: pH 8.5, organic matter 59.78%, total nitrogen content 1.138%, total phosphorus content 1107 mg/kg d.m., Fe 1248.3 mg/kg d.m., Mn 2.3 mg/kg d.m., N 14.7 mg/kg d.m., Pb 23.6 mg/kg d.m., Zn 233 mg/kg d.m. The volcanic tuff comes from the Mirșid quarry and has a 0.2-2mm granulation. Zea mays (corn) has been cultivated on every model, according to the adequate technology [9]. An autumn digging has been applied to the soil, at a 20 cm depth and the germination bed has been prepared before plantation through hoeing. This was also the moment when the fertilizers and tuff were introduced where necessary. The seed was sown on May 10.2017, on rows, with a 70 cm distance between rows and 25 cm distance between seeds. Thus, there were 25 plants on a row and 100 plants per experimental unit. During the vegetation period, only maintenance procedures were done, namely weed control procedures and soil loosening. The plants were harvested when the kernel was hard and the water content was under 30%.

Heavy metals in the aerial parts of mature plants were determined and compared to the Romanian standard. Plant tissues were thoroughly washed, 3x25 ml with de-ionized water, at room temperature, to remove any soil particles attached to plant surfaces. Plant sampling was performed in agreement with the standardized methodology (5g plant tissues were dried (105 °C) to constant weight. Plant samples with precise weight are then brought to 550 °C; to the residual materials 3.5ml of concentrated hydrochloric acid (d=1.189 kg/l) are added, samples are maintained 30 minutes on the dry sand bath. Then, 1.5ml hydrochloric acid (d =1.189kg/l): de-ionized water, 1:1 solution was added. After very slowly filtering on a paper filter, (filtration smooth type 640 de Mackerel-Nagel Germany), the samples were taken to calibrated flasks (25ml) with hydrochloric acid (d=1.189kg/l): de-ionized water, 1:1 solution. Heavy metals content from plant tissues extracts analysis was determined using an Atomic Absorption Spectrophotometer, GBC Avanta AAS, GBC Scientific Equipment Ltd. Company.

3. Results and discussion

The average quantities of cobs, ears and kernels, harvested from the experimental units are presented in Table 1. It is noticeable that the crops from the unfertilized soil are the least. The addition of sewage sludge in the absence of the amendment, in unit V_3 has caused an 11.2 % rise in cobs and 3, respectively 4% in ears and kernels compared to the quantities harvested off unfertilized lands. The addition of sewage sludge, in the presence of the amendment, in unit V_2, has caused a similar rise of the cob harvest to the harvests from fertilized soils, in the absence of amendment. On the other hand, the cob and

kernels harvests had a 17.5-19.1% rise compared to the quantities harvested off unfertilised lands.
It should be noticed that the cob and kernel harvests from the lands fertilized with sewage sludge are similar to the harvests from the soils fertilized with stable manure. It is also noticeable that the stable manure has led to the obtainment of a large quantity of cobs, compared to those from other units.

Table 1. Average quantities of cobs, ears and kernels harvested from experimental units (kg/variant)

Variant	Cobs	Ears	Kernels
M	13.45	8.00	6.55
V_1	15.20	9.70	8.10
V_2	15.15	8.25	6.85
V_3	19.00	10.6	9.00

Observation: Average quantity = sum of harvested quantities from the 3 identical replications of V_3

The average quantities of bio accumulated metals from the aerial parts of the plant, from the cobs and kernels harvested from the experimental units are presented in Table 2 and 3. Analyzing the data in Tables 2 and 3 it results that the aerial part of the plant, made of cobs, has accumulated larger quantities of metals than the kernels. Thus, iron bioaccumulation rose to 60.6-77.9%, for Mn it was 92.2-95.6% and for Cu 42.5-56.0%. In the case of Pb, there was a 50.8-52.9% bioaccumulation in plants harvested from fertilized units. Metal bioaccumulation of Zn can reach up to 50% in plants compared to kernels. Despite the Pb and Zn bioaccumulations, they were fit for the domains imposed by the EU.

Table 2. Average metal quantities bioaccumulated in the aerial parts of the plant and kernels, harested from the experimental variants [mg/kg d.m.]

Variant	Metals bioaccumulated in kernels					
	Cu	Fe	Mn	Ni	Pb	Zn
M	3.5	118.5	4.9	1.3	3.8	53.3
V_1	5.9	81.6	6.2	0.5	1.6	70.4
V_2	5.0	69.4	4.7	0.3	4.1	75.9
V_3	4.0	57.6	4.1	sld	2.9	64.3

In the European Union the maximum limits for Zn in feed were imposed at 250 mg/kg, and for Pb, they were limited at <10 mg/kg.
The aerial parts of the plant did not accumulate detectable levels of Cd and Cr.

Table 3. The average quantities of bioaccumulated metals in the aerial parts of the plant and cobs harvested from experimental units (mg/kg d.m.)

Variant	Bioaccumulated metal in cobs					
	Cu	Fe	Mn	Ni	Pb	Zn
M	7.0	300.7	62.7	1.6	4.7	77.4
V_1	11.9	263.9	139.6	sld	3.4	136.9
V_2	8.7	314.5	107.1	0.5	7.4	93.7
V_3	9.1	232.9	53.8	sld	5.9	116.0

As it can be noticed in Table 2 and Table 3, the values obtained, following the analysis of the aerial parts of the plant, cobs and kernels, were situated in the international limits imposed by the CMA.
The addition of fertilizing sewage sludge has generally led to metal bioaccumulation in the tissues of the plants cultivated on soil fertilized with stable manure. The addition of volcanic tuff on the soils fertilized with sewage sludge has caused and important reduction of metal bioaccumulation, such as Ni 61.5%, Pb 57.9% and Fe 31.1% compared to plants harvested from unfertilized test lands.

4. Conclusions

The addition of fertilizing sewage sludge has generally caused heavy metal bioaccumulation similar to the plants cultivated on the lands fertilized with stable manure. It should be mentioned that the addition of volcanic tuff on the lands fertilized with sewage sludge has led to an important reduction of metal bioaccumulations in kernels such as Ni 61.5% less, Pb 57.9% less and Fe 31.1% less, compared to the quantity of metal found in kernels harvested from the control variant. The aerial part of the plant, made of the cobs, accumulated a greater quantity of metals than the kernels, thus the Fe quantity was 60.0-77.9% higher, Mn was 92.2-95.6% higher and Cu was 42.5-56.0% higher. In what regards the Pb, metal bioaccumulation was between 50.8-52.9 % in the plants harvested from fertilized lands. For Zn, the metal bioaccumulations can reach 50% more compared to the quantity found in kernels. Despite these facts, the Pb and Zn bioaccumulations in the different aerial parts of the Zea mais plant were according to the limits imposed by the EU and there were no detectable traces of Cd and Cr in the aerial parts of the plants.

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