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Making agriculture greener

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Abstract

The application of sewage sludge has been a worldwide agricultural practice for many years. The present study aimed to investigate the effects of sewage sludge (Ss) has on the physiological parameters of maize (*Zea mays* L *cvs.* PR37NO1). 2 g dm^{-3} and 4 g dm^{-3} of sewage sludge were examined in hydroponic experiment. Some metal contents (Al, Cr, Mn, Na, Zn) in the shoots and roots of maize were taken. Living bacteria containing fertilizers (LBCF) were examined on how the treatments modify heavy metal uptakes. Dry matter accumulations in shoots and roots, length of shoots and roots of maize were measured. Chlorophyll contents were determined by using spectrophotometer methods. The dry matter accumulation and length of shoots decreased, the dry matter of roots increased in all of the treatments compared to the nutrient solution without treatment (control). Increased chlorophyll a, b and carotene contents were observed at 4 g dm^{-3} Ss and 4 g dm^{-3} Ss + bio fertilizer treatments.

Key words

Agriculture, Crop production, Maize, Sewage sludge

Introduction

In intensive cropping, the continuous use of high levels of chemical fertilizers often leads to a nutritional imbalance in the soil and a decline in crop productivity (Nambiar, 1994). The problem is more acute in acid lateritic soils because these are characteristically low in organic matter content (Mahapatra *et al.*, 1985) and deficient in available N, P, K, Ca and Mg as well as some micronutrients. A high availability of Fe, Al and Mn, and the deficiency in available plant nutrients in such soils can be overcome by liming and chemical fertilization. Both inputs are costly. Sewage sludge contains essential macronutrients including P, K, Ca, Mg, S and micronutrients, such as Fe, Mn, Zn, Cu, Co, B and Mo. Some sewage sludge are rich in heavy metals such as Cd, Pb, Ni.

Sewage sludge is an unwanted but inevitable by-product of wastewater treatment processes. Sedimentation – both before and after wastewater bio-treatment – produce sewage sludge. An increasing volume of wastewater is produced by the development of the industry and agriculture, and also as a concomitant of the improvement of human life. On the other hand, people are concerned about environmental protection more than ever, and the relevant legislation, regulations are becoming increasingly stricter and critical. The amount of wastewater treated, and consequently the amount of sewage sludge produced is to increase rapidly. The produced wastewater increased 2.5 times in Hungary in the last 30 years. This growth has been more intensive in larger countries, where the population increases rapidly.

Land application is the most commonly used method around the world and is being considered as one of the most economical methods of sludge disposal (Metcalf and Eddy, 2003). This is because the sludge is a freely and easily available nutrient source and hence it can save substantial costs, if applied to the soil as a fertilizer. Sewage sludge is characterized by high concentrations of N, P, other micronutrients (copper, zinc, molybdenum, boron, iron, magnesium and calcium) and organic matters which are beneficial to forestry, vegetation production and landscaping. The application of sewage sludge to agricultural land use results in improved physical, chemical and biological properties of the soil (Beck *et al.,* 1996).

The aim of this study was to examine the compensation effect of living bacteria containing fertilizer under sewage sludge conditions. Our hypothesis was that living bacteria containing fertilizer depending on applied quantity of sewage sludge can modify the effect of sewage sludge on maize. Special interest was given the changes in dry matter accumulation of root and shoot and elements uptake

Materials and Methods

Plant growth conditions : Maize (*Zea mays* L cvs. PR37NO1) seeds were sterilized with 18% hydrogen peroxide, and washed in distilled water, and placed in a 10 mM $CaSO_4$ solution for 4 hrs. Seeds were germinated on moistened filter paper at 25°C. The seedlings were transferred to a continuously aerated nutrient solution of the following composition: 2.0 mM $Ca(NO_3)_2$; 0.7 mM K_2SO_4; 0.5 mM $MgSO_4$; 0.1 mM KH_2PO_4; 0.1 mM KCl; 1µM H_3BO_3; 1µM $MnSO_4$; 1 µM $ZnSO_4$; 0.25 µM $CuSO_4$ and 0.01 µM $(NH_4)_6Mo_7O_{24}$. Iron was added as Fe (III)-EDTA at a concentration of 100 µM. The sewage sludge was added @ 2 and 4 g dm^{-3} to the nutrient solution.

The seedlings were grown under controlled environmental conditions (light/dark regime of 10/14 hr at 24/20°C, relative humidity of 65–70% and a photon flux density of 300 µmol m^{-2} s^{-1}) in the growth chamber. The volumes of experimental pots were 1.7 dm^{-3} with each pot containing 4 plants. The experiment was finished on the 11th day from the seed went on to receive the nutrient solution. LBCF (living bacteria containing fertilizer) containing two bacteria *Bacillus megaterium* and *Azotobacter chrococcum*, 1 ml dm^{-3} was added to the nutrient solution. The sewage sludge applied came from Alkaloida Chemicals Co. Ltd. (East Hungary). It was collected manually after the dewatering process.

Element contents : In order to find out the total element concentration, the plant materials were dried at 85°C and then digested as follows: 10 ml HNO_3 (65% v/v) was added to each gram of the samples for overnight incubation. Then, the samples were pre-digested for 30 min at 60°C. Finally, 3 ml H_2O_2 (30% m/m) was added for 90 min boiling at 120°C. The solution was made complete up to 50 ml, homogenised and filtered through an MN 640 W filter paper. The elements contents were checked with the use of an OPTIMA 3300DV ICP-OE Spectrophotometer.

Chlorophyll-a, b and carotenoid contents : The contents of chlorophyll a, b and carotene were measured with a spectrophotometer (Meterek SP 80). The data were obtained by using the spectrophotometrical formula proposed by Moran and Porath (1980).

Dry matter accumulation : The dry weight of shoots and roots were measured by thermal gravimetric analysis. Plant samples were dried at 85 ºC for 48 hr.

Results and Discussion

Boron, Fe, Mn, Cu, Zn and Mo are essential plant micronutrients that are present in sewage sludge, yet some of these elements can also be toxic to plants and animals if present in excessive amounts. Table 1 shows the element at content of the applied sewage sludge.

The accumulation of heavy metals in soil (Ozores *et al.*, 2005) and in crop plants grown on the soil amended with sludge has been reported by Wei and Liu (2005). The heavy metal accumulations are shown in Table 2.

The Al content decreased at 2 g dm^{-3} Ss and 2 g dm^{-3}Ss+ LBCF treatments as compared to control. Mn content decreased in the shoots as compared to single Ss treatments, when LBCF was added to Ss. Na content of decreased in all the treatments compared to control. The 4 g dm^{-3} Ss increased Al content in shoots of maize. Higher concentration of Al, Cr, Mn, Na and Zn were measured in roots than shoots.

The optimal nutrient supply in crop production is usually achieved by the application of fertilizers. The elements investigated can influence growth when accumulate in large or small quantities, therefore we measured the dry matter accumulation in shoots and roots, length of shoots and roots of maize.

The dry matter accumulation in shoots decreased significantly in all treatments, while that in roots increased in all the treatments. This increase was 69 % when 2 g dm^{-3} Ss was added to the nutrient solution. The increase was low when bio fertilizers were added to the sewage sludge. The length of the shoots decreased in all treatments significantly, while the length of the roots decreased significantly, by 8.6 cm, when 4 g dm^{-3} sewage sludge and bio-fertilizers were added to the nutrient solution.

Many researchers have studied the effect of sewage sludge on Cu and Zn level in corn (Bidwell and Dowdy, 1987; Cajuste *et al.,* 2000; Cripps *et al.,* 1992, Hinesly *et al.,* 1984; Logan *et al.,* 1997; Reddy *et al.,* 1989). Not so many studies have investigated the effect of sewage sludge on grass forage and the ones evaluating Cu and Zn include Sims (1990), while Soon *et al.* (1980), Warman (1986) and McBride and Evans (2002) also reported on Mn, and Tiffany *et al.* (2000) and Zebarth *et al.,* (2000) also evaluated Fe and Mn.

Table 1 : Element content (mg kg^{-1}) of sewage sludge

Elements	Content	Elements	Content
Aluminium	17,349	Manganese	496
Boron	31.6	Molybdenum	8.03
Calcium	1,23,988	Sodium	2,163
Cobalt	3.03	Nickle	24.5
Chromium	41.3	Phosphorus	21,289
Copper	109	Lead	70.1
Iron	21,098	Sulphur	16,000
Potassium	2,878	Strontium	195
Magnesium	5,548	Zinc	473

Table 2 : Effect of different treatments on some toxic-elements (Al, Cr, Mn, Na, Zn) uptake (mg kg^{-1}) n=3± S.E. (2: 2 g dm^{-3}, 4: 4 g dm^{-3} sewage sludge, Biofert.: living bacteria containing fertilizer)

Contents of elements in maize shoots					
			Treatments		
Elements	**Control**	**2**	**2+Biofert.**	**4**	**4+Biofert.**
Al	44.5±3.1	27.05±11.2	24.25±8.1	79.8±3.6	62.0±9.4
Cr	2.05±0.0	0.87±0.0	0.67±0.0	0.97±0.3	1.11±0.3
Mn	70.5±2.7	104.5±0.7	89.15±0.8	118.0±4.2	71.4±2.4
Na	1287.0±28.3	157.0±15.5	220.0±7.1	205.0±15.5	317.5±14.8
Sr	4.45±0.1	22.65±0.9	19.80±0.1	22.30±1.5	18.70±0.3
Contents of elements of maize roots					
			Treatments		
Elements	**Control**	**2**	**2+Biofert.**	**4**	**4+Biofert.**
Al	44.10±17.5	1905±159.0	2028.5±232.6	2663±219.0	1892±193.0
Cr	0.27±0.0	3.85±0.2	4.18±0.2	6.5±0.3	3.21±0.7
Mn	5.6±1.3	170.5±10.6	217±14.1	325±22.6	238±11.3
Na	1575±155.0	3086±33.9	3275.5±193.0	3212.5±19.1	3423.5±60.1
Sr	2.66±0.6	10.85±0.2	10.11±0.8	11.95±0.5	9.04±0.5

Table 3 : Effect of different treatments on some essential-elements (B, Ca, Cu, Fe, K, Mg, P, S, Zn) up-take (mg kg-1) n=3± S.E. (2: 2g dm-3, 4: 4 g dm-3 sewage sludge, Biofert.: living bacteria containing fertilizer)

Contents of elements of maize shoots					
			Treatments		
Elements	**Control**	**2**	**2+Biofert.**	**4**	**4+Biofert.**
B	6.83±0.1	16.80±2.3	14.1±0.00	21.15±0.3	18.35±1.5
Ca	7815±48.1	14744±377.6	12889±182.4	14206±349.3	11849±223.4
Cu	18.5±0.8	7.99±0.4	6.66±0.3	8.44±0.1	6.68±0.1
Fe	664.5±27.5	97.1±19.6	109±1.4	117.5±16.3	150.5±30
K	61775.5±215.4	10933±623.6	19140±459.4	11364±970	23339.5±362.7
Mg	4876.5±167.6	7787±123.0	6399.5±190.2	9104±360.6	5177±66.4
P	8916.5±338.7	11394±74.9	11058.5±249.6	13213±598	10102±237.6
S	7671.5±144.9	7487±86.3	6556±138.6	7468±15.5	5580±91.9
Zn	89.4±3.2	53.2±2.3	55.8±0.8	64.25±1.5	55.4±0.9
Contents of elements of maize roots					
			Treatments		
Elements	**Control**	**2**	**2+Biofert.**	**4**	**4+Biofert.**
B	2.97±0.6	5.87±0.6	5.86±0.6	8.33±0.1	6.6±0.2
Ca	1482.5±54.4	6492.5±238.3	5712±420.0	6587±445.5	4700±29.7
Cu	11.95±0.6	16.5±0.1	14.35±0.2	17.05±0.1	15.8±0.4
Fe	86.2±0.3	1726±240.4	2108±147.1	2706±169.7	2456±231.9
K	7638±161.1	6618.9±289.1	8974±391.6	7307±257.5	9971±357.4
Mg	455±1.0	7259.5±246.8	6334±376.2	7481±65.5	6917±83.4
P	3131±50.6	5873±2.8	7838±333.7	6692.5±147.8	8168±120.2
S	1448.5±195.9	11456.5±195.8	9885±95.8	11831.5±137.3	8703.5±98.3
Zn	61.7±2.1	133±7.1	130±9.8	172±9.8	144±2.8

Warnam and Termeer (2005) reported that application of sewage sludge to agricultural land usually increases Cu and Zn concentration in amended plants and normally the Zn content increased more substantially than Cu content. It is partly true as Cu content decreased in shoots of maize and increased in roots as compared to control. Similar trend was observed with Zn (Table 3).

Boron content decreased in shoots and roots when bio-fertilizers were added to the nutrient solution. Ca, Mg and S

Table 4 : Effects of different treatments on the dry matter accumulations of shoots and roots of maize (g $plant^{-1}$) and the length of shoots and roots (cm) n=12± S.E. Significant differences compared to the control: *p<0.05;***p<0.001

Treatments	Shoot (g $plant^{-1}$)	Root (g $plant^{-1}$)	Shoot (cm)	Root (cm)
Control	0.134± 0.0***	0.042± 0.0	26.60± 1.0	29.3± 6.7
2	0.089± 0.0***	0.071± 0.0***	21.64± 1.9***	37.9± 5.9*
2+Biofert.	0.069± 0.0***	0.047± 0.0*	19.23± 1.8***	28.4± 9.4
4	0.093± 0.0***	0.058± 0.0	22.57± 2.5***	28.2± 6.8
4+Biofert.	0.082± 0.0***	0.042± 0.0	20.98± 2.8***	20.7± 5.6*

Table 5 : Effects of different treatments on the relative chlorophyll (Rel.chl) contents, chlorophyll-*a* (chl-*a*), chlorophyll-*b* (chl-*b*) and carotene (car) (mg g^{-1}) n=3± S.E. Significant differences compared to the control: *p<0.05;***p<0.001.

Treatments	Rel.chl.	Chl-*a*	Chl-*b*	Chl-a/b ratio	Car.
Control	46.4± 2.1	15.1± 0.6	5.1± 0.5	2.9	10.2± 1.1
2	36.0± 4.4***	13.3± 1.3	4.3± 0.6	3.0	8.9± 0.6
2+Biofert.	38.7± 3.6***	14.3± 1.7	4.5± 0.9	3.1	9.7± 1.0
4	40.8± 2.8***	16.2± 0.8	5.9± 0.8	2.7	11.9± 0.4
4+Biofert.	40.3± 4.1***	16.2± 0.2	5.9± 0.2*	2.7	11.3± 0.5

contents in shoot and root increased at all the treatment, while K increased in treated plants.

Fe concentration is controversial issue in the 2 and 4 g dm^{-3} treatments. Fe content decreased in shoots when 2 g dm^{-3} sewage sludge was applied with bio fertilizer, and this value increased in roots as compared to control. Fe contents however, increased in shoots and decreased in roots when 4 g dm^{-3} sewage sludge was added to the nutrient solution with bio fertilizers.

P content decreased in shoot and increased in roots as compared to control, when biofertilizer was added to the nutrient solution. Low chlorophyll content affect photosynthetic activities. Decrease in dry matter accumulation can be explained by low level of chlorophyll content.

The relative chlorophyll and chlorophyll *a*, *b* contents also were measured in the 2nd leaf of maize. The total chlorophyll concentration is a unifying parameter for indicating the effect of specific intervention. However, it is important to record changes in the two components of chlorophyll a and chlorophyll b and especially in their ratios. This is due to the fact that heavy metals could affect each component at different levels creating changes in some part of plants physiology (Zengin and Munzuroglu, 2005).

Chlorophyll a, b and carotene contents were higher in 4 g dm^{-3} and 4 g dm^{-3} + bio-fertilizer treatments than control. The differences are not always significant (Table 5). Chlorophyll a increased by 1.1 mg. Chlorophyll b content was higher by 0.89 mg in 4 g dm^{-3} and 0.87 mg in 4 g dm^{-3} sewage sludge + LBCF treatment. The optimal chl-a/b ratio was 3. Therefore the treatments did not have unfavorable effect on chl-a/b ratios. The heavy metal accumulation responsible for the reduction in total chlorophyll concentration had negative effect on the ratio of chl-a to chl-b. This occured due to faster hydrolysis of chl-a compared with chl-b when plants were under stress (Schoch and Brown, 1987).

The effect of Ss were examined on some physiological parameters of maize. Al contents decreased in 2 g dm^{-3} Ss and 2 g dm^{-3} Ss + LBCF treatment as compared to control. Mn content in shoots decreased when LBCF was added to the sewage sludge as compared to sewage sludge treatments. High concentration of Al, Cr, Mn, Na and Zn was found in roots than in shoots. Dry matter accumulation in shoots significantly decreased at all the treatments. Dry matter content in roots increased at all treatments. Chlorophyll a,b and carotene content was higher in 4 g dm^{-3} and 4 g dm^{-3} + bio-fertilizer treatments than control. LBCF produced no convincing effect on the heavy metal uptake or on the physiological parameters of maize.

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