

## Agricultural Use of Sewage Sludge to Reduce Environmental Pollution

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**Abstract.** The application of sewage sludge has been a worldwide agricultural practice for many years. The aim of our study was to present results about some effects of sewage sludge (Ss) on the physiological parameters of maize (*Zea mays* L cvs. PR37NO1) in hydroponic experiment. Living bacteria containing fertilizer (LBCF) also was examined on how the treatments modify the heavy metal up-take. Some toxic elements content (Al, Cr, Mn, Na, Zn) were measured in the shoots and roots of maize. Higher concentration Al, Cr, Mn, Na and Zn were measured in the roots than in the shoots. The dry weight of shoots and roots were measured with thermal gravimetric analysis. The dry matter accumulation and length of shoots decreased, the dry matter of roots increased at all treatments compared to the control. Chlorophyll content was determined by spectrophotometer methods. Increased chlorophyll a, b and carotenes contents were observed at 4 g dm<sup>-3</sup> Ss and 4 g dm<sup>-3</sup> Ss + LBCE treatments.

**Keywords:** Agriculture, Crop production, Maize, Sewage sludge

### 1. Introduction

The agricultural application of sewage sludge is the most common disposal route in Europe and in China because sewage sludge contains N, P and several microelements such as Zn, Cu and Fe which are essential to plants growth. However, the widespread use of Ss in agriculture needs critical evaluation because it may contain different kind of contaminants such as heavy metals [1] which may accumulate in the soil, and can be taken up by plants and can also be harmful when entering into the human food chain. The Directive 86/278/EEC [2] set up serious control of Ss-use in fields which determines limit values for seven heavy metals.

In this study, the effect of SS on the heavy metal up-take was examined in case of maize (*Zea mays* L. cvs. PR37NO1). Living bacteria containing fertilizer was examined on how this modifies especially the heavy metal up-take.

### 2. Methods

#### 2.1. Plant Growth Conditions

Maize (*Zea mays* L cvs. PR37NO1) seeds were sterilized with 18% hydrogen peroxide, and washed in distilled water, and placed in 10 mM CaSO<sub>4</sub> for 4 hours. Seeds were germinated on moistened filter paper at 25°C. The seedlings were transferred to continuously aerated nutrient solution of the following composition: 2.0 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.7 mM K<sub>2</sub>SO<sub>4</sub>, 0.5 mM MgSO<sub>4</sub>, 0.1 mM KH<sub>2</sub>PO<sub>4</sub>, 0.1 mM KCl, 1μM H<sub>3</sub>BO<sub>3</sub>, 1μM MnSO<sub>4</sub>, 1 μM ZnSO<sub>4</sub>, 0.25 μM CuSO<sub>4</sub>, and 0.01 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>. Iron was added as Fe(III)-EDTA at a concentration of 100 μM. Sewage sludge was added at rate of 2 and 4 g L<sup>-1</sup> to the nutrient solution.

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Seedlings were grown under controlled environmental conditions (light/dark regime 10/14 h at 24/20°C, relative humidity of 65–70% and a photon flux density of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in controlled environmental room. The volume of experiment pots were 1.7 L, one pot contains 4 plants. The pH of nutrient solution was 4.4. The experiment was finished on the 11<sup>th</sup> from the seed to take on nutrient solution. LBCF contains two bacteria *Bacillus megaterium* and *Azotobacter chroococcum*, 1 ml  $\text{dm}^{-3}$  was added to the nutrient solution.

## 2.2. Element Contents

To measure the total elements concentration the plants materials were dried at 85°C and then digested as follows: 10 ml  $\text{HNO}_3$  (65v/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  $\text{H}_2\text{O}_2$  (30m/m%) was added for 90 min. boiling at 120°C. The solution were filled up to 50 ml, homogenised and filtered through MN 640 W filter paper. The elements contents were checked with the use of OPTIMA 3300DV ICP-OA Spectrophotometer.

## 2.3. Chlorophyll-a, b and Carotenes Contents

The contents of chlorophyll a, b and carotene were measured by spectrophotometer (Meterek SP 80). The data obtained after the spectrophotometry formula proposed by Moran and Porath [3].

## 2.4. Dry Matter Accumulation

The dry weight of shoots and roots were measured with thermal gravimetric analysis. Plant samples were drying at 85 C° for 48 h.

## 3. Results and Discussion

The accumulation of heavy metals in the soil [4] and in the crop plants grown on the soil amended with sludge has been reported by Wei and Liu [5].

Table1: Effect of different treatments on some toxic-element (Al, Cr, Mn, Na, Zn) up-take ( $\text{mg kg}^{-1}$ )  $n=3\pm$  S.E. (2: 2g  $\text{dm}^{-3}$ , 4: 4g  $\text{dm}^{-3}$  sewage sludge, LBCF: living bacteria containing fertilizer)

Contents of elements of maize shoots					
Elements	Treatments				
	Control	2	2+LBCF	4	4+LBCF
Al	44.5± 3.1	27.05± 11.24	24.25± 8.13	79.8± 3.62	62.0± 9.39
Cr	2.05± 0.02	0.87± 0.01	0.67± 0.05	0.97± 0.30	1.11± 0.34
Mn	70.5± 2.68	104.5± 0.71	89.15± 0.77	118.0± 4.24	71.4± 2.4
Na	1287.0± 28.28	157.0± 15.5	220.0± 7.07	205.0± 15.5	317.5± 14.8
Zn	89.4± 3.25	53.2± 2.26	55.8± 0.84	64.2± 1.48	55.4± 0.98
Contents of elements of maize roots					
Elements	Treatments				
	Control	2	2+LBCF	4	4+LBCF
Al	44.10± 17.5	1905± 159	2028.5± 232.6	2663± 219	1892± 193
Cr	0.27± 0.02	3.85± 0.21	4.18± 0.22	6.5± 0.27	3.21± 0.67
Mn	5.6± 1.3	170.5± 10.6	217± 14.14	325± 22.62	238± 11.31
Na	1575± 155	3086± 33.94	3275.5± 193	3212.5± 19.09	3423.5± 60.10
Zn	61.75± 2.05	133.0± 7.07	130.0± 9.89	172.0± 9.89	144.0± 2.82

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

The optimal nutrient supply is usually achieved by the application of fertilizers In crop production. The investigated elements can influence the growth when they accumulated in larger or smaller quantities in plants, so we measured the dry matter accumulation of shoots and roots, length of shoots and roots of maize.

Table 2: Effect of different treatments on the dry matter accumulation of shoots and roots of maize ( $\text{g plant}^{-1}$ ) and length of shoots and roots (cm)  $n=12 \pm$  S.E.

Treatments	Shoot ( $\text{g plant}^{-1}$ )	Root ( $\text{g plant}^{-1}$ )	Shoot (cm)	Root (cm)
Control	0.134 $\pm$ 0.01	0.042 $\pm$ 0.01	26.60 $\pm$ 1.03	29.33 $\pm$ 6.70
2	0.089 $\pm$ 0.01***	0.071 $\pm$ 0.01***	21.64 $\pm$ 1.87***	37.91 $\pm$ 5.89*
2+LBCF	0.069 $\pm$ 0.01***	0.047 $\pm$ 0.02	19.23 $\pm$ 1.85***	28.38 $\pm$ 9.41
4	0.093 $\pm$ 0.03**	0.058 $\pm$ 0.03	22.57 $\pm$ 2.52***	28.22 $\pm$ 6.84
4+LBCF	0.082 $\pm$ 0.02***	0.042 $\pm$ 0.02	20.98 $\pm$ 2.86***	20.70 $\pm$ 5.65*

Significant differences compared to the control: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

The dry matter accumulation of shoots significantly decreased at all treatments. The dry matter of root increased at all treatments. This increasing was 69 % when  $2 \text{ g dm}^{-3}$  was added to the nutrient solution. The bio-fertilizer addition to the sewage sludge the increase was lower then without bio-fertilizer. The length of shoots significantly decreased in all treatments, while the length of roots significantly increased with 8.5 cm when  $2 \text{ g dm}^{-3}$  sewage sludge was applied. This value significantly decreased with 8.6 cm when  $4 \text{ g dm}^{-3}$  sewage sludge and bio-fertilizer was added to the nutrient solution.

Low chlorophyll contents affect photosynthetic activities. The decreasing dry matter accumulation can be explained by the lower level of the chlorophyll contents. With respect to this later suggestion, we measured the relative chlorophyll and chlorophyll *a*-, *b* contents in the 2<sup>nd</sup> leaf of maize. Total chlorophyll concentration is a unifying parameter for indicating the effect of specific interventions. However, it is important to record changes in the two components of chlorophyll a (chl-a) and chlorophyll b (chl-b) and especially their ratio. This is due to the fact that heavy metals could affect each component at a different level creating changes in some part of plants physiology. The results are shown in Table 3.

Table 3: Effect of different treatments on the chlorophyll-*a* (chl-a), chlorophyll-*b* (chl-b) and carotene (car) ( $\text{mg kg}^{-1}$ )  $n=3 \pm$  S.E.

Treatments	Chl-a	Chl-b	Chl-a/b ratio	Car.
Control	15.09 $\pm$ 0.65	5.08 $\pm$ 0.47	2.97	10.16 $\pm$ 1.11
2	13.25 $\pm$ 1.26	4.34 $\pm$ 0.63	3.05	8.90 $\pm$ 0.64
2+LBCF	14.26 $\pm$ 1.71	4.55 $\pm$ 0.96	3.13	9.69 $\pm$ 1.01
4	16.19 $\pm$ 0.88	5.97 $\pm$ 0.76	2.71	11.96 $\pm$ 0.44
4+LBCF	16.19 $\pm$ 0.22	5.95 $\pm$ 0.21*	2.72	11.33 $\pm$ 0.54

Significant differences compared to the control: \* $p < 0.05$ ;

A, b chlorophyll and carotene contents were higher at the  $4 \text{ g dm}^{-3}$  and  $4 \text{ g dm}^{-3}$  + bio-fertilizer treatments than at the control. The a-chlorophyll increased by 1.1 mg. The b-chlorophyll contents were higher with 0.89 mg in the  $4 \text{ g dm}^{-3}$  and 0.87 mg in the  $4 \text{ g dm}^{-3}$  sewage sludge + LBCF treatment. The optimal chl-a/b ratio is 3. The results are around 3, so the treatments have not unfavorable effect on chl-a/b ratio. The heavy metal accumulation responsible for the reduction of total chlorophyll concentration had a similar negative effect in the ratio of chl-a to chl-b. This occurs due to a faster hydrolysis ratio of chl-a compared with chl-b when plants are under stress [6].

#### 4. Conclusion

The effect of Ss was examined on the some physiological parameters of maize. The content of Al decreased at the  $2 \text{ g dm}^{-3}$  Ss and  $2 \text{ g dm}^{-3}$  Ss+ LBCF treatments compared to the control. The Mn content in

the shoots decreased when LBCF was added to the SS compared to SS treatments. Higher concentration Al, Cr, Mn, Na and Zn were measured in the roots than in the shoots. The dry matter accumulation of shoots significantly decreased at all treatments. The dry matter of root increased at all treatments. A, b chlorophyll and carotene contents were higher at the 4 g dm<sup>-3</sup> and 4 g dm<sup>-3</sup> + bio-fertilizer treatments than at the control. LBCF has no convincing effect on the heavy metal up-take or on the change of measured physiological parameters of maize.

## 5. References

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