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Research Article

Micronutrient and heavy metal bioaccumulation in Bermuda grass (*Cynodon dactylon* L. Pers) grown in calcareous soil using stabilized and dried sewage sludge

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Abstract

This study examines the micronutrients and heavy metal bioaccumulation in Bermuda grass (*Cynodon dactylon* L. Pers), grown in two different types of calcareous soil (CS) with the application of stabilized and dried sewage sludge (SS). At the end of the growth period, the Fe, Zn, Mn and Cu (micronutrients) concentrations in the substrate have increased. Furthermore, it was determined that the total Fe, Zn, Mn and Cu concentrations were higher in CS_1 compared to CS_2. For the heavy metals the concentrations of Ni and Cr have increased, and the concentration of total Cd has slightly but significantly decreased. The total Pb concentration was below the detection limit (DL for Pb <0.03 mg kg^{-1}). It was determined that the total Ni, Cr and Cd concentrations were higher in CS_1 than in CS_2. In the aerial biomass of the Bermuda grass, the Fe and Zn concentrations have increased with increasing SS applications, whereas Mn concentrations have decreased. Ni, Cr, Cd and Pb concentrations were unaffected by SS applications for both types of CS. The bioconcentration factor (BCF) values for the shoot of the plant (BCF_{Fe}, BCF_{Zn}, BCF_{Mn}, BCF_{Cu}, BCF_{Ni}, BCF_{Cr}, BCF_{Cd}) were determined to be below the critical limit of 1. The application of sewage sludge has not resulted in bioaccumulation above critical limits in the shoot of the plant. However, considering Bermuda grass substrate, it can be suggested to add sewage sludge to the calcareous soil in amounts between 20–40 t ha^{-1}, considering its long-term remanence.

Keywords: Bermuda grass; bioaccumulation; calcareous soil; heavy metals; sewage sludge; micronutrient elements

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Introduction

Sufficient soil organic matter represents one of the most important elements of successful agricultural production. Organic matter is a necessary element of the soil for the sustainability of soil productivity (Gerke, 2022). An ideal agricultural land should contain about 5% organic matter. However, the organic content of the soil is closely related to the climate of the region. Generally, the organic matter quantities are higher in cooler climates as the decomposition rates are slower, and lower in warmer and arid climates (Conant *et al.*, 2011).

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Considering Turkey's climate zone, the organic content of the soil is low, being comprised between <1-3% for 87.93% of the land (Eyüpoğlu, 1999). About 45% of the mineral soils in Europe have low or very low organic carbon content (0-2%) and 45% have a medium content (2-6%) (Rusco *et al*., 2001). Among the most prominent methods used to prevent the insufficiency of organic matter in agricultural lands, it can be mentioned the application of farm manure (barnyard manure, poultry manure *etc*.), green manure (with legumes *etc*.) and compost (Johnston, 1999).

In Turkey in recent years, the local municipalities have invested largely in sewage infrastructures because of the changes in environmental policies, and thus the number of water treatment facilities has been increasing. According to Municipal Water Waste Statistics, in 2006 2.14 billion m^3 of 3.4 billion m^3 of wastewater was being refined, whereas in 2018 this number went up to 4.2 billion m^3 of 4.8 billion m^3 wastewater (Anonymous, 2018.). The increasing amounts of filtered water bring about an increase in the quantities of produced sewage sludge. After being decomposed, condensed, dehydrated and dried, this sludge is renamed as "stabilized and dried sewage sludge". Throughout Turkey, the amount of produced dried sewage sludge is around 1000 tons per day (Anonymous, 2013). Most of this waste is sent to cement factories to be used as additional fuel for thermal energy, whereas a part of is sent to private companies (Sanitary Landfills) to be stored for a certain price paid by the municipalities.

Urban waste sludge is expected to increase further due to the rapid increase in global population and growth in economic activities, and efforts are continuing to dispose of it in an environmentally friendly manner (Hoang *et al*., 2022). One of the alternative recycling methods for the stabilized and dried sewage sludge is agricultural usage (Bai *et al*., 2017; Balanica *et al*., 2018). This application is effective with the regulation of "The Usage of Home and City Sewage Sludge in Agriculture – 03.08.2010/27661" in Turkish legislation. In the European Union legislation, it is effective the regulation "Directive 86/278 - soil protection when sewage sludge is used in agriculture". It is essential to recommence scientific research on the values provided by these regulations for the agricultural use of sewage sludge without causing harm to the environment, crop production, or animal and human health. Researchers should take into consideration that it is important to evaluate the physical, chemical and biological properties of the soil (soil pH, soil salinity, texture, $CaCO_3$ and organic content, microorganism activities *etc*.) and the types of plant that will be grown when applying sewage sludge (Langdale *et al*., 1971; Singh and Agraval, 2008).

Bermuda grass (*Cynodon dactylon* L.) is a member of the cosmopolitan genus of grasses *Cynodon* and family *Poaceae*. It is a C4 grass that grows vegetatively from both rhizomes and stolons, as well as generatively by producing seeds. Due to its superior turf quality and outstanding durability, Bermuda grass is widely used for residential and recreational areas worldwide due to its ability to form a dense sward with a fine textured appearance. Bermuda grass is suitable for ornamental home lawns, golf course fairways/tees, athletic fields, industrial parks and soil erosion control as well (Shi *et al*., 2014; Noor *et al*., 2023). In previous studies, the application of sewage sludge was shown to enhance the growth of grass. For instance, research by Ok (2012) demonstrated that sewage sludge amendments improved the biomass production of perennial grasses by providing essential nutrients such as nitrogen, phosphorus, and potassium. Similarly, Zere Taskin and Bilgili (2023) reported that the application of treated sewage sludge resulted in significant yield increases in Lolium, highlighting its potential as a valuable nutrient source in agriculture. Many studies reported that applying sewage sludge had a favourable effect on the general aspect of the turfgrass (Cheng *et al.* 2007; Rezende *et al.* 2020).

In this study, stabilized and dried sewage sludge obtained from Hurma Wastewater Treatment Facility, which serves Antalya, one of the major tourist destinations in Turkey, was applied to two different calcareous soils with different levels of lime ($CaCO_3$) in increasing dosage. The biological material chosen was Bermuda grass because it is one of the most commonly used type of grass in building green fields in Antalya. Pursuing the purpose of agricultural usage of the stabilized sewage sludge in the substrate of Bermuda grass, the main aim of the study was to evaluate the soil and plant concentration and plant bioaccumulation of potentially toxic micronutrients (Fe, Zn, Mn and Cu) and heavy metals (Ni, Cr, Cd and Pb).

Materials and Methods

Experiment design and sampling

The experimental materials used in the present research were stabilized sewage sludge, soil with two different $CaCO_3$ contents and Bermuda grass plants. Sewage sludge was supplied from the domestic wastewater treatment plant in Antalya/Hurma in anaerobically digested, stabilized and dried form (Figure 1). Soils containing different $CaCO_3$ contents were collected from the Akdeniz University campus area. Bermuda grass (*Cynodon dactylon* L. Pers) was grown as an experimental plant. Some selected physico-chemical properties of soils and sewage sludge are presented in Table 1.

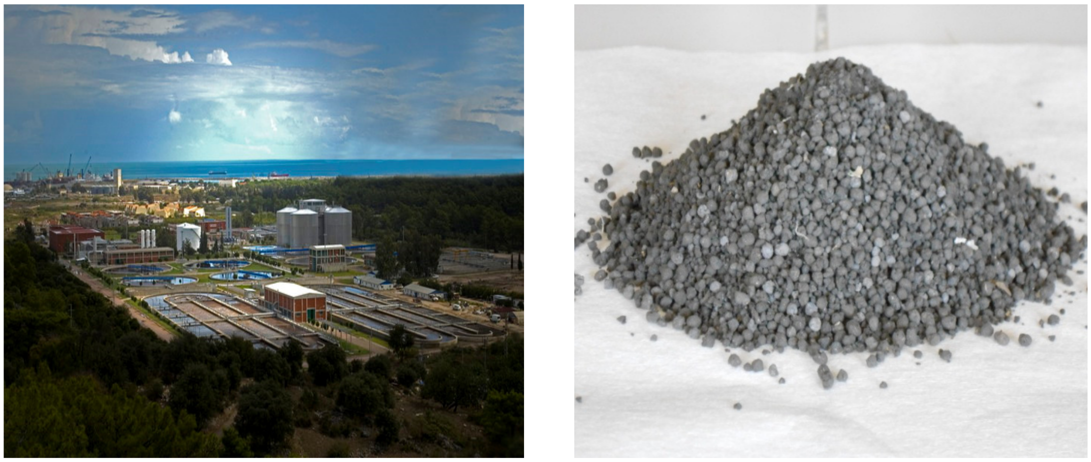

Figure 1. Antalya/Hurma wastewater treatment plant (left) and the stabilized and dried sewage sludge used in the experiment (right)

The research was conducted in randomized plot trial design with four replicates in a glass greenhouse and the experiment was conducted with a total of 48 variant. A total of 9 kg of soil was placed in pots. Soil samples containing two different levels of lime ($CaCO_3$), respectively 8% (CS_1) and 19.4% (CS_2) were mixed with stabilized and dried sewage sludge in the doses of 0 (SS_0), 5 (SS_{50}), 10 (SS_1), 20 (SS_2), 40 (SS_4), 80 (SS_8) t ha^{-1}. The substrate from the pots was watered evenly and incubated for 15 days (Figure 2).

Table 1. The physico-chemical properties of sewage sludge (SS) and calcareous soil (CS) used in the study

Parameters		Sewage sludge (SS)	Calcareous soil 1 (CS_1)	Calcareous soil 2 (CS_2)	CRM No: (RTC) CRM052	
					Reference values	Observed values
pH		6.33	7.06	7.31	-	-
EC, ds m^{-1}		5.0	0.29	0.26	-	-
$CaCO_3$, %		6.5	8	19.4	-	-
Texture		-	Loamy	Loamy	-	-
Organic matter, %		70	3.4	3.5	-	-
Total	Fe, mg kg^{-1}	4545	58650	49990	14700±632	13110
	Zn, mg kg^{-1}	582.1	185.3	180.2	94.3±2.95	91.07

	Mn, mg kg^{-1}	513.2	1140	816.2	217±11.8	198.9
	Cu, mg kg^{-1}	172.7	93.71	99.73	56.5±1.60	50.45
	Ni, mg kg^{-1}	159.7	94.25	62.38	50.8±1.42	56.24
	Cr, mg kg^{-1}	62.5	69.67	67.70	57.8±1.98	73.97
	Pb, mg kg^{-1}	20.41	*D.L.	*D.L.	82.9±2.52	54.89
	Cd, mg kg^{-1}	1.48	5.25	3.04	43±1.16	36.26

* <D.L. Below the detection limits of the device (for Pb D.L.<0.03 mg kg^{-1})

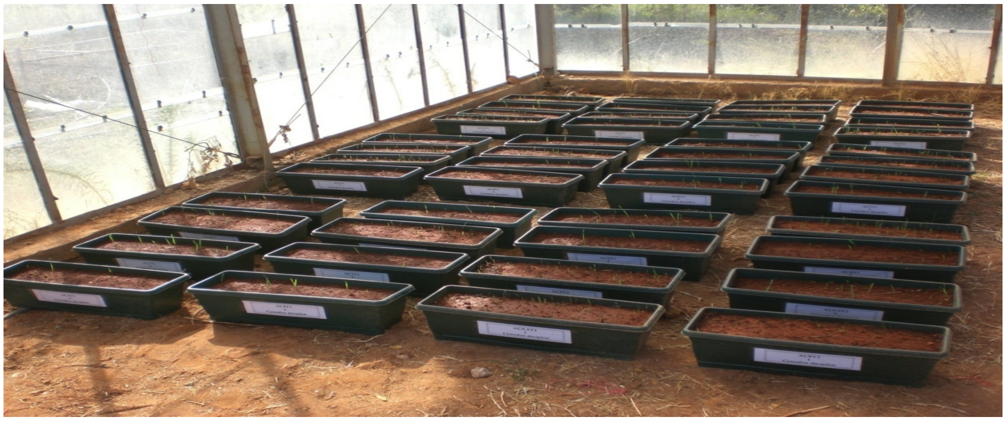

Figure 2. Substrate (the pots containing different mixtures of soil and sewage sludge) during the incubation period

At the end of the incubation period, Bermuda grass seeds was seeded at 80 g m^{-2} per pot. Plants were grown for 16 weeks with even watering. At the end of the experiment, the aerial biomass of the grass from each pot was cut for sample analyses (Figure 3) and the substrate samples were collected from each variant.

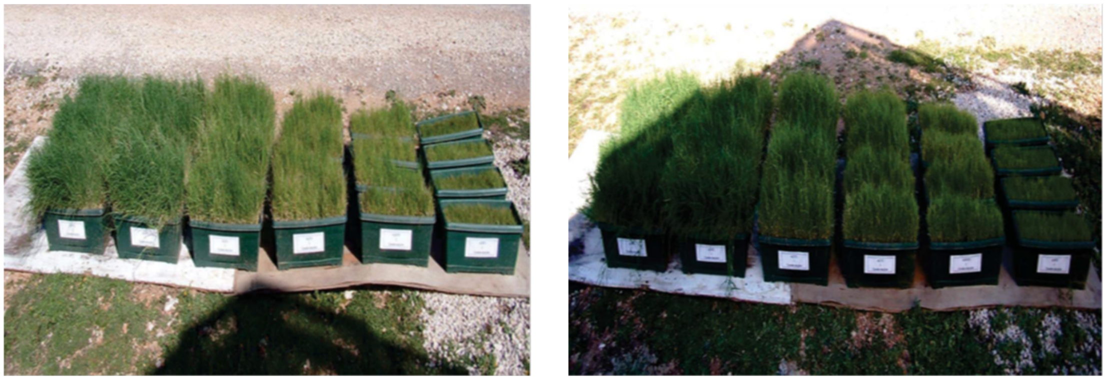

Figure 3. View of the experiment before the harvest of Bermuda grass (Left: CS$_1$- Right: CS$_2$; Applications of from left to right: SS$_8$-SS$_4$-SS$_2$-SS$_1$-SS$_0$.5-SS$_0$)

Laboratory analysis

The plant samples were washed with deionized water, and dried in oven at 65 °C until constant weight and dry matter (g pot^{-1}) was determined. After drying, the biomass was grounded and analyzed to determine some microelements (Fe, Zn, Mn and Cu) and heavy metal concentrations (Ni, Cr, Pb and Cd). In addition, plant substrate (sewage sludge and calcareous soil mixture) samples were also analyzed to determine total concentrations of the same microelements and heavy metals as in the case of Bermuda grass biomass samples.

The sampled soils, sewage sludge and substrate samples were air-dried and then passed through a 2 mm sieve. Soil texture was determined using hydrometer method (Bouyoucos, 1955), soil CaCO$_3$ content using

Scheibler calcimeter (Çağlar, 1949) and organic matter using modified Walkley-Black method (Black, 1965). The pH and EC (electrical conductivity) were measured in a 1:2.5 soil: deionized water mixture (Jackson, 1967). For the analysis of the concentration of total Fe, Zn, Mn, Cu, Ni, Cr, Pb and Cd was used 0.5 g of trial soils, sewage sludge and substrate samples. The samples of Bermuda grass biomass were 0.25 g weight, there being added 65% HNO_3 (9 ml) and 37% HCl (3 ml) and placed in microwave oven (Berghoff MWS-4 +) according to the U.S. EPA. (2007) and then wet combustion was carried out. The concentrations were calculated as ICP-OES (Perkin Elmer, Optima 7000 DV).

Bioaccumulation shows the capacity of a chemical substance to concentrate in the surrounding biological systems, and this feature is characterized by the bioconcentration factor (BCF). To calculate the bioconcentration factor of the microelements and heavy metals analyzed in the aerial biomass of Bermuda grass, we divided the concentration of each element in the above-ground parts of the plants by its total concentration in the growing medium, as described by Liu *et al.* (2009). This calculation was performed individually for each element.

$BCF = TC_{\text{aerial biomass}}/TC_{\text{substrate}}$

BCF: Bioconcentration Factor

TC: Total Concentration

Statistical analysis

The results obtained from the study were evaluated by variance analysis in the randomized plot trial design using the "SPSS 17.N 0" statistical package program and the Duncan Test, one of the multiple comparison methods, was used to determine the differences as a result of the variance analysis (SPSS, 2008).

Results and Discussion

The total Fe, Zn, Mn, and Cu concentrations of the substrate created due to the application of sewage sludge to soils containing different levels of lime increased. When the effect of the lime levels of the soils on the total Fe, Zn, Mn and Cu concentrations was examined, it was seen that the total Fe, Zn, Mn and Cu concentrations were higher in the substrate environments prepared with CS_1 soil compared to the substrate prepared with CS_2 soil (Table 2). Özyazıcı *et al.* (2012), in a study in which they examined the change in the Fe, Mn, Zn and Cu contents of the soil with the application of treatment sludge to the soil, stated that domestic and urban treatment sludges can provide significant contributions in increasing the amount of Fe, Zn and Mn from the nutrients needed by plants and in soils with alkaline reactions. They stated that treatment sludge application may be beneficial for micronutrient deficiency which is frequently seen in plants. Rigueiro-Rodriguez *et al.* (2012) also reported that fertilization with sewage sludge increased the total Zn in the soil over time but never exceeded the limit values and did not cause harmful effects in the grassland. In terms of heavy metals (non-nutritional elements), total Ni and Cr concentrations increased; a slight but significant decrease in total Cd concentrations was detected. The total Pb concentration was below the detection limit (D.L. <0.03 mg kg^{-1} for Pb). Total Ni, Cr and Cd concentrations in the substrate were found to be higher in CS_1 than CS_2 (Table 3).

Table 2. The microelement concentrations of the substrate

Sewage sludge (ha^{-1})	**Total Fe (mg kg^{-1})**			**Total Zn (mg kg^{-1})**			**Total Mn (mg kg^{-1})**			**Total Cu (mg kg^{-1})**		
	CS$_1$	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**
SS$_0$	22317d	18937d	20627	84.9f	79.6f	82.3	670.2d	425.0f	547.6	46.2de	34.5f	40.4
SS$_5$	35790b	30467c	33128	108.4e	104.1e	106.2	806.2c	527.0e	666.6	56.9c	42.9e	49.9
SS$_{10}$	41567a	33315bc	37441	126.4d	123.9d	125.2	827.0bc	540.8e	683.9	58.9bc	46.1de	52.5
SS$_{20}$	44050a	33967bc	39008	151.1b	131.5cd	141.3	876.8ab	559.4e	718.1	62.10ab	47.5de	54.8
SS$_{40}$	44875a	34502bc	39688	162.4ab	146.0bc	154.2	910.7a	566.9e	738.8	64.9a	48.2d	56.6
SS$_{80}$	45153a	32082bc	38617	173.1a	153.2b	163.2	895.0a	526.7e	710.8	65.5a	47.2de	56.4
Mean	38959	30545		134.4	123.1		831.0	524.3		59.1	44.4	
ANOVA												
Sewage sludge (SS)	***			***			***			***		
Soil (S)	***			**			***			***		
SS×S	***			***			***			***		

The values are the average of the four repeats. The differences between the values not denoted by the same letters are significant at 5%.
: Significant at 1% (P < 0.01). *: Significant at 0.1% (P < 0.001).

Table 3. The heavy metal concentrations of the substrate

Sewage sludge (t ha^{-1})	**Total Ni (mg kg^{-1})**			**Total Cr (mg kg^{-1})**			**Total Pb (mg kg^{-1})**			**Total Cd (mg kg^{-1})**		
	CS$_1$	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**
SS$_0$	65.1de	56.8e	61.0	63.4de	56.7e	60.0	<D.L.	<D.L.	<D.L.	4.4ab	2.7ef	3.5
SS$_5$	76.1cd	66.8de	71.5	73.0d	68.8d	70.9	<D.L.	<D.L.	<D.L.	4.9a	3.1de	4.0
SS$_{10}$	93.7ab	86.0bc	89.9	92.4bc	83.8c	88.1	< D.L.	<D.L.	<D.L.	3.9b	1.5g	2.7
SS$_{20}$	106.6a	87.1bc	96.8	99.9ab	85.6c	92.8	< D.L.	<D.L.	<D.L.	3.8bc	2.3f	3.0
SS$_{40}$	107.6a	91.2abc	99.4	101.2ab	91.7bc	96.4	<D.L.	<D.L.	<D.L.	4.0b	1.6g	2.8
SS$_{80}$	107.9a	98.5ab	103.2	104.03a	86.7c	95.3	<D.L.	<D.L.	<D.L.	3.3cd	2.1fg	2.7
Mean	92.8	81.1		88.98	78.9		<D.L.	<D.L.		4.1	2.2	
ANOVA												
Sewage sludge (SS)	***			***			-			***		
Soil (S)	**			***			-			***		
SS×S	***			***			-			***		

The values are the average of the four repeats. The differences between the values not denoted by the same letters are significant at 5%. **: Significant at 1% (P < 0.01). ***: Significant at 0.1% (P < 0.001). <D.L. : Below the detection limits of the device.

Total concentrations of nonessential heavy metals (Cd, Cr, Ni, and Pb) in the soils were well below the threshold values in Turkish legislation (Anonymous, 2001). Therefore, the currently used treatments can be regarded as environmentally safe.

The concentrations of the micronutrient elements (Fe, Zn, Mn and Cu) in the shoot of the Bermuda grass were presented in Table 4, together with dry matter efficiencies. The plant's dry matter efficiency, Fe concentration and Zn concentrations increased with sewage sludge applications, whereas the Mn concentration has decreased even though the changes were irregular. The changes in the plant's Cu concentration were not statistically significant.

Table 4. The microelement concentrations in the aerial biomass of the Bermuda grass and the dry matter yield

Sewage sludge (t ha^{-1})	Fe (mg kg^{-1})			Zn (mg kg^{-1})			Mn (mg kg^{-1})			Cu (mg kg^{-1})			Dry matter (g pot^{-1})		
	CS_1	CS_2	Mean	CS_1	CS_2	Mean	CS_1	CS_2	Mean	CS_1	CS_2	Mean	CS_1	CS_2	Mean
SS_0	233.5a	140.9de	187.2	15.83d	25.36cd	20.59	154.7c	154.1c	154.4	12.24	2.92	7.58	2.89g	5.13g	4.01
SS_5	228.1ab	167.3cde	197.7	28.94bc	23.67cd	26.30	80.03d	180.6bc	130.3	4.05	3.72	3.88	16.27f	20.23f	18.25
SS_{10}	171.4cde	211.2abc	191.4	19.39cd	29.30bc	24.34	60.98d	224.5ab	142.7	6.39	3.74	5.06	28.39e	38.14d	33.27
SS_{20}	204.4abc	127.5e	166.0	39.03ab	29.00bc	34.02	42.96d	140.8c	91.90	3.79	2.06	2.92	55.12c	49.10c	52.11
SS_{40}	180.4bcd	248.2a	214.3	41.06a	44.86a	42.96	62.38d	261.5a	162.0	3.49	3.84	3.67	61.86b	62.39b	62.12
SS_{80}	207.2abc	207.2abc	207.2	41.36a	42.03a	41.70	41.76d	220.5ab	131.1	2.99	5.68	4.33	75.65a	62.65b	69.15
Mean	204.5	183.7		30.94	32.37		73.80	197.0		5.49	3.66		40.03	39.61	
ANOVA															
Sewage sludge (SS)	*			***			**			N.S.			***		
Soil (S)	*			N.S.			***			N.S.			N.S.		
SS×S	***			***			***			N.S.			***		

The values are the average of the four repeats. The differences between the values not denoted by the same letters are significant at 5%.

*: Significant at 5% ($P < 0.05$). **: Significant at 1% ($P < 0.01$). ***: Significant at 0.1% ($P < 0.001$). NS: Not significant.

When the interaction between the soil and sewage sludge was evaluated, it was determined that the Fe concentrations and dry matter efficiencies were higher for the plants grown in CS_1 compared to CS_2. However, Zn and Mn concentrations were found to be higher in the plants grown in CS_2 compared to CS_1. It is important to note that the obtained values were very close to each other. Concerning the Cu concentration, the SSXT interaction was found to be statistically insignificant.

When the micronutrient element concentrations in the plant were evaluated according to Campbell (2000), it was determined that the values were within the reference values which are 50-250 mg kg^{-1} for Fe, 15-70 mg kg^{-1} for Zn, 20-300 mg kg^{-1} for Mn and 5-20 mg kg^{-1} for Cu. It is apparent that the sewage sludge applications have provided benefits for the Fe and Zn nutrition of the plants, as Fe and Zn concentrations of plants have increasingly moved away from the lower limit value given for sufficiency. Also, in general the Fe, Zn, Mn and Cu concentrations did not increase enough to surpass the biomass limit after sewage sludge applications; thus, it can be said that this application did not lead to any toxic effects. Even though these micronutrients are essential for the survival of the plant, excessive concentrations can result in toxic effects (Welch and Schuman, 1995). These findings can be a positive indicator for the usage of sewage sludge together with calcareous and alkaline soil.

The concentrations of Ni, Cr, Pb and Cd in the shoot of the Bermuda grass were presented in Table 5. The study has determined that sewage sludge and soil interaction did not have a significant effect on the plant's Ni, Cr and Cd concentrations. Sainger *et al.* (2011) studied the heavy metal tolerance of Bermuda grass growing on effluent discharge from electroplating industry and found bioaccumulation and translocation factor in order of Zn > Fe > Cu > Ni >Cr. The Pb concentration was below the detection limit of the measurement device. This result showed that Pb which is a non-nutritional element for plant life released from sewage sludge applied to soil did not lead a harmful effect on Bermuda grass.

Table 5. The heavy metal concentrations in the aerial biomass of the Bermuda grass

Sewage sludge (t ha^{-1})	Ni (mg kg^{-1})			Cr (mg kg^{-1})			Pb (mg kg^{-1})			Cd (mg kg^{-1})		
	CS$_1$	CS$_2$	Mean	CS$_1$	CS$_2$	Mean	CS$_1$	CS$_2$	Mean	CS$_1$	CS$_2$	Mean.
SS$_0$	1.20	1.00	1.10	2.16	2.16	2.16	0.267	0.347	0.307	0.373	0.242	0.307
SS$_5$	4.95	2.51	3.73	3.03	3.03	3.03	1.664	2.210	1.937	0.616	0.363	0.489
SS$_{10}$	6.64	1.74	4.19	2.47	2.47	2.47	0.382	0.385	0.384	0.068	0.111	0.090
SS$_{20}$	4.95	1.78	3.37	1.89	1.89	1.89	0.144	0.210	0.177	0.212	0.068	0.140
SS$_{40}$	1.13	3.37	2.25	2.37	2.37	2.37	0.175	0.27	0.101	0.027	0.063	0.045
SS$_{80}$	1.49	2.44	1.96	2.03	2.03	2.03	0.414	0.536	0.475	0.088	0.315	0.202
Mean	3.39	2.14		2.33	2.33		0.507	0.659		0.230	0.194	
ANOVA												
Sewage sludge (SS)	N.S.			N.S.			N.S.			N.S.		
Soil (S)	N.S.			N.S.			N.S.			N.S.		
SS×S	N.S.			N.S.			N.S.			N.S.		

The values are the average of the four repeats. The differences between the values not denoted by the same letters are significant at 5%.
NS: Not significant.

Bioconcentration factor (BCF) is an important expression of metal accumulation capacity as it takes into account the metal concentration rate in the plant and the environment in which the plant grows (Zayed *et al*., 1998; Odjegba and Fasidi, 2004; Liu *et al*., 2009; Wu *et al*., 2010). For plants, BCF is used as a measure of heavy metal accumulation, and values greater than 1 are indicated as an indicator of potential heavy metal phyto-hyperaccumulator species (Zhang *et al*., 2002). In this study, the bioconcentration factors calculated for both micronutrients and heavy metals in the upper part of Bermuda grass were lower than 1 (Tables 6 and 7).

Table 6. The bioconcentration factors of micro elements in the aerial biomass of Bermuda grass

Sewage sludge (t ha^{-1})	BCF$_{Fe}$			BCF$_{Zn}$			BCF$_{Mn}$			BCF$_{Cu}$		
	CS$_1$	CS$_2$	Mean	CS$_1$	CS$_2$	Mean	CS$_1$	CS$_2$	Mean	CS$_1$	CS$_2$	Mean
SS$_0$	0.010a	0.007b	0.009	0.188cd	0.318a	0.253	0.232c	0.365b	0.299	0.115ab	0.085abc	0.100
SS$_5$	0.006bc	0.005cd	0.006	0.262abc	0.228abcd	0.245	0.098d	0.340b	0.219	0.070abc	0.088abc	0.079
SS$_{10}$	0.004d	0.006bc	0.005	0.155d	0.235abcd	0.195	0.075d	0.420ab	0.248	0.035c	0.080abc	0.058
SS$_{20}$	0.005d	0.003d	0.004	0.263abc	0.223bcd	0.243	0.050d	0.250c	0.150	0.065bc	0.043c	0.054
SS$_{40}$	0.004d	0.007b	0.006	0.250abc	0.310ab	0.280	0.070d	0.463a	0.266	0.055c	0.080abc	0.068
SS$_{80}$	0.005d	0.006bc	0.006	0.243abcd	0.273abc	0.258	0.048d	0.420ab	0.234	0.048c	0.117a	0.083
Mean	0.005	0.006		0.227	0.265		0.096	0.376		0.065	0.082	
ANOVA												
Sewage sludge (SS)	***			N.S.			**			N.S.		
Soil (S)	N.S.			*			***			N.S.		
SS×S	***			**			***			**		

The values are the average of the four repeats. The differences between the values not denoted by the same letters are significant at 5%.

*: Significant at 5% (P<0.05). **: Significant at 1% (P<0.01). ***: Significant at 0.1% (P<0.001). NS: Not significant.

Table 7. The bioconcentration factors of heavy metals in the aerial biomass of Bermuda grass

Sewage sludge (t ha^{-1})	**BCF$_{Ni}$**			**BCF$_{Cr}$**			**BCF$_{Cd}$**		
	CS$_1$	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**	**CS$_1$**	**CS$_2$**	**Mean**
SS$_0$	0.011	0.018	0.015	0.034abcd	0.038abc	0.036	0.085	0.082	0.083ab
SS$_5$	0.063	0.039	0.051	0.040ab	0.045a	0.043	0.128	0.116	0.122a
SS$_{10}$	0.080	0.020	0.050	0.028bcd	0.030abcd	0.029	0.016	0.075	0.046b
SS$_{20}$	0.047	0.020	0.034	0.019d	0.022cd	0.021	0.056	0.029	0.043b
SS$_{40}$	0.011	0.037	0.024	0.024cd	0.026bcd	0.025	0.010	0.041	0.026b
SS$_{80}$	0.014	0.026	0.020	0.019d	0.023cd	0.021	0.029	0.146	0.088ab
Mean	0.038	0.027		0.027	0.030		0.043	0.064	
ANOVA									
Sewage sludge (SS)	N.S.			***			*		
Soil (S)	N.S.			N.S.			N.S.		
SS×S	N.S.			**			N.S.		

The values are the average of the four repeats. The differences between the values not denoted by the same letters are significant

*: Significant at 5% ($P<0.05$). **: Significant at 1% ($P<0.01$). ***: Significant at 0.1% ($P<0.001$). NS: Not significant.

This is an indication that these elements accumulate in the Bermuda grass plant and do not cause any toxicity. It has been stated in our previous studies that the chlorophyll content, dry matter yield and macronutrient nutrition of alfalfa and Bermuda grass plants and the organic matter content of the soils where it is applied increase with the application of sewage sludge (Ok and Orman, 2014; Ok and Orman, 2015; Ok and Orman, 2016).

Conclusions

According to the results obtained from two different calcareous soils applied with stabilized and dried sewage sludge taken from the wastewater treatment plant, at the end of the 16-week incubation period, the amount of Fe, Mn, Zn and Cu elements, which are in the class of nutrients essential for plant life, increased in the growing media. The fact that this increased rate was less in the soil with higher lime content than in the soil with lower lime content indicates the necessity of taking into consideration the lime rate of the soil where Bermuda grass will be grown in the treatment sludge application. The increase in Fe and Zn concentrations in the shoot of the grown Bermuda grass plant, with the application of sewage sludge, can provide a significant advantage in eliminating plant development disorders due to Fe and Zn deficiencies. When heavy metals that may have toxic effects on plants were evaluated, it was determined that Ni and Cr concentrations in the environment increased with the application of sewage sludge, Cd concentration decreased, and Pb concentration was below the detection limit. The noteworthy point here is that the heavy metal content harmful to the plant increased more in the soil with low lime content of the soil to which sewage sludge was applied, showing the importance of paying attention to the lime content in the soil to which sewage sludge will be applied. The bioconcentration factor values for the plant upper part (BCFFe, BCFZn, BCF$_{Mn}$, BCF$_{Cu}$, BCF$_{Ni}$, BCF$_{Cr}$, BCF$_{Cd}$) were found to be lower than 1, which is the critical limit value, meaning that the bioaccumulation in the plant upper part is within the allowed limits and does not have any negative effects. This indicates that it is possible to use stabilized sewage sludge safely in cultivation and that it is an important advantage that can provide useful disposal of sewage sludge. As a result, in the light of the data obtained, it can be recommended that when applying sewage sludge, the properties of the soil such as lime and pH, should be taken into consideration. Considering Bermuda grass substrate, it can be suggested to add sewage sludge to the calcareous soil in amounts between 20–40 t ha^{-1}, with considering its long-term effects. In addition, future studies should be conducted on the safe use of dried sewage sludge in different plant species and varieties.

Authors' Contributions

The experimental design, management, experiments and data analysis of this article, its preparation according to the journal writing rules, and its editing were done by HO, SO, IT, and IET. The authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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