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Effect of sewage sludge on metal content of grassland soil and herbage in semiarid lands

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Abstract: Utilization for agriculture is one of the main disposal methods for sewage sludge. Subirrigated pastures are close to settlements and under intensive usage. The objective of this study was to investigate the effects of sewage sludge on subirrigated pasture soil and plant metal concentrations, and the relationship between them. Activated sewage sludge was applied to surface grassland at 25, 50, 75, and 100 t ha^{-1} $year^{-1}$ treatments for 2 years. The concentration of all metals in topsoil (0–20 cm) increased depending on application dosages, but only Zn concentration increased in subsoil (20–40 cm) consistently. While in the second year the concentration of topsoil diethylene triamine pentaacetic acid (DTPA) metals increased significantly, it decreased in the subsoil. The ratio of organic matter increased in topsoil, but decreased in subsoil compared to the control. Doses of sewage sludge of 25 and 50 t ha^{-1} did not increase the metal content of herbage. However, the herbage's Cu, Zn, Pb, and Cd content increased at 75 and 100 t ha^{-1} treatments in comparison with both lower doses and the control. Similar to the soil concentration, the Zn content of herbage increased proportionally with higher sewage sludge application doses. Topsoil DTPA-extractable Zn, Cu, and Pb concentrations were correlated to herbage Zn, Cu, and Pb content significantly and positively. In subsoil, only DTPA-extractable Cu concentration was correlated to herbage Cu content significantly and negatively ($r = -0.53^{**}$). A 25 t ha^{-1} $year^{-1}$ application of sewage sludge can be recommended as an organic fertilizer in semiarid subirrigated pastures, as it increases topsoil organic matter and results in a low concentration of DTPA-extractable and plant metal content.

Key words: Heavy metals, organic matter, sewage sludge, subirrigated pasture

1. Introduction

Due to their closeness to residential areas, subirrigated pastures in Turkey are under pressure from early and intensive grazing from early spring to late autumn. Almost all rural areas and some urban areas with animal husbandry have subirrigated pastures, that is, common grasslands. They also have high groundwater levels, leading to weeds as a result of heavy grazing and compaction. It is necessary to improve the productivity of these areas because of intensive usage. Chemical fertilization does not contribute quickly to canopy cover and the physical properties of soil in meadows and ranges. Moreover, its applicability is minor due to such substantial problems as not being economical in large areas like meadows, and because its surface application increases the risk of eutrophication.

It is not possible to reduce sewage sludge enriched with plant nutrient and organic matters (Kidd et al. 2007) obtained inevitably as a result of treatment processes. Application of sewage sludge to farmland has been an effective disposal method widely used in Europe, the United States, and Canada for more than 40 years (Warman and Termeer 2005). Dumping sewage sludge in bodies of water is prohibited (EC 1991), and for economic and legal reasons (Ramirez et al. 2008), it has been suggested that sewage sludge cannot be reduced by landfilling and incineration (Eurostat 2011). Laws allowing the correct and safe use of sewage sludge in agriculture (EC 1986) have increased land application significantly. While the usage rate of total sewage sludge in agriculture in 2000 was 59% in the United Kingdom, 53% in Spain, and 14% in Poland, it increased to 74.2%, 82.6%, and 21% respectively in 2009. In France, this rate is approximately 50%. In Germany, which ranks first among European Union countries in total sewage sludge production despite recorded reduction in all disposal methods, no statistical information was available for this method of disposal (Eurostat 2011).

The residual effect of biosolids over the long term can be especially significant in many forage-based pastures, where only 50% of the million hectares of pastures are given inorganic nitrogen yearly (Sigua 2009). The lowest

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doses (25 t h^{-1}) of sewage sludge provided herbage with equal yield and equally high quality as the highest doses (200 kg N ha^{-1} + 100 kg P ha^{-1}) of chemical fertilizers in a subirrigated pasture in the semiarid region (Arvas et al. 2011b). Although sewage sludge contains lower amounts of nitrogen and phosphorus than chemical fertilizer does, it keeps more total nitrogen and available phosphorus in the soil at the end of the growing season under both irrigated and nonirrigated conditions (Arvas 2005; Arvas et al. 2011a). However, despite repeated applications of sewage sludge, it was found that higher nitrogen and phosphorus concentrations did not increase pollution risk (Sigua 2009). Application of sewage sludge as compost reduced phytotoxicity of organic compounds having bioaccumulator characteristics (Ramírez et al. 2008) and fully removed *Salmonellae* and fecal coliforms (Nasr 1997). Nevertheless, the fact that it caused accumulation of metals in soil and plant is the most crucial environmental concern (Hillman et al. 2004; Arvas 2005; Kidd et al. 2007; Neagoe et al. 2009; Torri and Lavado 2009; Keramati et al. 2010; Singh and Agrawal 2010), which has limited the widespread use of sewage sludge as fertilizer. The other limiting factor is ethical concern.

The aim of this study was to investigate the effect of high-dose applications of sewage sludge on the availability of metals in soil, the total amount of metals in herbage, the relation between organic matter and diethylene triamine pentaacetic acid (DTPA) metals, and metal uptake in grassland soil in semiarid regions, which are at risk of desertification.

2. Materials and methods

2.1. Study area

The experiment was conducted on heavily grazed grassland. The study area is located approximately 5 km northwest of Yüzüncü Yıl University, Van, Turkey (38°36′49″N, 43°14′03″E, altitude of 1654 m). The experiment was set up in the autumn of 2006. In the study area, the average annual precipitation was 387.4 mm, the average temperature was 8.9 °C, and the relative humidity was 58.8%. The average annual rainfall was respectively 424.1, 356.8, and 318.7 mm for the 3 consecutive years of the study. The average annual temperature was 10.1, 9.6, and 9.8 °C, and annual relative humidity was 58.8%, 60.7%, and 52.5%, respectively. Considering the averages over years, the experimental area was in a semiarid region according to the drought index. The experiment soil was alkaline and loamy (Table 1).

2.2. Experimental design

Activated and aerobically digested sewage sludge was stored for 2 years in a drying bed and air-dried before application. The Wastewater Treatment Plant of Yüzüncü Yıl University is located on the shore of Van Lake. Since the test material was exclusively domestic-based sewage sludge, the DTPA metal concentration was lower than the limit values specified under Directive 86/278/EEC of the European Union. Air-dried sewage sludge was uniformly spread on the surface soil of the experimental area at doses of 25, 50, 75, and 100 t ha^{-1}. The maximum application dose was determined to be 90 t ha^{-1}, similar

Table 1. Physicochemical properties of the sewage sludge and soil of experiment area.

Parameters	0–20 cm	20–40 cm	Sewage sludge
Texture	Loamy	Loamy	
Sand	36%	32%	
Silt	46%	48%	
Clay	18%	20%	
Organic matter	5.16%	4.25%	25%
pH (1:2 water)	8.42	9.3	6.20
*Iron (mg kg^{-1})	0.04	0.40	12.71
*Manganese (mg kg^{-1})	1.11	1.55	74.20
*Zinc (mg kg^{-1})	nd	nd	270.00
*Copper (mg kg^{-1})	0.52	0.86	11.16
*Lead (mg kg^{-1})	nd	0.02	0.98
*Cadmium (mg kg^{-1})	nd	nd	0.44

*Extractable; nd: not detected.

to that determined by Fish et al. (1995) for semiarid rangelands. Sewage sludge was applied to the plots, except for the control, in November for the 2 consecutive years of 2006 and 2007. Treatments were designated as SS_0 (for control), SS_1 (25 t ha^{-1}), SS_2 (50 t ha^{-1}), SS_3 (75 t ha^{-1}), and SS_4 (100 t ha^{-1}) sewage sludge treatments. Each treatment was repeated 3 times in a randomized complete block design. The experiment area was enclosed at the beginning of the study. As a result of overgrazing, the research area has been compressed, and plants like *Carex* and *Juncus*, *Cynedondactylon* L., *Poa bulbosa* L., *Medicago lupulina* L., *Melilotu* spp., and *Dactylis glomerata* L. have been observed predominantly in the vegetation.

2.3. Soil and herbage analyses

Soil samples were collected with a steel auger on 15 November 2007 and 2008 from topsoil (0–20 cm) and subsoil (20–40 cm) depths in triplicate from the replicate plots of each treatment. Soil analyses were carried out in duplicate on representative samples of initial soils and subsamples of bulk and rhizosphere soil in the cutting area (Kidd et al. 2007).

The samples were air dried, crushed, and passed through a stainless steel sieve with a 2-mm mesh size before analysis. Organic matter in the soil and sewage sludge samples was determined by the modified Walkley and Black rapid titration method (Walkley 1947).

Available fractions of micronutrients, heavy metals, and contents of soil and sewage sludge were extracted by mixing 10 g of air-dried sample with 20 mL DTPA (diethylene triamine pentaacetic acid) extracting solution (0.005 M DTPA, 0.01 M calcium chloride [$CaCl_2$], and 0.1 M triethanolamine [TEA], adjusted and buffered to pH 7.3) (Lindsay and Norvell 1978). After mixing, the solution bottles were shaken for 2 h on an automated mechanical shaker and then allowed to stand overnight. The solution was filtered using Whatman No. 42 filter paper.

Since the aboveground parts of plants are relevant to feeding, root studies of the plants were excluded. Samples were taken from 3 randomly placed quadrants, with 30 cm × 30 cm for each plot (Lopez-Mosquera et al. 2000). Herbage was oven-dried, ground in a stainless steel grinder, and passed through a sieve of 2-mm mesh size. After taking 0.5 g from plant samples and holding it for 1 night in an ash oven at 500 °C, the ash was left to cool by being taken out of the oven when it turned gray. The solution was brought up to 20 mL with deionized water after keeping for 10–15 min on a hot plate by adding 4 mL of 3 N HCL solution to the ash, which had been moistened with pure water. The solution that was kept standing was filtered with Whatman No. 42 filter paper after a period of 5–6 h.

Micronutrient and heavy metal concentrations of soil and herbage extracts were determined using an inductively coupled plasma mass spectrometer (XSERIES 2 ICP-MS, Thermo). Each set of the soil and herbage samples included a blank.

2.4. Statistical analyses

Data on herbage soil organic matter, herbage metals, and soil available metals were analyzed using ANOVA at $P < 0.05$ significance level with SPSS v. 17.0. Differences between means were determined by the post hoc Duncan's multiple range test. The significance of the correlation coefficient obtained is shown using * and ** to indicate the 95% and 99% probability levels, respectively.

3. Results

3.1. Metal contents of herbage

Sewage sludge treatment increased the metal content of grass, other than Mn, after the first year. Generally, an increase in metal content of herbage was observed in parallel with the increase in the application doses in the second year. However, the highest content of Fe was recorded in the control parcel. Sewage sludge applications did not increase the plants' Fe content compared to the control in both years (Table 2).

However, an increase was observed in herbage Fe contents proportionally with increasing sewage sludge doses, especially in the second year. The highest Cu, Zn, Pb, and Cd concentrations were determined in SS_3 and SS_4 treatments. There was a significant difference between these treatments only with regard to Pb concentration. There were no differences between SS_1 and SS_2 in terms of Cu, Zn, Pb, and Cd contents of herbage; the differences between them and the control were insignificant (Table 2). Year × sewage sludge was significant for other metals apart from Zn and Cd.

3.2. DTPA metals in topsoil

Sewage sludge increased the topsoil DTPA metal concentrations. The increase in the second year was significant compared to the first year. Unlike metal concentrations in sewage sludge (Zn > Mn > Fe > Cu > Pb > Cd), the metals' order was changed to Fe > Cd > Cu ≥ Pb > Zn > Mn, and the ratio increased by 5.3, 3.3, 2.2, 2.2, 2.0, and 1.8 in the second year compared to the first year, respectively (Table 3). Year × sewage sludge was significant for metals, except for Cu and Cd.

The highest concentrations of Fe, Zn, and Cd were detected in the SS_3 treatment, and the highest concentrations of Cu, Mn, and Pb were detected in the SS_2 treatment. In comparison with the SS_4 treatment, the SS_3 treatment increased the concentration of other metals significantly with the exception of Cu. While Zn increased in the SS_1 treatment compared to the control, Fe and Mn decreased, and no significant change was recorded for other metal concentrations.

Table 2. Effect of sewage sludge treatments on metal concentrations in herbage (mg kg^{-1}).

Treatment	Fe			Cu			Zn			Mn			Pb			Cd		
	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average
Control	239.03a	1098.70a	668.87a	13.63b	20.42b	17.02c	19.53c	19.28b	19.40b	64.75b	64.45	64.60b	13.88b	17.03b	14.46bc	0.017	0.033	0.025ab
SS_1	213.43bc	98.68c	156.06c	12.93bc	22.60b	17.76bc	27.88bc	66.18ab	47.03ab	128.63a	47.28	87.95a	10.93c	13.88b	12.41c	0.033	0.000	0.017b
SS_2	230.65ab	106.37c	168.51c	12.10bc	17.53b	14.82c	29.83bc	68.63ab	49.23ab	48.12b	53.50	50.81b	12.42bc	14.47b	13.44c	0.033	0.033	0.033ab
SS_3	193.30c	132.55c	162.93c	19.48a	25.00b	22.24ab	48.95a	91.00a	69.98a	73.93b	50.93	62.43b	19.35a	18.87b	19.11b	0.067	0.067	0.067a
SS^4	204.37c	252.04b	228.21b	10.83c	36.58a	23.70a	35.93ab	100.50a	68.21a	66.55b	49.23	57.99b	11.25c	36.54a	23.90a	0.020	0.050	0.035ab
Average	216.16 B	337.67 A		13.79 B	24.43 A		32.42 B	69.17 A		76.39 A	53.12 B		13.56 B	20.16 A		0.020	0.037	
General average			276.91			19.11			50.77			64.76			16.86			0.035

* Means with different letters in same column are significantly different ($P < 0.05$).

Table 3. Effect of sewage sludge treatments on organic matter (%) and DTPA-extractable metal concentrations in soil (mg kg^{-1}).

Treatment	Organic matter (%)			Fe			Cu			Zn			Mn			Pb			Cd		
	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average	2007	2008	Average
Soil depth of 0–20 cm																					
Control	5.22b	6.12d	5.67d	36.8b	156.7c	96.6c	23.9c	64.8b	44.4c	28.7d	37.2e	32.9e	110.7c	177.8b	144.3c	20.5b	53.2c	36.9c	0.10b	0.48	0.29b
SS_1	5.26b	6.50c	5.88c	24.9c	56.8d	40.8d	45.3ab	65.6b	55.5c	88.2c	219.3d	153.7d	90.6d	99.3c	95.3d	32.1ab	36.0d	34.0c	0.05b	0.30	0.18b
SS_2	5.37b	7.32b	6.34b	41.2ab	205.6bc	123.4bc	59.5a	143.9a	101.7a	122.1b	287.2c	204.7c	131.3b	292.0a	211.7a	49.8a	115.5a	82.6a	0.13b	0.57	0.35ab
SS_3	5.74a	7.85a	6.79a	45.7a	348.6a	179.2a	60.7a	114.5ab	87.6ab	311.3a	592.6a	452.0a	172.5a	218.0b	195.3a	47.3a	102.5ab	74.9a	0.33a	0.77	0.55a
SS_4	5.88a	7.67a	6.78a	40.1ab	235.0b	137.5b	34.4bc	101.5ab	68.0bc	283.4a	491.0b	387.2b	88.8d	279.2a	184.0b	32.6ab	87.8b	60.2b	0.11b	0.38	0.25b
Average	5.50B	7.09A		37.7B	200.5A		44.6 B	98.1 A		166.6B	325.5A		118.8 B	213.4 A		36.4 B	79.0 A		0.15 B	0.50 A	
General average			6.29			119.1			71.4			246.1			166.1			57.7			0.32
Soil depth of 20–40 cm																					
Control	5.53ab	5.81bc	5.67ab	171.4	52.6b	112.0a	102.2b	45.3d	73.7b	30.5d	29.5d	30.0e	239.4ab	102.1	170.8	81.6a	37.8b	59.7bc	0.18	0.12b	0.15bc
SS_1	5.14c	5.73bc	5.44c	139.7	34.7c	87.2b	69.5c	49.8cd	59.7c	51.1c	37.5c	44.3d	174.1b	133.8	154.0	56.7b	37.3b	47.0c	0.15	0.12b	0.13c
SS_2	5.05c	6.11a	5.58bc	149.3	77.8a	113.6a	93.4b	74.6a	80.0a	192.7b	52.3b	122.5c	318.3a	83.4	200.8	84.7a	54.1a	69.4ab	0.25	0.15ab	0.20ab
SS_3	5.34b	5.69c	5.51c	161.0	74.63a	117.8a	116.9a	59.8b	88.3a	369.1a	189.3a	279.2a	242.9ab	138.8	190.9	101.2a	56.5a	78.8a	0.25	0.20a	0.23a
SS_4	5.61a	5.92ab	5.76a	144.6	60.8ab	102.7a	97.5b	55.1bc	76.3b	125.2c	174.4a	149.8b	233.5ab	124.8	179.1	80.8a	51.3ab	66.1ab	0.23	0.10b	0.17bc
Average	5.34B	5.85A		153.2A	60.1 B		95.9 A	56.9 B		153.7A	95.6 B		241.7 A	116.6 B		81.0 A	47.4 B		0.21 A	0.14 B	
General average			5.59			106.7			76.4			125.1			179.1			64.2			0.18

Means with different letters in same column are significantly different ($P < 0.05$).

3.3. DTPA metals in subsoil

In contrast to topsoil, subsoil DTPA metal concentrations decreased significantly in the second year. The greatest decrease in the second year was observed in Fe concentration at 60.8% (Table 3).

Despite the increased sewage sludge doses, subsoil Fe concentration did not increase, but it decreased in the SS_1 treatment. In general, metal concentrations apart from Zn decreased in the SS_1 treatment compared to the control. According to the 2-year average, the decrease in Fe and Cu concentrations was significant, but the decrease in Pb and Cd concentrations was insignificant. The highest DTPA metals concentration, except for Mn in the SS_2 treatment, was observed in the SS_3 application. All DTPA metal concentrations decreased in the SS_4 treatment. Pb and Cd concentration increased significantly in the SS_3 treatment, but increased insignificantly in other treatments compared to the control (Table 3). However, Pb concentration decreased significantly in the SS_1 treatment. Year × sewage sludge was only significant for Zn and Cu.

When the correlation between topsoil DTPA metals and herbage metals contents was investigated, topsoil Zn, Cu, and Pb concentrations were related to herbage Zn, Cu, and Pb content significantly (r = 0.70**, r = 0.52**, r = 0.45*, respectively). However, there was no such relationship for Mn, Fe, and Cd concentrations (Table 4). In subsoil, while Cu concentration was related to herbage Cu content negatively and significantly (r = –0.53**), the relationship was found to be insignificant for other metals.

3.4. Organic matter

Sewage sludge increased topsoil organic matter (0–20 cm) in both years in comparison with the control, but this increase was especially high in the second year. The increase in the second year in both soil depths was significant compared to the first year. Except for the SS_2 and SS_4 treatments, subsoil (20–40 cm) organic matter decreased compared to the control according to the 2-year average. The effect of the SS_3 and SS_4 applications on topsoil organic matter was similar. While in topsoil the highest amount of organic matter was obtained with the SS_3 dose, in subsoil it was obtained with the SS_4 dose. After fencing the experiment area, an increase was recorded in organic matter in both soil depths of the control plots.

While topsoil organic matter was positively and significantly related to metals, the highest correlation coefficient was found to be related to Fe (r = 0.91) and the lowest was related to Cd (r = 0.75) (Table 5). Subsoil organic matter was related to Zn insignificantly, but was negative and significant for all other metals (Table 6).

Table 4. Correlation coefficients for relationship between both soil depths' DTPA-extractable metals and herbage metals.

		Herbage metal concentration					
		Fe	Cu	Mn	Zn	Pb	Cd
Topsoil DTPA-extractable metals	OM	ns	0.726**	–0.442*	0.706**	0.563**	ns
	Fe	ns	0.659**	–0.383*	0.634**	0.505**	ns
	Mn	ns	0.618**	ns	0.530**	0.593**	ns
	Cu	ns	0.524**	ns	.492**	ns	ns
	Zn	–0.505**	0.551**	ns	0.700**	0.485**	ns
	Pb	ns	0.552**	–0.383*	0.554**	0.453*	ns
	Cd	ns	0.522**	–0.364*	0.485**	0.420*	ns
Subsoil DTPA- extractable metals	OM	ns	0.484**	–0.410*	0.426*	0.367*	ns
	Fe	ns	–0.607**	ns	–0.460*	ns	ns
	Mn	ns	–0.489**	ns	–0.304	ns	ns
	Cu	–0.385*	–0.533**	ns	ns	ns	ns
	Zn	ns	Ns	ns	ns	ns	0.415*
	Pb	ns	–0.411*	ns	ns	ns	ns
	Cd	ns	–0.537**	ns	ns	ns	ns

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level; ns: not significant. (P > 0.05)

Table 5. Coefficients of correlation between organic matter and DTPA-extractable metals in topsoil (0–20 cm).

	Organic matter	Fe	Mn	Cu	Zn	Pb
Fe	0.905**					
Mn	0.766**	0.817**				
Cu	0.783**	0.751**	0.828**			
Zn	0.783**	0.599**	0.535**	0.599**		
Pb	0.835**	0.837**	0.884**	0.913**	0.640**	
Cd	0.748**	0.728**	0.679**	0.696**	0.552**	0.693**

**Correlation is significant at the 0.01 level.

Table 6. Coefficients of correlation between organic matter and DTPA-extractable metals in subsoil (20–40 cm).

	Organic matter	Fe	Mn	Cu	Zn	Pb
Fe	–0.671**					
Mn	–0.682**	0.735**				
Cu	–0.482**	0.888**	0.715**			
Zn	Ns	ns	0.395*	0.449*		
Pb	–0.503**	0.855**	0.798**	0.929**	0.579**	
Cd	–0.480**	0.653**	0.688**	0.706**	0.501**	0.768**

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level; ns: not significant.

4. Discussion

The organic matter content of sewage sludge (25%) increased the amount of topsoil organic matter significantly. Despite the increased topsoil organic matter depending on increased doses, no significant difference was determined between SS_3 (75 t ha^{-1}) and SS_4 (100 t ha^{-1}) treatments. This situation was attributed to the slowing down of decomposition in the application of high amounts of sewage sludge produced in aerobic conditions (Albiach et al. 2001). The significant increase of organic matter in the second year, and in both soil depths in the control plot, could be explained by the increase of root biomass as a result of the preserved experiment area not being grazed over after grass was mowed. However, it could be evaluated that the sewage sludge treatment's not increasing the subsoil organic matter compared to the control, and there being no significant difference between the maximum dose (100 t ha^{-1}) and the control, are the results of an important contribution of principles of land conservation to soil fertility and sustainability of flora in the form of preventing grazing in early spring and late autumn.. As in this study, in many other studies it was found that sewage sludge application increased soil organic matter (Kidd et al. 2007; Keramati et al. 2010; Singh and Agrawal 2010).

Sewage sludge increased metal concentrations in topsoil. Except for the highest treatment (100 t ha^{-1}), treatment levels of sewage sludge increased topsoil and subsoil DTPA Zn concentration gradually. This situation was considered to be due to high Zn concentration in sewage sludge, the alkaline soil of the experimental area (Singh and Agrawal 2008), and a weaker binding tendency to organic matter in comparison with other metals (Planquart et al. 1999). The highest DTPA metal concentration in both soil depths was detected in the 75

t ha^{-1} instead of the 100 t ha^{-1} application. As in organic matter, the greater the amount of sewage sludge, the slower the speed of decomposition (Albiach et al. 2001); it also causes a decrease in DTPA metal concentrations. With the 25 t ha^{-1} (SS_1) treatment, only Zn concentration was increased compared to the control in both soil depths. Barbarick et al. (1998) specified that there is an incompatibility between many metals and sewage sludge treatments under 20-cm soil depths and that the most compatible element with application doses is Zn. As a result of annually repeated sewage sludge applications over several years, it was established that the concentrations of Cu, Pb, Cd, and notably Zn metals increased up to the 40-cm soil depth (Barbarick et al. 1998; Baveye et al. 1999; Nyamangara and Mzezewa 1999; Singh and Agrawal 2008; Keramati et al. 2010). These results are consistent with the findings in this study. The concentrations of all metals in topsoil were related positively and significantly to topsoil organic matter. This situation in topsoil is thought to be responsible for the direct relationship between organic matter in surface application and DTPA metals concentration. Keramati et al. (2010) reported that the presence of organic matter increased the DTPA-extractability of metals. In contrast, all metals in subsoil were related to organic matter negatively and significantly, except for Zn. Low levels of organic matter and DTPA metals in subsoil might result from an insufficient amount of sewage sludge organic matter and low precipitation. It is suggested that the reason for the increase in the subsoil metals in the first year is that the rain, which was higher than in the second year, leached more metals from topsoil. In the second year, the decrease of metals in subsoil could be explained with low precipitation and the fixation of metals. In his research carried out in a similar subirrigated pasture with the same application doses and soil conditions with weekly irrigation, Arvas (2005) found that subsoil DTPA metals increased, but organic matter did not. In studies carried out in different locations with sewage sludge, it was established that only DTPA-Zn increased in underground soil without increasing organic matter (Sommers et al. 1991; Barbarick et al. 1998). Several years after the application of sewage sludge, the availability of metals decreased as insoluble precipitate and mineral complexes absorbed on the surface, with chelation of insoluble organic matter (Chaney 1973). This result explains the decrease in subsoil metals, except for Zn, determined in this study in the second year of the experiment.

According to the results, it can be argued that precipitation of about 400 mm and below cannot pose a threat for leaching of metals under the 20-cm depth in grassland soil. At the end of the experiment, DTPA-Fe, -Cu, -Zn, -Mn, -Pb, and -Cd concentrations detected in both soil depths were found to be lower than the limit values defined in Directive 86/278/EEC, which could be explained with the soil pH being alkaline and the metal concentrations of sewage sludge being lower than limit values under the Directive.

Sewage sludge application decreased Fe content dramatically compared to the control. Plant Zn content increased insignificantly in lower treatments and significantly in higher treatments. Despite the inconsistency of Cu, Mn, Pb, and Cd contents of herbage with application doses, these metals increased with the increasing of doses, apart from Mn. While topsoil DTPA Zn, Cu, and Cd concentrations were related positively and significantly to herbage Zn, Cu, and Cd concentrations, Cu concentration was related negatively and significantly ($r = -0.53^{**}$) in subsoil only. It was reported that surface application of solid sewage sludge increased metal concentration of grass by metals adhering on leaves' surfaces, Cu being the most concentrated (Hillman et al. 2004). However, in research done in a pasture having the same levels of treatment and with weekly sprinkler irrigation, it was determined that Cu content was lower compared to the control in all treatment levels (Arvas 2005). Regarding the plant *Lolium perenne*, to which 150 t ha^{-1} sewage sludge (air-dried) was applied, it was suggested that Zn content increased significantly in every fourth cutting, Cu content increased only in the third cutting, and Cd and Pb metals remained under allowable limits (Torri and Lavado 2009). These results support the findings obtained in this study. In the second year of the application, the metal content of herbage, except for Mn, increased proportionally with topsoil metal concentrations. It is argued that high P content of sewage sludge leads to the slowing of Mn uptake (Kabata-Pendias and Pendias 1995). In another article in which the findings obtained in this experiment were published, it was established that sewage sludge treatment increased available soil P content in semiarid grassland (Arvas et al. 2011).

In conclusion, sewage sludge increased the metal content of soil and plants, as well as organic matter content. An application dose of sewage sludge, with similar characteristics, of 25 t ha^{-1} year^{-1} can be recommended as organic fertilizer for subirrigated pastures having 400 mm of precipitation due to the significant increase it causes in the organic matter of topsoil as well as low DTPA and plant metal concentration.

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