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Effects of Sewage Sludge and Nitrogen Fertilizer Application on Nutrient and Heavy Metal Concentrations in Grass-legume Mixtures

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ABSTRACT: The study was conducted in 2004-2007 years in the experimental area of Agricultural Faculty of Yüzüncü Yıl University, in East Anatolia Region of Turkey. We harvested once times in 2005, 2006 and 2007, because of dry conditions. The aim of this study was to assess the potential of using sewage sludge as an alternative to nitrogen fertilizer, and to determine nutrients and heavy metals of the plant in Grass-legume Mixtures. Sewage sludge was applied in spring or autumn in two separate periods. The experimental design was Split-plot, completely randomized blok design with 4 replication. Main-Plot was determined by application time (spring and autumn), and also sub-plot was determined by the application (control, N-fertilizer, Sewage Sludge-1, Sewage Sludge-2, Sewage Sludge-3).

Application time (spring and Autumn) of sewage sludge was not an effective factor on dry matter yield, and N, P, K, Ca, Mg, Fe, Mn, Cu, Pb, Cd, Cr and Ni content. Years and application time interaction of dry matter yield was statistically significant. The highest dry matter yield was obtained in the 3rd year and the autumn application of sewage sludge. According to control and nitrogen fertilizers, Sewage sludge increased the dry matter yield, N, K, Zn, Cu, Pb, Cd and Cr contents in Grass-legume Mixtures. This study showed that sewage sludge may be used as a nitrogen source for Grass-legume Mixtures production.

Keywords: Grass-legume mixtures, heavy metal, nitrogen fertilizer, sewage sludge

Buğdaygil-baklagil Karışımlarında Besin Maddesi ve Ağır Metal Konsantrasyonları Üzerine Azot Gübresi ve Arıtma Çamuru Uygulamasının Etkileri

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ÖZET: Araştırma, 2004-2007 yılları arasında Yüzüncü Yıl Üniversitesi Ziraat Fakültesi deneme alanında kurulmuştur. Deneme kurak şartlarda kurulduğundan dolayı 2005-2006 ve 2007 yıllarında yılda bir biçim yapılmıştır. Bu araştırmanın amacı, buğdaygil-baklagil karışımlarının ağır metal ve besin maddesi birikimlerine azot gübrelemesine alternatif olarak kullanılan arıtma çamurunun etkilerini belirlemektir. Arıtma çamuru ilkbahar ve sonbahar olmak üzere iki ayrı dönemde uygulanmıştır. Deneme 4 tekerrürlü Bölünmüş Parseller deneme desenine göre kurulmuştur. Ana parsellere uygulama zamanı (İlkbahar ve Sonbahar), Alt parsellere ise uygulamalar (Kontrol, Azot gübresi, Arıtma çamuru 1, Arıtma çamuru 2, Arıtma çamuru 3) olarak belirlenmiştir.

Arıtma çamuru Uygulama zamanı (İlkbahar ve Sonbahar), kuru madde verimi ve bitkinin N, P, K, Ca, Mg, Mn, Cu, Pb, Cr ve Ni içeriğine etkisi önemli olmamıştır. "Yıl x Uygulama zamanı" interaksiyonu kuru madde verimi üzerine etkisi önemli olmuştur. En yüksek kuru madde verimi denemenin 3. yılında ve sonbaharda uygulanan arıtma çamuru uygulamasından elde edilmiştir. Kontrol ve Azot uygulamasına göre, arıtma çamuru buğdaygil-baklagil karışımlarının kuru madde verimi ve bitkinin N, K, Zn, Cu, Pb, Cd ve Cr içeriğini artırmıştır.

Bu araştırma, arıtma çamurunun buğdaygil-baklagil karışımlarında bir azot kaynağı olarak kullanılabileceğini göstermektedir.

Anahtar kelimeler: Buğdaygil-baklagil karışımı, ağır metal, azotlu gübreleme, arıtma çamuru

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INTRODUCTION

Sewage sludge has been applied to agricultural land for centuries (Follet et al., 1981). Sewage sludge application to agricultural lands has been a widely accepted practice during recent years. Its use in agricultural land is promoted; because it is considered that it will not only solve the problem of disposal but also increase productivity in agriculture. Their typical characteristics and value as fertilizers have been reviewed by Sommers (1977) and Pomares (1982), and there are a very large number of articles reporting results obtained with different crops, soil and application procedures. However, negative effects of sewage sludge such as elevated heavy metal levels resulting from the usage of sewage sludge must also be taken into consideration (Smith, 1996).

A typical sewage sludge may contain 3% N, 2.2 % P and 0.3 % K (Ott and Foster, 1997). It was reported that plant-available nutrient such as nitrogen, phosphourous and organic material contained in sludge could be regarded as soil improvers and might replace conventional fertilizer in agricultural production (Moreda et al., 1988; Elsgaard et al., 2001; Moreno et al., 1997; Casado-vela et al., 2006). Organic matter can increase water infiltration, water-holding capacity, soil granulation and the ability of soil or surface material to retain nutrients, and reduce soil erosion, and soil compaction, and also provide nutrients for plant growth and food and energy for beneficial soil micro-organisms. All these beneficial properties make biosolids a good choice for homeowners, farmers and foresters. In addition, farmers can benefit from biosolids application which reduces their fertilizer cost (Matthews, 1984; Su and Wong, 2003).

The rate at which municipal sewage sludge may be applied to land is based on a number of factors including concentrations of heavy metals, pathogens, toxic organic compounds and nutrients. Thirty to forty percent of sludge organic nitrogen could be assumed to mineralize during the first year of application (Reed et al., 1991; Cogger et al., 2001). Therefore, the differences in the soil, climate, sludge composition, and management factors require more specific estimates for different climatic regions or different cropping systems (Binder et al., 2002).

Sewage sludge as a fertilizer is used by many researchers. These researchers have stated that sewage sludge increases dry matter yield and none of the heavy metals of plant tissue reached either phytotoxic or toxic levels (Bozkurt et al., 2009; Roudsari and Pishdar, 2007; Akdeniz et al., 2009; Keskin et al., 2009; Cogger et al., 2001; Shober et al., 2003).

The aim of this study was to assess the potential use of sewage sludge as an alternative to nitrogen fertilizer, and to determine dry matter yield and and nutrients and heavy metals content of forage in grass-legume mixture under dryland condition in high elevation areas.

MATERIALS AND METHODS

The study was conducted in the sub irrigated degraded pasture experimental area of Agricultural Faculty of Yüzüncü Yıl University, in East Anatolia Region of Turkey, for 4 years between 2004 and 2007. The altitude was 1725 m. Total precipitation was 426, 337, 427 and 349 mm, respectively. Annual average temperature were 9.5, 9.9, 10.0 and 9.6 ^{o}C for experimental years, respectively (Anonymous, 2007).

Plant, Sewage Sludge and Soil analysis Methods: The N content of plant samples was determined by the Kjeldahl metod. Phosphorus was measured by spectrophometer. K, Ca, Mg, Fe Mn, Cd, Cr, Cu, Pb and Zn contents were determined using flame atomic absorbtion spectrophotometry (Kacar and İnal, 2008).

Organic matter in sewage sludge was measured by the dry combustion method (Nelson and Sommers, 1982). Total P in sludge was measured spectrophotometrically. Total metals in sludge were determined using flame atomic absorbtion spectrophotometry following extraction by nitric-hydrochloric acid digestion (Khan and Frankland, 1983).

Soil samples were dried and sieved (2 mm) for analytical purposes. Textural analysis was performed using the hydrometer method (Bouyoucos 1965). Soil pH was determined in a 1:2.5 soil water suspension (Jackson, 1958). Electrical conductivity (EC) was determined acording to Richard (1954). Total N was measured by the Kjeldahl method. Available P was determined by the Olsen procedure for calcareous soil (Olsen et al., 1954). Calcium carbonate was measured with a calcimeter. Organic matter was analysed colorimetrically using the modified Walkley-Black method (Houba et al., 1989).

Exchangeable K, Ca and Mg were measured by atomic absorbtion spectroscopy after an ammonium acetate extraction (Thomas, 1982). The concentrations of soil Fe, Mn, Cd, Cu, Pb and Zn were determined in DTPA extract using AAS (Lindsay and Norvell, 1978).

Soil and Sewage Sludge Properties: Properties of experimental site soil and sewage sludge used in the experiment are given in Table 1.

The soils at the experimental site were sandy loam, pH was 8.77, organic matter content was 1.41 % and DTPA extractable of Fe, Mn, Zn, Cu, Cd, Ni and Pb in the upper 30 cm of soil were 3.30, 7.32, 0.27, 1.10, 0.038, 0.61 and 0.60 (mg kg^{-1}), respectively.

Chemical characteristics of sewage sludge are given in Table 1. pH was 6.97, organic matter content was 47.2 %, total N, P, K was 2.20, 0.45 and 0.49 %, respectively. Total metal concentrations of Fe, Mn, Zn, Cu, Cr, Cd and Pb were 9578, 427, 795, 84, 130, 1.37 and 47 (mg kg^{-1}), respectively. DTPA extractable metals of Fe, Mn, Zn, Cu, Cr, Cd and Pb was 160, 20, 150, 15, 0.67, 0.35 and 10.7 (mg kg^{-1}), respectively.

Permissible maximum heavy metal contents in stabilized sewage sludge are given in Table 2 (Anonymous, 2010). As is shown in Table 2, the sewege sludge used in the experiment contained lower value than internationally permitted limit values.

Field Applications : The experimental design was randomized blok design with the treatments in split plot arrangement with 4 replications. Main-plot was determined by application times (spring and autumn), and sub- plot was determined by nitrogen applications (0 and 60 kg ha^{-1}) and Sewage Sludge rates (4.1, 8.2 and 16.4 t ha^{-1}). The experimental plot was 1.8 x 5 m = 9 m^2 in size Grass-legume mixtures was sown 6 May 2004 with 30 cm row space. The seedling rate was 20

Table 1. Characteristics of soil and sewage sludge (dry weight basis)

Properties	**Soil**	**Properties**	**Sewage sludge**
Texture	Sandy-loam	pH	6.97
CaCO$_3$ (%)	15.7	EC (mS cm^{-1})	4.31
EC (mS cm^{-1})	0.27	Organic Matter (%)	47.2
pH (1:2.5)	8.77	Total N (%)	2.20
N-Kjeldahl (g kg^{-1})	0.105	Total P (%)	0.45
P-Olsen (mg kg^{-1})	9.0	Total K (%)	0.49
Organic Matter (%)	1.41		
Extractable Cations (mg kg^{-1})		**Total Metal Concentrations (mg kg^{-1})**	
K	300	Fe	9578
Fe	3.30	Mn	427
Mn	7.32	Zn	795
Zn	0.27	Cu	84
Cu	1.10	Cr	130
Cd	0.038	Cd	1,37
Ni	0.61	Pb	47
Pb	0.60	**DTPA extractable metals (mg kg^{-1})**	
		Fe	160
		Mn	20
		Zn	150
		Cu	15
		Cr	0.67
		Cd	0.35
		Pb	10.7

Table 2. Permissible maximum heavy metal contents in stabilized sewage sludge (mg kg^{-1}) (Anonymous, 2010)

Heavy Metal (Total)	**Maximum permissible heavy metal content in sewage sludge (mg kg^{-1} dry weight basis)**
Cadmium (Cd)	10
Chromium (Cr)	1000
Copper (Cu)	1000
Lead (Pb)	750
Zinc (Zn)	2500

Table 3. Plants of Grass-legume Mixtures and the rates

Species	% Mixing Ration
Lolium perenne	15
Festuca arundinacea	15
Trifolium repens	10
Dactylis glomerata	20
Agropyron cristatum	20
Bromus inermis	20

kg ha^{-1} for all species. Plant species and their ratio in the mixture were given in Table 3.

The experiment included five applications: a Zero-N (control), a inorganic nitrogen fertilization rate (60 kg N ha^{-1}) and three sewage sludge rates (4.1, 8.2 and 16.4 t ha^{-1}). Cumulative sludge doses (3 years) were 12.3, 24.6 and 49.2 t ha^{-1}. Also, 80 kg ha^{-1} triple P_2O_5 were applied in control plots and plots of inorganic nitrogen applications.

Sewage sludge was applied mixed in the depth of soil by using a hand hoe in early spring in the 1st year of the experiment. Other applications of sewage sludge were applied to the soil surface and mixed with a hand hoe in the soil. Spring application of sewage sludge was applied by april, and autumn application of sewage sludge was applied by September.

We harvested once time in 2005, 2006 and 2007, because of dry conditions. Grass-legume mixtures were harvested at full flowering with a reaping hook on June 20th 2005; June 23rd 2006; and June 28th 2007. The harvested Grass-legume mixtures from each plot were weighed fresh and a 3000 gr subsample was collected and dried at 70 ^{0}C for 24 h (Jones 1981) for determination of nutrient and heavy metal concentration of Grass-legume mixtures.

The SPSS for Windows program was used for the statistical analysis. Year, Application times and Application means were compared with Duncan test at ($P < 0.05$).

RESULTS

The effects of nitrogen fertilizer and sewage sludge applications on nitrogen, P, K, Ca, Mg, Fe, Mn, Zn, Cu, Pb, Cd, Cr and Ni contents of Plant in Grass-legume mixtures and dry matter yield were investigated and the results were given in Table 4 and 5.

Sewage sludge was applied in spring and autumn in two separate periods. And also, its effects on N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, Pb, Cd, Cr and Ni contents of plants and dry matter yield of these application time were investigated and the obtained results are given in Table 4 and 5.

Dry matter Yield

Sewage sludge and N fertilizer application increased the dry matter yield of Grass-legume mixtures. The highest dry matter yields were obtained at the high and middle sewage sludge applications (16.4 and 8.2 t ha^{-1}) The lowest dry matter yields were obtained from control (Table 4).

The effect of years on dry matter yield in Grass-legume mixtures was statistically significant. The highest dry matter yields were obtained in the second year (2006) and third year (2007). This shows that increasing sewage sludge application increased dry matter yield.

Nutrient and Heavy Metal Contents of Grass-legume mixtures

When Table 4 and 5 are examined, the effect of applications on P, Ca, Mg, Fe Mn and Ni content of plants was insignificant. The effects of applications on N, K, Zn, Cu, Pb Cd and Cr content of plants were found significantly.

The effect of N, P, K, Mg, Pb, Cd, Ca, Fe and Mn contents of years (2005, 2006 and 2007) was statistically significant. The highest N, P, K, Mg, Pb, Cd contents of grass-legume mixtures was obtained during the third year (2007) period (Table 4 and 5). Nutrient and heavy metals of grass-legume mixtures can increase as a result of long-term sewage sludge application. On the other hand, long-term sewage sludge application decreased Ca, Fe and Mn content of the grass-legume mixtures (Table 4 and 5).

The applications significantly affected the plant nitrogen content. The highest nitrogen content was

Table 4. Effect of sewage sludge and N fertilizer on dry matter yield and N, P, K, Ca, Mg content of plants in Grass-legume mixtures

	Rate	**Dry Matter Yield**	**N**	**P**	**K**	**Ca**	**Mg**
Applications (A)	**kg ha^{-1}**	**kg ha^{-1}**			**%**		
Control	0	2479.9 c	1.254 c	0.150	0.777 b	0.770	0.208
N-fertilizer	60	2714.9 b	1.408 a	0.146	0.803 b	0.769	0.213
Sewage sludge-1	4100	2695.5 b	1.293 bc	0.154	0.785 b	0.785	0.222
Sewage sludge-2	8200	2954.3 a	1.322 b	0.151	0.870 a	0.747	0.217
Sewage sludge-3	16400	3023.7 a	1.393 a	0.149	0.917 a	0.755	0.212
Application Times (A.T.)							
Spring		2733.0	1.32	0.149	0.828	0.767	0.219
Autumn		2814.3	1.34	0.151	0.832	0.763	0.210
Years(Y)							
2005		2666.9 b	1.276 b	0.152 a	0.794 b	0.758 b	0.232 a
2006		2848.2 a	1.301 b	0.143 b	0.810 b	0.812 a	0.170 b
2007		2805.9 a	1.426 a	0.155 a	0.888 a	0.725 c	0.241 a
Variance Source				**F value and Significant**			
Year		5.786**	43.106**	15.534**	14.145**	14.463**	66.532**
Application		3.190 ns	2.333 ns	0.812 ns	0.066 ns	0.062 ns	2.858 ns
Application Time		18.424**	17.001**	1.639 ns	12.261^{xx}	1.002 ns	0.703 ns
Y x AT		6.988**	3.848*	0.400 ns	3.297 ns	2.088 ns	1.256 ns
Y x A		1.533 ns	2.120 ns	1.020 ns	1.947 ns	1.198 ns	0.590 ns
A x AT		0.966ns	0.153 ns	0.357 ns	1.099 ns	0.245 ns	0.248 ns
Y x A x AT		2.167 ns	0.963 ns	1.867 ns	1.175 ns	0.409 ns	0.203 ns

Means with a column followed by a different letter are significantly different ($P < 0.05^{*}$, $P < 0.01^{**}$), ns= Non-Significant

Table 5. Effect of sewage sludge and N fertilizer on Fe, Mn, Zn, Cu, Pb, Cd, Cr, Ni content of plants in Grass-legume mixtures

	Rate	**Fe**	**Mn**	**Zn**	**Cu**	**Pb**	**Cd**	**Cr**	**Ni**
Applications (A)	**kg ha^{-1}**	**mg kg^{-1}**							
Control	Zero-N	79.29	58.07	11.62 bc	2.71 c	0.42 b	0.086 b	0.69 ab	0,95
N-fertilizer	60	83.37	58.07	11.10 c	2.97 bc	0.41 b	0.087 b	0.64 b	0,95
Sewage sludge-1	4100	82.29	59.82	13.03 a	2.73 c	0.45 ab	0.086 b	0.72 a	1,09
Sewage sludge-2	8200	84.00	54.55	12.29 ab	3.20 b	0.45 ab	0.087 b	0.74 a	1,21
Sewage sludge-3	16400	81.75	52.78	12.37 ab	3.55 a	0.48 a	0.101 a	0.74 a	1,18
Application Times (A.T.)									
Spring		82.96	56.45	11.92	3.11	0.39	0.09	0.756 a	1,05
Autumn		81.31	56.86	12.25	2.95	0.49	0.08	0.664 b	1,11
Years (Y)									
2005		87.27 a	71.29 a	11.79	3.39 a	0.43 b	0.08 b	0.74 a	
2006		86.02 a	57.42 b	12.10	2.38 b	0.41 b	0.05 c	0.64 b	
2007		73.12 b	41.27 c	12.36	3.32 a	0.49 a	0.12 a	0.73 a	
Variance Source		**F value and Significant**							
Year		10.566**	104.72**	1.74 ns	42.45**	11.30**	181.1**	10.35**	
Application Time		0.352 ns	0.059 ns	1.73 ns	2.66 ns	44.35**	0.99 ns	21.98**	0,55 ns
Application		0.343 ns	2.327 ns	7.14**	9.97**	2.40 ns	3.66**	3.33*	1.18 ns
Y x AT		2.092 ns	0.497 ns	8.11**	18.08**	4.61 ns	23.96**	0.66 ns	
Y x A		1.192 ns	1.294 ns	1.85 ns	2.53**	1.05 ns	2.54 ns	1.09 ns	
AT x A		0.046 ns	0.774 ns	1.39 ns	0.24 ns	1.42 ns	1.94 ns	0.42 ns	0,74 ns
Y x AT x A		0.372 ns	0.403 ns	1.36 ns	0.48 ns	0.61 ns	0.90 ns	0.53 ns	
*Phytotoxic Levels (mg kg^{-1})				100-400	20-100	30-300	5-30	5-30	10-100

Means with a column followed by a different letter are significantly different ($P < 0.05^{*}$, $P < 0.01^{**}$); ns= Non-Significant; *(Lopez et al., 2000)

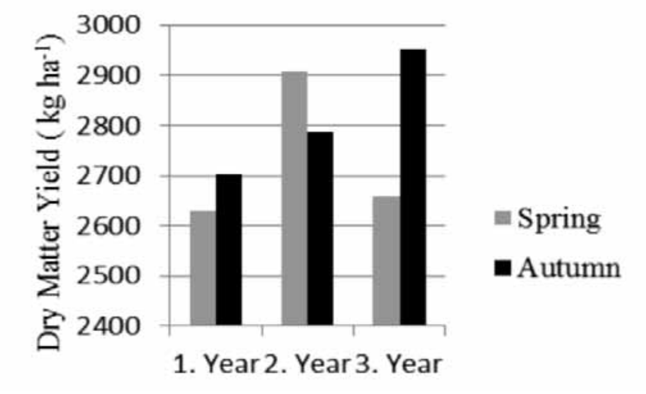

Figure 1. Effect of Year and Application Time on Dry Matter Yield (kg ha^{-1})

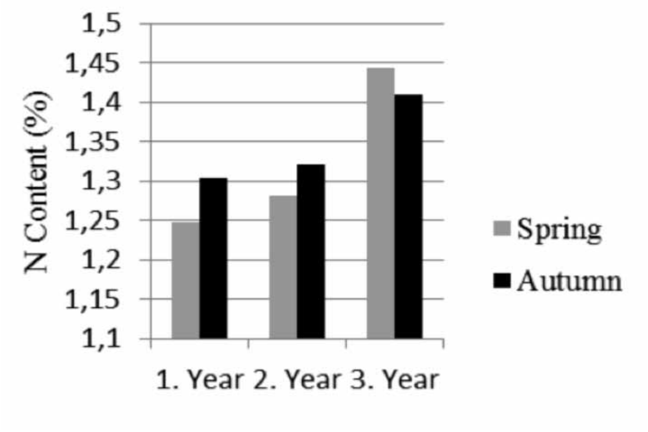

Figure 2. Effect of Year and Application Time on N Content (%)

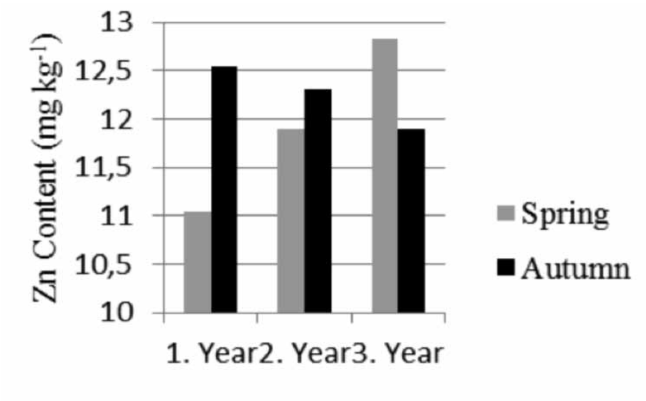

Figure 3. Effect of Year and application Time on Zn Content (mg kg^{-1})

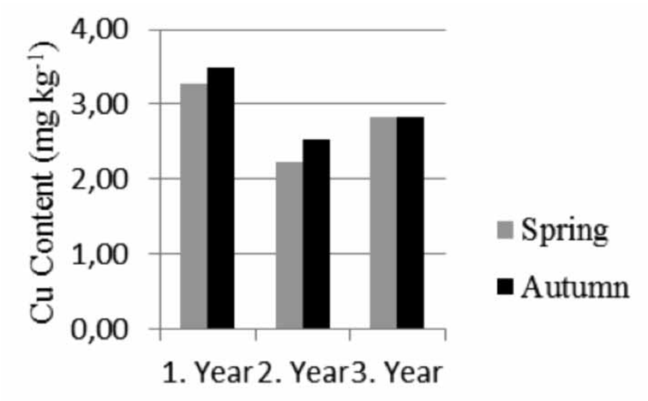

Figure 4. Effect of Year and Application Time on Cu Content (mg kg^{-1})

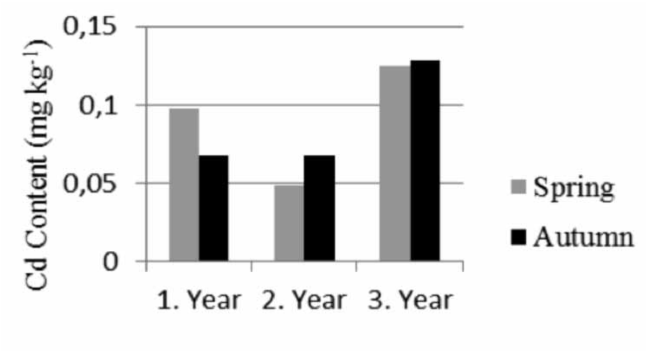

Figure 5. Effect of Year and Application Time on Cd Content (mg kg^{-1})

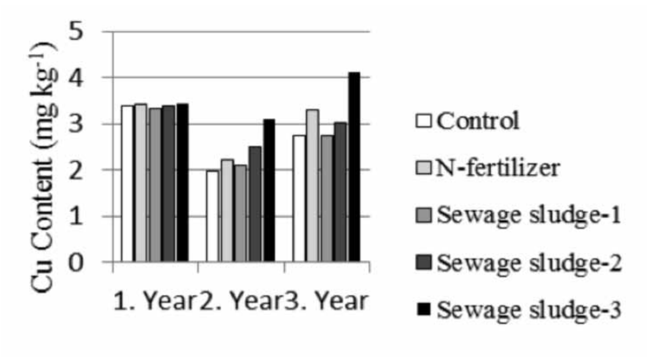

Figure 6. Effect of Year and Application on Cu Content (mg kg^{-1})

obtained in the high and middle sewage sludge applications. Heavy metal (Zn, Cu, Pb, Cd, Cr) contents of Grass-legume mixtures increased with sewage sludge application. In general, compared to control, the highest heavy metals obtained in the highest sewage sludge. Nickel content of plants were measured only in 2007. Compared to the control and nitrogen fertilization, although sewage sludge increased nickel content of plants, this increase was not statistically significant.

Application Times (spring and autumn)

Sewage sludge was applied on Grass-legume mixtures in two different periods in spring and autumn.

The effects of application time on N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, Cd and Ni contents of plant were not significant. On the other hand, Year x Application Time interactions of dry matter yield and N, Zn, Cu and Cd contents were statistically significant. The highest dry matter yield was obtained in the third year and autumn (Figure 1). When compared to Spring application of sewage sludge, Autumn application of sewage sludge increased dry matter yield of Grass-legume mixtures. The highest N and Zn contents were obtained in the third year and spring (Figure 2 and Figure 3). The highest Cu content was obtained in the first year and autumn (Figure 4). The highest Cd content was obtained in the third year and autumn (Figure 5).

Years and application interactions of Cu content in grass-legume mixtures were statistically significant. The highest Cu content was obtained in the third year with the highest sewage sludge applications (Figure 6).

DISCUSSION

Sewage sludge improves the physical and chemical properties of infertility soil and increases the fertility (Akdeniz et al., 2006; Penn and Sims, 2002). Organic matter (47.2 %), total N (2.20 %) and other nutrients (P, K, Fe, Mn, Zn, Cu) were high in sewage sludge used in this research (Table 1). The sewage sludge might increase dry matter yield as it improves the soil and provides nutrients to the plants.

There are many other studies indicating that dry matter yield increased with increasing nitrogen fertilizer (Akdeniz et al., 2006; Keskin et al., 2009; Bozkurt et al., 2006; Cogger et al., 2001; Shober et al., 2003) and sewage sludge (Bozkurt et al., 2009; Akdeniz et al., 2009; Pietz et al., 1989; Cogger et al., 2001; Kresse and Naylor, 1983; Binder et al., 2002; Shober et al., 2003).

In general, researchers reported that N content of plant tissue increased with sewage sludge application (Akdeniz et al., 2009; Keskin et al., 2009; Bozkurt et al., 2006;) and N fertilizer (Akdeniz et al., 2009; Roudsari and Pishdar, 2007).

According to the control and nitrogen fertilizer application, sewage sludge application increased potassium content of the plant. Some studies showed that potassium content of plant tissue increased with N fertilizer (Akdeniz et al., 2009) and sewage sludge application (Akdeniz et al., 2009; Keskin et al., 2009; Roudsari and Pishdar, 2007). In contrast, most researchers reported that K content of plant tissue was not affected by N fertilizer (Akdeniz et al., 2006; Keskin et al., 2009) and Sewage sludge application (Shober et al., 2003; Bozkurt et al., 2009; Akdeniz et al., 2006; Keskin et al., 2009; Dowdy et al., 1994).

Some researchers reported that sewage sludge incresed Cu content (Akdeniz et al., 2009; Keskin et al., 2009; Cogger et al., 2001), Zn content (Akdeniz et al., 2006; Wen et al., 2002; Soon et al., 1980), Pb content (Bozkurt et al., 2009; Bozkurt et al., 2006), Cr content (Bozkurt et al., 2006; Logan et al., 1997) and Cd content (Bozkurt et al., 2006) of plant tissue. In contrast, other researcher showed that sewage sludge did not affect Cu (Shober et al., 2003), Zn (Shober et al., 2003), Pb (Akdeniz et al., 2006; Roudsari and Pishdar, 2007) content of plant tissue.

Sewage sludge usually contains high levels of organic matter (47.2 %) and also it is rich in nutrients and heavy metals (Table 1). In general, high organic matter decreased soil pH. For this reason, plant tissue uptook most heavy metal in soil.

Researchers reported that commercial N fertilizer affected Cu content (Akdeniz et al., 2009), Zn content (Akdeniz et al., 2009; Keskin et al., 2009) and Pb content (Akdeniz et al., 2009) of plant tissue. In contrast, some studies showed that N fertilizer did not affect Cu content (Akdeniz et al., 2006), Pb content (Akdeniz et al., 2006; Keskin et al., 2009), Cr content (Akdeniz et al., 2009; Akdeniz et al., 2006; Keskin et al., 2009) and Cd content (Akdeniz et al., 2009; Akdeniz et al., 2006; Keskin et al., 2009) of plant tissue.

Sewage sludge and N fertilizer application positively affected dry matter yield of Grass-legume mixtures. The highest dry matter yields were obtained at the highest sewage sludge (16.4 t ha^{-1}). This study showed that sewage sludge may be used as a nitrogen source for Grass-legume mixtures. Application time (spring and Autumn) of sewage sludge was an effective factor on dry matter yield, and N, P, K, Ca, Mg, Fe, Mn, Cu, Pb, Cd, Cr and Ni contents. Years and application time interaction of dry matter yield was statistically significant. The highest dry matter yield was obtained in the third year and autumn. Dry matter yield increased in the autumn applications of sewage sludge more than the spring applications. Sewage sludge application increased N, K, Zn, Cu, Pb, Cd and Cr contents of plants in Grass-legume mixtures. None of the heavy metals of plant tissue reached either phytotoxic or toxic levels (Table 5; Lopez et al., 2000). The application did not effect P, Ca, Mg, Fe, Mn and Ni contents of Grass-legume mixtures.

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