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The Effects of Sewage Sludge Application Doses and Times on Extractable Metal Concentrations in a Calcareous Pasture Soil

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ABSTRACT
The objective of this study was to determine the effect of the increasing doses of sewage sludge and different application times on DTPA (diethylenetriaminepentaacetic acid) extractable nutrients and heavy metal concentration in calcareous soil for three years. For this purpose, sewage sludge was applied at different rates (0, 4.1, 8.2, 16.4 t ha-1) and periods (spring and autumn) to meet the nitrogen fertilizer requirement of grass-legume mixtures. Experiment results showed that cumulative sludge applications significantly increased DTPA extractable metal concentrations for 0-20 cm. Sludge application in spring period reduced the soil pH and increased the extractable metal concentrations compared to the autumn. Sludge application doses increased DTPA-Zn concentration for all soil depths and years. At the end of the experiment, it was determined that the heavy metal contents in the soil did not reach the levels that would have a negative effect on plant production. The fact that Van soil is rich in lime and alkaline pH of the soil, where the experiment was carried out, caused the solubility of heavy metals from sewage sludge to be low and toxic effect not to be seen. However, long-term sludge application should be followed in soil.

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Kireçli Bir Mera Toprağında Arıtma Çamuru Dozlarının ve Uygulama Zamanının Ekstrakte Edilebilir Metal Konsantrasyonuna Etkisi

ÖZET
Bu çalışmanın amacı artan oranlarda arıtma çamuru dozlarının ve uygulama zamanının üç yıl süreyle kireçli bir toprakta DTPA (dietilen triamin penta asetikasit) ile ekstrakte edilebilir besin elementi ve ağır metal konsantrasyonuna etkisini belirlemektir. Bu amaçla, mera karışımının azotlu gübre ihtiyacını karşılamak için arıtma çamuru farklı oranlarda (0, 4.1, 8.2 ve 16.4 ton ha^{-1}) ve dönemlerde (ilkbahar ve sonbahar) uygulanmıştır. Elde edilen sonuçlara göre, artan çamur uygulamaları 0-20 cm derinlikte DTPA ile ekstrakte edilebilir metal konsantrasyonunu önemli düzeyde artırmıştır. Sonbahara göre ilkbahar dönemi çamur uygulaması toprak pH'sını azaltmış ve ekstrakte edilebilir metal konsantrasyonlarını artırmıştır. Artan arıtma çamuru dozları ile DTPA Zn konsantrasyonu tüm toprak derinliklerinde her iki yıl artmıştır. Deneme sonunda, toprakta belirlenen ağır metal içeriklerinin bitkisel üretim açısından olumsuz etki oluşturacak düzeylere ulaşmadığı belirlenmiştir. Denemenin yürütüldüğü Van yöresi topraklarının yüksek kireç ve alkalin pH'ya sahip olması çamurdan gelen ağır metallerin çözünürlüğünün düşük olmasına ve toksik etki görülmemesine neden olmuştur. Buna rağmen, mera topraklarında uzun dönem çamur uygulamalarının etkisi takip edilmelidir.

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INTRODUCTION

Sewage sludge is the residue product of wastewater treatment and has been used in agriculture for many years as a fertilizer. This organic waste is typically rich in organic matter and contains high level nitrogen, phosphorus, potassium and micronutrients that are required for plant growth. Sewage sludge, also known biosolid, can be used to improve the growth and nutritional quality of crops and forage grasses while reducing the need for chemical fertilizer (Sharma et al., 2017; Mohamed et al., 2018; Neves et al., 2018). Moreover, sludge application to agriculture lands can increase water holding capacity, decrease bulk density, stimulate aeration and root penetrability and increase soil microorganism activity (Kukal et al. 2012; Wolna-Maruwka et al., 2018). However, one concern on application of sewage sludge is its relatively high heavy metal contents, which can potentially cause soil pollution and negative effect on plant growth.

Heavy metals in sewage sludge may limit the use of sewage sludge as a soil amendment. Rigueiro-Rodriguez et al. (2012) observed 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. Wolejko et al. (2015) stated that municipal sewage sludge application did not significantly affect the content of heavy metals both in the grass and in the soil. Malinowska (2016) reported that the highest application of sewage sludge resulted in the highest concentration of Zn, Cu and Cd in the grass while the highest concentration of Ni and Pb was in the soil. Because of the presence of both beneficial (nutrient and organic matter) and nonbeneficial (heavy metal and soluble salts) components in sewage sludge, it is necessary to systematically study the impacts of sewage sludge application on plant growth and on the soil physical and chemical properties to arrange sewage sludge dose.

Although much research has been done on the beneficial and harmful effects of sludge application doses, there has not been enough research on the time of sludge application. The time of sewage sludge application is very important because, if the sewage sludge is applied in autumn, nutrients can be leached as the plant roots are not able to absorb them because of the low temperatures. However, if applied too late, nutrient use is delayed and the grown season could be shortened. When the mineralization process of the sewage sludge is taken into consideration, it is difficult to determine the optimum application time (Rigueiro-Rodriguez et al., 2010). Sorensen and Rubaek (2012) compared the effect of manure application timing and soil texture on the nutrient leaching. According to experiment results, it is determined that solid manure should not be applied to winter wheat on sandy and sandy loam soils under irrigated conditions.

The objective of this study was to determine the effect of sewage sludge doses and application times on DTPA extractable nutrient and heavy metal concentrations in calcareous pasture soil for three years.

MATERIAL and METHODS

The experiment was conducted on grass-legume mixtures in Van Turkey, 1725 m above sea level, during 2004-2007. Rainfall and mean temperature in area is around 380 mm and 8.8 °C. Annual total rainfall and average temperature during the 3 years experimental period (2004-2006) were 426 mm, 337 mm, 427 mm and 9.5 °C, 9.9 °C, 10.0 °C, respectively (Anonymous, 2019).

The sludge used in this experiment was obtained from Van Municipality Wastewater Treatment Plant. Aerobically stabilized sewage sludge was dried in holding pools. In this process, organic matter in the sludge decomposes and pathogen microorganisms reduce. As a mean 3 years, physico-chemical properties of soil and sewage sludge used in this experiment are given at Table 1. Field experiment was conducted using split plot design with 4 replications. Main plot was determined by application times (spring and autumn) and sub-plot was determined by sludge doses (0, 4.1, 8.2, 16.4 t ha^{-1}) and nitrogen fertilizer application (60 kg N ha^{-1}). Experimental plot was 1.8X5 m= 9 m^2 in size. Grass-legume mixtures was sown 6 May 2004 with 30 cm row space.

There were five application rates and two application times (spring and autumn) in the experiment design. Five application rates:

1. Control
2. Chemical nitrogen fertilizer: 60 kg N ha^{-1}
3. $Sludge_1$: 4.1 t sewage sludge ha^{-1}
4. $Sludge_2$: 8.2 t sewage sludge ha^{-1}
5. $Sludge_3$: 16.4 t sewage sludge ha^{-1}

Cumulative sludge rates (3 years) were 12.3, 24.6 and 49.2 t ha^{-1}. Sludge applications were made for three years (2004-2006) and soil samples were taken in the last two years of experiment (2005-2007). Soil samples were taken from the various depths (0-20 cm, 20-40 cm, 40-60 cm) and about 6 months after sludge application. The last soil samples at the experiment were taken in April 2007. The annual nitrogen requirement of grass-legume mixtures was accepted as 60 kg N ha^{-1}. Sludge doses were determined to be half, full and double of the plant nitrogen requirement. It was accepted that about 30-35 % of the total nitrogen in the sludge was available in the first year (Cogger et al., 2001). Nitrogen fertilizer was applied only in the spring season and in the form of ammonium sulphate for both application times. Triple superphosphate at 80 kg P_2O_5 ha^{-1} was applied to control and nitrogen fertilizer plots. Sewage sludge was mixed in the early spring with a shovel to a depth about 20 cm before sowing. The other sludge applications were made on surface soil and mixed with a hand hoe. The spring applications of the sewage sludge were carried out in April, and the autumn applications were carried out in September.

Table 1. Some characteristics of soil and sewage sludge used in experiment (dry weight basis)
Çizelge 1. Denemede kullanılan toprak ve çamurun bazı özellikleri (kuru ağırlık esasına göre)

Properties	Soil	Sewage Sludge	Permitted values for sewage sludge (Anonymous, 2005)
Texture	Sandy clay loam		
$CaCO_3$, %	15.7		
EC, mS cm^{-1}	0.270	4.74	
pH, 1:2.5	8.77	6.97	
Organic Matter, %	1.58	51.4	
Total N, %	0.105	2.20	
Total P, %		0.45	
Total K, %		0.49	
Olsen P, mg kg^{-1}	9.0		
Extractable K, mg kg^{-1}	300		
DTPA Fe, mg kg^{-1}	3.30		
DTPA Mn, mg kg^{-1}	5.80		
DTPA Zn, mg kg^{-1}	0.27		
DTPA Cu, mg kg^{-1}	1.10		
Total Fe, %		0.96	
Total Mn, mg kg^{-1}		427	
Total Zn, mg kg^{-1}		795	4000
Total Cu, mg kg^{-1}		84	1750
Total Cd, mg kg^{-1}		1.37	40
Total Cr, mg kg^{-1}		130	1200
Total Pb, mg kg^{-1}		47	1200
Total Ni, mg kg^{-1}		100	400

In the soil of the experiment area, texture was determined using the hydrometer method for making particle size analysis. Soil pH was determined in a 1:2.5 soil-water suspension. Organic matter was analyzed colorimetrically with the modified Walkley Black method. Calcium carbonate was measured with calcimeter. Extractable K was measured by atomic absorption spectroscopy (AAS) with using ammonium acetate extraction. Total N was measured by the Kjeldahl method and available P was determined by the Olsen procedure for calcareous soil. DTPA (diethylenetriaminepentaacetic acid) extractable Fe, Mn, Zn, Cu, Pb, Cd, Cr and Ni were extracted using a solution (pH=7.3) containing 0.005 DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ with 2 hours shaking time using AAS. Total heavy metals in soil and sewage sludge were determined using flame atomic absorption spectroscopy following extraction by nitric-hydrochloric acid digestion (Kacar, 1994; Khan and Frankland, 1983).

Statistical analysis was performed by using the analysis of variance procedure for each year. If the F-value indicated significant differences ($P<0.05$), means compared using Duncan's multiple range test (Gomez and Gomez, 1984).

RESULTS

Effects of Sewage Sludge Application Doses

The effects of sludge application doses on the soil pH , DTPA extractable nutrient and heavy metal concentrations for different soil depths are given in Table 2 and Table 3. Soil pH is very important factor determining directly about the possibility of plant growth, nutrients and heavy metals uptake and biological processes. The increasing sewage sludge applications slightly decreased the soil pH for all depths. The soil pH changed from 8.85 to 8.75 in sludge doses for 0-20 cm soil depth. But, this change is not important statistically. Changes on the soil pH was smaller for 20-60 cm depth. The greater pH as soil depth increases is due to a calcareous soil horizon.

While DTPA extractable Mn concentration did not show any significant change in the second year of experiment, it increased significantly with the addition of sewage sludge for 0-20 cm soil depth in the third year. The highest Mn concentration was obtained at the application dose of $sludge_1$ for 0-20 cm. For other soil depths, the effects of sludge application was not significant (Table 2 and Table 3). DTPA extractable Fe concentration increased significantly with sewage sludge application for 0-20 cm depth in the third year.

Table 2. Effect of different sewage sludge doses on the DTPA extractable nutrient and heavy metal concentrations (mg kg^{-1}) for 0-60 cm soil depth in the second year of experiment

Çizelge 2. Denemenin ikinci yılında 0-60 cm toprak derinliği için DTPA ile ekstrakte edilebilir besin elementi ve ağır metal konsantrasyonlarına farklı çamur dozlarının etkisi

Depth	Treatment	Mn	Zn	Cu	Pb	Cd	Cr
0-20 cm	Control	3.85	0.18 c	0.92	0.40	0.028 b	0.020
	Nitrogen fertilizer	4.01	0.17 c	0.90	0.40	0.027 b	0.020
	$Sludge_1$	4.17	0.35 b	1.02	0.45	0.036 a	0.020
	$Sludge_2$	4.16	0.45 b	0.94	0.40	0.033 ab	0.020
	$Sludge_3$	4.51	0.92 a	1.00	0.46	0.039 a	0.021
F value		ns	68.3^{***}	ns	ns	6.0^{**}	ns
20-40 cm	Control	4.26	0.16 b	0.96	0.39	0.028	0.023
	Nitrogen fertilizer	4.05	0.15 b	0.90	0.42	0.027	0.019
	$Sludge_1$	4.26	0.22 b	0.99	0.45	0.027	0.019
	$Sludge_2$	3.78	0.23 b	0.84	0.36	0.030	0.021
	$Sludge_3$	4.31	0.34 a	0.89	0.37	0.030	0.021
F value		ns	7.1^{***}	ns	ns	Ns	ns
40-60 cm	Control	4.24	0.15 b	0.96	0.38	0.027	0.021
	Nitrogen fertilizer	3.76	0.13 b	0.89	0.40	0.028	0.019
	$Sludge_1$	3.84	0.15 b	0.97	0.41	0.030	0.020
	$Sludge_2$	3.72	0.15 b	0.79	0.38	0.029	0.021
	$Sludge_3$	3.84	0.22 a	0.84	0.39	0.030	0.021
F value		ns	5.3^{**}	Ns	ns	Ns	ns

$Sludge_1$: 4.1 t ha^{-1}, $Sludge_2$: 8.2 t ha^{-1}, $Sludge_3$: 16.4 t ha^{-1}, **: P<0.01, ***:P<0.001, ns: not significant. Means followed by the same letter within the same column and depth are not statistically different (Duncan test, P<0.05)

Table 3. Effects of different sewage sludge doses on soil pH, DTPA extractable nutrient and heavy metal concentrations (mg kg^{-1}) for 0-60 cm soil depth in the third year of experiment

Çizelge 3. Denemenin üçüncü yılında 0-60 cm toprak derinliği için PH, DTPA ile ekstrakte edilebilir besin elementi ve ağır metal konsantrasyonlarına farklı çamur dozlarının etkisi

Depth	Treatment	Soil pH	Mn	Fe	Zn	Cu	Pb	Cd	Cr	Ni
0-20 cm	Control	8.85	6.59 c	2.97 c	0.30 d	1.15 b	0.50 b	0.018 b	0.027	0.31 b
	Nitrogen fertilizer	8.83	7.24 bc	2.60 c	0.31 d	1.14 b	0.47 b	0.017 b	0.029	0.32 b
	$Sludge_1$	8.81	8.76 a	3.80 b	1.06 c	1.38 a	0.67 a	0.019ab	0.031	0.47 a
	$Sludge_2$	8.75	7.96 ab	4.43ab	1.93 b	1.26ab	0.65 a	0.020ab	0.028	0.44 a
	$Sludge_3$	8.77	7.66abc	4.51 a	2.68 a	1.34 a	0.70 a	0.022 a	0.028	0.38ab
F value		ns	14.7^{***}	14.7^{***}	72.5^{***}	3.83^{*}	12.8^{***}	3.59^{*}	ns	3.23^{*}
20-40 cm	Control	8.96	6.23	2.81	0.26 c	1.15	0.44 b	0.018	0.028	0.32
	Nitrogen fertilizer	8.89	6.43	2.65	0.27 c	1.10	0.42 b	0.017	0.030	0.34
	$Sludge_1$	8.88	7.68	2.91	0.36 b	1.38	0.56 a	0.017	0.027	0.44
	$Sludge_2$	8.93	6.40	2.72	0.47 a	1.18	0.47ab	0.018	0.029	0.37
	$Sludge_3$	8.88	5.95	2.79	0.49 a	1.15	0.51ab	0.019	0.030	0.39
F value		ns	ns	ns	11.7^{***}	Ns	3.15^{*}	ns	ns	ns
40-60 cm	Control	8.99	6.03	2.98	0.21 c	1.13	0.49 b	0.018	0.030	0.35
	Nitrogen fertilizer	8.92	4.38	2.84	0.21 c	0.97	0.45 b	0.016	0.032	0.33
	$Sludge_1$	8.95	6.80	2.84	0.29ab	1.32	0.57 a	0.019	0.031	0.35
	$Sludge_2$	8.96	4.80	2.57	0.26bc	1.04	0.50 b	0.018	0.027	0.32
	$Sludge_3$	8.96	5.03	2.58	0.31 a	1.05	0.50 b	0.019	0.027	0.38
F value		ns	ns	ns	7.4^{***}	ns	3.32^{*}	ns	ns	ns

$Sludge_1$: 4.1 t ha^{-1}, $Sludge_2$: 8.2 t ha^{-1}, $Sludge_3$: 16.4 t ha^{-1}, *: P<0.05, ***:P<0.001, ns: not significant. Means followed by the same letter within the same column and depth are not statistically different (Duncan test, P<0.05)

Extractable Fe concentration increased from 2.97 mg kg^{-1} to 4.51 mg kg^{-1} at the highest sludge application dose for 0-20 cm (Table 3).

Among extractable metals, the most notable changes were observed in Zn concentration. Sewage sludge doses increased significantly DTPA-Zn for all depths and years. The most important increases on the DTPA-Zn were obtained for 0-20 cm depth in third year. DTPA-Zn increased significantly from 0.30 mg kg^{-1} to 2.68 mg kg^{-1} for 0-20 cm at the highest sludge dose. The highest sludge application showed 9 fold increase in DTPA-Zn (Table 3).

Sewage sludge application doses did not cause a significant change in DTPA-Cu concentration for all depths in the second year. However, increasing sludge doses increased significantly DTPA-Cu for 0-20 cm in the end of experiment. Extractable Cu concentration increased from 1.15 mg kg^{-1} to 1.34 mg kg^{-1} at the sludge$_1$ dose (Table 2 and Table 3). While DTPA-Pb concentration did not show any significant change in the second year of experiment, it increased significantly with the addition of sewage sludge for all depths in the third year. Extractable Pb concentration at the highest sludge dose increased significantly from 0.50 mg kg^{-1} to 0.70 mg kg^{-1} for 0-20 cm soil depth (Table 3).

DTPA extractable Cd concentration increased significantly from 0.028 mg kg^{-1} to 0.039 mg kg^{-1} in the second year and from 0.018 mg kg^{-1} to 0.022 mg kg^{-1} in the third year with increasing sludge doses for 0-20 cm depth, respectively. For 20-60 cm soil depths, sewage sludge applications did not cause a significant change in DTPA-Cd (Table 2 and Table 3). Also, sludge applications did not affect DTPA extractable Cr concentration for all depths and years. DTPA-Ni concentration increased significantly with the addition of sewage sludge for 0-20 cm in the end of experiment. The highest DTPA-Ni was obtained at the sludge$_1$ dose. For other soil depths, the effect of sludge application was not significant (Table 3). Soil pH and DTPA-Fe and Ni concentrations were determined only in the last year of experiment.

Effects of Sewage Sludge Application Time

The effect of sewage sludge application time on the soil pH, DTPA extractable nutrient and heavy metal concentrations for different soil depths are given in Table 4 and Table 5. The effect of sludge application time on the soil pH was found to be important for all depths after three years of sludge application. Sludge application in the spring period resulted in lower soil pH than in the autumn period for all depths in the end of experiment. Soil pH, which determined as 8.70 sludge application in the spring period, rose to 8.90 in the autumn period (Table 5).

The effect of sludge application time on the DTPA extractable Mn concentration was not statistically significant for all soil depths in the second year of experiment. However, this effect was important significantly for 0-20 cm in the third year. DTPA-Mn concentration increased significantly to 8.14 mg kg^{-1} with sewage sludge application in the spring period (Table 4 and Table 5).

The effect of sludge application time on the DTPA-Fe concentration was important for 0-20 cm depth. In the spring period, DTPA-Fe concentration was found to be significantly higher than the application of the sludge in the autumn. Extractable Fe concentration increased to 4.07 mg kg^{-1} with sewage sludge application in the spring period. Effect of dose × time interaction was found to be significant for 0-20 cm. The effect of application time on the DTPA-Fe was not significant for 20-60 cm soil depth (Table 5).

Table 4. Effect of sewage sludge application time on the DTPA extractable nutrient and heavy metal concentrations (mg kg^{-1}) for 0-60 cm depth in the second year of experiment

Çizelge 4. Denemenin ikinci yılında 0-60 cm toprak derinliği için DTPA ile ekstrakte edilebilir besin elementi ve ağır metal konsantrasyonlarına çamur uygulama zamanının etkisi

Depth	Application time	Mn	Zn	Cu	Pb	Cd	Cr
0-20 cm	Spring	4.06	0.38 b	0.93	0.40	0.032	0.020
	Autumn	4.26	0.45 a	0.98	0.44	0.033	0.021
F value		ns	5.1*	Ns	ns	ns	ns
F value	Dose×Time interaction	ns	3.1*	Ns	ns	ns	ns
20-40 cm	Spring	4.03	0.21	0.90	0.38	0.027	0.019
	Autumn	4.23	0.23	0.94	0.41	0.030	0.022
F value		ns	ns	Ns	ns	ns	ns
F value	Dose×Time interaction	ns	ns	Ns	ns	ns	ns
40-60 cm	Spring	3.69	0.15	0.85	0.40	0.027	0.019 b
	Autumn	4.07	0.17	0.93	0.38	0.030	0.022 a
F value		ns	ns	Ns	ns	ns	4.50*
F value	Dose×Time interaction	ns	3.0*	Ns	ns	ns	ns

*: P<0.05, ns: not significant. Means followed by the same letter within the same column and depth are not statistically different (Duncan test, P<0.05)

Table 5. Effect of sewage sludge application time on the soil pH, DTPA extractable nutrient and heavy metal concentrations (mg kg-1) for 0-60 cm soil depth in the third year of experiment

Çizelge 5. Denemenin üçüncü yılında 0-60 cm toprak derinliği için pH, DTPA ile ekstrakte edilebilir besin elementi ve ağır metal konsantrasyonlarına çamur uygulama zamanının etkisi

Depth	Application Time	Soil pH	Mn	Fe	Zn	Cu	Pb	Cd	Cr	Ni
0-20 cm	Spring	8.70 b	8.14 a	4.07 a	1.61 a	1.32 a	0.64 a	0.020a	0.028	0.45a
	Autumn	8.90 a	7.15 b	3.25 b	0.91 b	1.19 b	0.56 b	0.018b	0.029	0.32b
F value		33.0***	8.3**	16.8***	41.2***	7.12*	9.8**	7.59*	ns	12.5**
F value	Dose×Time interaction	ns	ns	3.9*	16.4***	3.14*	ns	4.75**	ns	ns
20-40 cm	Spring	8.83 b	6.98	2.87	0.35	1.22	0.53 a	0.019a	0.028	0.41a
	Autumn	8.98 a	6.10	2.69	0.38	1.17	0.43 b	0.017b	0.030	0.33b
F value		16.7***	ns	ns	ns	Ns	10.8**	7.40*	ns	6.68*
F value	Dose×Time interaction	ns	ns	ns	ns	Ns	ns	ns	ns	ns
40-60 cm	Spring	8.90 b	5.90	2.89	0.24	1.13	0.56 a	0.019a	0.029	0.37
	Autumn	9.01 a	4.92	2.63	0.27	1.07	0.45 b	0.017b	0.030	0.32
F value		18.1***	ns	ns	ns	Ns	27.0***	8.23**	ns	ns
F value	Dose×Time interaction	ns	ns	ns	3.1*	Ns	ns	3.83*	ns	ns

*: P<0.05, **: P<0.01, ***:P<0.001, ns: not significant. Means followed by the same letter within the same column and depth are not statistically different (Duncan test, P<0.05)

DTPA-Zn concentration was the most affected by the sludge application time among all metals. The effect of sludge application time on the DTPA-Zn concentration was found to be important for 0-20 cm depth in both years. Moreover, effect of dose × time interaction was important for 0-20 and 20-40 cm soil depths. The higher DTPA-Zn concentration was obtained in spring period for third year while it was obtained in the autumn for second year. However, the dose × application time interaction showed that the higher DTPA-Zn was in the spring period for both years. DTPA-Zn concentration, which was 0.91 mg kg^{-1} in autumn, increased to 1.61 mg kg^{-1} by spring application for 0-20 cm (Table 4 and Table 5).

While the effect of sludge application time on DTPA-Cu concentration was not significant in the second year, it was found significant for 0-20 cm depth in the end of experiment. Copper concentration increased considerable to 1.32 mg kg^{-1} with sewage sludge application in the spring period. Effect of dose × application time interaction on the extractable Cu concentration was found to be significant for 0-20 cm. The highest DTPA-Cu was obtained at sludge$_3$ application in spring period.

DTPA-Pb and Cd concentrations did not show any significant change due to the effect of sludge application time in the second year. However, sludge application time significantly affected the DTPA-Pb and Cd for all soil depths in the end of experiment. Sludge application in the spring period resulted in higher DTPA-Pb and Cd than in the autumn period for all soil depths. DTPA-Pb and Cd for 0-20 cm increased to 0.64 mg kg^{-1} and 0.020 mg kg^{-1} with sludge application in the spring period, respectively. Effect of the dose × application time interaction on the DTPA-Cd concentration was important. The highest DTPA-Cd was obtained in the spring sludge application in third year (Table 5). Effect of sludge application time on the DTPA extractable Cr concentration was not significant for all depths. DTPA-Ni concentration changed significantly with sludge application time for 0-40 cm depth. In the spring period, DTPA-Ni was found to be significantly higher than sludge application in the autumn. Extractable Ni increased to 0.45 mg kg^{-1} with sludge application in the spring period for 0-20 cm soil depth (Table 5).

DISCUSSION and CONCLUSION

The toxic effect of heavy metals from sewage sludge on agricultural soils is largely dependent on factors such as the heavy metal concentration of sludge, amount of sludge applied, soil pH, rate of sludge decomposition, climatic and soil properties (Singh and Kumar, 2014; Kitczak et al., 2016; Zahedifar et al., 2017). Compared with the allowable critical values, the heavy metal content of the sludge used in the study is found to be low under Turkey law (Anonymous, 2005). Zinc was the element with the highest proportion with respect to the limit indicated by law as dangerous but was only 20 % of the total recommendation.

An important parameter used in determining heavy metal level in the agricultural soils is the DTPA extractable heavy metal concentrations. In this regards, while the total heavy metal concentration provides information on potential pollution, extractable heavy metals provide information on active pollution. Many investigators prefer to use the DTPA-extractable amounts of soil to determine the heavy metal pollution (Wu et al., 2012; Bansal et al., 2014; Ullah and Khan, 2015; Tziachris et al., 2017).

The results of research showed that the increasing sludge doses over a 3-years period had no significant effect on soil pH. This may be due to the lime and buffering power of the soil. However, the effect of sludge application time on the soil pH was found to be significant. Mohamed et al. (2018) reported that soil pH was significantly affected by sewage sludge, becoming less alkaline compared to the control. On the contrary, Mosquera-Losada et al. (2001) reported that sewage sludge application did not cause a significant change in soil pH of silvopastoral system. The pH of the sludge used in the study was determined to be 6.97. At the end of the experiment, the effect of sludge application doses on soil pH can be explained by the neutralization of the sludge pH used in the experiment and the low sludge application doses. It is also likely that the autumn-winter season in the Van Region is cold compared to the spring-summer period, resulting in a low level of decomposition in the sludge organic matter a low effect on soil pH. Rigueiro-Rodriguez et al. (2010) argued that the effect of sludge applications at different times was not very clear and that it would be more appropriate to apply it in February.

Extractable Zn and Cd concentrations increased significantly with increasing sludge application to a calcareous soil in the second year of experiment while DTPA-Mn, Cu, Pb and Cr concentrations were not affected. The effect of increasing sludge application became more pronounced in the end of the experiment. All extractable metal concentrations except Cr were increased by the sludge addition for 0-20 cm depth. Extractable Zn and Pb concentrations increased for all soil depths. The concentrations of extractable Mn, Fe, Zn, Cu, Pb, Cd and Ni were significantly changed by the effect of sludge application time at 0-20 cm depth in the end of experiment. Higher nutrient and heavy metal concentrations were obtained in the spring period application.

Probably, the high decomposition rate of organic matter and lower soil pH due to the higher temperature in spring-summer period resulted in higher concentration of extractable nutrients and heavy metals. Similarly, it is reported that the high temperature increases the decomposition rate of sludge organic matter and the availability of heavy metals (Wolejko et al., 2014). It was determined that the treatment of sewage sludge on urban lawn grasses did not significantly affect the heavy metal concentration in the soil (Wolejko et al., 2015). Various studies on different ecological conditions showed that sewage sludge applications increased the heavy metal concentration in soil and plant. But, these increases did not cause harmful effects (Keskin et al., 2012; Silva et al. 2013; Kwon et al., 2014). Probably, the high content of lime and pH of experiment soil prevented it from increasing the availability of heavy metals. However, policies with respect to the application of sewage sludge as a fertilizer must regulate heavy metal availability in soil because environmental risk is better evaluated in this way. In addition, the high lime content, high pH and low available Fe and Zn concentrations of Van Soils, where the research is carried out, make the sludge application more attractive in this soil.

In conclusion, at the end of the 3-years study, Van Municipal sewage sludge application at increasing rates did not cause a significant change in soil pH. On the other hand, sludge application at different times significantly affected soil pH. Soil pH was significantly reduced with the application of spring period. At the end of the experiment, DTPA extractable Mn, Fe, Zn, Cu, Pb, Cd and Ni concentrations increased significantly by the sludge addition for 0-20 cm soil depth. For Zn and Pb concentrations, this increase was found to be important for all soil depths. In spring, DTPA extractable Mn, Fe, Zn, Cu, Pb, Cd and Ni concentrations were significantly increased by sludge application compared to in metal concentrations in the soil, while not reaching toxic level. As stated in Table 1, lime content and pH value of the experiment soil are high and DTPA-Fe and Zn concentrations are low. In this case, the addition of sludge which enriches the soil with Fe and Zn may be useful.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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