DETERMINING THE EFFECTS OF SEWAGE SLUDGE AND *RHIZOBIUM* INOCULATION ON NUTRIENT AND HEAVY METAL CONTENT OF LENTIL (*LENS CULINARIS* MEDIC.)

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Abstract. This study investigates the effects of increasing doses of sewage sludge with *Rhizobium* inoculation on lentil (*Lens culinaris* Medic.) straw and grain macro, micro and heavy metal contents in 2003-2004 and 2004-2005 growing seasons. Experiment was conducted as a randomized complete block design with 3 replications. At the end of the study, it was seen that *Rhizobium* inoculation affects the N, Mg, and Pb contents of the lentil straw on by $P<0.05$, P and Ca contents by $P<0.01$ in the first year; the N, Fe, and Zn contents on $P<0.05$ level, on the Pb content $P<0.01$ level in the second year. Lentil grains were affected only on the K, Fe, Pb, and Cd contents by $P<0.05$. Sewage sludge applications were used to detect the effects on the N, Mg, Fe, Mn and Cu contents of the lentil straw by $P<0.01$, Co content by $P<0.05$, and Pb content by $P<0.01$ in first year; on the N, K, Zn and Pb contents by $P<0.01$, P and Mn contents by $P<0.01$, on the Mg, Fe, Cu, and Co contents by $P<0.05$ in the second year. Effects received by the lentil grains are as follows: The N, K, and Cu contents by $P<0.01$, on the Pb, Fe, P, and Mg contents by $P<0.01$, on the Ca and Zn contents by $P<0.05$ in the first year; on the P, K, Ca, Mg, Fe, Mn, Zn, Cu and Co contents by $P<0.01$, on the Pb content by $P<0.05$ in the second year. The effects of inoculation on the lentil straw and grain, regarding the nutrient and heavy metal contents were determined to be insignificant. This result may be a result of weak inoculation application and the heavy metal content of the sewage sludge, as well as the negative effects of *Rhizobium* inoculation.
Keywords: *Cadmium, macro nutrients, legume, Rhizobium, municipal waste sludge*

Introduction

One of the preferred methods to remove sewage sludge in industrialized and populous countries is to use sewage sludge in agricultural production under controlled conditions. Particularly, sewage sludge derived industrial waste cause health problems because of the potential toxic substance like heavy metals, phenolics (Mueller et al., 1989; Van Assche and Clijsters, 1990). Moreover, the organic substance of soils is low. In order to achieve steady agricultural production, and taking into account the limited organic fertilizer resources – used for preserving or increasing the organic substance content of soils – scientists have been using sewage sludge.

High or low of organic substance extent of soils have effects on physical, chemical and biological specification directly and indirectly. The organic fertilizer source used includes nutrient elements (stable manure, poultry manure, refuse compost, sewage sludge) and the content of potential toxic elements affect the frequency of application in agricultural production. In addition, the pH of the soil affects the amount and place of application of the sewage sludge (Logan and Chaney, 1983).

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Generally, it is preferred that sewage sludge is applied to the soil which has 6 pH or more. Because sewage sludge contains elements especially like Cd, Zn and Ni. These have high activity on acidic soils. Using sewage sludge in agricultural production improves physical characteristic of the soil and it functions as a source of nutrition elements for plants (Smith, 1996). The important thing here is that we should be careful in the harvest period after sewage sludge applied. Because after sewage sludge is applied, there should be a delay period before harvesting and therefore, sewage sludge applications must be done considering the harvest periods (Lovell et al., 1995).

As sewage sludge includes potential toxic substances, it has serious effects on macro and micro flora of soils (Sterritt and Lester, 1980; Brynhildsen and Rosswall, 1997; Athar and Ahmad, 2002). So, some researchers had investigated and found out what the sewage sludge effects on the soil development and soil microorganism are (Sönmez, 2003; Tüfenkçi et al., 2006a). According to the research, microorganisms had developed some mechanism against the toxic substances, but *Rhizobium* bacteria (Haferburg and Kothe, 2007), was discovered to be especially vulnerable to them (Chaudri et al., 1993, 2000). Therefore, soil organisms especially *Rhizobiums* where sewage sludge are applied face some important problems. In the area where sewage sludge is applied, heavy metal loading in different organs of plants has been monitored (Xian, 1989; Nellesen and Fletcher, 1993; Yürük and Bozkurt, 2006). The legumes used in the rehabilitation of contaminated areas with heavy metals (Zribi et al., 2012), and especially the presence of microbial density produced by plants in the rhizosphere and root exudates are very important (Glick, 2004). It has been reported that it has a negative effect on the inoculation of cadmium, copper and zinc *Rhizobium* in areas where treatment sludge has been applied for many years (Charlton et al., 2016).

In this study, we aimed to determine the effects of *Rhizobium* budding on the nutrition element of the straw and grain and monitor the changes on the heavy metal content after the application of sewage sludge on the lentil plant.

Materials and Methods

This study was carried out on the experiment area of Van Yüzüncü Yıl University in Eastern Anatolia in Turkey (between latitudes 35^o 57’ 84 N and longitudes 42^o 74’ 61 E at an elevation of 1725 m) in 2003-04 and 2004-05 growing seasons using one red-seeded nationally registered lentil cultivar (cv. Sazak-91) with 1000-seed weight of 30 g. Annual amounts of rainfall were 349.7 mm and 421.2 mm in 2003-04 and 2004-05, respectively. Average temperatures were 9.9^{0}C in 2003-04 and 9.6^{0}C in 2004-05 (*Table 1*). The results of the elemental analysis of the treatment area soil and the sewage sludge used in the experiment were given in *Table 2*.

Seeds were inoculated using peat inoculants which included a mixture of nodule-forming strains of *Rhizobium leguminosarum biovar viciae* specific to lentil. Peat inoculant was prepared with commercial peat cultures provided by the Soil and Fertilizer Research Institute, Ankara, Turkey, according to the method of Somesagaran and Hoben (1994). Peat inoculants were kept in a refrigerator at +4 ^{0}C until they were used. It was separately provided from the Soil and Fertilizer Research Institute in 2003-04 and 2004-05 winter seasons. Content and activity of peat inoculants were checked before the trials in 2003-04 and 2004-05. Peat inoculants was used after cell count was adjusted to 1 x 10^8 *Rhizobium* cells g^{-1} so the content of peat culture was standardized by diluting peat inoculants. The most probable number (MPN) method was used for estimating viable cells of *Rhizobium* (Somesagaran and Hoben, 1994).

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***Table 1.** Meteorological data for the growing seasons of 2003-2004, 2004-2005 and long-term averages in Van, Turkey (TSMS, 2006)*

Months	Precipitation (mm)			Average temperature (ºC)			Relative humidity (%)		
	2003-04	2004-05	LTA*	2003-04	2004-05	LTA*	2003-04	2004-05	LTA*
September	16.4	-	13.0	17.0	18.0	17.2	64.5	48.7	44.0
October	23.6	48.1	45.2	13.0	12.0	10.6	71.0	64.1	58.0
November	59.6	102.4	47.9	4.5	4.6	4.4	74.3	75.1	66.0
December	14.9	41.0	37.3	0.2	-3.7	-0.8	76.7	73.8	69.0
January	25.0	34.4	35.4	-0.9	-3.3	-3.6	78.8	77.1	68.0
February	39.6	27.2	32.5	-0.6	-3.3	-3.2	76.1	73.7	69.0
March	69.9	59.1	45.7	3.7	2.5	0.9	72.3	70.9	68.0
April	26.9	55.9	56.6	6.9	8.9	7.4	66.4	64.1	62.0
May	68.7	35.8	45.0	12.4	13.3	13.0	67.8	62.5	56.0
June	3.1	13.0	18.5	18.5	18.7	18.0	57.8	55.9	50.0
July	2.0	0.3	5.2	21.4	24.1	22.2	52.7	51.3	44.0
August	-	4.0	3.4	22.2	23.4	21.8	46.5	62.1	41.0
Total	349.7	421.2	385.7						
Average				9.9	9.6	9.0	67	64.9	57

*LTA = Long-term average (1979-2006)

Four doses of trial sewage sludge (SS0; 0 (control), SS10; 10, SS20; 20 and SS40; 40-ton ha^{-1}) were applied as inoculated and non-inoculated in parcels with 3 repetitions. Sowing was done by hand with 30 cm row spacing during late October in both years (20 October 2003 and 25 October 2004). The seeding rate was 140 kg ha^{-1} with the large-grained Sazak 91. A basal dose of 140 kg ha^{-1} di-ammonium phosphate (DAP) was given to each parcel at the time of sowing. Seeds were inoculated with *Rhizobium leguminosarum biovar viciae* bacteria at the predetermined rates before sowing in all parcels, except for the uninoculated control (Vincent, 1970). Application of the peat inoculant on the seeds was conducted using water which contains 2% sugar. The parcel size was 1.5 x 5 m. The experiment was carried out as rain fed. Parcels were hand-weeded twice each season. Plants were harvested during late June in both years (22 June 2004 and 27 June 2005).

The levels of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), cadmium (Cd) and lead (Pb) were analyzed in dried and grinded samples that straw and grain extracts were obtained from each repetition to be in two parallel according to the methods reported by Kacar and İnal (2008) (*Table 3*).

At the end of the experiment, as in Jackson (1958), soil samples were taken from 0-20 cm depth on each repeating and brought to the laboratory and analyzed. The rest was done as follows: Texture, (Bouyoucous, 1951) soil reaction, (Jackson, 1958); total salt, (Richards, 1954): lime, (Hızalan and Ünal, 1966); organic substance, according to modified Walkley Black Method (Walkley, 1947); nitrogen, according to Kjehldahl Method (Kacar, 1994); available phosphorus, according to sodium bicarbonate method (Olsen et al., 1954); exchangeable potassium, calcium and magnesium, according to Thomas (1982) with 1 N ammonium acetate; micro elements and heavy metals found in soil samples were retrieved after shaking with 0.05 M DTPA (Diethylene Triamine Penta Acetic acid) adjusted to pH 7.3 (Lindsay and Norvel, 1978). These residuals were specified in Atomic Absorption Spectrometer. The values obtained at the end of the experiment were analyzed with Costat statistics program (Düzgüneş et al., 1987).

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Table 2. *The results of the chemical and physical analysis of the testing area soil and the sewage sludge*

Source	Years	Salt	OM	Texture	Lime	N	pH	P	K	Ca	Mg	Fe	Mn	Zn	Cu	Cd	Co	Pb
		%			%			mg kg^{-1}										
Soil	1.Year	0.03	0.82	SL	4.99	0.08	7.93	3.92	360	2130	201	10.37	19.0	1.57	1.61	-	0.132	-
	2.Year	0.03	0.80	SL	5.01	0.07	7.95	3.87	351	2201	211	9.7	17.6	1.03	1.52	0.11	0.098	0.06
S.S.	1.Year	0.04	29.4	-	-	1.32	7.98	1544	2430	2100	567	956	440	560	149	0.96	4.56	2.89
	2.Year	0.05	30.2	-	-	1.40	8.01	1645	2301	2081	497	889	413	529	152	1.02	4.12	3.01
	Permissible limit (pH>6)*													300	140	3	20	300

SS: Sewage sludge, OM: Organic matter, *: Anonymous (2003)

Table 3. *Results of some chemical and physical analysis of application parcels after testing*

R	SS, ton ha^{-1}	Salt	OM	Lime	N	pH	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		%					mg kg^{-1}							
R+	0	0.026*	0.85	6.2	0.071	7.49	3.7	499	2125	208	17.2	25.5	2.9	3.1
	10	0.027	0.94	3.7	0.115	7.49	3.8	470	2109	195	18.7	22.7	2.6	1.6
	20	0.023	0.97	5.2	0.119	7.47	4.3	327	2160	197	19.5	20.0	1.2	1.8
	40	0.025	1.03	4.2	0.136	7.58	6.0	411	2144	184	16.4	19.7	0.4	1.4
R-	0	0.021	0.82	5.0	0.080	7.73	3.9	360	2130	201	10.4	19.0	4.6	1.6
	10	0.020	0.92	3.6	0.098	7.83	4.2	305	2067	161	17.6	18.0	1.8	1.4
	20	0.023	0.96	2.7	0.106	7.63	4.6	311	2051	159	16.8	20.1	1.0	1.4
	40	0.018	1.04	2.7	0.132	7.73	5.3	352	2040	175	15.5	17.1	0.4	1.3

R: *Rhizobium*, R+: *Rhizobium* inoculate, R-: *Rhizobium* non-inoculate, SS: Sewage Sludge, OM: Organic Matter, *: values are the average of 3 replications

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Results

Effects of the application in the content of straw and grain macro elements

As it can be seen in *Table 4*, inoculation had a significant effect on both the first and the second year lentil straw N ($p<0.01$) and Ca ($p<0.01$) values, while it had a significant effect on P and Mg ($p<0.01$) values only on the first year. Its effect on potassium was found to be insignificant in both years. It was seen that while the sewage sludge affected the nitrogen ($p<0.01$) on both years, it was discovered that it affected P, K and Ca ($p<0.01$) only on the second year and Mg ($p<0.01$) only on the first year. It was observed that the interaction only affects nitrogen with a value of $p<0.05$ in both years (*Table 4*). While the sewage sludge application on the grain nitrogen content had a significant effect on the first year ($p<0.01$), it had a significant effect on the P, K, Ca, and Mg elements in both years, respectively as $p<0.01$, $p<0.01$, $p<0.05$ / $p<0.01$ and $p<0.01$. Inoculation had a significant in grain nitrogen and phosphorus in both years ($p<0.01$ and $p<0.05$), in potassium and calcium ($p<0.01$ and $p<0.05$), it had a significant effect only in the first year. It was detected that the interaction affected the K and the Mg content of the grain macro nutrients on the second year with a level of $p<0.05$ (*Table 5*).

Table 4. *Effects of Rhizobium and sewage sludge on macro element contents of lentil straw*

Treatments	Nitrogen (%)		Phosphorus (mg kg^{-1})		Potassium (%)		Calcium (%)		Magnessium (%)	
	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05
Sewage Sludge (ton ha^{-1}) (SS)										
SS0	1.189 c**	1.533 c**	1315 b ^{ns}	1300 b**	2.224 b ^{ns}	2.127 d**	3.95 b ^{ns}	4.60 b**	2.446 c**	2.522 b*
SS10	1.290 b	1.568 c	1420 ab	1417 b	2.419 ab	2.427 c	4.18 ab	4.92 b	3.507 bc	2.567 ab
SS20	1.270 bc	1.651 b	1452 ab	1762 a	2.530 ab	2.799 b	4.30 ab	5.32 a	3.538 ab	2.687 ab
SS40	1.402 a	1.728 a	1578 a	1907 a	2.597 a	2.931 a	4.48 a	5.48 a	3.578 a	2.598 a
***Rhizobium* (R)**										
R(+)	1.412 a*	1.693 a*	1701 a**	1610 ^{ns}	2.537 ^{ns}	2.594 ^{ns}	4.443 a**	5.32 ^{ns}	0.551 a*	2.568 ^{ns}
R(-)	1.164 b	1.547 b	1183 b	1580	2.347	2.548	4.018 b	4.79	0.484 b	2.559
Interactions (RxSS)										
R(-)xSS0	1.072 d *	0.433 d **	1110 ns	1291 ns	2.182 ns	2.089 ns	3.606 ns	4.503 ns	2.410 ns	2.515
R(-)xSS10	1.199 cd	0.455 d	1168	1402	2.343	2.362	3.938	4.750	2.475	2.551
R(-)xSS20	1.167cd	0.599 c	1205	1833	2.392	2.835	4.134	4.935	2.512	2.610
R(-)xSS40	1.185 cd	0.703 ab	1246	1794	2.473	2.906	4.393	5.008	2.540	2.562
R(+)xSS0	1.307 bc	0.633 bc	1520	1311	2.266	2.164	4.297	4.697	2.481	2.530
R(+)xSS10	1.349 b	0.682 ab	1673	1417	2.495	2.492	4.426	5.094	2.540	2.582
R(+)xSS20	1.372 b	0.703 ab	1700	1694	2.668	2.763	4.474	5.703	2.565	2.587
R(+)xSS40	1.619 a	0.752 a	1910	2023	2.721	2.957	4.575	5.955	2.616	2.575

ns: not significant; *: Significant at P<0.05 level, **: Significant at P<0.01 level, #: Values followed by the different letters are significantly different, SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R(-): *Rhizobium* non-inoculation, R(+): *Rhizobium* inoculation

When the change in the content of straw macro element of lentil at sewage sludge application was investigated, it was seen that the contents of N, P, K, Ca and Mg increased considerably in both years according to control. The highest N, P, K, Ca and Mg values were reached respectively as 1.402%, 1578 mg kg^{-1}, 2.597%, 4.48% and 3.578% at the application of 40-ton ha^{-1} of sewage sludge in the first year (*Table 4, Figure 1*).

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Table 5. *Effects of Rhizobium and sewage sludge on macro element contents of lentil grain*

Treatments	**Nitrogen (%)**		**Phosphorus (mg kg^{-1})**		**Potassium (%)**		**Calcium (%)**		**Magnessium (%)**	
	2003-04	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**
Sewage Sludge (ton ha^{-1}) (SS)										
SS0	4.339 c**	3.681 b ^{ns}	5139 b**	5328 c**	1.072 c**	1.277 d**	0.934 b**	1.135 c**	0.145 c**	0.126 b**
SS10	4.453 bc	3.840 ab	5689 a	5553 b	1.133 c	1.419 c	1.173 ab	1.450 b	0.188 b	0.135 b
SS20	4.481 b	3.960 ab	5866 a	5612 b	1.291 b	1.611 b	1.287 a	1.510 ab	0.201 b	0.136 b
SS40	4.629 a	4.170 a	5996 a	5833 a	1.463 a	1.846 a	1.416 a	1.589 a	0.254 a	0.157 a
***Rhizobium* (R)**										
R(+)	4.541 ns	4.057 ns	5844 ns	5638 ns	1.300 a*	1.519 ns	1.311 ns	1.459 ns	0.205 ns	0.140 ns
R(-)	4.411	3.768	5501	5532	1.179 b	1.558	1.093	1.503	0.189	0.138
Interactions (RxSS)										
R(-)xSS0	4.246 ns	3.607 ns	4880 ns	4208 ns	1.020 ns	1.228 b*	0.0797 ns	^{s}0.1363 ns	0.132 ns	0.126 ns
R(-)xSS10	4.346	3.724	5699	4506	1.078	1.457 b	0.0988	0.1469	0.185	0.141
R(-)xSS20	4.411	3.862	5704	4561	1.298	2.708 a	0.1239	0.1544	0.187	0.143
R(-)xSS40	4.638	3.879	5722	4855	1.320	1.840 ab	0.1347	0.1634	0.251	0.149
R(+)xSS0	4.431	3.753	5398	4448	1.124	1.327 b	0.1070	0.1386	0.158	0.127
R(+)xSS10	4.560	3.956	5680	4601	1.188	1.382 b	0.1358	0.1431	0.191	0.129
R(+)xSS20	4.551	4.059	6027	4663	1.283	1.515 b	0.1334	0.1476	0.216	0.129
R(+)xSS40	4.620	4.461	6269	4840	1.605	1.852 ab	0.1484	0.1545	0.257	0.165

ns: not significant; *: Significant at $P<0.05$ level, **: Significant at $P<0.01$ level, $^{\#}$: Values followed by the different letters are significantly different, SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R(-): *Rhizobium* non-inoculation, R(+): *Rhizobium* inoculation

Same results were reached also in the second year, but only the content of Mg value was reached as 2.687% at application of 20-ton ha^{-1} of the sewage sludge. At the application of 40-ton ha^{-1} of sewage sludge, the highest values of N, P, K, Ca and Mg were determined as 1.728%, 1907 mg kg^{-1}, 2.931%, 5.48% and 2.569%, respectively. The lowest value of both years was determined in control parcels (*Table 4, Figure 1).*

After statistical analyses, straw macro element of lentil in inoculated parcels increased in both years as compared to non-inoculated parcels. It was seen that N content in this increase was significant in both years; P, Ca and Mg are significant only in the first year. Increase in potassium content was not significant (*Table 4*). Inoculation resulted in significant increases in the nitrogen, phosphorus, calcium and magnesium content of lentils. A significant increase was detected for N in both years; for P, Ca and Mg it was only detected in the first year. It was seen that the decrease and increase in potassium content was statistically insignificant (*Table 4, Figure 1*).

At the application of 40-ton ha^{-1} of sewage sludge, the highest value was reached in both years. The lowest value in both years was determined in control parcels. The highest value on grain of N, P, K, Ca and Mg elements were respectively detected as 4.629%, 0.599%, 1.463%, 0.142% and 0.254% in first year, as 4.170%, 0.583%, 1.846%, 0.159% and 0.157%, respectively in second year (*Table 5, Figure 1*).

The effect of application to the straw and grain micro element content of lentil

The results of variance analysis and the results of the averages of the effect of straw and grain micro element content of lentil and *Rhizobium* applications are given in *Table 5, 6* and *Figure 2*.

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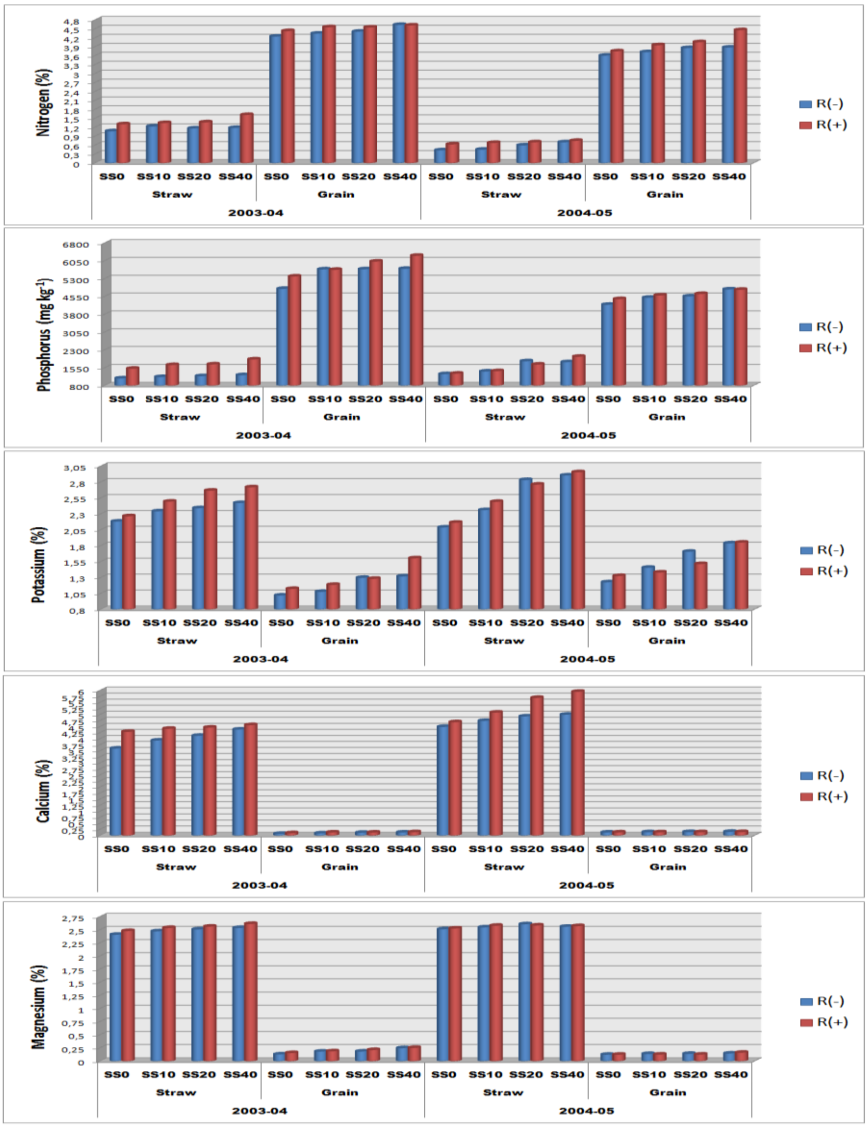

Figure 1. *Effects of applications on lentil straw and grain macro element contents SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R-: Rhizobium non-inoculation, R+: Rhizobium non-inoculation*

The application of sewage sludge affected the content of straw iron value of lentil by P<0.01 in first year, by P<0.05 in the second year. It affected the manganese content by P<0.01 in both years; zinc content by P<0.01 in the second year while it was insignificant in the first year. Copper content was affected by P<0.01 in the first year, by P<0.05 in the second year. Inoculation affected the content of manganese and copper in both years, while it affected the content of iron and zinc by P<0.05 in the second year. It was seen that its effect on iron and zinc application in the first year was insignificant (*Table 6*).

Inoculation effect on the grain micro element content was significant for Fe and Zn (p<0.01) in both years and for Mn in the second year (p<0.01). The sewage sludge application was significant for Fe, Zn and Cu (p<0.01 / p<0.05) in both years and for Mn (p<0.01) in the second year. The effect of the interaction was detected as significant in the second year only in Mn (p<0.01) (*Table 7*).

Table 6. *Effects of Rhizobium and sewage sludge on micro element contents of lentil straw*

Treatments	Iron (mg kg^{-1})		Manganese (mg kg^{-1})		Zinc (mg kg^{-1})		Copper (mg kg^{-1})	
	2003-04	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**
Sewage Sludge (ton ha^{-1}) (SS)								
SS0	50.6 c**	28.9 b*	54.8 b **	44.8 b**	6.07 a ns	6.29 c**	7.12 b**	7.84 b*
SS10	57.6 bc	34.4 ab	61.5 a	46.0 b	6.42 a	7.42 b	9.55 a	9.23 ab
SS20	68.3 ab	36.9 ab	64.2 a	46.7 b	6.53 a	8.03 ab	10.48 a	9.39 a
SS40	72.9 a	42.9 a	67.7 a	56.3 a	6.57 a	8.66 a	11.07 a	10.12 a
***Rhizobium* (R)**								
R(+)	63.5 ns	39.7 a*	65.2 ns	47.4 ns	5.79 ns	7.81 ns	9.54 ns	9.15 ns
R(-)	61.2	31.9 b	58.9	49.5	6.86	7.39	9.57	9.15
Interactions (RxSS)								
R(-)xSS0	49.5 ns	24.5 ns	52.5 ns	46.2 **	6.33 ns	6.26 ns	7.28 ns	8.01 ns
R(-)xSS10	54.2	31.9	56.4	47.7	6.56	6.97	10.13	9.09
R(-)xSS20	68.8	31.8	50.4	47.9	7.37	7.77	9.90	9.32
R(-)xSS40	72.3	39.5	66.3	56.2	7.18	8.55	10.97	10.15
R(+)xSS0	51.8	33.4	57.2	43.2	5.79	6.31	6.96	7.67
R(+)xSS10	60.8	36.9	66.6	44.4	6.3	7.87	8.97	9.37
R(+)xSS20	67.8	42.0	68.0	45.5	5.8	8.29	11.06	9.46
R(+)xSS40	73.2	46.4	68.9	56.4	5.38	8.76	11.17	10.09

ns: not significant; *: Significant at P<0.05 level, **: Significant at P<0.01 level, #: Values followed by the different letters are significantly different, SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R(-): *Rhizobium* non-inoculation, R(+): *Rhizobium* inoculation

Table 7. *Effects of Rhizobium and sewage sludge on micro element contents of lentil grain*

Treatments	Iron (mg kg^{-1})		Manganese (mg kg^{-1})		Zinc (mg kg^{-1})		Copper (mg kg^{-1})	
	2003-04	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**	**2003-04**	**2004-05**
Sewage Sludge (ton ha^{-1}) (SS)								
SS0	48.1 c**	67.9 d**	10.7 ns	11.0 c**	16.37 b*	11.85 c**	10.18 c**	7.85 c**
SS10	54.2 bc	95.2 c	11.3	14.3 b	17.39 ab	13.65 b	10.39 bc	8.42 b
SS20	58.8 b	123.3 b	11.2	14.8 b	17.81 ab	14.49 b	11.08 ab	9.09 a
SS40	81.8 a	166.2 a	10.0	16.7 a	18.98 a	16.57 a	11.70 a	9.16 a
***Rhizobium* (R)**								
R(+)	66.1a**	121.7 ns	10.8 ns	13.5 ns	18.41 ns	13.18 ns	10.87 ns	8.56 ns
R(-)	55.3 b	104.6	10.8	14.9	16.87	15.10	10.81	8.69
Interactions (RxSS)								
R(-)xSS0	42.8 ns	63.8 ns	10.7 ns	10.7 c**	15.17 ns	12.29 ns	9.83 ns	7.61 ns
R(-)xSS10	46.7	86.1	12.2	14.2 b	16.74	14.58	11.08	8.37
R(-)xSS20	53.6	107.9	9.8	15.5 b	17.35	15.17	10.35	9.33
R(-)xSS40	78.3	160.5	6.5	19.2 a	18.19	18.37	11.98	9.46
R(+)xSS0	53.5	72.0	10.6	11.4 c	17.57	11.40	10.54	8.08
R(+)xSS10	61.7	104.2	10.4	14.1 b	18.03	12.73	11.08	8.47
R(+)xSS20	64.0	138.6	11.7	14.3 b	18.28	13.81	10.42	8.85
R(+)xSS40	85.3	171.8	10.6	14.3 b	19.76	14.76	11042	8.85

ns: not significant; *: Significant at P<0.05 level, **: Significant at P<0.01 level, # : Values followed by the different letters are significantly different, SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R(-): *Rhizobium* non-inoculation, R(+): *Rhizobium* inoculation

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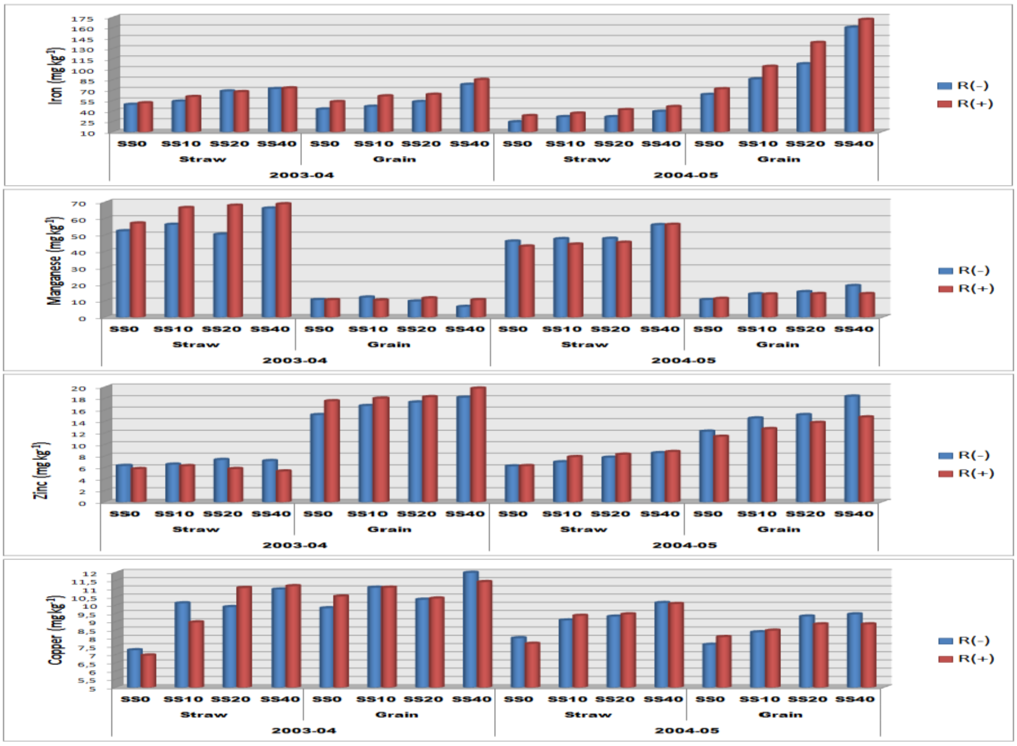

Figure 2. *Effects of the applications on straw and grain micro element contents of lentil SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R-: Rhizobium non-inoculation, R+: Rhizobium non-inoculation*

The highest value of straw content of Fe, Mn, Zn and Cu were reached at the application of 40-ton ha^{-1} of sewage sludge in both years and the lowest value level was detected at control parcels. The highest value was reached as 72.9 mg kg^{-1}, 67.7 mg kg^{-1}, 6.57 mg kg^{-1} and 11.07 mg kg^{-1} respectively in first year; as 42.9 mg kg^{-1}, 56.3 mg kg^{-1}, 8.66 mg kg^{-1} and 10.12 mg kg^{-1} respectively in second year. The alteration in content of straw micro element depending on inoculation was variable. Others were found to be insignificant while only iron was insignificant in the second year (*Table 6, Figure 2*).

Even though inoculation had insignificant effects on the grain micro element content of lentil in both years, except for Fe, it was seen that sewage sludge had very important effects on Fe, Zn and Cu in both years, on Mn in the second year only (*Table 7, Figure 2*).

According to control, depending on applications of sewage sludge, Mn increased only in the second year, while Fe, Zn and Cu increased highly in both years. The highest value was reached at the application of 40-ton ha^{-1} of sewage sludge as 81.8 mg kg^{-1}, 18.98 mg kg^{-1} and 11.70 mg kg^{-1} respectively in the first year and 166.2 mg kg^{-1}, 16.7 mg kg^{-1}, 16.57 mg kg^{-1} and 9.16 mg kg^{-1} respectively in the second year (*Table 7, Figure 2*).

The effect of the applications to the straw and grain heavy metals content of lentil

The results of variance analysis and the results of the averages of the effect of sewage sludge and *Rhizobium* applications on the straw and grain heavy metal contents of lentil are given in *Table 8, 9,* and *Figure 3*.

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Rhizobium application had a significant effect on the straw Co content of the lentil in the second year ($p<0.05$) and on the Pb content in both years ($p<0.01$). The effect of the sewage sludge application and interaction was significant only on Pb content in both years ($p<0.01$). While the inoculation on grain heavy metal content of lentil had a significant effect on the content of Pb ($p<0.01$) and Cd ($p<0.05$) in the first year, the sewage sludge application had a significant effect on Co in the second year ($p<0.05$) and on Pb ($p<0.01$/$p<0.05$) in both years. The effect of interference on grain heavy metal content was found to be insignificant (*Table 8, 9*).

As the doses of sewage sludge are increased, the content of straw heavy metal of lentil also increased. The highest value in Co, Pb, and Cd contents were reached at the application of 40-ton ha^{-1} of sewage sludge in both years as 2.714 mg kg^{-1}/0.999mg kg^{-1}, 0.618 mg kg^{-1}/0.602 mg kg^{-1}, 0.201 mg kg^{-1}/1.027 mg kg^{-1} respectively, and the lowest value was reached at control parcels. Inoculation only affected Pb content. Lead content decreased in the second year while it increased with inoculation in the first year (*Table 8, Figure 3*).

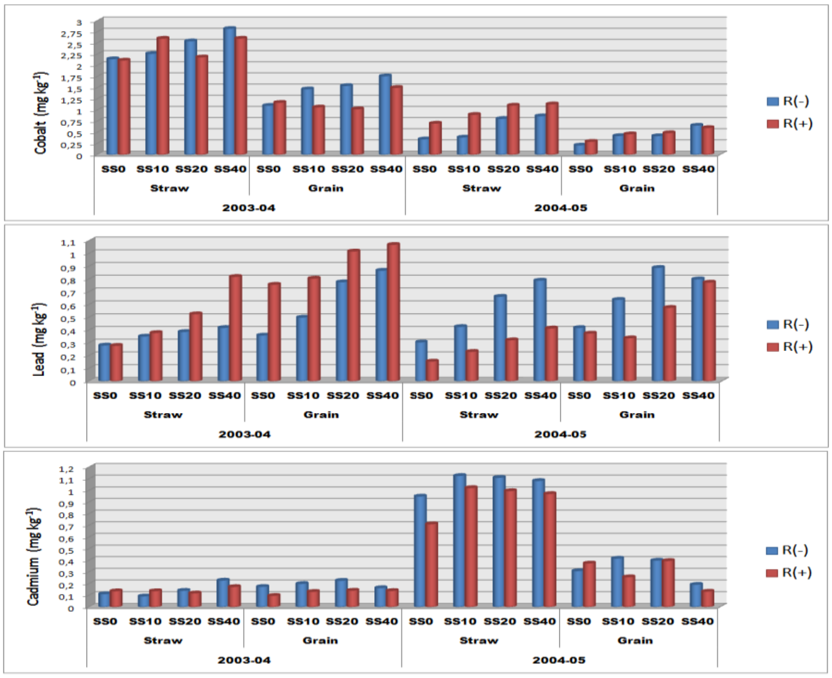

***Figure 3.** Effects of the applications on straw and grain micro element contents of lentil SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R-: Rhizobium non-inoculation, R+: Rhizobium non-inoculation*

It was seen that sewage sludge had a statistically important effect on the grain heavy metal content of lentil with regards to Co in the second year and Pb in both years but its effect on Cd was insignificant (*Table 9, Figure 3*).

Co and Pb content increased significantly with the application of residual sewage sludge according to control in both years. The highest value was reached as 1.631 mg kg^{-1} and 0.968 mg kg^{-1} respectively in the first year; as 0.628 mg kg^{-1} and 0.686 mg kg^{-1} respectively in the second year. *Rhizobium inoculation* decreased the taking of Cd, while it increased the taking of Pb seed in the first year (*Table 9, Figure 3*).

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Table 8. *Effects of Rhizobium and sewage sludge on heavy metal contents of lentil straw*

Treatments	Cobalt (mg kg^{-1})		Lead (mg kg^{-1})		Cadmium (mg kg^{-1})	
	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05
Sewage Sludge (ton ha^{-1}) (SS)						
SS0	2.129 b*	0.524 b*	0.280 d**	0.230 d**	0.124 ab*	0.831 ns
SS10	2.432 ab	0.644 ab	0.365 c	0.329 c	0.115 b	1.075
SS20	2.364 ab	0.956 a	0.456 b	0.492 b	0.130 ab	1.053
SS40	2.714 a	0.999 a	0.618 a	0.602 a	0.201 a	1.027
***Rhizobium* (R)**						
R(+)	2.376 ns	0.959 ns	0.500 a*	0.281 b**	0.142 ns	0.925 ns
R(-)	2.443	0.603	0.359 b	0.546 a	0.143	1.068
Interactions (RxSS)						
R(-)xSS0	2.1437 ns	0.3483 ns	0.2808 d**	0.3047 d**	01117 ns	0.9500 ns
R(-)xSS10	2.2620	0.3897	0.3508 cd	0.4267 c	0.0925	1.1267
R(-)xSS20	2.5443	0.8067	0.3867 cd	0.6623 b	0.1408	1.1100
R(-)xSS40	2.8227	0.8657	0.4183 c	0.7889 a	0.2283	1.0833
R(+)xSS0	2.1133	0.7007	0.2783 d	0.1551 e	0.1367	0.7117
R(+)xSS10	2.6027	0.8973	0.3787 cd	0.2313 de	0.1375	1.0225
R(+)xSS20	2.1843	1.1043	0.5258 b	0.3218 d	0.1185	0.9967
R(+)xSS40	2.6043	1.1323	0.8183 a	0.4146 c	0.1733	0.9717

ns: not significant; *: Significant at P<0.05 level, **: Significant at P<0.01 level, #: Values followed by the different letters are significantly different, SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R(-): *Rhizobium* non-inoculation, R(+): *Rhizobium* inoculation

Table 9. *Effects of Rhizobium and sewage sludge on heavy metal contents of lentil grain*

Treatments	Cobalt (mg kg^{-1})		Lead (mg kg^{-1})		Cadmium (mg kg^{-1})	
	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05
Sewage Sludge (ton ha^{-1}) (SS)						
SS0	1.133 b ns	0.253 c**	0.558 b**	0.393 c*	0.136 ns	0.342 ns
SS10	1.267 ab	0.443 b	0.652 b	0.488 bc	0.166	0.275
SS20	1.282 ab	0.454 b	0.897 a	0.732 ab	0.184	0.398
SS40	1.631 a	0.628 a	0.968 a	0.787 a	0.151	0.225
***Rhizobium* (R)**						
R(+)	1.188 ns	0.462 ns	0.912 a*	0.515 ns	0.128 b*	0.291 ns
R(-)	1.468	0.427	0.625 b	0.686	0.191 a	0.330
Interactions (RxSS)						
R(-)xSS0	1.0990 ns	0.2113 ns	0.3590 ns	0.4183 ns	0.174 ns	0.309 ns
R(-)xSS10	1.4693	0.4223	0.4992	0.6383	0.200	0.417
R(-)xSS20	1.5430	0.4190	0.7761	0.8888	0.227	0.400
R(-)xSS40	1.7603	0.6547	0.8667	0.7992	0.164	0.195
R(+)xSS0	1.1670	0.2937	0.7567	0.3743	0.098	0.375
R(+)xSS10	1.0637	0.4627	0.8050	0.3373	0.132	0.258
R(+)xSS20	1.0217	0.4880	1.0174	0.5758	0.142	0.397
R(+)xSS40	1.5010	0.6017	1.0690	0.7738	0.139	0.133

ns: not significant; *: Significant at P<0.05 level, **: Significant at P<0.01 level, #: Values followed by the different letters are significantly different, SS0: 0 ton ha^{-1}, SS10: 10 ton ha^{-1}, SS20: 20 ton ha^{-1}, SS40: 40 ton ha^{-1}, R(-): *Rhizobium* non-inoculation, R(+): *Rhizobium* inoculation

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Discussion

In our study, it was determined that the increasing applications of sewage sludge increase the amount of lentils' straw and grain macro, micro and heavy metal contents in both years. The highest values were reached in 40-ton sewage sludge ha^{-1} applied parcels. Increases in N, P, K, Ca, Mg, Fe, Mn, Cu, Co, and Pb contents of the lentil straw in the first year were respectively 17.9%, 19.7%, 16.8%, 13.4%, 5.4%, 44.1%, 23.5%, 55.5%, 27.5%, 120.7 and in the second year they 12.7%, 47.7%, 37.8%, 19.1%, 3.0%, 48.4%, 25.9%, 29.1%, 90.7%, 161.7%, respectively. The straw zinc content increased by 37.7% in the second year and the cadmium content increased by 62.1% in the first year. The first and second year increases in the grain nutrient and heavy metal contents were respectively N; 6.7%-13.3%, P; 16.5%-9.8%, K; 36.5%-44.6%, Ca; 52.7%-16.1%, Mg; 75.2%-24.6%, Fe; 70.1%-144.8%, Mn; 51.8%, Zn; 15.9%-39.8%, Cu; 14.9%-16.7%, Co; 43.9%-148.2% and Pb; 73.5%-100.3%. Even though there was a decrease in the grain Cd content, this was found to be statistically insignificant (*Table 4, Table* 5, *Figure 1*).

In similar studies, it has been reported that plants' nutrient and heavy metal contents go up as a result of increasing sewage sludge applications (Sönmez and Bozkurt, 2005; Tüfenkçi et al., 2006b; Yürük and Bozkurt, 2006; Singh and Agrawal, 2010). This is probably because of the changes in the nutrient element composition of soils with sewage sludge. Indeed, Antolin et al. (2005) reported in their study that with the addition of long-term sewage sludge, there is a decrease in the pH of the soil and increase in the total organic carbon and DTPA extractable heavy metal content. In other relevant studies, it was reported that there was an improvement in the physical and chemical properties of the soil with the addition of sewage sludge. The amount of product and the nutrient element contents increased too (Barzegar et al., 2002; Speir et al., 2003; Sönmez and Bozkurt, 2005; Tüfenkçi et al., 2006b). With a high rate of sewage sludge addition, the cation exchange capacity (CEC) of soils increases (Soon, 1981) and the bioavailability of heavy metals become limited (Kladivko and Nelson, 1979; Kim and Kim, 1999).

With *Rhizobium* inoculation; the phosphorus, potassium, calcium and magnesium contents of straw and grain nitrogen have increased within the years. These increases were 21.3%-9.4%, 44.1%-1.9%, 8.9%-1.8%-10.5%-11.9% and 2.6%-0.3% respectively in lentil straw and 2.9%-7.6%, 6.2%-2.0%, 10.3%-2.6%, 20.2%-3.4% and 8.5%-1.5% respectively in lentil grain (*Table 4, Table 5, Figure 1*). It is reported by some researchers that *Rhizobium* inoculation provides significant increases, especially in the nitrogen content, as well as in plant growth (Marschner, 1995; Al-Karaki, 1999; Kacar and Katkat, 1999; Soumaya et al., 2016; Tüfenkçi et al., 2006a). The physical and chemical properties of soils have a significant effect on microorganism activities (Ham, 1980; Lowendorf, 1980; Haktanır and Arcak, 1997). In addition to being a good source of organic fertilizers with high organic matter and nutrients, sewage sludge should be used with caution due to the high amount of heavy metals they contain (Fernandes, 2005; Bozkurt et al., 2006; Carbonell et al., 2009; Fernandez et al., 2009). Applied sewage sludge affects the physical, chemical and biological properties of soil and therefore affects the microorganism activities. This is also seen in the results reported by some researchers (Chaudri et al., 2008; El-Azhari et al., 2012; Charlton et al., 2016). As a matter of fact, Mattanaa et al. (2014) reported that the effects of sewage sludge on soil microorganisms are different according to the methods applied to the sludge before.

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Antolin et al. (2010) stated that the addition of sewage sludge has no significant effect on nodulation.

The effect of inoculation on grain and straw micro element content compared to non-inoculated plants is evident in iron values. Manganese and zinc were detected only in the grain in the second year. Iron increases in the first and second year straw and grain are respectively 3.8% -24.5% and 19.5% -16.4%. In the second year, 10.4% increase in grain manganese and 14.6% decrease in zinc were determined. It was seen that inoculation in copper did not make a significant difference (*Table 6*, *Table 7, Figure 2*). Zribi et al. (2012) reported that *Medicago sativa-sinorhizobium* symbiosis could be used for phytoremediation in medium polluted areas with Cd, Cu, Pb and Zn.

The significant effect of *Rhizobium* inoculation on the heavy metal content was determined as an increase in the cobalt content of the lentil straw in the second year and lead in both years by 59.0%, 39.3% and 94.3%, respectively. The effect of inoculation on the grain heavy metal content of lentil is designated in lead and cadmium contents. Despite the increase in lead content in both years, it was seen that only the first year was important. It was seen that there was an increase of 45.9% compared to non-inoculated plants. The cadmium content was decreased with inoculation by 49.2% in the first year and 13.4% in the second year. The decrease with inoculation in the cobalt content during the first year was 23.6%, but this decrease was deemed insignificant (*Table 8, Table 9, Figure 3*). Akhtar et al. (2018) reported that *Rhizobium* inoculation has a positive effect on metal contaminated areas. Ghnaya et al. (2018) reported that *Rhizobium* inoculation in cadmium-contaminated environments increased the root cd content of *Medicago sativa* L. and increased the transport to the surface of the plant.

Conclusion

Rhizobium inoculation is particularly recommended in legumes. Due to rapid population increase and technological developments, the amount of treatment sludge is increased. It is currently being investigated for the disposal of sewage sludge, which is an important problem for use in agricultural areas. In this study, the efficiency of treatment sludge use and *Rhizobium* inoculation in legumes was investigated. As a result, *Rhizobium* inoculation has been found to have a positive effect on both the lentil and the nutrient element content.

In increasing applications of sewage sludge, *Rhizobium* inoculation has been shown to have a more pronounced effect on the straw element content of lentils, especially in the first year trials than in *Rhizobium* non-inoculation.

The most important disadvantage of the use of sewage sludge is the risk of heavy metal contents of soil and plant. In this study, *Rhizobium* inoculation has been shown to have a negative effect on the absorption of heavy metals against increasing sludge applications. The chemical composition of the treatment sludge to be used in such studies should be considered and accordingly the application dose should be adjusted.

As a result, we have determined that the use of sewage sludge is not a risk for heavy metal in terms of both soil and plant and it can be used with *Rhizobium* inoculation.

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