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ORIGINAL PAPER

EFFECTS OF URBAN SEWAGE SLUDGE ON BEAN (*PHASEOLUS VULGARIS* L.) SEEDLING EMERGENCE RATE AND DEVELOPMENT

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Abstract

This study was carried out to determine the emergence rate and development of bean seedlings in response to the treatment with urban sludge in increasing doses (0%, 25%, 50%, 75%, 100%) applied to a dwarf (Efsane) and pole (Öz Ayşe) bean cultivar grown under climate chamber conditions. The following traits were investigated: first emergence and first leaf formation time, seedling length, root length, leaf color, leaf area, number of leaves, seedling age weight, seedling dry weight, root age weight and root dry weight of plant morphological properties and Na, Ca, K, Mg, Cu, Fe, Zn, Mn, Co, Ni, Cr, Cd concentrations. Morphological measurements show that 25 % and 50% sludge treatment doses have a positive effect on the seedling emergence and the first true leaf formation but sewage sludge is not effective at 75% and 100% doses. A significant increase in the content of heavy metals, such as Cu, K, Mg, Mn and Zn, could be expected under increasing doses of sludge, whereas in fact there was a decrease in Ni, Cd, Co, Cr, Fe, Na and Ca values. It was determined that the Ca content of plants was beneficial for the plant growth. Concentrations of the heavy metals Cu, Cd, Ni, Mn, Zn and Cr were high enough in some cases to cause toxic effects, which affected adversely the plant growth. In addition, it was determined that there is no plant emergence at the 100 % sludge treatment dose. It has been determined that the resistance of the dwarf variety Esfane to the application of sewage sludge in increasing doses is higher than that of the Öz Ayşe variety. The analyses showed that the dwarf variety contained more heavy metals than the pole type and in both types the content of heavy metals was above the toxic thresholds.

Keywords: dwarf bean, pole bean, sewage sludge.

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.), genetically originating from America and South Asia, is a warm climate plant that is well adapted to warm temperate regions. However, it is sensitive to hot weather during germination and to relative humidity and drought during flowering (Şehirali 1998). Bean, which is one of the most important edible legumes produced and consumed by developing countries, is a carbohydrate- and protein-rich food which is produced and consumed significantly by the population in our country. Being a member of the legume family, this plant contains 22-30% protein in its grains and is therefore seen as a crop of great importance in human nutrition. It is rich in calcium, potassium, magnesium, phosphorus and carbohydrates and vitamins. It is also one of the plant sources of proteins (Akçin 1988). Considering the nutritional content of edible legumes, the bean provides 22% of vegetable proteins and 7% of carbohydrates, while 38% of proteins and 5% of carbohydrates in animal nutrition originate from legumes. Beans have an important place in the canned and frozen food industry, especially recently when making an effort to meet the protein and carbohydrate demand required in human diet (Wery, Grinac 1983). Beans are beneficial in animal nutrition and hence, indirectly, in human nutrition, which is why they have an important place in animal husbandry. A bean plant has lumpy protrusions called nodules on its roots. By means of these nodules, nodosite bacteria (*Rhizobium phaseoli*) transform free nitrogen of the air into a form that the plant can use, thus enriching the soil with nitrogen. Due to this feature of bacteria, nodosite bacteria fix 30-50 kg of pure nitrogen in the soil in one hectare of bean planting during the growing period (Şehirali 1998). The bean plant, which has different varieties, has two types, pole and dwarf, depending on the growth of the stem. It has been determined that the number of knuckles on the main stems of the dwarf types varies between 3-10, their length is 20-60 cm, the length of the pod is 8-12 cm, the pod width is 7-25 cm and the weight of a thousand grain varies between 200-300 g (Sepetoğlu 2002).The limiting factor in the spread of beans is temperature. In places where the average temperature of the summer months is below 10°C, the pods do not fully mature, and they shed flowers in places where the average daily temperature is above 32°C (Şehirali 1998). It has become inevitable to move agricultural production to a higher level than the current one in order to provide food and raw materials to the agricultural industry for the rapidly increasing human population worldwide. Increasing agricultural production should be in the form of either growing plants that yield the most per unit area or improving production areas. Today, our agricultural lands have reached the upper limits in our country, same as in many other countries. For this reason, technologies to achieve more efficient production per unit area are sought and scientific studies are developing in this direction every day. The problems caused by the ecosystem's inability to regenerate itself can aggravate ecological issues, which is manifested by certain natural events and disasters observed nowadays. The increasing amounts of waste generated due to the population growth and rapid urbanization cannot undergo any process to be recycled in nature. Therefore, it is inevitable that the life mechanism will be damaged as a result of accumulated waste. The World Health Organization (WHO) has revealed in its research that biologically sourced waste materials adversely affect human health. In the WHO report, it was concluded that 160,000 people died in the last 10 years due to diseases such as typhoid and dysentery (ANONYMOUS 2019).

One of the types of waste generated in increasing quantities due to rapid urbanization is biological sewage sludge. A significant abiotic stress that has caused environmental pollution in recent years is triggered by heavy metal accumulation in sewage sludge (CASTRO 2011). These metals, unlike organic pollutants, do not decompose through biological processes and are not converted into harmless compounds. Heavy metals remain in the environment for a long time. Furthermore, heavy metals can penetrate the food chain. A common feature of environmental stresses is the ability to produce toxic oxygen derivatives (ICHIBAN 2011). Storage and burning operations are carried out for the disposal of sewage sludge. Sludge disposal always constitutes a significant part of the wastewater management cost. Since this problem is not solved with current methods, it has become necessary to conduct new research on this subject. In many countries, regulations have become effective to govern certain sewage sludge disposal practices in the last 20 years. For example, legal restrictions have been imposed on the discharge of waste into the oceans and their use in landfills, which has created an alternative perspective to the use of sewage sludge in agricultural fields. Considering its application in agriculture, the research carried out so far has proven that sludge has an economic value in crop cultivation. However, uncontrolled discharge of sewage sludge or inability to find alternative usage areas pose great risks. According to the Environmental Protection Agency, one of the most serious risks is non-recyclable biological waste, which negatively affects animals, plants, and soil.

This study was carried out in order to determine the effects of plant nutrients and heavy metal content in sewage sludge on pole and dwarf bean varieties. With this study, it is aimed to indicate to what extent sewage sludge can be used in order to apply this biological waste successfully in areas where pole and dwarf bean cultivation is carried out. Another goal has been to reveal the possibility of transforming this pollution source into a safe and useful resource.

MATERIAL AND METHODS

Material

Preparation of plant material

In the experiment, the varieties of pole (Öz Ayşe) and Dwarf (Efsane) bean (*Phaseolus vulgaris* L.) were used as plant material. The seeds used in the study were obtained from Yuzuncu Yil University, Faculty of Agriculture, Department of Field Crops. During the study, the development of morphological traits in the plants was observed, and the leaf samples taken from the plants were dried and made ready for the analysis of plant nutrients and heavy metals. Seeds were prepared for planting in order to determine the development status of bean varieties grown in sewage sludge according to their genetic structures.

Preparation of the growth medium

Sewage sludge obtained from Van Edremit Biological Sewage Directorate was used as solid material in the study. Freshly supplied sewage sludge was kept outside in an airy environment for 3 months and then applied to the pots at the specified doses after dried to crumbly texture. In terms of physical properties, sewage sludge is an odorous solid waste, obtained from processing urban waste and produced in either a solid or liquid form. It can be produced in various amounts depending on the applied sewage processes, and generally contains 0.25-12% of solid particles (Durak 2005). In our experiment, the dry matter content was determined in accordance with the Regulation on The Use of Domestic and Urban Sewage Sludge in Soil, issued by the Ministry of Environment and Forestry. It defines solid matter as the amount of solid material remaining after drying sewage sludge in a drying oven at 70°C to constant weight in order to prevent the loss of organic matter. It was determined that the porosity of the sewage sludge used as a solid medium in the experiment was high and the particles were large. In line with these characterestics, the water holding capacity of that sewage sludge was low. The content of Na, Ca, K, Mg, P, Cu, Fe, Zn, Mn, Co, Ni, Cd in sewage sludge was determined by Atomic Absorption Spectrophotometery (Khan, Frankland 1983) – Table 1. The salinity value was measured in a sample diluted at a ratio of 1: 2.5, and the alkalinity was determined by measuring (%) the volume of CO_2 released from $CaCO_3$ reacting with HCl (Richards 1954). Samples diluted at pH value (1: 2.5) were prepared (Greweling, Peech 1960). The prepared samples were analyzed in the Soil Department of Van Yuzuncu Yil University.

Table 1

Some chemical analysis results of sewage sludge with 90% dry matter content

Results of analyses	$CaCO_3$	pH	E.C (µS cm^{-1})	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Co (mg kg^{-1})	Ni (mg kg^{-1})	Cd (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Cr (mg kg^{-1})
	14.30%	7	1582.6	5464.7	5815.0	31465.7	10138.8	5392.5	388.1	1771.5	99.96	5983.3	7832.3	1896.7

METHOD

In order to determine the effects of urban sewage sludge used in the study on bean varieties, 0%, 25%, 50%, 75% and 100% doses of sewage sludge were applied to pots with beans, in 4 replications, according to a completely randomized experimental design. The research was conducted in 40 paperboard pots (330 cc) measuring 5.5 cm in bottom diameter x 11 cm height x 8 cm top diameter. The pots were placed in a climate room of the Yuzuncu Yil University Field Crops Department. The planting was carried out by placing 2 seeds in each pot, and the water requirement of the plants was met periodically by supplying pure water through tubes. After planting, the emergence rate for both varieties was assessed, and the experiment was terminated when the plants produced 4-5 real leaves. The effects of the applications of increasing sewage sludge amounts on the dwarf and pole varieties were analyzed, and the differences detected between the varieties were evaluated comparatively.

Some morphological traits, such as the emergence time, first true leaf formation period, seedling length, seedling wet weight, root length, leaf number, leaf area, leaf color, seedling dry weight, root dry weight, and root wet weight, were observed in this study according to Tosun (2015). With respect to the plant mineral matter and heavy metal content, analyses of the elements Na, Ca, K, Mg, Cu, Fe, Zn, Mn, Co, Ni, Cr and Cd in leaves from a total of 40 plants, in 4 replications, were performed by the Atomic Absorption Spectrophotometeric method (Kacar 1984).

Statistical analyses of data

The findings obtained from the research were analyzed according to the completely randomized experimental design (CRD) with the Costat V.6.0 program, and the differences between the treatments were evaluated according to the least significant differences (LSD test).

RESULTS AND DISCUSSION

Plant morphological analysis

In this study, it has been observed that there was no emergence of plants of the bean varieties due to the high toxic effect of heavy metals in the 100% sewage sludge treatment. The mean values of the observed parameters and the LSD analysis of the effects of the sewage sludge doses used and the values achieved for variety and sludge dose interactions are given in Tables 2-5.

First emergence time

The average first emergence time in plants was 6.47 days in Efsane and 5.98 days in Öz Ayşe. When the sewage sludge dose averages were examined, it was determined that the earliest first emergence (10.13 days and 9.44 days) was seen in the second and third doses, while the latest was obtained from the treatments where the first and fourth doses of sewage were used, except for the fifth dose (Table 2). It has been determined that 25% and 50% doses of sewage sludge accelerate the emergence time in beans, while the 75% share has a negative effect on the first emergence time and the 100% sludge sewage treatment completely prevents germination. As known, sewage sludge (100%) is rich in plant nutrients, such as nitrate, phosphate and metals (Kumar, Chopra 2014). Fibeli (2005) maintains that high doses of sewage sludge prevent germination and development of the plant due to their high ammonia content. In another study, Taşatar (1997) reported that the prolongation of the plant germination time and the cessation of the growth of plants when sewage sludge was applied were caused by the heavy metals accumulated within the plant.

First leaf formation

When the data in Table 2 are examined, the average of first leaf formation was 7.90 days for the Efsane variety and 7.45 days for Öz Ayşe variety, and no statistically significant difference observed between the varieties (P>0.05). However, there were statistically significant differences (P<0.01) between the applied sewage sludge doses and it was determined that the earliest first leaf formation appeared in response to the second and third doses (7.92 and 8.00 days), while the latest leaf formations (11.75 and 10.72 days) appeared when the first and fourth doses were applied, again with the exception of the fifth dose. It was observed that there were statistically significant differences (P<0.05) between type x sewage sludge interactions (Table 2). It was determined that the earliest (7.00 days) true leaf formation was observed in the second highest sewage sewage sludge treatment with the Özayşe variety, while the latest (12 days) was detected in the fourth highest sewage sewage sludge dose with the Efsane variety, again excluding the fifth dose, where no emergence was observed. While the earliest first leaf formations were seen at 25% and 50% sewage sludge doses, it was determined that the leaf formation day time increased at the 75% dose (Table 2). TAŞATAR (1997), in a study investigating the effects of heavy metals on plant leaf formation, stated that sewage sludge containing high doses of heavy metals reduced plant leaf formation.

Table 2

The first emergence time, first leaf formation, seedling length, average leaf area values, and LSD comparison groups obtained as a result of the experiment

Variation sources		Sludge doses (%)	First emergence (day)	First leaf formation (day)	Seedling length (cm)	Root length (cm)	Leaf area (cm^2)
Varieties x Sludge[III]	**Efsane**	**0**	9.50	11.00*ab*	15.85	11.20	13.08*a-d*
		25	6.00	7.50*de*	10.95	10.35	13.64*a-c*
		50	6.33	9.00*b-d*	9.13	6.55	17.96*a*
		75	10.50	12.00*a*	11.35	8.15	14.46*ab*
		100	0.00	0.00*f*	0.00	0.00	0.00*e*
		average[I]	**6.47**	**7.90**	**9.46B**	**7.25**	**11.83A**
	Öz Ayşe	**0**	10.75	12.50*a*	19.48	11.88	12.32*b-d*
		25	5.75	8.33*cd*	16.27	8.67	8.82*d*
		50	5.00	7.00*e*	10.90	6.67	9.02*cd*
		75	8.38	9.44*bc*	14.88	4.76	9.24*cd*
		100	0.00	0.00*f*	0.00	0.00	0.00*e*
		average[I]	**5.98**	**7.45**	**12.30A**	**6.39**	**7.88B**
Sludge Doses[II]		**0**	10.13*A*	11.75*A*	17.66*A*	11.54*A*	12.70*A*
		25	5.88*B*	7.92*B*	13.61*B*	9.51*A*	11.23*A*
		50	5.67*B*	8.00*B*	10.02*C*	6.61*B*	13.49*A*
		75	9.44*A*	10.72*A*	13.11*B*	6.46*B*	11.85*A*
		100	0.00*C*	0.00*C*	0.00*C*	0.00*C*	0.00*B*
LSD for varieties (V)			**0.785**	**0.743**	**1.842***	**1.400**	**1.897****
LSD for sludge doses (SD)			**1.241****	**1.175****	**2.913****	**2.213****	**2.999****
CV			**19.534**	**14.987***	**26.218**	**31.772**	**29.809***

* Significant at the level of 5%, ** Significant at the level of 1%;

[I] The difference between the means shown in the same column with the same lowercase letters is not statistically significant (P>0.05).

[II] The difference between the means shown in the same column with the same capital bold letters is not statistically significant (P>0.05).

[III] The difference between the means in the same column with the same capital italic letter is not statistically significant (P>0.05).

Seedling length (cm)

Table 2 shows that the differences between varieties were statistically significant at the level of 5%, although there were also very significant differences between sewage sludge doses at the level of 1%, and the differences between varieties and sewage sludge doses were statistically insignificant ($p>0.05$). The average seedling length of the Öz Ayşe variety was measured as 12.30 cm long, and the average seedling length of the Efsane variety was 9.46 cm (Table 2). In the study, it was determined that the height of the seedling of the pole variety was much above that of the dwarf variety. Akbulut, Karakurt, Tonguç (2014) attributed such differences to the genetic structural differences between plants. In the study, when the seedling lengths were examined relative to sewage sludge doses, it was found that the highest mean seedling length (17.66 cm) was obtained from the control sewage doses (Table 2). It was determined that both varieties were negatively affected by increasing sewage sludge doses at the same rate, regardless of genetic differences between the varieties. These results confirm the observation of Taşatar (1997) that sewage sludge with a high heavy metal content limits the growth of plants.

Root length (cm)

As seen in Table 2, the differences between the varieties were found to be statistically insignificant ($P>0.05$), while the differences between sewage sludge doses were statistically significant ($P<0.01$). In the study, it was determined that the difference between the variety x sewage sludge doses was statistically insignificant ($P>0.05$), as the cultivars showed the same response to sewage sludge doses. The average root length of the Efsane variety was 7.25 cm, while the average root length of the Öz Ayşe variety was 6.39 cm (Table 2). When root length results were collated in Table 2, it was found that the highest root length average values (11.54 and 9.61 cm) were obtained from the control and first sewage sludge dose. Korboulewsky, Bonin, Massiani (2002) reported that increased sewage sludge doses had a positive effect on plant root development in their study on *Diplotaxis erucoides*. Contrary to these results, it was determined in this experiment that doses of sewage sludge had a negative effect on root development in both bean varieties due to the heavy metal concentrations it contained.

Leaf area (cm²)

As seen in Table 2, statistically significant differences ($P<0.01$) were found between varieties in terms of leaf areas and sewage sludge doses. The interactions between variety x sewage sludge doses were found to be statistically significant ($P<0.05$). The average leaf area of the Öz Ayşe variety was 11.83 cm^2 and the average leaf area of the Efsane variety was 7.88 cm^2 (Table 2). When the sewage sludge doses were taken into account, it appeared that the average values were approximately the same under all doses except the highest, 100% sewage sludge dose. It was determined that sewage sludge had no positive effect on the leaf area compared to the control. However, contrary to the results reported by Türkmen, Şensoy, Çirka (2001), who claimed that increasing sewage sludge doses under the cucumber plant significantly increased the leaf area compared to the control. This discrepancy between the two experiments may be due to the different plant species studied and the different properties of applied sewage sludge. The different responses of the bean varieties to sewage sludge doses in terms of leaf area meant that the variety x sewage sludge interaction was found to be important. Thus, the largest leaf area in the Efsane variety (17.96 cm^2) appeared in the 50% sewage sludge variant, while the largest leaf area in the Öz Ayşe variety (12.32 cm^2) was determined when the 0% dose had been applied.

Leaf number

There were statistically significant (P<0.05) differences between the cultivars and also significant (P<0.01) differences between sewage sludge doses regarding this trait. It was observed that the differences due to the variety x sewage sludge dose interactions were significant (P<0.01) – Table 3. As seen in Table 3, the average number of leaves was 8.07 for the Efsane variety, and 6.74 for the Öz Ayşe variety. As for the sewage sludge doeses, the highest one (100%) was again excluded while the 50% dose led to a statistically significantly reduced number of leaves compared to the control (Table 3). The Efsane variety grew the maximum number of leaves (12.50) in the 0% sewage sludge treatment, while the Öz Ayşe Variety produced the maximum number of leaves (9.38) in the 75% sewage sludge treatment. In contrast to these results, Türkmen, Şensoy, Çirka (2001) reported that when increased sewage sludge doses were used to grow cucumber plants, a positive effect on the number of leaves of the plants compared to control was observed.

Seedling fresh weight (g)

As can be seen in Table 3, it was determined that the statistically determined difference between the cultivars and the differences between sewage sludge doses are very significant (P<0.01). Moreover, it was determined that the differences between the variety x sewage sludge dose interactions were statistically significant (P<0.05). The average fresh weight of seedlings was measured at 2.46 g for the Efsane variety and 1.40 g for the Öz Ayşe variety. Among the sewage sludge treatments, it was determined that 0% and 75% doses resulted in the highest mean seedling fresh weight values, such as 2.46 and 2.74 g, respectively (Table 3). It was noticed that the dwarf variety reached higher weight values than the pole variety. The highest fresh weight value of 3.82 g was observed for the Efsane variety in the 75% sewage sludge dose treatment, while the highest fresh weight value of 1.93 g was determined for the Öz Ayşe variety in the 0% dose treatment. Korboulewsky, Bonin, Massiani (2002) stated that the sewage sludge applied in increasing doses under the plant *Diplotaxis erucoides* stmulated the growth of the plant stem and increased the plant weight relative to the dose of sewage sludge. Çakir, Çimrin (2018), in a study investigating the effects of increasing doses

Table 3

Number of leaves, seedling fresh weight, seedling dry weight, root fresh weight, root dry weight values, and LSD comparison groups obtained as a result of the experiment

Variation sources		Sludge doses (%)	Number of leaves (number)	Seedling fresh weight (g)	Seedling dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Varieties x Sludge doses[III]	Efsane	**0**	12.50*a*	3.60*ab*	1.85*ab*	1.65*a*	0.85
		25	10.00*ab*	2.70*bc*	1.35*bc*	0.55*de*	0.38
		50	6.33*d*	2.17*cd*	0.97*cd*	0.53*de*	0.27
		75	11.50*a*	3.82*a*	1.89*a*	0.66*cd*	0.34
		100	0.00*e*	0.00*e*	0.00*e*	0.00*f*	0.00
		average[I]	**8.07A**	**2.46A**	**1.21A**	**0.68A**	**0.37**
	Öz Ayşe	**0**	7.00*cd*	1.93*cd*	1.60*ab*	1.15*b*	0.80
		25	8.33*bc*	1.63*d*	1.10*cd*	0.80*c*	0.50
		50	9.00*b*	1.80*d*	0.93*cd*	0.53*de*	0.33
		75	9.38*b*	1.66*d*	0.86*d*	0.40*e*	0.34
		100	0.00*e*	0.00*e*	0.00*e*	0.00*f*	0.00
		average[I]	**6.74B**	**1.40B**	**0.90B**	**0.58B**	**0.39**
Sludge Doses[II]		**0**	9.75*A*	2.76*A*	1.73*A*	1.40*A*	0.83*A*
		25	9.17*A*	2.17*B*	1.23*BC*	0.68*B*	0.44*B*
		50	7.67*B*	1.98*B*	0.95*C*	0.53*B*	0.30*B*
		75	10.44*A*	2.74*A*	1.38*B*	0.53*B*	0.34*B*
		100	0.00*C*	0.00*C*	0.00*D*	0.00*C*	0.00*C*
LSD for varieties (V)			**0.906***	**0.339****	**0.187***	**0.095 ***	**0.094**
LSD for Sludge doses (SD)			**1.432****	**0.536****	**0.295****	**0.150****	**0.149****
CV			**18.945****	**27.198***	**27.401***	**23.377****	**38.338**

* Significant at the level of 5%, ** Significant at the level of 1%;

[I] The difference between the means shown in the same column with the same lowercase letters is not statistically significant (*P*>0.05).

[II] The difference between the means shown in the same column with the same capital bold letters is not statistically significant (*P*>0.05).

[III] The difference between the means in the same column with the same capital italic letter is not statistically significant (*P*>0.05).

of sewage sludge on the corn, reported that sewage sludge had positive effects on the wet weight of the plant and that all applications increased the yield compared to the control group, although the yield began to decrease after the application of a 75% dose.

Seedling dry weight (g)

The data in Table 3 show that the difference between the cultivars was statistically significant at a 5% level and the difference between sewage sludge doses was very significant ($P<0.01$). The differences between variety x sewage sludge dose interactions were found to be statistically significant ($P<0.01$). It was determined that the dry weight average (1.21 g) of the legendary variety was significantly higher than the dry weight average (0.9 g) of the Öz Ayşe variety. When the differences between sewage sludge doses were examined, it was determined that the average dry weight of the control applications (1.73 g) was higher than that of the other doses (Table 3). The maximum average dry weight of both cultivars was found to be 1.85 g in the Efsane variety and 1.60 g in the Öz Ayşe variety, respectively, from the control application. It was observed that the dry weight of the plant decreased as the doses of sewage sludge increased and it has been determined that the dwarf variety contains more dry weight than the pole variety. The result of the study is partially in line with the results reported by Çakir, Çimrin (2018).

Root fresh weight (g)

In Table 3, it can be seen that the difference between the cultivars was statistically significant ($P<0.05$), while the differences between sewage sludge doses and variety x sewage sludge interactions were statistically very significant ($P<0.01$). It was determined that root fresh weight (0.68 g) of the Efsane variety was higher than that of the Öz Ayşe variety (0.58 g). Among the applied doses, it was observed that the highest value of the trait appeared in control applications (Table 3). The highest root age weight values of the Efsane and Öz Ayşe cultivars were 1.65 and 1.15 g, respectively, in the control application. In this study, contrary to the results of Türkmen, Şensoy, Çirka (2001), negative effects of sewage sludge on the bean root parts were observed. It has been determined that increasing amounts of sewage sludge reduce root growth.

Root dry weight (g)

As indicated in Table 3, it was been determined that the differences between the varieties and between the varieties x sewage sludge interactions were statistically insignificant ($P>0.05$), and the differences between sewage sludge doses were statistically very significant ($P<0.01$). It was determined that the root dry weight averages for the Efsane and Öz Ayşe varieties were 0.37 and 0.39 g, respectively. When data in Table 3 were examined, it was seen that root dry weight decreased due to the increase in sewage sludge doses. It was determined that the highest root dry weight (0.83g) was achieved in the control and this weight decreased when sewage sludge was applied in increasing doses, which is contrary to the results of Çimrin, Bozkurt, Erdal (2000).

Mineral substance and heavy metal content in plants

The pole and dwarf bean varieties were characterized according to their content of some macro- and micro-elements. Tables 4-5 show, in terms of the observed elements, that the difference between the cultivars was statistically very significant (*P*<0.01), except for Na and Cr (*P*<0.05). The differences between sewage sludge doses and the variety x sewage sludge interactions were statistically very significant (*P*<0.01). The mineral element content of the pole bean variety was higher thanthat of the dwarf bean variety. The dwarf bean variety was found to accumulate higher concentrations in all the observed elements than the pole bean variety. It was found that both bean types were affected by the sewage sludge at the same rate (Tables 4-5).

In the study, while concentrations of Ca, Cd, and Co were positively affected until the second sewage sludge dose, they were observed to have been adversely affected by the other, higher doses. Ouzounidou (1994) reported that the Cu element in the plant enters the plant body by separating the Ca element in the cell wall of the plant and binding it here. In this case, the cell wall loses its elasticity, which means that the plant's turgor deteriorates. Thus, it reduces Ca transition from roots to leaves.

In most of the previous studies conducted on both beans and other plants, high doses of sewage sludge had a negative effect on the element concentrations in plants after a certain dose Ouzounidou (1994), Zengin, Munzuroğlu 2003, Asri, Sönmez 2006, Xu et al. 2006. In a study by Asri, Sönmez (2006), it was determined that copper reveals its toxic effect on the root system in plants exceeding the toxic limit. They stated that the toxic effect negatively affects the membrane permeability in the root system, preventing the nutrient intake of the plants. Çimrin, Bozkurt, Erdal (2000), in their study investigating the effect of increasing doses of sewage sludge on the corn plant, reported that potassium, known as the turgor regulator, positively affected the root development and the useful amount for the plant was 5610 mg kg^{-1}. Doses above the useful amount reduce the turgor pressure in the roots of the plant and prevent passage of nutrients. Demirkan, Söğüt (2018) stated that manganese has toxic effects over 1000 mg kg^{-1}, where the useful value for the plant is between 400-1000 mg kg^{-1}. Zengin, Munzuroğlu (2003) reported that an increased zinc content in a bean plant had a negative effect on root, stem and leaf growth, and that it prevented the plant from absorbing nutrients by binding to the cell membranes in the stem region. Taşatar (1997) stated that a dose of 100% sewage sludge prevented the first root development of the plant due to its heavy metal content, thus halting the plant growth. In the current study, it was determined that the plant root formation resembled that reported in other, similar studies, but the plant manifested a response to toxic levels of heavy metals with the increase in applied doses of sewage sludge. In addition, high amounts of both vital and non-vital heavy metals in the soil can cause toxicity and growth retardation in most plants (Dong 2010).

Table 4

The content of Ca, Cu, K, Mg, Cd, Ni, and LSD comparison groups obtained as a result of the experiment

Variation sources		SD	Ca (mg kg^{-1})	Cu (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Cd (mg kg^{-1})	Ni (mg kg^{-1})
Varieties x Sludge doses[III]	Efsane	**0%**	12 930.9*e*	194.47*c*	7483.28*d*	5515.02*c*	41.02*abc*	404.81*a*
		25%	16 737.9*a*	242.51*a*	7823.72*c*	8681.52*b*	41.83*ab*	269.87*c*
		50%	13 339.1*c*	191.49*c*	8497.53*b*	10910.42*a*	37.96*d*	109.31*f*
		75%	4 418.1*h*	187.34*c*	8967.13*a*	4721.08*d*	39.89*c*	97.94*g*
		100%	0.00*i*	0.00*g*	0.00*g*	0.00*i*	0.00*f*	0.00*h*
		Average[I]	**9 485.2A**	**163.16A**	**6554.33*A***	**5965.61A**	**32.14A**	**176.39A**
	Öz Ayşe	**0%**	10 046.6*f*	113.38*e*	6482.31*e*	3141.26*g*	40.91*bc*	299.55*b*
		25%	14 679.6*b*	143.62*d*	5225.11*f*	4112.28*e*	42.23*a*	163.16*d*
		50%	13 047.3*d*	211.92*b*	6423.23*e*	3400.30*f*	37.54*d*	152.42*e*
		75%	5 902.3*g*	103.63*f*	5148.39*f*	2574.86*h*	29.60*e*	106.19*f*
		100%	0.00*i*	0.00*g*	0.00*g*	0.00*i*	0.00*f*	0.00*h*
		Average[I]	**8 735.16B**	**114.51B**	**4655.81B**	**2645.74B**	**30.06B**	**144.26B**
Sludge doses[II]		**0%**	11 488.7*C*	153.92*C*	6982.80*B*	4328.14*C*	40.96*A*	352.18*A*
		25%	15 708.8*A*	193.06*B*	6524.42*C*	6396.90*B*	42.03*A*	216.51*B*
		50%	13 193.2*B*	201.70*A*	7460.38*A*	7155.36*A*	37.75*B*	130.86*C*
		75%	5 160.2*D*	145.48*D*	7057.76*B*	3647.97*D*	34.74*C*	102.07*D*
		100%	0.0*E*	0.00*E*	0.00*D*	0.00*E*	0.00*D*	0.00*E*
LSD for varieties (V)			**14.448****	**2.943****	**56.659****	**76.774****	**0.698****	**1.835****
LSD for sludge doses (SD)			**22.845****	**4.653****	**89.586****	**121.390****	**1.103****	**2.902****
CV			**0.246****	**3.282****	**1.565****	**2.761****	**3.475****	**1.772****

* Significant at the level of 5%, ** Significant at the level of 1%;
[I] The difference between the means shown in the same column with the same lowercase letters is not statistically significant (*P*>0.05).
[II] The difference between the means shown in the same column with the same capital bold letters is not statistically significant (*P*>0.05).
[III] The difference between the means in the same column with the same capital italic letter is not statistically significant (*P*>0.05).

Almaz (2017) determined the sodium content in the plant to reach 1390.5 mg kg^{-1} in a study investigating the effects of sewage sludge on heavy metals in corn. It was observed that increasing amounts of sewage sludge caused a decrease in the amount of Na in the bean plant. Radha, Srivastava, Madan (2000) found that the presence of 500 mg kg^{-1} chromium in soil reduced the germination of bean seeds by 48%. They also found that the first physiological event affected by chromium reaching a toxic level in the plant was the germination of the seed and that chromium inhibits seed germina-

Table 5

The content of Co, Mn, Zn, Na, Cr, Fe, and LSD comparison groups obtained as a result of the experiment

Variation sources		SD	Co (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})	Cr (mg kg^{-1})	Fe (mg kg^{-1})
Varieties x Sludge doses [III]	Efsane	**0%**	91.44*c*	3787.03*b*	481.37*g*	788.81*a*	430.07*a*	976.45*aa*
		25%	117.65*a*	4653.57*a*	637.61*e*	579.23*d*	279.40*c*	566.25*d*
		50%	98.49*b*	2996.30*c*	1080.06*d*	555.43*e*	200.89*e*	721.19*b*
		75%	39.44*f*	2692.70*cd*	2668.08*b*	631.97*c*	231.87*d*	689.08*c*
		100%	0.00*h*	0.00*g*	0.00*i*	0.00*h*	0.00*f*	0.00*g*
		average [I]	**69.40A**	**2825.92A**	**973.42A**	**511.09A**	**228.45A**	**590.59A**
	Öz Ayşe	**0%**	71.79*d*	2298.65*e*	228.56*h*	784.92*a*	371.77*b*	701.01*c*
		25%	89.31*c*	2411.68*de*	562.33*f*	750.37*b*	223.70*d*	565.12*d*
		50%	26.36*e*	3678.00*b*	1159.82*c*	466.97*g*	283.71*c*	287.83*e*
		75%	26.36*g*	1727.88*f*	3159.16*a*	504.51*f*	229.64*d*	209.57*f*
		100%	0.00*h*	0.00*g*	0.00*i*	0.00*h*	0.00*f*	0.00*g*
		average [I]	**42.76B**	**2023.24B**	**1021.97B**	**501.35B**	**221.76B**	**352.71B**
Sludge doses [II]		**0%**	81.61*C*	3042.84*B*	354.96*D*	786.86*A*	400.92*A*	838.73*A*
		25%	103.48*A*	3532.62*A*	599.97*C*	664.80*B*	251.55*B*	565.69*B*
		50%	62.42*B*	3337.15*A*	1119.94*B*	511.20*C*	242.30*C*	504.51*C*
		75%	32.90*D*	2210.29*C*	2913.62*A*	568.24*D*	230.75*D*	449.32*D*
		100%	0.00*E*	0.00*D*	0.00*E*	0.00*E*	0.00*E*	0.00*E*
LSD for varieties (V)			**0.875****	**179.660****	**17.360****	**6.739***	**5.786***	**7.331****
LSD for sludge doses (SD)			**1.384****	**284.074****	**27.449****	**10.656****	**9.148****	**11.591****
CV			**2.250****	**11.474****	**2.694****	**2.061****	**3.980****	**2.407****

* Significant at the level of 5%, ** Significant at the level of 1%;
[I] The difference between the means shown in the same column with the same lowercase letters is not statistically significant (P>0.05).
[II] The difference between the means shown in the same column with the same capital bold letters is not statistically significant (P>0.05).
[III] The difference between the means in the same column with the same capital italic letter is not statistically significant (P>0.05).

tion by accelerating the protease action and formation of amylase. In this study, it was determined that the first emergence was irregular compared to the control group and, similarly to other studies, the plant manifested signs of toxicity at certain doses of sewage sludge. Kabata-Pendias (2010) stated that the amount of Fe in a plant in a range of 2-200 mg kg^{-1} is an acceptable limit in plant physiology. In this study, contrary to previous studies, it was determined that the Fe content in the plant decreased with increasing sewage sludge doses (Tables 4-5). This is thought to be due to the high levels of other antagonistic metals, such as K, Ca, Mg, Mn, Zn and Cu, that suppress Fe uptake. The results obtained in the study showed that the Fe content decreased in parallel with the increase in the doses of sewage sludge, which is in contrast to results from other, similar studies.

In comparison with the results of previous studies summarized above, this study showed that the decrease in the amount of Ca after the application of the 25% sewage sludge dose was due to the increase in the Cu element entering the plant. It was determined that Ni, Na, Cr, and Fe concentrations measured in plants showed a linear decrease depending on the increasing sewage sludge doses compared to the control application (Tables 4-5).

CONCLUSION

In the study, the effect of sewage sludge on some morphological parameters and chemical element concentrations in two different bean types (pole and dwarf) was investigated. Unfortunately, in both bean types, no plant emergence was obvserved due to the toxic effect of heavy metals when the 100% sewage sludge dose was applied. It was observed that both bean types with different genetic structures were affected by sewage sludge approximately to the same degree. It was determined that sewage sludge had a negative effect on most of the properties observed after germination, except for the first leaf formation, leaf area, seedling fresh weight, and seedling dry weight, compared to the control treatment. When the effect of sewage sludge was examined in terms of chemical composition of both bean types, it was observed that it had a positive effect on the examined elements except Cd, Ni, Cr, and Fe. It should be taken into account that sewage sludge application in agriculture may adversely affect human health due to the heavy metal content and pathogens it contains. In this context, it would be more appropriate to use it as plant growth media after stabilization processes in order to eliminate the negative effects of the toxic amount of heavy metals detected in urban sewage sludge.

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