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# Arıtma Çamurunun Kadife Çiçeği (*Tagetes erecta* L.) ve Yer Minesi (*Verbena hybrida*) Bitkileri ile Toprağın Besin Elementleri ve Ağır Metal Üzerine Etkisi

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## ÖZET

Bu çalışma, kadife çiçeği (*Tagetes erecta* L.), Yer minesi (*Verbena hybrida*) süs bitkilerinin Biyokonsantrasyon faktörü (BCF), bitki besin elementi ve ağır metal konsantrasyonları ile hasattan sonra her saksıdan alınan topraktaki ağır metal konsantrasyonunu üzerine arıtma çamurunun etkilerini belirlemek amacıyla yapılmıştır. Saksı denemesi tesadüf blokları deneme desenine göre 3 tekrarlı olarak yürütülmüştür. Arıtma çamuru/toprak karışımları (w/w) aşağıdaki şekilde karıştırılmıştır: %0 arıtma çamuru+%100 toprak (kontrol), %3 arıtma çamuru+%97 toprak (%3 SS), %6 arıtma çamuru+94 toprak (%6 SS) ve %9 arıtma çamuru +%91 toprak (%9 SS). BAC değerlerine göre Marigold Zn, Cd elementleri ve Garden mineçiçeği ise Zn elementi için hiperakümülatör bitki olabilir. Kadife çiçeği ve Yer minesinin N, P, K, Mg ve Ca makro bitki besin elementleri en yüksek değerleri sırasıyla %9 SS ve %6 SS uygulamalarından almıştır. Kadife çiçeği, en büyük Na (784 mg kg$^{-1}$) %9 SS, Fe (2236 mg kg$^{-1}$) %9 SS, Cu (7. 4 mg kg$^{-1}$) %9 SS, Zn (136 mg kg$^{-1}$) %6 SS, Mn (142 mg kg$^{-1}$) %6 SS ve B (42 mg kg$^{-1}$) kontrol uygulamalarından elde edilmiştir. Yer minesi bitkisinde, en büyük Na (696 mg kg$^{-1}$) %6 SS uygulamasından, Fe (1700 mg kg$^{-1}$) %6 SS, Cu (12 mg kg$^{-1}$) %6 SS, Zn (115 mg kg$^{-1}$) %6 SS, Mn (100 mg kg$^{-1}$) %3 SS ve B (47 mg kg$^{-1}$) kontrol uygulamalarından saptanmıştır. Ağır metaller (Ni, Cd, Cr, Pb, As ve Hg) bakımından kadife çiçeği ve bahçe mineçiçeği bitkilerinde toksisite etkilerine rastlanmamıştır. Toprak ağır metal (Ni, Cd, Pb, As ve Hg) seviyeleri limit değerlerin altında belirlenmiştir.

**Toprak Bilimi**

**Araştırma Makalesi**

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**Anahtar Kelimeler**
Ağır metal
Arıtma çamuru
Biyokonsantrasyon faktörü
Bitki besin elementleri
Süs bitkileri

# Effect of Sewage Sludge on Marigold (*Tagetes erecta* L.) and Garden Verbena (*Verbena hybrida*) Plant and Soil Nutrient Elements and Heavy Metal

## ABSTRACT

This study was carried out to determine the effects of sewage sludge (SS) treatments on bioconcentration factor (BCF), plant nutrients, heavy metal marigold (*Tagetes erecta* L.), garden verbena (*Verbena hybrida*) ornamental plants and soil taken from the pot after harvest is to determine heavy metal concentration. Pot experiments conducted randomized blocks design with 3 replications. Sewage sludge/soil mixtures (w/w) was arranged: 0% SS+%100 soil (control), 3% SS+ 97% soil (3 % SS), 6% SS+94 % soil (6% SS) and 9% SS +91% soil (9% SS). According to BAC values Marigold can be for Zn, Cd and Garden verbena can be hyperaccumulator plant for Zn element. Marigold and garden verbena, the greatest N, P, K, Mg, and Ca plant nutrients were respectively obtained from 9% SS and 6% SS treatments. Marigold, the greatest Na concentration (784 mg kg$^{-1}$) was obtained from 9% SS, Fe (2236 mg kg$^{-1}$) 9% SS, Cu (7.4 mg kg$^{-1}$) 9% SS, Zn (136 mg kg$^{-1}$) 6% SS, Mn (142 mg kg$^{-1}$) 6% SS and B (42 mg kg$^{-1}$) control treatments. Garden verbena, the greatest Na concentration (696 mg kg$^{-1}$), was

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obtained at 6% SS, Fe (1700 mg kg$^{-1}$) 6% SS, Cu (12 mg kg$^{-1}$) 6% SS, Zn (115 mg kg$^{-1}$) 6% SS, Mn (100 mg kg$^{-1}$) 3% SS and B (47 mg kg$^{-1}$) control treatments. Heavy metals (Ni, Cd, Cr, Pb, As and Hg) toxicity impacts were not encountered on marigold and garden verbena plants. Soil heavy metal (Ni, Cd, Pb, As and Hg) levels were below the threshold values.

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## INTRODUCTION

Together with the global increase in population, food demands are also increasing. To meet such increasing needs, agricultural production should be increased. The world population is expected to rise by 9.3 billion by the year 2050. Thus food demand is expected to increase by about 60% within the next 30 years (Lee, 2011). Urbanization and industrialization increase sewage sludge generation of these processes. Just because of human and environmental health concerns, proper methods should be used in the disposal of sewage sludge. Sewage sludge (SS) is an inevitable by-product of wastewater treatment and a severe pollution source if not disposed of properly (Song et al., 2014). There are three primary methods used in the disposal of sewage sludge: soil application, storage and incineration. In some cases, agricultural use as a fertilizer is seen as the best option for disposal of sewage sludge (Sanchez et al., 2004). Increasing inorganic fertilizer costs and a large quantity of SS generations worldwide have led researchers to investigate the potential use of the land application as an alternative disposal method for sewage sludge (Petersen et al., 2003). In recent years, it has been observed that treatment sludge began to be used as a source of organic matter in the creation of landscapes and green areas in the world. Agricultural use of sewage sludge not only offers an affordable means of disposal but also improves soil fertility and physical properties, thereby enhancesing crop productivity. Moreover, it facilitates nutrient recycling and reduces the cost of cultivation (Swain et al., 2021). Sewage sludge use in agriculture has been recognized as an environment-friendly management technique (Beidokhti et al., 2019). It acts as a vital source of organic matter (OM) for agricultural soils; moreover, it provides essential macro and micronutrients for plant growth and development (Latare et al., 2018; Karkush & Aljorany, 2019). The positive response of various crops, such as rice and wheat (Latare et al., 2017), spinach (Golui et al., 2014), Grass-legume (Bozkurt et al., 2020) apple trees (Bozkurt & Yarılgaç, 2003) and Maize (*Zea mays* L.) (Çakır & Çimrin, 2020) sewage sludge application has been reported. Sewage sludge was applied as 20 kg m$^2$ year-1 dose on plant growth of Liquidambar Orientalis species (Demirkan & Söğüt, 2018). The sewage sludge (sludge and soil mixed at a volume ratio of 1:10, 3:10 and 5:10) was applied to the Sedum lineare ornamental plant at different ratios (Peng et al., 2017). Ornamental plants are the best choices because they provide environment-enhancing green spaces and offer commercial products (Mir et al., 2019). On the other hand, being an ornamental crop due to the interest in its flowers reduces the risk of contamination of the food chain (Chitraprabha & and Sathyavathi, 2018).

The aim of this study was to evaluate the effects of different doses of municipal sewage sludge applications on Bioconcentration factors (BCF), nutrient concentration (N, P, K, Mg, Ca, Na, Fe, Cu, Zn, Mn and B) and heavy metal levels of concentration (Ni, Cd, Cr, Pb, As and Hg) ornamental plants of marigold (Tagetes erecta L.) and garden verbena (Verbena hybrida) and soil taken from each pot after harvest is to determine the heavy metal concentration (Zn, Cu, Ni, Pb, Cd, As and Hg ) in the samples according to the applications.

## MATERIAL and METHOD

### Material

This study started in the April 2015 pot experiments under greenhouse conditions. Bornova town of Izmir province (38° 27' 12.5'' N, 27° 13' 40.2'' E). The soil used in this study was taken from a depth of 0-20 cm in a farm in the city of the Research, Application and Production Farm of Ege University Agriculture Faculty and transferred to the laboratory and dried at laboratory temperature. The physical and chemical characteristics of soil are presented in Table 2. After air drying, the soil samples were passed through a 4 mm sieve. The soil is classified as Typic xerofluvent (Soil Survey Staff, 2010). The physicochemical properties of soil amended by municipal sewage sludge (SS) and growth of Marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants were grown in two separate plots in amended soil with pot experiments under greenhouse (1.50 m x 21 m) conditions. The granulated dry municipal sewage sludge was supplied from the Wastewater Treatment Plant of İzmir Greater City Municipality. Sewage sludge was applied at different doses to Marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants. Analysis results of treated sewage sludge and experiments soil are given in table 1 and table 2.

Table 1. Analysis results of sewage sludge
*Çizelge 1. Arıtma çamurunun analiz sonuçları*

| Properties | Values | Properties | Values |
|---|---|---|---|
| pH | 7.18±0.14 | Na (mg kg$^{-1}$) | 1391±22.11 |
| EC (dS m$^{-1}$) | 1.95±0.11 | Fe (mg kg$^{-1}$) | 12755±124.53 |
| C:N ratio | 9.90±0.51 | Cu (mg kg$^{-1}$) | 177±7.21 |
| $C_{org}$ (%) | 29.66±1.18 | Zn (mg kg$^{-1}$)<br>Mn (mg kg$^{-1}$) | 1377±32.14<br>350±20.12 |
| N(%) | 2.99±0.50 | Ni (mg kg$^{-1}$) | 69.73±3.01 |
| P (%) | 0.23±0.12 | Pb (mg kg$^{-1}$) | 17.44±1.11 |
| K (%) | 0.34±0.18 | Cr (mg kg$^{-1}$) | 112.5±2.32 |
| Ca (%) | 6.36±1.15 | Cd (mg kg$^{-1}$) | 2.83±0.21 |
| Mg (%) | 2.04±0.21 | B (mg kg$^{-1}$) | 16.10±0.34 |

According to the Turkish directives, the heavy metal limits (mg kg$^{-1}$) for sewage sludge use in agriculture are as follows: Cd 10, Cr 1000, Ni 300, Pb 750, Cu 1000 and Zn 2500. The sludge used for this study contains the heavy metal concentrations (mg kg$^{-1}$) as follows: Cd (2.83±0.21), Cr (112.5±2.32), Ni (69.73±3.01), Pb (17.44±1.11), Cu (177±7.21) and Zn (1377±32.14). Thus, the concentrations of sewage sludge heavy metals in this study were lower than the permitted limits (Anonymous, 2010). Since sewage sludge was brought to suitable physical conditions to be used in plant production, it was directly used in growing medium mixtures without any further processing. Experiments were conducted in randomized blocks design with 3 replications. Soil and sewage sludge mixtures prepared at specific proportions were placed on top of this drainage layer. To measure the performance of sewage sludge as a growing medium, essential fertilizers were not used in the present experiments. About 5 cm gravel drainage layer was placed beneath the sowing boxes. Soil and sewage sludge mixtures were placed on top of this drainage layer. Fertilizers were not applied to measure the sole performance of sewage sludge as a growing medium. Marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants were grown in two separate plots. Five seedlings were planted in each plot, and a total of 20 seedlings (5 seedlings x 4 replicates) were planted on 28.04.2015.

Table 2. Analysis results of experimental soil
*Çizelge 2. Deneme toprağının analiz sonuçları*

| Properties | Values | Properties | Values |
|---|---|---|---|
| pH | 7.64±0.12 | Ca (mg kg$^{-1}$) | 2162±25.83 |
| EC (dS m$^{-1}$) | 0.44±0.10 | Na (mg kg$^{-1}$) | 31.17±3.16 |
| Texture | Sandy-loam | Fe (mg kg-1) | 2.65±0.51 |
| Sand (%) | 55.84 | Cu (mg kg$^{-1}$) | 0.89±0.11 |
| Silt (%) | 31.44 | Zn (mg kg$^{-1}$) | 2.08±0.15 |
| Clay (%) | 12.72 | Mn (mg kg$^{-1}$) | 1.83±0.32 |
| $CaCO_3$ (%) | 4.74±0.32 | Ni (mg kg$^{-1}$) | 53.09±2.73 |
| $C_{org}$ (%) | 0.876±0.21 | Pb (mg kg$^{-1}$) | 13.34±1.21 |
| N (%) | 0.081±0.04 | Cr (mg kg$^{-1}$) | 25.57±1.54 |
| P (mg kg$^{-1}$) | 14.66±2.13 | Cd (mg kg-1) | 0.75±0.18 |
| K (mg kg$^{-1}$) | 237±10.20 | B (mg kg$^{-1}$) | 1.07±0.08 |

In this experiment, pots with a top diameter of 40 cm, the height of 40 cm, and a capacity of 36 kg were used. Soil moisture content was maintained at 70% water holding capacity (WHC). Experiments had different treatments sewage sludge/soil mixtures (v/v) was mixed with soil in the following proportions: 0%

sewage sludge+%100 soil (36 kg) (applications control), 3% sewage sludge (0.850 kg)+ 97% soil (35.15 kg) (applications 3 % SS), 6% sewage sludge (1.94 kg)+94 % soil (34.06 kg) (applications 6% SS) and 9% sewage sludge (3.03 kg)+91% soil (32.97 kg) (applications 9% SS). Throughout the experiments, irrigations were performed based on soil field capacity. Pots were watered every 4 days with deionized water to maintain soils close to 70% of WHC throughout the experimental period and the leaked water returned into the pots in order to prevent losses. Chemicals were applied against louse afid and pests. The study was carried out between 28 April and 15 July 2015. The total duration of the experiment was 79 days.

## Method

The soil material used in the present experiments was sieved and made available to be used in mixtures. Three wooden sowing boxes with 12 cells were used in the present experiments. Each cell has dimensions of 40x40x20 cm and was filled with 4 different growing media all, including gravel, garden soil and sewage sludge. Soil samples were air-dried and passed through a 2 mm sieve. pH was measured in (1:2.5 soil: water) extract, and soil salinity (EC, dS $m^{-1}$) was measured in 1:2.5 soil: water extracts. Organic matter ($C_{org}$) was determined using the Modified Walkler-Black method (Jackson, 1973). Lime ($CaCO_3$) was measured with a Scheibler calcimeter (Nelson, 1982). The texture was determined with the hydrometer method (Bouyoucos, 1962). Total nitrogen (N) with modified Kjeldahl method (Bremner, 1965). Available phosphorus was determined by Olsen method (Olsen & Dean, 1965). Soil available Na, K and Ca concentration were determined using 1 N ammonium acetate extraction ($NH_4OAc$, pH=7) (Pratt, 1965). Sample K, Ca, and Na were determined in a flame photometer. Soil Fe, Cu, Zn and Mn were determined through the extractions with DTPA solution (Lindsay and Norvell, 1978). Soil Fe, Cu, Zn and Mn concentrations were determined in atomic absorption spectrophotometer (AAS) (Hanlon, 1992). Soil total Zn, Cu, Ni, Pb, Cd, Cr, As and Hg concentrations were determined in HCl and $HNO_3$ (aqua regia 3:1, v/v) extracts, and Hg concentration was determined in cold-vapor atomic absorption spectrophotometer (Kacar & İnal, 2008). Boron concentration was determined by hot-water extract with azomethine-H method (Wolf, 1971). The principal chemical properties of sewage sludge sample In particular, the pH was measured on mixtures of sewage sludge: water 1:5; the EC was measured on a 1:5 sewage sludge sample: water ratio extract and the organic matter ($C_{org}$) Modified Walkler-Black method (Jackson, 1973). Total nitrogen (N) with modified Kjeldahl method (Bremner, 1965). Sewage sludge samples after nitric and perchloric acid digestion($HNO_3$; $HClO_4$; 4:1, v/v). Total P spectrophotometric analysis with the use of vanadomolibdo phosphoric yellow color method (Lott et al., 1956). Total K, Ca, and Na concentrations were determined in a flame photometer, Mg, Fe, Zn, Cu, Mn, Ni, Cd, Cr, Pb, As and Hg measurements were performed by using cold vapour atomic absorption spectrophotometer (AAS) (Kacar & İnal, 2008). Following dry-ashing, sample B concentration was determined spectrophotometrically with the use of an azomethine-H method (Wolf, 1971). The plants were harvested carefully for analysis after 13 weeks of planting. Leaves were collected from the mid-third sections of each of the plants (Coelho et. al., 2017). All samples were washed to remove any adhering soil particles and rinsed with distilled water. The plant samples were dried at 65-70 °C for 48 h and then grinded and made ready for analysis. Total Nitrogen (N) analysis was conducted by modified Kjeldahl method (Bremner, 1965). Plant samples acid-digestion ($HNO_3$; $HClO_4$; 4:1, v/v) was performed before nutrient analysis. Total P spectrophotometric analysis with the use of vanadomolibdo phosphoric yellow color method (Lott et al., 1956). Total K, Ca and Na concentration were determined in a flame photometer, and Mg, Fe, Zn, Cu, Mn, Ni, Cd, Cr, Pb, As were determined by atomic absorption spectrophotometer. Mercury (Hg) measurement was performed by using a cold vapour atomic absorption spectrophotometer (Kacar & İnal, 2008). Boron (B) concentration dry-ashing was determined spectrophotometrically with the azomethine-H method (Wolf, 1971).

Bioconcentration Factors (BCF); Metal loads were calculated using the bioconcentration factor (BCF) as: BCF=metal concentration in the plant (mg $kg^{-1}$dw)/metal concentration in soil (Chang et al., 2014).

Statistical Analysis; Statistical analyses were conducted with the use of SPSS Statistics 20.0 software in accordance with randomized blocks design. Significant means were compared with the use of Duncan's multiple range test at α=0.05 significance level. Differences between the treatments were significant at $P < 0.01$ or $P < 0.05$.

## RESULTS and DISCUSSION

### Bioconcentration Factors (BCF)

In Marigold (*Tagetes erecta* L.) plants, bioconcentration factors (BCF) were identified as Zn (1.11±0.11-1.34±0.16), Cu (0.27±0.05-0.34±0.02), Ni (0.17±0.02-0.28±0.05), Pb (0.10±0.04-0.14±0.04), Cd (0.16±0.08-1.17±0.21), As (0.02±0.005-0.03±0.006) and Hg (0.22±0.10-0.43±0.12). Bioconcentration factor (BCF) was as follows for Marigold (*Tagetes erecta* L.): Zn>Cd>Hg>Cu>Ni>Pb>As. In garden verbena (*Verbena hybrida*) plants, bioconcentration factors (BCF) were identified as Zn (1.04±0.10-1.17±0.15), Cu (0.34±0.03-0.52±0.07), Ni (0.16±0.04-0.19±0.03), Pb

(0.10±0.03-0.14±0.04), Cd (0.22±0.11-0.27±0.14), As (0.03±0.001-0.04±0.005) and Hg (0.19±0.10-0.31±0.12) (Table 3). Bioconcentration factor (BCF) was as follows for garden verbena (*Verbena hybrida*): Zn>Cu>Hg>Cd>Ni>Pb>As. In both plants, the highest BAC value was determined for Zn element and the lowest BAC value for As. Analyzing the results of the bioconcentration factor (BCF) for the tested plants, it was noted that the Marigold plant accumulated Zn and Cd more easily, followed by Hg, Cu, Ni and Pb, and then As to a lesser extent. The values of factors for Cd and Zn were correlated with the high mobility of these elements compared to other metals and their relatively easy plant uptake. The lowest values of the BCF found for Pb and As are due to the lowest mobility of Pb and As from soil to plant tissues (Pusz et al., 2021). The content of metals in plants can vary depending on their ability to move from soil to aboveground parts (Awa & Hadibarata, 2020). Khan et al. (2008) also suggested that the BCFs of Cd and Zn were high in brassica plants. Cd is the metal most susceptible to accumulation from the soil by plants (Kabata-Pendias & Mukherjee, 2007). Translocation factor and BCF are a vital indices to determine the phytoremediation capability of plants, and it was well reported that plants revealing BCF values >1 could be favorable for phytoextraction (Chanu & Gupta, 2016).

BCF of less than 1 means more heavy metals concentration in soil than those taken up by plants (Hellen and Othman, 2016). It further enables the categorization of plants as accumulators (BAC>1) or excluders (BAC<1) of trace elements (Olowoyo et al., 2010).

In the present study for Zn and Cd, BCF was>1. Therefore, marigold plant can be a hyperaccumulator plant for Zn, and Cd elements and the Garden verbena plant can be a hyperaccumulator plant for Zn element (Biswal et al., 2022).

Table 3. Average BCF values of plants
Çizelge 3. Bitkilerin ortalama BCF değeri

| Plants | Treatments | Zn | Cu | Ni | Pb | Cd | As | Hg |
|---|---|---|---|---|---|---|---|---|
| Marigold | Control | 1.17±0.10 | 0.34±0.02 | 0.25±0.04 | 0.11±0.04 | 0.16±0.08 | 0.02±0.005 | 0.39±0.11 |
| | % 3 SS | 1.11±0.11 | 0.31±0.02 | 0.17±0.02 | 0.10±0.03 | 0.62±0.12 | 0.02±0.005 | 0.34±0.10 |
| | % 6 SS | 1.34±0.16 | 0.28±0.04 | 0.27±0.04 | 0.14±0.04 | 0.99±0.14 | 0.03±0.006 | 0.22±0.10 |
| | % 9 SS | 1.12±0.14 | 0.27±0.05 | 0.28±0.05 | 0.12±0.04 | 1.17±0.21 | 0.03±0.006 | 0.43±0.12 |
| G.verbena | Control | 1.04±0.10 | 0.34±0.03 | 0.16±0.04 | 0.14±0.04 | 0.22±0.11 | 0.03±0.001 | 0.26±0.11 |
| | % 3 SS | 1.05±0.10 | 0.45±0.05 | 0.16±0.05 | 0.10±0.03 | 0.23±0.12 | 0.04±0.005 | 0.25±0.11 |
| | % 6 SS | 1.17±0.15 | 0.52±0.07 | 0.18±0.04 | 0.13±0.05 | 0.27±0.13 | 0.03±0.002 | 0.31±0.12 |
| | % 9 SS | 1.13±0.12 | 0.43±0.08 | 0.19±0.03 | 0.13±0.04 | 0.27±0.14 | 0.03±0.003 | 0.19±0.10 |

## Plant nutrients

Experimental treatments had significant (P<0.01) effects on plant leaf total nitrogen (N) concentration. In marigold (*Tagetes erecta* L.) plants, the most significant N (%) concentration (3.0±0.13) was obtained from 9% SS and the lowest (2.4±0.10) from the control treatments. In garden verbena (*Verbena hybrida*) plants, the greatest value (3.0±0.14) was obtained from 9% SS treatments and the lowest (2.3±0.11) from the control treatments (Table 4).

Table 4. Macro plant nutrient concentrations of marigold and garden verbena plants
*Çizelge 4. Kadife çiçeği ve yer minesi bitkilerinin makro bitki elementi konsantrasyonları*

| Plants | Treatments | N | P | (%)<br>K | Mg | Ca |
|---|---|---|---|---|---|---|
| Marigold | Control | 2.4±0.10b | 0.39± 0.13 | 2.00±0.14ab | 0.42±0.10b | 3.2±0.11c |
| | 3% SS | 2.7±0.11ab | 0.34±0.10 | 1.97±0.16 b | 0.43±0.11b | 3.8±0.14bc |
| | 6% SS | 2.9 ±0.11a | 0.37±0.11 | 2.30± 0.10a | 0.60±0.12a | 5.2 ±0.20a |
| | 9% SS | 3.0 ±0.13a | 0.42±0.15 | 2.33 ±0.22a | 0.61±0.24a | 5.0±0.15ab |
| | S. level | ** | ns | * | * | * |
| G.verbena | Control | 2.3 ±0.11b | 0.26±0.12c | 2.27±0.17ab | 0.60±0.11b | 3.5±0.10 |
| | 3% SS | 2.5 ±0.10b | 0.33±0.13bc | 2.30±0.20ab | 0.64±0.12ab | 3.9±0.12 |
| | 6% SS | 2.6±0.12ab | 0.43±0.16ab | 2.60±0.23a | 0.67±0.10ab | 4.3±0.16 |
| | 9% SS | 3.0 ±0.14a | 0.51±0.21a | 2.20± 0.11b | 0.70± 0.14a | 4.8±0.20 |
| | S. level | ** | ** | ** | * | ns |

Significant level (S. level),* p<0.05 ** p<0.01 ns: not significant

Applications of the marigold plant on phosphorus (P) concentration had no significant effect. Plant P (%) concentration varied between 0.34±0.10-0.42±0.15. On the other hand, treatments had significant effects on the P concentration of garden verbena plants ($P<0.01$), with the greatest value (0.51±0.21) in 9% SS treatments and the lowest value (0.26±0.12%) in the control treatments. Marigold potassium (K), magnesium (Mg) and calcium (Ca) concentrations were significantly affected by treatments ($P<0.05$). The greatest plant K (2.33±0.22) and Mg (0.61±0.24) concentration were obtained from 9% SS treatments, followed by 6% SS treatments. Both treatments (6% SS and 9% SS) were placed into the same statistical group. The greatest Ca (%) concentration (5.2±0.20) was obtained from 6% SS treatments, followed by 9% SS treatments (5.0±0.15). On the other hand, in garden verbena plants, treatments had a significant effects on plant K concentration at significant ($P<0.01$) level and on Mg at ($P<0.05$) significant level but did not have significant effects on plant Ca. Plant K (%) concentration varied between 2.20±0.11-2.60±0.23 with the greatest value in 6% SS treatments and Mg (%) varied between 0.60±0.11-0.70±0.14 with the greatest value in 9% SS treatments. The most remarkable plant Ca (%) (4.80±0.20) was obtained from 9% SS and the lowest concentration (3.5±0.10) value was obtained from control treatments. Increasing SS doses had significant effects on Na concentrations of marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants significant ($P<0.01$) level. In marigold and garden verbena plants, the lowest Na (mg kg$^{-1}$) concentration was obtained from the control (349±11.93) treatments and the greatest values (377±15.36) were obtained from 9% SS treatments of marigold plants (784±26.41) and 6% SS treatments of garden verbena plants (696±25.16). Microelement concentration of marigold and garden verbena plants also increased with increasing SS doses (Table 5).

Table 5. Micro element concentrations of marigold and garden verbena plants

*Çizelge 5. Kadife çiçeği ve yer minesi bitkilerinin mikro element konsantrasyonları*

| Plants | Treatments | Na | (mg kg$^{-1}$) Fe | Cu | Zn | Mn | B |
|---|---|---|---|---|---|---|---|
| Marigold | Control | 349±11.93c | 1698±35.41 | 7.3±0.21 | 95±10.17c | 104±11.82b | 42±10.21 |
| | 3% SS | 493±13.57c | 1503±28.52 | 7.3±0.25 | 102±12.25bc | 109±14.26ab | 39±8.14 |
| | 6% SS | 625±15.12ab | 2300±50.95 | 7.3±0.25 | 136±16.32a | 142±20.74a | 39±9.63 |
| | 9% SS | 784±26.41a | 2236±40.24 | 7.7±0.30 | 130±14.50ab | 129±15.21ab | 37±8.95 |
| | S. level | ** | ns | ns | * | ** | ns |
| G.verbena | Control | 377±15.36c | 1521±25.38 | 8 ±0.11b | 84±10.66b | 74±12.64 | 47±11.15 |
| | 3% SS | 456±18.72bc | 1587±30.64 | 10±0.15ab | 101±11.23ab | 100±15.26 | 46±9.28 |
| | 6% SS | 696±25.16a | 1700±50.42 | 12± 0.21a | 115±14.18a | 96±20.51 | 46±10.65 |
| | 9% SS | 611±20.51ab | 1423±26.45 | 10±0.18ab | 108±13.25a | 92±18.40 | 46±12.84 |
| | S. level | ** | ns | * | ** | ns | ns |

Significant level (S. level),* $p<0.05$ ** $p<0.01$ ns: not significant

Plant Fe (mg kg$^{-1}$) concentrations varied between 1503±28.52-2300±50.95 in marigold plants and between 1423±26.45-1700±50.42 in garden verbena plants. The highest amount of Fe was determined in 6% SS applications for the marigold plant (2300±50.95) and the G.verbena plant (1700±50.42), respectively. Applications of the marigold plant had no significant effect on Cu (mg kg$^{-1}$) concentrations and varied between 7.3±0.21-7.7±0.30. Treatments had significant effects on G.verbena plant Cu concentration at $P<0.05$ level. The lowest Cu (mg kg$^{-1}$) concentration was found in the control (8 ±0.11) treatment, and the highest value was 6% SS (12± 0.21) application. The effect of applications on Zn in the marigold plant was found to be significant ($P<0.05$) level. The most minor Zn (mg kg$^{-1}$) concentration was determined in control (95±10.17) and the highest in 6% SS (136±16.32) application. Treatments had significant ($P<0.01$) effects on Zn concentration in garden verbena plants. The smallest Zn concentration in the control (84±10.66) and the highest value (115±14.18) in the 6% SS treatment. Marigold plant Mn (mg kg$^{-1}$) concentrations significantly ($p<0.01$) increased with increasing SS treatments. The lowest Mn concentration was obtained from the control treatment (104±11.82), and the most excellent Mn value (142±20.74) was obtained from the 6 % SS treatment. G.verbena Mn concentrations did not significantly change with SS treatments. The highest Mn was obtained from the 3 % SS treatment (100±15.26) and the lowest value (74±12.64) from the control treatment. In both plant species, the effects of sewage sludge treatments on plant B (mg kg$^{-1}$) concentration were not significant. The highest B concentration in the marigold plant was seen in the control treatment (42±10.21) and the lowest value in the 9% SS treatment (37±8.95 mg kg-1). In the G.verbena, plant the greatest B concentration (47±11.15) was obtained from control treatments, followed by 3% SS, 6% SS and 9% SS treatments. Afonso et al. (2018) reported plant nutrients for lemon verbena. The sufficiency ranges set for the macronutrients (%) N, P, K, Ca, and Mg were respectively 2.80–4.30, 0.09–0.38, 1.00–2.80, 0.75–3.00, and 0.20–0.80. The sufficiency ranges found for the micronutrients B, Cu, Fe, Zn, and Mn were, respectively, 35–200, 7–22, 60–300, 25–125, and 40–250 mg kg$^{-1}$. Present study, N and Fe elements differed according to these values, especially in the Fe element. Soares et al. (2018) concentration of Na (321-513 mg kg$^{-1}$), but Amed et al. (2012) differed from the Fe (836.74 mg kg$^{-1}$) concentration value. The herb is a good source of Fe as well, where roots contain notably higher quantity (1763 mg kg$^{-1}$) than leaves (836.74 mg kg$^{-1}$), stems (324.39 mg kg$^{-1}$), seeds (893.25 mg kg$^{-1}$) and seeds husk (476.21 mg kg$^{-1}$) (Amed et al., 2012). Eaton et al. (2013) reported plant nutrients for the marigold plants. The sufficiency ranges set for the macronutrients (%) N, P, K, Ca, and Mg were respectively 2.40–5.70, 0.30–1.40, 0.90–5.30, 1.70–3.60, and 0.60–1.90. The sufficiency ranges found for the micronutrients (mg kg$^{-1}$) B, Cu, Fe, Zn, and Mn were, respectively, 30-53, 8–23, 61–233, 67–229 and 44–541. Present study, Fe elements differed according to these values, especially in terms of Fe element. Similar to Na (595 mg kg$^{-1}$) element, as stated by Sonmez et al (2017), but differed from Fe (971.6 mg kg$^{-1}$). These differences are thought to be due to soil characteristics, sewage sludge treatments (12755±124.53 mg kg$^{-1}$ Fe) and plant variety. It can be concluded that Marigold (*Tagetes erecta* L.) and Garden verbena (*Verbena hybrida*) plants have high Fe concentration, and these plants can be hyper accumulator for Fe element.

## Plant heavy metal concentration

Treatments had significant ($P<0.05$) effects of marigold Ni (mg kg$^{-1}$) concentration. The most significant value (13±1.23) was obtained from 9% SS treatments and the lowest (8±1.35) in 3% SS treatments. In garden verbena plants, the greatest Ni (8.9±0.93) was obtained from 9% SS treatments and the lowest (7.0±0.52) in the control treatments (Table 6).

Table 6. Heavy metal concentrations of marigold and garden verbena plants
*Çizelge 6. Marigold ve garden verbena bitkilerinin ağır metal konsantrasyonları*

| Plants | Treatments | Ni | Cd | Cr | Pb | As | Hg |
|---|---|---|---|---|---|---|---|
| | | | (mg kg$^{-1}$) | | | | (µg kg$^{-1}$) |
| Marigold | Control | 11±0.71ab | 0.16±0.08b | 19±0.28b | 2.5 ±0.10b | 0.35±0.02 | 48.79±2.34 |
| | 3% SS | 8±1.35b | 0.69±0.10ab | 24±0.32ab | 2.7±0.16ab | 0.32±0.05 | 43.11±8.32 |
| | 6% SS | 12±1.12a | 1.08±0.06ab | 25± 0.43a | 3.8±0.21a | 0.49±0.06 | 45.61±9.11 |
| | 9% SS | 13±1.23a | 1.21± 0.09a | 28± 0.51a | 3.2±0.20ab | 0.52±0.07 | 61.82±10.13 |
| | S. level | * | ** | * | * | ns | ns |
| G.verbena | Control | 7.0±0.52 | 0.3±0.05 | 7.2 ±0.46b | 3.0±0.21 | 0.40±0.03 | 27.04±3.46 |
| | 3% SS | 7.3±0.61 | 0.3±0.06 | 8.2±0.62ab | 2.6±0.15 | 0.52±0.06 | 28.59±4.73 |
| | 6% SS | 8.3±0.75 | 0.4±0.07 | 9.4±0.81ab | 2.9±0.20 | 0.42±0.04 | 37.11±5.30 |
| | 9% SS | 8.9±0.93 | 0.4±0.09 | 10.0±1.02a | 3.2±0.28 | 0.49±0.05 | 24.15±9.28 |
| | S. level | ns | ns | * | ns | ns | ns |

Significant level (S. level), * $p<0.05$ ** $p<0.01$ ns: not significant

Experimental treatments had significant effects on marigold Cd (mg kg$^{-1}$) concentration ($P<0.01$), with the greatest value (1.21±0.09) in the 9% SS treatments and the lowest value (0.16±0.08) in the control treatments. Effects of SS treatments on garden verbena Cd concentration were not found to be significant. Plant Cd value 0.3±0.05-0.4±0.09. Marigold plant Cr (mg kg$^{-1}$) concentration significantly ($P<0.05$) varied between 19±0.28–28±0.51, the lowest value in the control treatment and the greatest value in 9% SS application dose. Effects of SS treatments on garden verbena Cr concentrations were not found to be significant. Plant Cr concentration 7.2±0.46-10.0±1.02. There were significant ($P<0.05$) differences in Marigold Pb (mg kg$^{-1}$) concentration. The most significant plant Pb concentration (3.8±0.21) was obtained from 6% SS treatments and the lowest (2.5±0.10) in the control treatments. On the other hand, in garden verbena plants, the greatest Pb concentration (3.2±0.28) was obtained from 9% SS treatments and the lowest (2.6±0.15) from 3% SS treatments. Sewage sludge treatments did not have significant effects on marigold As and Hg concentration. Plant As (mg kg$^{-1}$) concentration varied between 0.32±0.02-0.52±0.07 and Hg (µg kg$^{-1}$) concentration range from 43.11±8.32 to 61.82±10.13. Similarly, the effects of SS treatments on garden verbena As and Hg value were not found to be significant. Plant As concentration 0.40±0.03-0.52±0.06 and Hg concentration 24.15±8.28-37.11±5.30. Present As the concentration of marigold and garden verbena plants were lower than the EU threshold values for As in fodder (2-4 mg kg$^{-1}$) (Adamse et al., 2017; Dradrach et al., 2020). Acoding to Kabata-Pendias & Pendias (2011) Normal values of total heavy metals in plants (mg kg$^{-1}$) 0.02-5.0 Ni, 0.1-2.4 Cd, 0.03-14.0 Cr and 0.2-20.0 Pb concentration in plant. Marigold plant concentrations of Ni, Pb and Garden verbena plant Ni concentration were determined to be higher than the specified average values. Mercury concentrations determined in Marigold and G. verbena plants were below the maximum (0.5 mg kg$^{-1}$) limit value specified for contaminated food by the World Health Organization (WHO, 2004).

## Soil heavy metal concentration

The effect of sewage sludge applications on the total Zn of marigold plant soils after harvest was statistically significant ($P<0.05$) and differed according to the applications. The smallest Zn (mg kg$^{-1}$) value was determined at 81.38±1.22 in the control application, and the highest Zn value was determined at 116.58±2.47 in the 9% SS application. The applications did not have a significant effect on the total Zn of garden verbena plant soils after harvest. The lowest Zn concentration was 80.47±2.73 in the control application and the highest value was obtained at 97.97±3.2 in the 6 % SS application respectively. The effect of the treatments on marigold soil the total Cu was statistically significant ($P<0.05$), with the highest Cu (mg kg$^{-1}$) value being 28.49±1.14 in 9% SS application and the lowest Cu value in control application with 21.23±2.15. The applications did not have a significant effect on the total Cu garden verbena soil. The concentration of Cu (mg kg$^{-1}$) was determined in the range of 22.28±2.20-23.35±2.12. The effect of sewage sludge doses on the total Ni concentration of the soil was not significant. In marigold soil, the lowest soil Ni (mg kg$^{-1}$) concentration (44.32±1.95) was determined in control, and the highest Ni concentration (46.70±2.02) was determined in 3% SS application. In garden verbena soil, the lowest Ni (mg kg$^{-1}$) value (43.95±1.28) was obtained from the control and the greatest Ni concentration (45.88±1.10) from 9% SS treatments. These values were below the toxic level of 100 mg kg$^{-1}$ specified by Özbek et al. (1995). Soil total Pb significantly increased with increasing SS doses ($P<0.05$) compared to the control. In marigold soil, the lowest Pb (mg kg$^{-1}$) concentration (22.42±1.66) was obtained from the control and the greatest (27.58±0.39) from 9% SS treatments. In garden verbena soil, the lowest value (21.63±0.87) was obtained from the control and the greatest (25.13±2.88) from 3% SS treatments (Table 7).

Table 7. Heavy metal concentration of post-harvest soils according to applications
Çizelge 7. Hasat sonrası toprakların uygulamalara göre ağır metal konsantrasyonları

| Plants | Treatments | Zn | Cu | Ni | Pb<br>(mg kg$^{-1}$) | Cd | As | Hg<br>(µg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Marigold | Control | 81.38±1.22b | 21.23±2.15b | 44.32±1.95 | 22.42±1.66b | 1.02±0.15 | 17.76±0.83 | 125.71±21.77 |
| | 3% SS | 91.57±1.14ab | 24.22±2.41ab | 46.70±2.02 | 25.75±0.45a | 1.12±0.17 | 18.81±0.17 | 128.04±13.47 |
| | 6% SS | 101.86±3.14ab | 25.62±2.12ab | 44.98±1.12 | 27.04±1.05a | 1.09±0.26 | 19.41±0.10 | 207.79±59.37 |
| | 9% SS | 116.58±2.47a | 28.49±1.14a | 46.58±1.45 | 27.58±0.39a | 1.03±0.38 | 19.81±0.05 | 143.33±3.90 |
| | S. level | * | * | ns | * | ns | ns | ns |
| G.verbena | Control | 80.47±2.73 | 23.35±2.12 | 43.95±1.28 | 21.63±0.87 | 1.38±0.02 | 14.39±0.10 | 104.92±12.27 |
| | 3% SS | 95.74±2.81 | 22.28±2.20 | 45.17±1.27 | 25.13±2.88 | 1.28±0.13 | 14.62±1.32 | 114.25±6.54 |
| | 6% SS | 97.97± 3.24 | 23.12±3.41 | 45.58±0.46 | 22.79±0.96 | 1.47±0.02 | 14.93±0.72 | 121.13±12.87 |
| | 9% SS | 95.46±3.12 | 23.32±3.56 | 45.88±1.10 | 23.71±3.37 | 1.50±0.01 | 15.15±2.44 | 126.04±25.31 |
| | S. level | ns | ns | ns | ns | ns | ns | ns |

Significant level (S. level) * $p<0.05$ ** $p<0.01$ns: not significant

Present Pb values were all below the toxic level of 100 mg kg$^{-1}$ specified by Kabata-Pendias & Pendias (1992). Total Cd (mg kg$^{-1}$) concentration in marigold soil and garden verbena soil did not significantly change with increasing SS doses. Soil total Cd varied between 1.02±0.15-1.12±0.17 in marigold soil and 1.28±0.13-1.50±0.01 in garden verbena soil. Present Cd values were within the normal limits of 3 mg kg$^{-1}$ specified by Kabata-Pendias (2011). Soil total Cd, Ni and Pb values were different from the values of Delibacak & Ongun (2018), probably because of varying treatment doses and soil characteristics. Soil pH also influences the presence and uptake of heavy metals (Khan et al., 2015; Eid & Shaltout, 2016). In marigold soil, the lowest soil total As (mg kg$^{-1}$) concentration (17.76 ±0.83) was obtained from the control and the greatest concentration (19.81±0.05) from 9% SS treatments. In garden verbena soil the lowest value (14.39±0.10) was obtained from the control and the greatest concentration (15.15±2.44) from 9% SS treatments. Present As values were all below standard value of 20 mg kg$^{-1}$ of WHO and FAO (Chiroma et al., 2014). Soil total Hg (µg kg$^{-1}$) concentration did not change significantly with increasing SS doses. In marigold soil, the lowest soil Hg concentration (125.71±21.77) was obtained from the control and the greatest concentration (207.79±59.37) from 6% SS treatments. In garden verbena soil, the lowest value (104.92±12.27) was obtained from the control and the greatest concentration (126.04±25.31) from 9% SS treatments. Present Hg values were below the standard limit of 1.5 mg kg$^{-1}$ of Turkey (Anonymous, 2010; Chiroma et al., 2014). Threshold values of heavy metals (mg kg$^{-1}$) in soil were given in Turkey as 200 Zn, 100 Cu, 70 Ni, 100 Pb, and 1.5 Cd, respectively (Anonymous, 2010). Soil heavy metal (Zn, Cu, Ni, Cd, Pb, As and Hg) concentrations were below the allowable limits. Depending on the treatment sludge application dose, it has been reported that EC and exchangeable Na increase in soils in long-term soil application (Cucina et al., 2019). The positive relationship between sludge addition and EC increase has been reported by several studies (Dhanker et al., 2021). The soil EC significantly increased with sludge dosage. Since the organic acids produced during the decomposition of solid waste caused the accumulation of dissolved salts (Hamdi et al., 2019). Sewage Sludge application did not affect total Cd, Pb and Ni concentrations in treated soils; total Hg, Zn and Cu accumulated proportionally with the amount of sewage sludge applied (Cucina et al., 2019). The total concentration of trace elements showed the following variation in the soil over the 5 yr (mg kg$^{-1}$): Cd (16.8–20.0), Co (19.5–21.5), Cr (98.2–125.7), Cu (8.1–17.1), Mn (62.9– 85.7), Ni (20.3–35.0), Pb (27.0–52.4), and Zn (20.3–35.8) (Chagas et al. 2020). It was stated that Sarğın (2011), seven years after the treatment sludge application, the heavy metal Zn, Cu, Pb, Ni and Cd concentrations in the soil were determined below the limit values for all soil depths (0-30 cm, 30-60 cm and 60-100 cm). Changes in heavy metal accumulation in soils depend primarily on the characteristics of the sewage sludge, the rate applied and soil texture (Wu et al., 2012). Heavy metals bioavailability in soils after amendment varies according to soil characteristics, such as soil pH, and organic matter content (Achiba et al., 2009).

## CONCLUSION

The BCF values of marigold for Zn, Cd element and garden verbena for Zn were >1. Thus, marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) were considered as a potential accumulator plants and can be used for the remediation of contaminated soils. In terms of heavy metals (Ni, Cd, Cr, Pb, As and Hg), toxicity symptoms were not encountered in marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants. Soil heavy metal (Ni, Cd, Pb, As and Hg) were below the limit values. Based on the present findings, it is recommended that sewage sludge treatments could be applied at 3% SS for marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrid*) plants.

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### Statement Contribution of The Authors

Authors declares the contribution of the authors is equal.

### Statement of Conflict of Interest

Authors have declared no conflict of interest.

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