Turkish Journal of Agriculture and Forestry

Volume 47 | Number 6 Article 4

12-1-2023

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Turkish Journal of Agriculture and Forestry
http://journals.tubitak.gov.tr/agriculture/
Research Article
Turk J Agric For
(2023) 47: 828-841
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doi:10.55730/1300-011X.3131

Effects of sewage sludge treatments on plant nutrients, heavy metals and tall fescue (*Festuca arundinacea* Schreb.)

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Received: 24.08.2022 • **Accepted/Published Online:** 15.05.2023 • **Final Version:** 01.12.2023

Abstract: This study was conducted to investigate the effects of anaerobically stabilized sewage sludge (SS) converted into 90% dryness granules at different doses of control, 3% SS, 6% SS, and 9% SS on elemental composition of *Festuca arundinacea* Schreb. and physicochemical properties of soil. Color measurements were made with the CIE L* a* b* method unit; the highest color (4.10) and the highest coating score (5.7) were calculated for the 6% SS treatment. The highest biomass (9.11 g) was obtained from the 3% SS and the lowest (7.67 g) from the 9% SS dose. Plant height measurements were listed as follows: 3% SS (9.5 cm) > 6% SS (8.8 cm) > control (8.7 cm) > 9% SS (8.1 cm). Average bioaccumulation factor for the present heavy metals were ordered as: Cd (0.542) > Hg (0.452) > Cr (0.448) > Ni (0.246) > Pb (0.076). The lowest geoaccumulation index (I_{geo}) was obtained in Cr (−1.35) and the highest value in Cd (1.69). Available P, K, Ca, Mg were analyzed with ammonium acetate extraction method, and Fe, Zn, Cu, and Mn in the soil extracted with DTPA were determined. In the soil samples, total N (0.180%) and available elements (mg kg^{-1}) P (101.393), Ca (6502.570), Mg (609.433), Na (259.44), Fe (13.61), Cu (4.05), Zn (17.76), and B (1.00) were determined at high values in the 9% SS application. Heavy metals (mg kg^{-1}) were extracted with aqua regia. Nickel (48.87), Pb (26.83), Cd (0.97), and Hg (153.12 µg kg^{-1}) were determined at high values in the 9% SS application. Plant nutrients in the plant samples were extracted from acid digestion, and N (3.93%), P (0.51%), Mg (0.59%), Ca (1.77%), Zn (127 mg kg^{-1}), and B (15 mg kg^{-1}) were determined at high values in the 9% SS application. Heavy metals' (Ni, Cd, Cr, Pb, and Hg) content toxic effects were not observed in the plants.

Key words: Bioconcentration factor, clipping, heavy metals, geoaccumulation index, sewage sludge

1. Introduction

Sewage sludge (SS) is highly rich in organic carbon and organic matter; thus, it is largely used in agricultural practices as a fertilizer. Agriculture offers an alternative disposal method of wastewater and sludge treatment. Safe disposal and recycle of SS are major environmental concerns worldwide. There are three primary methods used in disposal of sewage sludge: incineration, landfills, and agricultural applications (Cristina et al., 2020). Agricultural use of sewage sludge not only offers an affordable means of disposal but also improves soil fertility and physical properties, thereby enhancing crop productivity. Moreover, it facilitates nutrient recycling and reduces the cost of cultivation (Swain et al., 2021). Consumption of mineral fertilizers, expressed as the main nutrient at the global level ($N + P_2O_5 + K_2O$) was 191.5 million tons in 2017–2018, and this is an increase of 9.7% compared to 2011–2012 (Nutrien, 2018) and the upward trend will continue (International Fertilizer Association, 2019). sewages sludge gives an opportunity to use the fertilization potential of alternative raw materials in agriculture (Chojnacka et al., 2020). Recycling these wastes in terms of fertilization makes it possible to increase sustainable productivity by reducing the dependence on imported fertilizer sources (Kominko et al., 2021). The application of sewage sludge to the soil is an important waste disposal method, both economically and environmentally (Ren et al., 2017). Application of sewage sludge to soil has been practiced worldwide and much research has reported the positive influence of sewage sludge on soil and crop production, including increasing the nutrient and organic matter content of soil (Arlo et al., 2022). However, sewage sludge may contain high levels of pollutants, organic and/ or heavy metals (Rastetter and Gerhardt, 2017). Therefore,

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an effective management strategy of SS is required because SS may lead to environmental pollution if not dealt with appropriately (Singh and Agrawal, 2008). At the same time, when an effective waste management is applied, the occurrence of eutrophication is prevented (Scholz et al., 2013). Sewage sludge can serve as an intriguing alternative in the production of organomineral fertilizers (Antille et al., 2017; Rodrigues et al., 2021). Several studies have evaluated the effects of composted sewage sludge on barley and found a positive influence on crop productivity (Pasqualone et al., 2017; Penido et al., 2019). Composting and anaerobic digestion have emerged as better options for managing food waste and sewage sludge (Yaser et al., 2022).

Grasses play a crucial role in phytostabilization due to their fast growth, substantial biomass, and robust root systems. They effectively cover the soil surface and exhibit high resistance to heavy metal toxicity. Consequently, grasses excel in soil stabilization compared to trees or shrubs (Radziemska et al., 2017). Primary grass species, such as tall fescue (*Festuca arundinacea* Schreb.), native to Europe and North Africa, are widely used for forage in extensive livestock operations in mild climates. This cool-season grass boasts a deep root system, various physiological traits, significant drought resistance, and a remarkable ability to adapt to harsh conditions (Qu et al., 2021). Numerous studies have explored the phytoremediation potential of *F. arundinacea* for both organic (Mendarte-Alquisira et al., 2017) and inorganic contaminants (Wasilkowski et al., 2019). The bioaccumulation factor (BCF) serves as a vital quantitative indicator for crop contamination and has found widespread use in predicting metal transfer from soil to plants (Garcia et al., 2009). Similarly, the geoaccumulation index (I_{geo}) is extensively employed in estimating contamination levels of heavy metals in soils (Adimalla and Wang, 2018). According to Turkish directives, the allowable limits for heavy metals (mg kg^{-1}) in sewage sludge used in agriculture are as follows: Cd 10, Cr 1000, Ni 300, Pb 750, and Hg 10 mg kg^{-1} (Ministry of Environment and Forestry, 2010). Consequently, the concentrations of heavy metals in the sewage sludge studied were within the permissible limits. The application of sewage sludge to the soil provides both economically and environmentally acceptable methods for its management.

This study aimed to investigate the impact of municipal sewage sludge dosage on tall fescue (*Festuca arundinacea* Schreb.), covering aspects like coverage class score, plant clip biomass and plant height, color change unit (ΔE) value, bioconcentration factor (BCF), geoaccumulation index (I_{geo}), soil heavy metal levels (Ni, Pb, Cd, Cr, and Hg), plant nutrients (N, P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, and B), plant heavy metals (Ni, Pb, Cd, Cr, and Hg), and available plant elements in the soil (Na, Fe, Cu, Zn, Mn, and B).

2. Materials and methods

2.1. Material

The pot experiments began in April 2015 under greenhouse conditions in Bornova Town, İzmir Province (38° 27′12.5″ N, 27° 13′40.2″ E). The study focused on the physicochemical properties of soil amended with municipal sewage sludge and the growth of tall fescue (*Festuca arundinacea* Schreb.) under greenhouse conditions (1.50 m × 21 m). The region experiences a Mediterranean climate. Meteorological data from the experimental area revealed an average annual minimum temperature of 13.7 °C and a maximum of 22.3 °C, with a monthly average precipitation of 59.2 mm (Table 1) provides a summary of the climatological data sourced from the Turkish State Meteorological Service, 2022. The granulated and 90% dry municipal wastewater treatment sludge was obtained from the Wastewater Treatment Plant of İzmir Greater City Municipality and stabilized under anaerobic conditions (Table 2). Sewage sludge was applied at different doses to tall fescue (*Festuca arundinacea* Schreb.) plants. The soil used in this study was gathered from a depth of 0 to 20 cm on the grounds of the Research Application and Production Farm at Ege University's Faculty of Agriculture. It was then transported to the laboratory and subjected to drying at a temperature of 20 °C. Table 3 provides an overview of the physical

Table 1. Climate data of the experimental area.

Minimum temperature (°C)												
2015 month	5.3 J	6.3 F	8.2 M	9.7 A	16.4 M	19.2 J	23.0 J	23.8 A	21.0 S	15.5 O	11.1 N	5.4 D	13.7 Ave.
Maximum temperature (°C)													
2015 month	11.9 J	12.5 F	15.2 M	19.1 A	27.0 M	28.3 J	33.5 J	33.3 A	30.1 S	23.6 O	19.7 N	13.4 D	22.3 Ave.
Precipitation (mm)													
2015 month	173.3 J	100.6 F	92.0 M	30.6 A	25.6 M	52.1 J	0.0 J	37.5 A	7.2 S	87.3 O	103.8 N	0.2 D	59.2 Ave.

Table 2. Characterization of sewage sludge material.

Properties	Values	Properties	Values
pH[a] $_{1:2.5\ water}$	7.18	Na[e] ($mg\ kg^{-1}$)	1391
EC[b] ($dS\ m^{-1}$)	1.95	Fe[e] ($mg\ kg^{-1}$)	12755
C:N ratio	9.90	Cu[e] ($mg\ kg^{-1}$)	177
C_{org}[c] (%)	29.66	Zn[e] ($mg\ kg^{-1}$) Mn[e] ($mg\ kg^{-1}$)	1377 350
N[d] $_{kjeldahl}$ (%)	2.99	Ni[e] ($mg\ kg^{-1}$)	69.73
P[e] (%)	0.23	Pb[e] ($mg\ kg^{-1}$)	17.44
K[e] (%)	0.34	Cr[e] ($mg\ kg^{-1}$)	112.5
Ca[e] (%)	6.36	Cd[e] ($mg\ kg^{-1}$)	2.83
Mg[e] (%)	2.04	Hg[e] ($mg\ kg^{-1}$) B[f] ($mg\ kg^{-1}$)	0.34 16.10

[a]: 1:2.5 water extract,**[b]:** Electrical conductivity, w:v, 1:5 water, **[c]:** Organic carbon,
[d]: kjeldahl, **[e]:** total HNO_3+$HClO_4$ extract, [f]: ash were determined by azomethine-H methods.
Each value is the mean of three replicates and on an oven-dry (105 °C) basis.

Table 3. Analysis results of experimental soil.

Properties	Values	Properties	Values
pH[a] $_{1:2.5\ water}$	7.64	Ca[f] ($mg\ kg^{-1}$)	2162
EC[b] ($dS\ m^{-1}$)	0.44	Na[f] ($mg\ kg^{-1}$)	31.17
Texture	Sandy-loam	Fe[g] ($mg\ kg^{-1}$)	2.65
Sand (%)	55.84	Cu[g] ($mg\ kg^{-1}$)	0.89
Silt (%)	31.44	Zn[g] ($mg\ kg^{-1}$)	2.08
Clay (%)	12.72	Mn[g] ($mg\ kg^{-1}$)	1.83
$CaCO_3$ (%)	4.74	Ni[h] ($mg\ kg^{-1}$)	53.09
C_{org}[c] (%)	0.876	Pb[h] ($mg\ kg^{-1}$)	13.34
N[d] (%)	0.081	Cr[h] ($mg\ kg^{-1}$)	25.57
P[e] ($mg\ kg^{-1}$)	14.66	Cd[h] ($mg\ kg^{-1}$) Hg[h] ($mg\ kg^{-1}$)	0.75 61.53
K[f] ($mg\ kg^{-1}$)	237	B[i] hot water ($mg\ kg^{-1}$)	1.07

[a]: w:v, 1:2.5 water, [b]: Electrical conductivity w:v, 1:2.5 water, **[c]:** Organic carbon [d]: total Kjeldahl,
[e]: available Olsen,[f]: available 1N NH_4OAc extract; [g]: available DTPA extract; [h]: total HCl + HNO_3 extract,
[i]: hot water extracts were determined by azomethine-H methods. Each value is the mean of three replicates

and chemical characteristics of the experimental soil. Following the air-drying process, the soil samples were sieved through a 4-mm mesh. The soil was categorized as a typic xerofluvent according to the Soil Survey Staff (2010) classification. The heavy metal content in the stabilized sewage sludge (SS) used in this study fell below the limits established by the European directive 86/278/CEE (CEC, 1986) and Turkish regulatory guidelines (Ministry of Environment and Forestry, 2010). As the sewage sludge had been adjusted to suitable physical conditions for crop production, it was directly incorporated into growing medium mixtures without any further treatment.

Festuca arundinacea grass species were used as the plant material for the experiments. Grass seeds were sown in wooden sowing pots with a top diameter of 40 cm, a height of 40 cm, and a capacity of 36 kg. In each sowing pot, a 5-cm thick gravel layer was placed beneath to allow for proper drainage. Soil and sewage sludge mixtures prepared at certain proportions were placed on top of this drainage layer. The performance of sewage sludge as a growing medium was assessed. Soil moisture content was maintained at 75% water holding capacity (WHC). Four different treatments of sewage sludge / soil mixtures (w /

w) were set up as follows: 0% sewage sludge + 100% soil (36 kg) (control application), 3% sewage sludge (0.850 kg) + 97% soil (35.15 kg) (3% SS application), 6% sewage sludge (1.94 kg) + 94% soil (34.06 kg) (6% SS application), and 9% sewage sludge (3.03 kg) + 91% soil (32.97 kg) (9% SS application). A randomized block design with three replications was employed. Seeds were sown on April 28, 2015 at a uniform rate of 60 g seeds m^{-2}. After sowing, the seeds were covered with a 1-cm thick layer of silt. All pots were watered every 4 days with deionized water to maintain soils close to 70% of WHC throughout the experimental period. Leaked water was returned into the pots to prevent any losses. The experiment was conducted in a controlled greenhouse with 70% ± 5% relative humidity, 24 ± 2 °C day temperature, and 20 ± 2 °C night temperature. The study was carried out between April 28 and July 15, 2015, with the total duration of the experiment being 79 days. The first grass germinated on May 04, 2015, after sowing on April 28, 2015. Mechanical weed control was practiced, and chemicals were applied against aphids and pests. When the *F. arundinacea* reached a length of 7–8 cm after planting, it was cut with shears to leave a stubble height of 4–5 cm.

2.2. Method

At the end of the experiment, soil samples were extracted from the growing pots (boxes). These samples were air-dried and sifted through a 2-mm sieve. The total soil content of Ni, Pb, Cd, and Cr was determined in aqua regia (HCl : HNO3 3:1) extracts, while Hg content was measured using a cold-vapor atomic absorption spectrophotometer (AAS) (Kacar and İnal, 2008). Soil-available Fe, Cu, Zn, and Mn were leached using a DTPA (diethylenetriaminepentaacetate) solution (Lindsay and Norvell, 1978), and subsequently analyzed via AAS (Hanlon, 1992). Boron content was assessed through hot-water extractions using a spectrophotometer (Wolf, 1971).

Plant height (cm), which measures the distance from the soil surface to the top of the panicle, was recorded. The average plant heights of 10 specimens and the yield from each pot after clipping were documented weekly starting from May 29 (Uzun and Bilgili, 2011). Throughout the experiment, each plot was mowed seven times using hand-shears. Prior to oven drying, leaves were thoroughly rinsed with distilled water to eliminate any adhering debris. For dry biomass determinations, leaves were separated during harvest and then oven-dried at 70 °C for 48 h. Leaf dry weight was recorded at the conclusion of each clipping.

Plant samples were ground using a stainless-steel mill for plant nutrient analysis. The results were reported on a dry matter (105 °C) basis. Macro- and microelements, along with heavy metal analyses in the plant (Festuca arundinacea), were conducted using the last clipping (seventh) plant samples. Total nitrogen (N) analysis followed the modified Kjeldahl method (Bremner, 1965). Wet plant samples were digested with a mixture of concentrated HNO_3 and concentrated $HClO_4$ at a 4:1 (v / v) ratio for plant nutrient analysis (Lou et al., 2013). Spectrophotometric analysis for total P was performed using the vanadomolibdo phosphoric yellow color method (Lott et al., 1956). Total concentrations of K, Ca, and Na were determined using a flame photometer. Total Mg, Fe, Zn, Cu, Mn, Ni, Pb, Cd, and Cr were measured using AAS. Mercury (Hg) levels were assessed using the cold vapor AAS (Kacar and İnal, 2008). Boron (B) concentration was determined spectrophotometrically using the azomethine-H method (Wolf, 1971).

Bioconcentration factors (BCF) were calculated using the formula proposed by Chang et al. (2014):

BCF = metal concentration in the plant (mg kg^{-1}dw) / metal concentration in the soil

Color measurements (ΔE) were conducted using a Konica Minolta spectrophotometer. Measurements were taken from eight different areas of the plant in accordance with CIE Lab (1976) standards. Color differences were determined between the treated and untreated SS treatment under the CIE 100 standard observer angle and CIE standard D65 light source. In the components of the CIE Lab color space, L* represents the lightness of color, while a* and b* indicate the color (Mclaren, 1976). The color difference (ΔE), which indicates the total color difference, was calculated as follows after Fairchild and Pirrotta (1991) and Brainard (2003): $\mathbf{\Delta E} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$(1).

The coverage class score scale (1–9) was determined according to Morris and Shearman (1998).

The geoaccumulation index (I_{geo}) is a commonly used method for assessing soil contamination (Chung and Chon, 2014). It compares current element concentrations with preindustrial concentrations to identify increasing metal contamination levels. The geoaccumulation index was calculated using the following equation, as proposed by Müller (1969) and Adimalla et al. (2019):

$I_{geo} = \log_2(Ci / 1.5 Cb)$

where Ci represents the concentration of heavy metal in the soil, and Cb is the concentration of the heavy metal in the background sample.Müller (1969) divided the geoaccumulation index into seven classes, which are as follows: ($I_{geo} \leq 0$) practically uncontaminated; ($0 < I_{geo} < 1$) uncontaminated to moderately contaminated; ($1 < I_{geo} < 2$) moderately contaminated; ($2 < I_{geo} < 3$) moderately to heavily contaminated; ($3 < I_{geo} < 4$) heavily contaminated; ($4 < I_{geo} < 5$) heavily to extremely contaminated, and ($5 \leq I_{geo}$) extremely contaminated.

2.3. Statistical analysis

Analysis of variance for a randomized block design was used to test for significant differences between treatment means. The LSD post hoc test was then used to determine which means differed. A p-value of <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS Statistics 20.0 software.

3. Results and discussion

3.1. Coverage score level

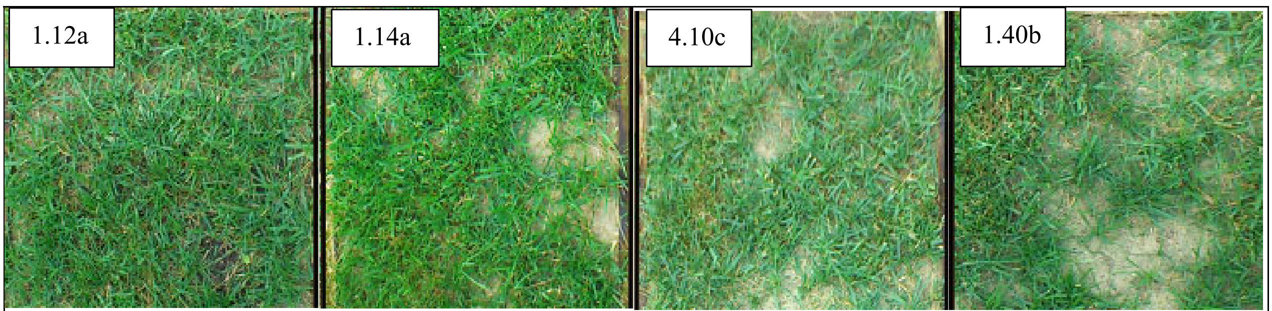
The coverage scores were listed as follows: 6% SS (5.7) > 3% SS (5.3) > 9% SS (4.7) = C (4.7). A coverage score of 5 corresponds to a coverage area of 40%–60%. It was suggested that the variation in coverage class score of one cool-season turfgrass species, tall fescue (*Festuca arundinacea* Schreb.), could be affected by temperature (Zere and Bilgili, 2022). These findings suggest that tall fescue showed better growth in soil with 6% SS during the early stages, while long-term growth was better in those with 3% SS (Figure 1). In a study conducted by Nematollahi et al. (2018), the increase in sludge dose from 5% to 10% resulted in a decreasing trend in coverage scores (from 7.05 to 5.86) and color scores (ranging from 6.94 to 6.80) on a scale of 1 to 9, which is consistent with our results. Instead of counting the number of plants in the plots, the coverage percentage after sowing was determined using a 45-day coverage class score scale (1–9).

3.2. Color unit (ΔE) value

A color difference was considered to exist when the color difference (ΔE) value exceeded one. The color values (ΔE) for the control, 3% SS, and 9% SS applications were closer to each other, while the color difference for 6% SS was greater. The highest ΔE value was recorded in the 6% SS application with a ΔE of 4.10, while the smallest was in the control application with a ΔE of 1.12 (Figure 2). All ΔE values exceeded one, indicating that the color change increased with higher ΔE values. A color ΔE value between 1.0 and 2.0 suggests a minimally detectable color change, while a value between 4.0 and 5.0 indicates a significant color difference (Mokrzycki and Tatol, 2011). Due to the high organic matter content (51.13%) in the sewage sludge, the nitrogen content is also elevated. Nitrogen is a crucial element for turfgrass color and growth rate. Bilgili and Acikgoz (2005) demonstrated that turf color and quality were influenced by SS treatments, and that increased nitrogen significantly enhanced color. The highest color and quality values were achieved at lower temperatures in spring and autumn, which can be attributed to temperature being a critical factor limiting the growth of cool-season

Control (4.7 score)	3% SS (5.3 score)	6% SS (5.7 score)	9% SS (4.7 score)

Figure 1. Effects of application on coverage class score scale on *F. arundinacea* Schreb.



Control (** p < 0.01)	3% SS (** p < 0.01)	6% SS (** p < 0.01)	9% SS (** p < 0.01)

Figure 2. Results of visual *F. arundinacea* Schreb. color unit (ΔE).

grasses (Jiang and Huang, 2001). The decrease in color ΔE parameter in the 9% SS application may be related to the heavy metal content. Liao and Huang (2002) reported that heavy metal toxicity increased gradually with the rising concentration of low molecular weight organic acids, leading to a reduction in chlorophyll content and biomass of ryegrass.

3.3. Plant clip biomass and plant height

Sewage sludge doses had varying effects on biomass yields; the highest biomass yield (9.11 g) was obtained with the 3% SS dose, while the lowest (7.67 g) was observed with the 9% SS dose (Table 4). The aboveground biomass yields of tall fescue (*Festuca arundinacea* Schreb.) in soil amended with different levels of SS applications. The tall fescue biomass peaked with the addition of 3% SS, reaching a maximum of 9.11 g. This represented an 11% increase compared to the control. Conversely, biomass tended to decrease with increasing SS dose, reaching a minimum value of 7.67 g with the 9% SS application. This result differed significantly from that of Cheng et al. (2007), who found that ryegrass biomass reached its maximum with a 20% sludge compost amendment. Differences in soil and SS characteristics may account for the disparity in research results. The clipping yield exhibited high variability and was dependent on seasons, application times, and N sources (Bilgili et al., 2011). Therefore, treated SS can support plant growth depending on the application dose, owing to its relatively higher nutrient content (Toze, 2006). The effect of sewage sludge on biomass is also noted by Hua et al. (2008), as it provides a source of plant nutrients for production. Plant biomass production research studies by Gubisova et al. (2020) reported yield increases due to the addition of sewage sludge. The increase in biomass yields with increasing SS treatment doses could be attributed to the rise in soil organic matter content and improved soil physical properties (Eid et al., 2017). The highest biomass was obtained in the 6th clipping, while the smallest biomass was recorded in the 1st clipping. As shown in Table 5, the plants with the 3% SS dose had the highest average grading value, followed by 6% SS, the control group, and finally, 9% SS, listed in the following order: 3% (9.5 cm) > 6% (8.8 cm) > control (8.7 cm) > 9% (8.1 cm). Gubisova et al. (2020) reported that the height of the plants gradually increased with the SS dose.

3.4. Bioconcentration factor (BCF) and geoaccumulation index (I_{geo})

The bioconcentration factors (BCF) for the studied heavy metals were as follows: Ni (0.20–0.28), Cd (0.24–0.78),

Table 4. Clipping yield of *F. arundinacea* Schreb. under different sewage sludge (SS)

Clipping time								
Applications	1	2	3	4	5	6	7	Total (g)
Control	1.08 b	1.14 b	1.11 b	1.16 b	1.28 b	1.25 b	1.18 ab	8.20 ab
3% SS	1.15 a	1.17 a	1.21 a	1.41 a	1.48 a	1.45 a	1.25 a	9.11 a
6% SS	1.07 b	1.09 b	1.10 b	1.14 b	1.26 b	1.24 b	1.15 b	8.05 b
9% SS	1.04 b	1.07 b	1.08 ab	1.08 ab	1.15 c	1.15 c	1.10 b	7.67 b
Significant	*	*	*	*	**	**	**	**

* $p < 0.05$, ** $p < 0.01$

Table 5. Effects of sewage sludge application on plant height.

	1	2	3	4	5	6	7	8	
Applications	29.05.2015	05.06.2015	12.06.2015	19.06.2015	26.06.2015	03.07.2015	10.07.2015	15.07.2015	Average (cm)
Control	5.0	13.1	11.0	10.0	8.2	9.8	6.3	6.0	8.7
3% SS	5.0	13.8	13.2	10.7	10.7	11.5	5.5	5.8	9.5
6% SS	5.0	12.9	12.5	10.0	10.3	9.8	4.8	5.3	8.8
9% SS	5.0	13.4	12.8	9.2	7.8	7.3	4.0	4.8	8.1

Cr (0.37–0.54), Pb (0.06–0.09), and Hg (0.34–0.70). On average, the BCFs for the heavy metals were ordered as Cd (0.542) > Hg (0.452) > Cr (0.448) > Ni (0.246) > Pb (0.076) (Table 6). The lower BCF values observed for Pb are likely due to its lower mobility from soil to plant tissues (Pusz et al., 2021). Cadmium displayed the highest average BCF for *F. arundinacea*, with a value of 0.54. Higher BCF values indicate a greater ability for metal elements to migrate into plants. The BCF values of metals indicated that differences in the study area depended not only on the soil properties but also on the biological characteristics of the species and variations between species (Cai et al., 2019). In the soil samples taken after the experiment, the pH changes according to the SS applications were listed as follows: soil pH: C (7.69) > 3% SS (7.47) > 6% SS (7.26) > 9% SS (7.10). Soil pH had an effect on the bioconcentration factor (BCF) values. It has been noted that the bioconcentration (BCF) value of the leaf in alkaline soil is higher than in acidic soil (Liu et al., 2016). This further enables the categorization of plants as accumulators (BCF > 1) or excluders (BCF < 1) of trace elements (Olowoyo et al., 2010). The bioconcentration factor is a crucial index for assessing the phytoremediation capability of plants. It has been well-documented that plants with BCF values greater than 1 are likely to be effective for phytoextraction (Chanu and Gupta 2016). A bioconcentration factor of less than 1 indicates a higher concentration of heavy metals in the soil than that taken up by plants (Hellen and Othman, 2016). Our findings showed that all schemes had low BCF values for Ni, Cd, Cr, Pb, and Hg, indicating that heavy metals were lower in plant samples than in the soil samples.

Geoaccumulation index (I_{geo}) values varied between 0.55 and 0.70 for Ni, between −0.30 and 0.25 for Pb, between 1.53 and 1.69 for Cd, between −1.35 and 0.81 for Cr, and between −0.30 and 0.54 for Hg. All present I_{geo} values ranged from −1.35 to 1.69 (Table 6). The lowest geoaccumulation index was obtained for Cr and the highest for Cd. Geoaccumulation index values indicated moderate to high contamination in experimental soils, including control plots. The geoaccumulation index analysis indicates that no Ni, Cd, Cr, Pb, and Hg exist in soils from the study area. I_{geo} for Pb and Cr in the studied soils were negative. The negative I_{geo} values show that the soils studied are practically uncontaminated with Pb and Cr. This implies that the Pb and Cr content of the soils is not from anthropogenic sources but rather from natural sources. The difference in our results compared to Radomirovic et al. (2020), (Ni = 1.6, Cd = 1.8, Cr = 1.9, Pb = 3.3, and Hg = 1.4) average I_{geo} value can be explained by SS applications and soil pH. The maximum I_{geo} value was 1.69, indicating moderate soil contamination according to Müller (1969) ($1 < I_{geo} < 2$: moderately contaminated). All the I_{geo} values, except for those of Cd, were < 1, meaning that all the treatments fell under the unpolluted to moderately polluted category. The geoaccumulation index (I_{geo}) of cadmium was higher than that of the other metals, perhaps due to the use of chemical fertilizers in the farmlands. This result was supported by the observations made by Karimi et al. (2020). Geoaccumulation index (I_{geo}) is used as an indicator to identify and quantify the degree of elemental pollution and to assess the intensity of anthropogenic contaminants accumulated in sediment (Barbieri 2016).

3.5. Soil total high metals

Total heavy metal levels in the soil differed significantly among the sewage sludge applications ($p < 0.05$). Compared to the control, Ni concentration was significantly higher ($p < 0.05$) in the SS treatments (Table 7). As the SS dose

Table 6. Effects of application on bioconcentration factor (BCF) and geoaccumulation index (I_{geo}).

Applications	Ni	Cd	Cr	Pb	Hg
Control	0.20	0.24	0.43	0.08	0.70
3% SS	0.28	0.41	0.45	0.06	0.34
6% SS	0.25	0.74	0.37	0.08	0.43
9% SS	0.25	0.78	0.54	0.09	0.34
Average	0.246	0.542	0.448	0.076	0.452
The geoaccumulation index (I_{geo})					
Control	0.55	−0.30	1.53	−1.35	−0.30
3% SS	0.64	−0.05	1.63	−1.15	0.32
6% SS	0.66	0.04	1.63	−0.81	0.41
9% SS	0.70	0.25	1.69	−0.83	0.54
Average	0.64	−0.02	1.62	−1.04	0.24

increased, total Ni contents increased from 44.13 mg kg^{-1} in the control plot to 48.87 mg kg^{-1} in the 9% SS treatment. Soil total Pb concentration significantly ($p < 0.05$) increased with increasing SS treatments. Soil total Pb content increased from 18.337 mg kg^{-1} in the control plot to 26.83 mg kg^{-1} in the 9% SS treatments. The phytotoxic threshold for soil total Pb is reported as 100 mg kg^{-1} (Kabata-Pendias, 2011). Soil total Cd concentration was not significantly affected by the SS dose. Soil total Cd content ranged from 0.87 to 0.97 mg kg^{-1}. These values were quite below the threshold value of 3 mg kg^{-1} reported by Kabata-Pendias (2011). Soil total Cr content also significantly ($p < 0.05$) increased with increasing SS dose. The highest value was obtained from the 6% SS treatment (50.80 mg kg^{-1}), and the lowest from the control treatment (35.20 mg kg^{-1}). Sewage sludge percentage significantly influenced soil Hg concentrations ($p < 0.05$). Soil Hg concentration varied between 84.803 and 153.125 µg kg^{-1}. The threshold values of soil heavy metals reported in Türkiye are: 100 mg kg^{-1} for Pb, 1.5 mg kg^{-1} for Cd, 100 mg kg^{-1} for Cr, 70 mg kg^{-1} for Ni, and 1.5 mg kg^{-1} for Hg (Ministry of Environment and Forestry, 2010)

3.6. Plant macro- and microelement content

Sewage sludge dosage had a significant effect on the total nitrogen (N) content of plants ($p < 0.05$). The highest plant N content (3.93%) was obtained from the 9% SS treatment (Table 8).

Akviros et al. (2006) observed a difference in nitrogen content of tall fescue plants at a dose of 50 t ha^{-1}, which is the highest application of sewage sludge. In our study, we were able to detect a difference in the test plant for sewage sludge to which we applied a dose of approximately 230 t ha^{-1}.

The plant phosphorus (P) content was significantly influenced by the SS dose ($p < 0.05$). The highest plant P content (0.51%) was obtained from the 9% SS treatment, and the lowest (0.34%) from the control treatment. The effects of SS dose on plant potassium (K) and magnesium (Mg) contents were not significant. Plant K contents varied between 2.57% and 2.93%, with the highest value in the 6% SS treatment. Plant Mg content ranged from 0.55% to 0.59%, with the highest value in the 9% SS treatments.

Sewage sludge dosage significantly influenced plant calcium (Ca) content ($p < 0.01$). The highest plant Ca content (1.77%) was obtained from the 9% SS dose. The effect of SS dose on sodium (Na) content of *F. arundinacea* plants was significant ($p < 0.05$). The highest plant Na content (1029 mg kg^{-1}) was obtained from the 3% SS dose, and the lowest (752 mg kg^{-1}) from the control treatment.

The effects of SS dose on plant iron (Fe) and copper (Cu) contents were not significant. Plant Fe content varied between 361 and 506 mg kg^{-1}, and Cu contents varied between 7 and 10 mg kg^{-1}. Madyiwa et al. (2002) reported Cu content of grass plants grown in SS-treated soils between 34 and 45 mg kg^{-1}. Values recorded in this study were within the sufficient range as indicated by Jones (1980) (5–20 mg kg^{-1}). The highest plant Fe content was obtained from the 3% SS dose.

Plant zinc (Zn) content increased with SS dose. Significant differences were observed in Zn content of *Festuca* plants among different SS doses ($p < 0.05$). The highest plant Zn content (127 mg kg^{-1}) was obtained from the 9% SS dose. According to the sufficient plant Zn levels of Jones (1980) (22–30 mg kg^{-1}), present values were sufficient. Similar to our findings, Madyiwa et al. (2002) reported plant Zn content between 22 and 210 mg kg^{-1}.

Sewage sludge dosage had a significant effect on plant manganese (Mn) content ($p < 0.05$). The highest plant Mn content (82 mg kg^{-1}) was obtained from the control treatment, and the lowest (48 mg kg^{-1}) from the 9% SS dose. Bennett (1996) reported sufficient plant Mn levels as being between 20 and 300 mg kg^{-1}, and all the values recorded in this study were within this sufficient range. Mn values were also below the toxic level of 300 mg kg^{-1}.

The effects of SS dose on boron content of *Festuca* plants were not significant. The highest plant boron content (17 mg kg^{-1}) was obtained from the 9% SS dose, followed by the 3% SS dose (16 mg kg^{-1}).

Table 7. Value of soil total heavy metal according to application.

Applications	Ni	Pb	Cd	Cr	Hg
	(mg kg^{-1})				(µg kg^{-1})
Control	44.13 b	18.33 b	0.87	35.20 c	84.80 b
3% SS	46.86 ab	21.58 ab	0.93	40.06 ab	131.16 ab
6% SS	47.66 ab	23.21 ab	0.93	50.80 a	139.33 a
9% SS	48.87 a	26.83 a	0.97	49.67 a	153.12 a
Significant	*	*	ns	*	*

* $p < 0.05$, ** $p < 0.01$, ns: not significant

The increasing SS rates significantly increased the concentrations of total N, P, K, Mg, and Ca in *Festuca arundinacea* compared to the control. The 6% SS application increased the total N concentration to 3.70%, while the 9% SS application increased it to 3.93%. Applications of increasing SS rates significantly increased the plant P concentrations about two-fold at the highest sludge rate (from 0.34% to 0.51%). Increasing SS rates applications significantly increased the K concentration in *Festuca arundinacea* compared to the control, increasing from 2.57% to the highest value of 2.93% in the 6% SS applications. Applications of *F. arundinacea* plant had no significant effect on Mg (%) concentrations and varied between 0.55 and 0.59. The highest plant Ca (1.77%) was obtained from 9% SS, while the lowest concentration (0.97%) value was obtained from control treatments. The increased concentrations of N, P, K, Ca, K, Na, Fe, Mn, Cu, Zn, and B can be attributed to more significant plant biomass growth, possibly related to better root system development and physical soil properties of the 3% SS application rate (Arlo et al., 2022). Plant nutrients were found to be at a sufficient level according to the reference values of Jones (1980), N (2.75–4.2%), P (0.3–0.55%), K (1.0–2.5%), Ca (0.5–1.25%), Mg (0.2–0.6%), Fe (30–100 mg kg^{-1}), Mn (20–150 mg kg^{-1}), Zn (20–55 mg kg^{-1}), B (10–60 mg kg^{-1}), Cu (5–20 mg kg^{-1}). Singh and Agrawal (2008) reported that the nutrient accumulation pattern in crops grown in sludge-amended soils varies with the type of soil, species, phenology, and the chelating effect on metals.

3.7. Plant heavy metal contents

Experimental treatments had significant effects on the plant nickel (Ni) content ($p < 0.05$). The highest Ni content in plants (13 mg kg^{-1}) was obtained from the 3% SS treatment, while the lowest (9 mg kg^{-1}) was from the control treatment (Table 9). Michalk et al., (1996) reported Ni contents in grass plants grown in SS-treated soils ranging from 4.0 to 10.0 mg kg^{-1}. Kabata-Pendias (2011) suggested an optimal plant Ni content range of 0.02–5 mg kg^{-1}. The values obtained in this study exceeded this range of optimal values.

SS treatments also had a significant effect on plant cadmium (Cd) content ($p < 0.01$). The highest value (0.76 mg kg^{-1}) was obtained from the 9% SS treatment, while the lowest (0.21 mg kg^{-1}) was from the control treatment. Plant chromium (Cr) content ranged from 15 to 27 mg kg^{-1}, and the effects of SS treatments on plant Cr content were significant ($p < 0.01$). The highest value was observed in the 9% SS treatment, and the lowest in the control treatment. Mengel and Kirkby (1987) reported critical Cr levels between 1.0 and 2.0 mg kg^{-1}. The values recorded in this study exceeded these critical levels.

Sewage sludge treatments significantly influenced plant lead (Pb) content ($p < 0.05$). The highest plant Pb content was obtained from the 9% SS treatment. Plant Pb contents recorded in this study were similar to those reported in Madyiwa et al. (2002) (ranging from 1.0 to 1.5 mg kg^{-1}).

The effects of SS treatments on mercury (Hg) content of *F. arundinacea* plants were not significant. Plant Hg content varied from 44.62 to 59.45 µg kg^{-1}. The increase in

Table 8. Effects of application on macro- and micronutrients of *Festuca arundinacea* Schreb.

Applications	N	P	K	Mg	Ca
	(%)				
Control	3.70 b	0.34 b	2.57	0.55	0.97 b
%3 SS	3.73 ab	0.40 ab	2.70	0.56	1.33 ab
%6 SS	3.67 b	0.46 a	2.93	0.56	1.40 ab
%9 SS	3.93 a	0.51 a	2.67	0.59	1.77 a
Significant	*	*	ns	ns	**
Applications	Fe	Cu	Zn	Mn	B
	(mg kg^{-1})				
Control	361	10	102 bc	82 a	15
%3 SS	506	8	117 ab	67 ab	16
%6 SS	349	9	93 c	63 ab	15
%9 SS	404	7	127 a	48 b	17
Significant	ns	ns	*	*	ns

* $p < 0.05$, ** $p < 0.01$, ns: not significant

the SS rate significantly increased the concentrations of Ni, Pb, Cd, Cr, and Hg, which was important in comparison to the control soil. This observation may be explained by the decrease in soil pH (Sukreeyapongse et al., 2002), which made the heavy metals more available with the increase in SS rates (Eid et al., 2017).

3.8. Soil available plant microelements (Na, Fe, Cu, Zn, Mn, and B)

Soil available Na contents significantly ($p < 0.05$) increased with increasing SS doses, except for 3% SS, compared to the control plots. The highest available Na concentration was obtained from the 9% SS treatment (259.44 mg kg^{-1}), while the lowest was in the 3% SS treatment (128.15 mg kg^{-1}) (Table 10). These findings are consistent with the results of Kumar and Chopra (2014), who reported increased salinity levels with SS treatments. Sodium might have affected EC values (Cristina et al., 2020).

Soil available Fe concentrations significantly ($p < 0.05$) increased with increasing SS doses compared to the control plots. The highest available Fe concentration was seen in the 9% SS treatment (13.61 mg kg^{-1}), while the lowest was in the control treatment (4.74 mg kg^{-1}). These findings on available Fe contents were considerably lower than the values reported by Bıyıklı et al. (2020), ranging from 24.8 to 28.7 mg kg^{-1}.

Increasing SS treatments significantly ($p < 0.01$) increased soil available Cu concentrations compared to the control. With increasing SS doses, soil available Cu contents increased from 1.07 mg kg^{-1} in the control plots to 4.05 mg kg^{-1} in the 9% SS treatment. The available Cu levels likely increased due to the higher organic matter (OM) contents with SS treatments. Positive correlations were reported between organic matter content and available Cu level in the soils (Sipkova et al., 2014).

Increasing SS doses had a positive effect ($p < 0.01$) on available Zn contents. With increasing SS doses, soil available Zn contents increased from 2.90 mg kg^{-1} in the control plots to 17.76 mg kg^{-1} in the 9% SS treatment. Available Mn concentrations did not significantly change with SS treatments. The highest available Mn was obtained from the 9% SS treatment (5.85 mg kg^{-1}), while the lowest was from the 3% SS treatment (4.69 mg kg^{-1}). The highest available Cu concentration was seen in the 9% SS treatment (4.05 mg kg^{-1}), and the lowest in the control treatment (1.07 mg kg^{-1}).

Sewage sludge increased Cu content. Present SS treatments did not pose any health risks or show any pollution risk. The lowest B content was obtained from the control treatment (0.32 mg kg^{-1}), and the greatest value was obtained from the 9% SS treatment. Bozkurt and

Table 9. Effects of application on high metals of *Festuca arundinacea* Schreb.

Applications	Ni	Cd	Cr	Pb	Hg
	(mg kg^{-1})				(µg kg^{-1})
Control	9 b	0.21 b	15 b	1.4 b	59.08
3% SS	13 a	0.38 ab	18 ab	1.4 b	44.62
6% SS	12 a	0.69 ab	19 ab	1.9 ab	59.45
9% SS	12 a	0.76 a	27 a	2.4 a	51.34
Significant	*	**	**	*	ns

*$p < 0.05$, ** $p < 0.01$, ns: not significant

Table 10. Available of soil micronutrients according to application

Applications	Na	Fe	Cu	Zn	Mn	B
	(mg kg^{-1})					
Control	143.78 ab	4.74 b	1.07 b	2.90 c	5.22	0.32 b
3% SS	128.15 b	7.34 b	2.14 ab	8.57 bc	4.69	0.53 ab
6% SS	171.91 ab	8.87 ab	3.23 ab	13.31 ab	5.72	0.84 a
9% SS	259.44 a	13.61 a	4.05 a	17.76 a	5.85	1.00 a
Significant	*	*	**	**	ns	*

* $p < 0.05$, ** $p < 0.01$, ns: not significant

Cimrin (2003) reported increasing available Fe, Mn, Zn, and Cu concentrations with increasing SS treatment doses. Elements such as Cu, Fe, Mn, and Zn become more soluble under acidic conditions (Eid et al., 2017).

4. Conclusion

Our findings indicate that the 3% SS treatment was the best application for plant growth and development. For heavy metals (Ni, Cd, Cr, Pb, and Hg), toxicity symptoms were not observed in *F. arundinaceae* plants. The soil heavy metal content was also below the specified thresholds or toxic levels. It was concluded that sewage sludge treatments positively affected the growth and development of *F. arundinaceae*. In terms of geoaccumulation index (I_{geo}), soil heavy metal values were all at normal levels.

Acknowledgments

We are grateful to Ege University Planning and Monitoring Coordination of Organizational Development and Directorate of Library and Documentation for their support in editing and proofreading services for this study. This study was financially supported by İzmir Water and Sewerage Administration General Directorate (İZSU) of İzmir Metropolitan Municipality. The authors would like to express their gratitude for this support.

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