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The Effect of Biological Secondary Treated Domestic Wastewater on Agronomic Properties and Element Content of Some Forage Plants

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ABSTRACT
This study was carried out to determine the effect of advanced biological treated domestic wastewater on the agronomic properties and nutritional content of some forage crops, as a result of the increasing interest in using treated wastewater in agriculture. The study of diluting biologically treated domestic wastewater with varying amounts of pure water 25%, 50%, and 75% pure water, and applied to crested wheatgrass (Agropyron cristatum Geartn), orchard grass (Dactylis glomerata L.) and alfalfa (Medicago sativa L.) at field capacity. The trial plants used were alfalfa, orchard grass, and crested wheatgrass. The application of the diluted wastewater influenced the height of all three plants. After the first cutting, both alfalfa and orchard grass had increased green and dry weights per pot, with the most significant increase observed in alfalfa. The application of wastewater resulted in an increase in nutrient and metal concentrations in all three plants. Additionally, the concentration of selenium decreased in alfalfa and crested wheatgrass. The diluted wastewater did not affect the calcium concentration in alfalfa. However, it had a positive effect on the relationship between orchard grass and all elements, except for selenium which had a negative effect. Moreover, the application of wastewater led to an increase in soil organic matter and electrical conductivity, while decreasing the pH with increasing wastewater ratio. Moreover, the application of wastewater led to an increase in soil organic matter and electrical conductivity, while decreasing the pH with increasing wastewater ratio. The concentration of nutrients and metals was significantly affected by the application of diluted domestic water. This was due to the increased uptake of plant elements, which in turn was associated with responses in soil organic matter and electrical conductivity. It may be advisable to conduct long-term field studies to determine the dilution rate at which the salinity risk threshold can be established.

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İleri Biyolojik Evsel Atık Suyunun Bazı Yem Bitkilerinin Tarımsal Özellikleri ve Element İçeriklerine Etkisi

ÖZET
Bu çalışma, arıtılmış atık suların tarımda kullanımına olan ilginin artmasına sonucunda, evsel ileri biyolojik atık suyunun bazı yem bitkilerinin tarımsal ve besin içeriğine etkisini belirlemek amacıyla yürütülmüştür. Biyolojik işlemlerle arıtılan evsel atık su, saf su ile seyreltilerek otlak ayrığı (Agropyron cristatum Geartn), domuz ayrığı (Dactylis glomerata L.) ve yoncanın (Medicago sativa L.) bazı tarımsal özellikleri ve element içerikleri üzerine etkileri araştırılmıştır. Atık su %25, %50 ve %75 oranlarında saf su ile seyreltilerek tarla kapasitesine göre saksılara uygulanmıştır. Seyreltilmiş atık su uygulamasının otlak ayrığı, domuz ayrığı ve yoncanın bitki boyuna etkisi önemli olmuştur. İlk biçmeden itibaren yonca ve domuz ayrığının saksı başına yeşil ve kuru ağırlıkları artmış, en belirgin artış ise yoncada belirlenmiştir. Seyreltik arıtma suyu oranları üç bitkide de besin elementi ve metal

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konsantrasyonlarının artmasına neden olurken, yonca ve otlak ayrığında Se konsantrasyonunun azalmasına neden olmuştur. Her iki buğdaygil türünden farklı olarak atıksu oranı yonca Ca konsantrasyonunu önemli ölçüde etkilememiştir. Seyreltik atık su domuz ayrığının tüm elementlerle ilişkisini önemli ve pozitif olarak etkilerken, Se ile negatif yönde etkilemiştir. Atık su seyreltme oranlarının artmasıyla toprağın organik maddesi ve elektriksel iletkenlik ile toprak pH'sı düşmüştür. Seyreltilmiş evsel su uygulaması, toprağın organik maddesi ve elektriksel iletkenlik tepkileriyle ilişkili olarak bitki elementi alımının artmasına aracılık ederek besin ve metal konsantrasyonlarında önemli rol oynamıştır. Tuzluluk risk eşiğinin belirlenebileceği sulandırma oranı için, uzun süreli arazi çalışmalarının yapılması gerekmektedir.

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INTRODUCTION

Due to the rapid development and population growth in Turkey, there has been an increase in industrial, urban, and agricultural activities. As a result, pollutants from these activities have contaminated water sources (Khan & Avari, 2014). The country's population exceeded 85 million in 2023 (TUİK, 2023), which presents a significant challenge in meeting the demand for drinking, utility, and agricultural water. According to recent research conducted by Phasinam et al. (2022) and Wu et al. (2022), it has been found that more than 70% of the world's water resources are utilized for irrigation purposes. As agricultural activity continues to grow, globally water resources is also increasing, both on a global scale and within our country. The World Economic Forum has identified the water crisis as one of the five most significant global risks. Currently, it is a fact that a quarter of the world's population faces significant water stress (WRI, 2019). If water consumption continues at the current rate, it is predicted by Du et al. (2022) that two-thirds of the global population will live in regions with severe water stress by 2050. Therefore, the use of treated wastewater for agricultural irrigation has become increasingly important, as highlighted by Perez et al. (2015) and Aşık & Özsoy (2016). Local administrators and policymakers have made efforts to use recycled water for irrigation instead of discharging it into natural receiving environments, as noted by Rahman et al. (2016). This approach can contribute to the health of freshwater ecosystems. According to FAO (2017), the annual amount of N, P, and K used in agriculture globally was 186.67 million tons in 2015, and it is expected to reach 201.66 million tons by the end of 2020. The impact of treated wastewater on plant growth is influenced by the chemical properties of the water and the type of plant (Erdoğan et al., 2009; Shahrivar et al., 2019). Additionally, it is worth noting that treated wastewater can serve as a viable alternative to chemical fertilizers, particularly in terms of macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sodium, which have both economic and ecological importance (Arvas & Çelebi, 2023). However, it is worth noting that domestic wastewater treatment may result in higher soil salt and Na concentrations compared to other cations, which could potentially have a negative impact on plant growth and yield (Becerra-Castro et al., 2015; Arvas et al., 2022; Ngara et al., 2012; Levy et al., 2013).

To investigate the impact of the environmental pollutants of wastewater treatment facility water mixed with certain proportions of distilled water, as an alternative to chemical fertilizers with high cost, this study examined various dilute wastewater rates (25%, 50%, and 75%) on crested wheatgrass, orchard grass and alfalfa.

MATERIAL and METHOD

Trial Plants

Alfalfa (*Medicago sativa* L. 'Bilensoy 80' cultiv.) is a perennial forage legume plant that is resistant to winter, drought, and lodging except in the year of planting, and can be harvested in the first week of June under Anatolian conditions. Having the ability to grow after mowing increases the number of mowings. (TIGEM, 2024)

Orchard grass (*Dactylis glomerata* L. 'Amba' cultv.) is a long-life, drought-resistant cool seasonal grass forage plant and the best in terms of vegetative and generative shoots length, number of shoots/bush, and foliar area/shoot. (Corches & Moisuc, 2010).

Crested Wheatgrass (*Agropyron cristatum* L. (gaertn) 'Fairway' cultv.) is a long-lived with a deep root system, drought-resistant cool seasonal grass forage plant. It is used extensively for reclamation, stabilization and erosion control. introduced/released 1983, from Ankara Turkey. (Great Basin Seed, 2024).

Biological Treated Domestic Wastewater

The wastewater used in the research was taken from the Biologically Treated Domestic Wastewater (BTDW) discharge point after removal of carbon, nitrogen, and phosphorus at the Ministry of Environment and Urbanization, Housing Development Administration (TOKİ) housing located at 34° 59′ 14.30″ E and 42° 53′ 265.20″ N (Table 1).

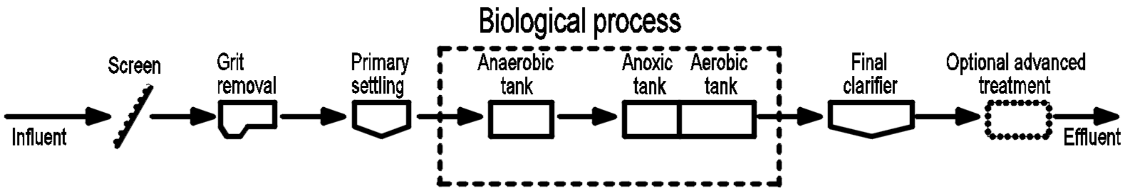

Figure 1. The flow of chart biologically treated process wastewater treatment (Van Metropolitan Municipality, VASKİ, Arvas et al., 2022)
Şekil1. Atıksu arıtma tesisinin biyolojik arıtım işlemi akış şeması (Van Büyük Şehir Belediyesi, VASKİ, Arvas et al., 2022).

Table 1. Some characteristics and limit values for wastewater and soil in the research
Çizelge 1. Çalışmada kullanılan toprak ve atıksuyun bazı özellikler ve sınır değerler

Measurement	Soil	Wastewater	Limit value*
Texture class	Loamy	-	-
Sand (%)	29.20	-	-
Clay (%)	42.80	-	-
Silt (%)	28.0	-	-
pH	8.45	7.81	6-9
EC (dS/m)	0.285	0.654	0.250-3
Organic matter (%)	1.27	-	-
Nitrate (ppm)	-	226.27	5-20
Ca (%)	0.627	0.013	75-200
Mg (%)	0.147	0.006	50-150
Na (%)	0.045	0.013	0.025
Fe (ppm)	102.2	0.004	0.3-1
Cu (ppm)	0.113	4.41	0.2-5
Zn (ppm)	0.213	8.84	2-10
Mn (ppm)	2.471	9.02	0.2-10
Mo (ppm)	nd**	nd**	0.01-0.05
Se (ppm)	nd**	nd**	0.02-0.2
As (ppm)	0.116	0.891	0.1-2
Pb (ppm)	0.026	0.159	5-10

*Regulation Amending the Regulation on Surface Water Quality Management, April 15, 2015, and Official Gazette No. 29327 of Turkish Republic, converted from the European Union legislations.
** nd: non detected

Plant Growth Condition

The experiment was carried out in three repetitions in pots with a height of 22.5 cm, a base diameter of 7.5 cm, and a top diameter of 10 cm, according to the randomized plot design. The soil used for the pots was obtained from a field that had previously been used for alfalfa production, at a depth of 0-30 cm. Wastewater (WW) was diluted by 75%, 50%, and 25% with distilled water (DW). 30 kg N ha^{-1} and 80 kg P_2O_5 ha^{-1} were applied to the control pot (100% pure water) according to the volume calculation (Kacar & Katkat, 2009). Clover, crested wheatgrass, and orchard grass were sowed separately in each pot and thinned to five plants per pot after emergence. The pots were subsequently moved to a climate-controlled room, where the temperature was set to 22/18 ℃ (day/night), the humidity was maintained at 65%, and a 16/8-hour day/night photoperiod was established. After the wastewater taken from the discharge point was diluted with distilled water, it was applied to the potting soil with 20% field capacity with 400 ml at 8-day intervals. Since the aim of the study was accumulating nutrients and the vegetative development of the species, the vernalization process has been neglected. The buds formed in the root crown of grasses form more vegetative parts without being vernalized. Plant morphology was affected by vernalization via a decrease in biomass resulting from a reduced tiller number, the time to panicle emergence and the number of leaves on the main stem were reduced by the chilling treatment in the field and greenhouse experiments (Chauvel et al, 2002).

The mowing was started one month after sowing and then repeated at 15-day intervals. Plant height was measured, and then plants were cut to a height of 3 cm to simulate grazing by sheep. The total green weight of the plant was measured in the pot, and then the plant was dried at 65 °C until a fixed weight was reached. The dry weight was calculated as g/pot (Zhao et al., 2020). The trial lasted for four months, during which nutrient and heavy metal concentrations in the potting soil were identified before and after the trial.

Physical and chemical analysis of plants and soil

After soil samples were dried in the open air and passed through 2 mm sieves, the soil reaction (McLean., 1983), electrical conductivity (EC) (Richard, 1954), and composition (Bouyoucos, 1951) were identified. The total macro and microelements and metals were extracted from soil and plant samples using a mixture of 67-70% nitric acid (HNO_3) and 70% $HNO_3/4HClO_4$ perchloric acid ($HClO_4$) (Khan et al., 1983). Due to the low concentration of molybdenum (Mo) in filters, it was converted to ppm after being detected with ICP-MS (X II SERIES). The concentrations of other elements such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), arsenic (As) and lead (Pb) were detected using ICP-OES (ICAP6300 DUO).

Wastewater analysis

Wastewater samples were collected separately for each irrigation and stored at a temperature of -18°C for analysis. The pH and electrical conductivity of the wastewater were measured using the methods described by McLean (1983) and Richard (1954), respectively. At the end of the trial, the collected wastewater samples were mixed and diluted 8 times and element content was determined via ICP-MS and ICP-OES. Due to low concentration, Mo was converted to ppm after being detected with ICP-MS (X II SERIES). The concentrations of nitrate (NO_3), Ca, Mg, sodium (Na), Fe, Cu, Zn, Mn, Se, As, and Pb in solution were determined with ICP-OES (ICAP6300 DUO).

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics Version 22.0 software (IBM corp.). The data obtained was presented as mean ± standard deviation and analyzed using analysis of variance (ANOVA). Differences between means were determined using the Duncan Multiple Range Test. Pearson correlation coefficients were used to identify the correlation between wastewater ratio and element contents.

RESULTS AND DISCUSSION

Plant Height

The study found that the impact of dilute wastewater and the number of cuts, as well as their interaction, had a significant effect ($P<0.01$) on the plant height of alfalfa, crested wheatgrass, and orchard grass. Crested wheatgrass had the highest plant height measured with 50%DW+50%WW irrigation in the first cutting period (22.0 cm). According to the experiment results, orchard grass had the highest height when irrigated with a combination of 50%DW+50%WW, measuring 20.00 cm during the eighth cut. The control group, which was irrigated with 100%DW, had the lowest plant height at the first cut. As for alfalfa, the highest plant height was observed during the 2^{nd} and 7^{th} cuttings when irrigated with 50%DW+50%WW, measuring 16.67 cm and 15.33 cm, respectively. On the other hand, the lowest plant height was observed when irrigated with 25%DW+75%WW, measuring 5.00 cm at the first cut (Table 3). According to Arvas et al. (2022) and Lv et al. (2023), it was found that high nitrate content in wastewater had a positive effect on the plant height of alfalfa and orchard grass. Additionally, Altın et al. (2009) suggested that the lack of difference in crested wheatgrass for cuts after the 1^{st} and 2^{nd} cuts was due to slower emergence, emplacement, and development periods after planting.

Green Weight

The dilute wastewater significantly increased the green weight of the crested wheatgrass, orchard grass, and alfalfa plants ($P<0.01$). Additionally, the interaction between the number of cuts and wastewater proportions was also found to be very significant ($P<0.01$). For the control irrigation (chemical fertilizer + distilled water) and later cuts of crested wheatgrass, the green weight significantly reduced, while the weight increased with increasing wastewater proportions. The highest green weight for crested wheatgrass was observed during the 2nd cut with the use of 50% DW+50%WW and 25% DW+75%WW applications (Table 3).

Green weight increased significantly at all irrigation rates and in subsequent cuttings of orchard grass. The highest mean weight was observed for the 25%

DW+75%WW irrigation at the 3rd and 8th cuts (0.130 and 0.126 g/pot, respectively).

The dilute wastewater had a more significant effect on increasing the alfalfa green weight compared to the other two plants. Despite frequent cutting, there was a progressive increase in green weight with progressive cuts, with the highest increase observed for all irrigation applications at the 8th cut (0.100, 0.270, 0.361, and 0.334 g/pot, respectively). This increase is attributed to the variable cations (K, Ca, Mg, Na) found in wastewater which encourage the development of alfalfa (Arvas et al., 2022). In general, frequent mowing and irrigation are the two main factors that cause low yield and withdrawal of alfalfa. Repeated mows once a month led to a stepped increase in the yield of the alfalfa in the planting year, while subsequent cutting in the second year caused a reduction in yield (Erbeyi, 2022). This result supports our findings. In the interaction of mowing numbers and wastewater rates, the highest alfalfa green weight was at the 8th mowing of 50% DW + 50% WW application (0.361 g/pot) and the average highest green weight (0.185 g/pot) was 75% WW + 25%DW application.

Significant weight increases were determined in orchard grass and alfalfa plants with diluted wastewater ratios in subsequent mowing (Table 2). The increase in plant height increased the weight per plant and height is accepted as a measure of yield and weight of pasture and forage (Avcioğlu, 1996). The findings of this study are consistent with previous research on orchard grass. Koç and Gökkuş (1996) found that good care conditions and abundant leaves led to good development. Similarly, Avcı and Doğrusöz (2012) observed that frequent and deep cuts resulted in abundant spikelets and increased weight with height.

Dry Weight

The effects of wastewater and mowing on the dry weight of crested wheatgrass, orchard grass, and alfalfa plants were significant($P<0.01$).

The results show that crested wheatgrass reached its highest dry weight during the 2nd cut when irrigated the 50% DW+50%WW irrigation (0.013 g/pot). Conversely, the lowest mean weight was observed for the control group (100%DW) and 25% WW+75%DW irrigations. For orchard grass, all cuts had increases in dry weights, with the highest dry weight observed during the 4th and 8th mows when irrigated with 25%DW+75%WW irrigation (0.043 and 0.042 g/pot, respectively). The lowest weight (0.05 g/pot) was obtained for the 1st cut with 75%DW+25%WW irrigation. The effect of irrigation on the dry weight of orchard grass was significant, with the highest average dry weight (0.028 g/pot) obtained with 25%DW+75%WW irrigation.

The effect on alfalfa was more pronounced compared to the other two grass plants, with all irrigations having weight increases in the 8th mows compared to previous mows. As the number of mowing increased in the alfalfa, more Duncan groupings formed. The highest dry weight was obtained from the 8th mow with 50%DW+50%WW irrigation (0.120 g/pot), with the lowest dry weight at the 1st mow with 25%WW+75%DW irrigation. Municipal wastewater was reported to increase dry matter significantly for alfalfa, corn, elephant grass, and oxtail millet (Aghtape et al., 2011; Matheyarasu et al., 2017).

Nutrients and Heavy Metal Content of Species

The concentrations of nutrients and metals examined were significant in terms of plants and irrigation water ratios ($P<0.01$). The highest P, Fe, Cu, Mn, and Pb concentrations in alfalfa were obtained with 25%DW+75%WW irrigation, and the highest Mg, Zn, Mo, and As concentrations were obtained with 50%DW+50%WW and 25%DW+75%WW applications, respectively (Table 3). However, the highest Se concentrations were determined in the control (100%DW+chemical NP). The lowest P, Mg, Fe, Mn, and As concentrations were obtained with control and 75%DW+25%WW irrigations. The lowest Zn, Cu, Mo, and Pb were obtained with the control irrigation. Diluted wastewater ratios did not affect the Ca concentration in alfalfa, but numerically.

For crested wheatgrass, the highest P, Mg, Cu, Zn, Cu, and Pb concentrations were identified with 25%DW+75%WW irrigation. There were no differences between 50%DW+50%WW and 25%DW+75%WW irrigations for Ca, Fe, Mn, and As concentrations. The highest Se concentration was detected with the control irrigation and 75%DW+25%WW. The lowest P and Mg were with the control and 75%DW+25%WW irrigations. The Lowest Ca, Fe, Zn, Cu, Mo, Mn, As, and Pb concentrations were obtained from control and 75%DW+25%WW application. The wastewater did not affect the Se concentration of crested wheatgrass.

Diluted wastewater and chemical fertilizer (control) applications to orchard grass affected the concentration of all elements in the plant by significant levels, apart from selenium. The highest Mg, Fe, Zn, Cu, Mn, As, and Pb concentrations were determined with 25%DW+75%WW irrigation, with the highest P, Ca, and Mo concentrations determined with 25%DW+75%WW and 50%DW+50%WW irrigations.

The lowest P and Mg concentrations were determined with control and 75%DW+25%WW irrigations, with the lowest Ca, Fe, Zn, Cu, Mo, Mn, As, and Pb from the control.

Table 2. The effects of wastewater rates and mowing numbers on some agronomic properties of crested wheatgrass, orchard grass and alfalfa (mean ± sd)*.

Çizelge 2. Arıtma suyu oranları ve biçim sayılarının otlak ayrığı, domuz ayrığı ve yoncanın bazı tarımsal özellikleri üzerine etkisi (mean ± sd).*

		Plant height (cm) Bitki Boyu (cm)			**Green weight (g pot^{-1}) Yaş ağırlık (g saksı$^{-1}$)**			**Dry weight (g pot^{-1}) Kuru ağırlık (g saksı$^{-1}$)**		
WR	**MN**	***A. cristatum***	***D. glomerata***	***M. sativa***	***A. cristatum***	***D. glomerata***	***M. sativa***	***A. cristatum***	***D. glomerata***	***M. sativa***
W_1	1	20.66 ± 1.53^{a}	10.00 ± 4.58^{c}	6.00 ± 1.73^{b}	0.016 ± 0.00^{c}	0.023 ± 0.00^{d}	0.036 ± 0.01^{e}	0.005 ± 0.000^{c}	0.008 ± 0.00^{d}	0.012 ± 0.00^{e}
	2	20.33 ± 3.06^{a}	13.67 ± 2.89^{bc}	11.33 ± 1.53^{ab}	0.018 ± 0.00^{b}	0.022 ± 0.00^{d}	0.048 ± 0.11^{c}	0.006 ± 0.000^{b}	0.007 ± 0.00^{d}	0.016 ± 0.00^{c}
	3	13.00 ± 3.00^{b}	16.00 ± 1.73^{ab}	7.67 ± 0.58^{ab}	0.022 ± 0.00^{a}	0.045 ± 0.00^{b}	0.039 ± 0.00^{e}	0.007 ± 0.000^{a}	0.015 ± 0.00^{b}	0.013 ± 0.00^{e}
	4	10.33 ± 2.31^{b}	13.67 ± 0.58^{bc}	8.00 ± 2.65^{ab}	0.007 ± 0.00^{d}	0.023 ± 0.00^{d}	0.059 ± 0.00^{b}	0.002 ± 0.000^{d}	0.008 ± 0.00^{d}	0.020 ± 0.00^{b}
	5	9.00 ± 2.00^{b}	14.33 ± 0.58^{bc}	8.00 ± 4.36^{ab}	0.007 ± 0.00^{d}	0.032 ± 0.00^{c}	0.047 ± 0.00^{cd}	0.002 ± 0.000^{d}	0.011 ± 0.00^{c}	0.016 ± 0.00^{cd}
	6	10.33 ± 0.58^{b}	16.67 ± 1.16^{ab}	7.67 ± 2.08^{ab}	0.006 ± 0.00^{d}	0.041 ± 0.00^{b}	0.045 ± 0.01^{cd}	0.002 ± 0.000^{d}	0.014 ± 0.00^{b}	0.015 ± 0.00^{cd}
	7	9.67 ± 1.15^{b}	19.00 ± 2.00^{a}	10.00 ± 4.36^{ab}	0.005 ± 0.00^{d}	0.043 ± 0.00^{b}	0.057 ± 0.01^{b}	0.002 ± 0.000^{d}	0.015 ± 0.00^{b}	0.019 ± 0.00^{b}
	8	9.00 ± 2.00^{b}	15.33 ± 2.31^{ab}	9.00 ± 1.73^{ab}	0.006 ± 0.00^{d}	0.069 ± 0.01^{a}	0.100 ± 0.01^{a}	0.002 ± 0.000^{d}	0.023 ± 0.00^{a}	0.033 ± 0.00^{a}
Mean		13.71 ± 4.56^{c}	12.79 ± 1.35^{d}	8.25 ± 2.07^{c}	0.011 ± 0.00^{c}	0.037 ± 0.02^{c}	0.054 ± 0.02^{d}	0.003 ± 0.00^{c}	0.013 ± 0.01^{c}	0.018 ± 0.01^{d}
W_2	1	21.67 ± 4.51^{a}	13.00 ± 1.00^{bcd}	5.67 ± 1.53^{d}	0.022 ± 0.00^{a}	0.016 ± 0.00^{d}	0.026 ± 0.00^{e}	0.007 ± 0.00^{a}	0.005 ± 0.00^{d}	0.009 ± 0.00^{e}
	2	18.33 ± 0.58^{a}	11.33 ± 1.16^{d}	12.67 ± 1.53^{a}	0.022 ± 0.00^{a}	0.021 ± 0.00^{d}	0.084 ± 0.01^{bc}	0.007 ± 0.00^{a}	0.007 ± 0.00^{d}	0.028 ± 0.00^{c}
	3	12.00 ± 2.00^{b}	15.00 ± 1.00^{ab}	7.67 ± 1.53^{cd}	0.019 ± 0.00^{a}	0.041 ± 0.00^{bc}	0.068 ± 0.00^{cd}	0.006 ± 0.00^{a}	0.014 ± 0.00^{bc}	0.023 ± 0.00^{cd}
	4	9.67 ± 1.16^{b}	13.33 ± 1.16^{bcd}	8.67 ± 3.06^{bcd}	0.011 ± 0.00^{bc}	0.037 ± 0.00^{bc}	0.082 ± 0.00^{cd}	0.004 ± 0.00^{bc}	0.012 ± 0.00^{bc}	0.027 ± 0.00^{dc}
	5	9.33 ± 1.16^{b}	12.67 ± 1.53^{cd}	7.33 ± 2.52^{d}	0.008 ± 0.00^{c}	0.036 ± 0.00^{bc}	0.054 ± 0.00^{d}	0.003 ± 0.00^{c}	0.012 ± 0.00^{bc}	0.018 ± 0.00^{d}
	6	8.67 ± 2.08^{b}	13.33 ± 0.58^{bcd}	9.00 ± 3.00^{abcd}	0.005 ± 0.00^{d}	0.035 ± 0.00^{c}	0.068 ± 0.00^{cd}	0.002 ± 0.00^{d}	0.012 ± 0.00^{b}	0.023 ± 0.00^{dc}
	7	12.00 ± 1.00^{b}	13.67 ± 1.16^{bc}	11.33 ± 1.53^{abc}	0.007 ± 0.00^{c}	0.047 ± 0.00^{b}	0.144 ± 0.02^{b}	0.002 ± 0.00^{d}	0.016 ± 0.00^{b}	0.048 ± 0.01^{b}
	8	11.67 ± 0.58^{b}	16.67 ± 1.53^{a}	12.00 ± 1.00^{ab}	0.013 ± 0.01^{b}	0.070 ± 0.02^{a}	0.270 ± 0.03^{a}	0.004 ± 0.00^{b}	0.023 ± 0.01^{a}	0.090 ± 0.01^{a}
Mean		13.04 ± 3.88^{d}	13.63 ± 1.56^{c}	9.50 ± 2.52^{b}	0.013 ± 0.01^{b}	0.038 ± 0.02^{c}	0.100 ± 0.07^{c}	0.004 ± 0.00^{c}	0.013 ± 0.01^{c}	0.033 ± 0.02^{c}

Table 2. Continuation of the table
Çizelge 2. Çizelgenin devamı

W_3	1	22.00±1.73^{a}	10.33±2.52^{c}	5.33±0.58^{c}	0.025±0.01^{b}	0.030±0.00^{e}	0.079±0.00^{d}	0.008±0.00^{b}	0.010±0.00^{e}	0.026±0.00^{d}
	2	20.67±1.53^{a}	15.33±4.16^{abc}	16.67±3.06^{a}	0.039±0.00^{a}	0.031±0.00^{e}	0.066±0.01^{d}	0.013±0.00^{a}	0.010±0.00^{e}	0.022±0.00^{d}
	3	12.67±2.08^{b}	17.33±4.04^{ab}	9.67±2.08^{b}	0.025±0.00^{b}	0.066±0.00^{b}	0.118±0.01^{c}	0.008±0.00^{b}	0.022±0.00^{b}	0.039±0.00^{c}
	4	11.33±0.58^{b}	14.00±4.58^{abc}	9.67±2.08^{b}	0.017±0.00^{c}	0.048±0.00^{cd}	0.080±0.00^{d}	0.006±0.00^{c}	0.016±0.00^{cd}	0.027±0.00^{d}
	5	10.00±3.46^{b}	13.33±2.8^{bc}	9.33±3.51^{b}	0.008±0.00^{d}	0.040±0.00^{de}	0.083±0.02^{d}	0.003±0.00^{d}	0.014±0.00^{de}	0.028±0.01^{d}
	6	14.00±9.53^{b}	17.33±0.58^{ab}	12.67±0.58^{ab}	0.009±0.00^{d}	0.046±0.02^{cd}	0.133±0.02^{c}	0.003±0.00^{d}	0.015±0.01^{cd}	0.044±0.01^{c}
	7	12.00±1.00^{b}	19.00±2.65^{ab}	15.33±2.52^{a}	0.011±0.00^{cd}	0.059±0.00^{bc}	0.181±0.02^{b}	0.004±0.00^{cd}	0.020±0.00^{bc}	0.060±0.01^{b}
	8	11.67±1.16^{b}	20.00±3.61^{a}	15.00±1.00^{a}	0.013±0.00^{cd}	0.100±0.01^{a}	0.361±0.02^{a}	0.004±0.00^{cd}	0.032±0.00^{a}	0.120±0.01^{a}
Mean		14.63±4.52^{a}	14.46±2.40^{b}	11.38±3.17^{a}	0.019±0.01^{a}	0.052±0.02^{b}	0.138±0.09^{b}	0.006±0.00^{b}	0.017±0.01^{b}	0.046±0.03^{b}
W_4	1	20.00±6.00^{a}	10.67±4.04^{b}	5.00±1.00^{b}	0.019±0.00^{cd}	0.045±0.02^{d}	0.091±0.00^{e}	0.007±0.00^{cd}	0.015±0.01^{d}	0.030±0.00^{e}
	2	20.00±6.00^{a}	13.33±3.51^{ab}	13.33±2.08^{a}	0.032±0.00^{a}	0.086±0.02^{c}	0.133±0.02^{d}	0.011±0.00^{a}	0.029±0.01^{c}	0.044±0.01^{d}
	3	17.00±1.00^{ab}	16.67±1.53^{ab}	11.33±0.58^{a}	0.021±0.00^{bc}	0.130±0.00^{a}	0.170±0.01^{c}	0.007±0.00^{bc}	0.043±0.00^{a}	0.057±0.00^{c}
	4	10.67±3.21^{c}	13.67±1.53^{ab}	9.67±5.51^{ab}	0.018±0.00^{cd}	0.081±0.00^{c}	0.131±0.01^{d}	0.006±0.00^{cd}	0.027±0.00^{c}	0.044±0.00^{d}
	5	9.67±2.88^{c}	15.33±3.22^{ab}	11.00±2.65^{ab}	0.009±0.00^{f}	0.050±0.01^{d}	0.190±0.02^{c}	0.003±0.00^{f}	0.017±0.00^{d}	0.063±0.01^{c}
	6	9.33±2.08^{c}	17.67±3.06^{a}	9.00±3.46^{ab}	0.014±0.00^{e}	0.056±0.01^{d}	0.138±0.01^{cd}	0.005±0.00^{e}	0.019±0.00^{d}	0.046±0.00^{d}
	7	12.00±3.61^{bc}	17.670±3.06^{a}	14.33±4.93^{a}	0.017±0.00^{d}	0.104±0.01^{b}	0.2910.02±^{b}	0.006±0.00^{d}	0.035±0.00^{b}	0.097±0.01^{b}
	8	10.00±2.65^{c}	17.67±4.16^{a}	13.67±2.08^{a}	0.022±0.00^{a}	0.126±0.00^{a}	0.334±0.02^{a}	0.007±0.00^{a}	0.042±0.00^{a}	0.111±0.00^{a}
Mean		14.13±4.58^{b}	15.75±1.62^{a}	11.67±2.99^{a}	0.019±0.01^{a}	0.085±0.03^{a}	0.185±0.08^{a}	0.006±0.00^{a}	0.028±0.01^{a}	0.062±0.03^{a}

* Means with different letters in the same column are significantly different ($P < 0.05$), based on Duncan's multiple range test.
**W: Water ratios; W_1: Control; W_2: 25 % WW +75 % DW; W_3: 50 % WW + 50 % DW; W_4: 75 % WW + 25 % DW
***WW: Wastewater; DW: Diluted Wastewater
****MN: Mowing Number

Table 3. The effects of wastewater rates on the some nutrient and metal (ppm) contents of crested wheatgrass, orchard grass and alfalfa (mean ± sd)*.

Çizelge 3. Arıtma suyu oranlarının otlak ayrığı, domuz ayrığı ve yoncanın bazı besin elementleri ve metal içeriğine etkisi (mean ± sd).*

Factors		P	Ca	Mg	Fe	Zn	Cu	Mo	Se	Mn	As	Pb
A	W1	18589.00±166.1[c]	44587.67±279.8	21927.67±364.1[b]	0.28±0.01[c]	35.78±0.78[c]	56.86±0.17[d]	0.01±0.00[c]	0.73±0.05[a]	318.10±4.49[c]	2.39±0.14[b]	0.01±0.00[d]
	W2	18846.33±113.5[c]	44958.67±328.9	21918.33±60.0[b]	0.29±0.00[c]	50.19±0.81[b]	62.89±0.19[c]	0.02±0.00[a]	0.64±0.01[b]	311.50±1.44[c]	2.51±0.16[b]	0.39±0.01[c]
	W3	19405.67±178.4[b]	44848.00±120.8	22276.33±356.8[ab]	0.32±0.01[b]	59.58±0.72[a]	67.62±0.33[b]	0.01±0.00[b]	0.62±0.01[b]	338.80±5.91[b]	2.92±0.12[a]	0.44±0.01[b]
	W4	19877.00±190.5[a]	44921.67±57.5	22810.00±58.4[a]	0.39±0.01[a]	60.95±0.89[a]	71.67±0.30[a]	0.02±0.00[a]	0.65±0.01[b]	363.57±5.13[a]	2.87±0.11[a]	0.49±0.01[a]
Mean		**19179.50±536.0[a]**	**44829.00±245.1[a]**	**22233.08±435.5[a]**	**0.321±0.04[b]**	**51.62±10.5[a]**	**64.76±5.8[c]**	**0.01±0.00[c]**	**0.66±0.05[a]**	**332.99±21.6**	**2.67±0.26[a]**	**0.33±0.19[b]**
CW	W1	15708.00±327.1[d]	31374.33±155.1[c]	15734.00±77.8[c]	0.29±0.01[c]	31.43±0.41[d]	61.66±0.33[d]	0.01±0.00	0.47±0.03[a]	310.50±1.00[c]	2.12±0.05[b]	0.01±0.00[d]
	W2	16451.67±315.5[c]	32618.33±104.2[b]	15854.00±148.3[c]	0.34±0.01[b]	33.20±0.68[c]	69.58±0.17[c]	0.02±0.00	0.47±0.01[a]	317.17±0.55[b]	2.26±0.05[b]	0.46±0.02[c]
	W3	17477.33±338.2[b]	32987.67±11.0[a]	17585.00±270.5[b]	0.74±0.03[a]	45.28±0.39[b]	73.44±0.47[b]	0.02±0.00	0.43±0.00[b]	366.20±1.57[a]	2.69±0.06[a]	0.54±0.01[b]
	W4	18410.33±455.2[a]	33198.67±67.8[a]	18969.67±334.9[a]	0.71±0.01[a]	48.72±0.34[a]	78.53±0.13[a]	0.02±0.00	0.45±0.01[b]	363.43±3.37[a]	2.74±0.11[a]	0.67±0.012[a]
Mean		**17011.83±1112.5[c]**	**32544.75±743.2[b]**	**17035.67±1408.5[c]**	**0.519±0.21[a]**	**39.66±7.8[c]**	**70.80±6.4[a]**	**0.02±0.00[b]**	**0.45±0.02[b]**	**339.33±26.8**	**2.46±0.28[b]**	**0.42±0.26[a]**
OG	W1	16966.33±168.0[b]	30364.67±203.4[c]	18521.00±161.0[c]	0.30±0.00[d]	32.32±0.87[d]	59.61±0.13[d]	0.01±0.00[c]	0.42±0.01	321.90±1.00[d]	2.37±0.05[d]	0.01±0.00[d]
	W2	17186.67±168.3[b]	31228.67±82.7[b]	18707.00±323.8[c]	0.37±0.02[c]	38.65±0.11[c]	62.66±0.11[c]	0.02±0.00[b]	0.39±0.01	327.53±2.51[c]	2.47±0.05[c]	0.44±0.01[c]
	W3	18323.33±176. 9[a]	32596.33±122.2[a]	19530.00±231.1[b]	0.57±0.03[b]	50.21±0.79[b]	68.80±0.27[b]	0.02±0.00[a]	0.41±0.01	337.83±1.14[b]	2.66±0.01[b]	0.57±0.02[b]
	W4	18325.67±587.7[a]	32684.67±90.7[a]	21743.67±158.9[a]	0.67±0.02[a]	58.99±0.97[a]	74.77±0.22[a]	0.02±0.00[a]	0.40±0.01	348.30±1.22[a]	2.91±0.06[a]	0.66±0.02[a]
Mean		**17700.50±714.3[b]**	**31718.58±1021.3[c]**	**19625.42±1351.9[b]**	**0.479±0.16[a]**	**45.04±10.7[b]**	**66.460±6.1[b]**	**0.02±0.00[a]**	**0.41±0.01[c]**	**333.89±10.6**	**2.60±0.22[a]**	**0.42±0.26[a]**

*Means with different letters in the same column are significantly different ($P < 0.05$), based on Duncan's multiple range test.
**W: Water ratios; W_1: Control; W_2: 25 % WW +75 % DW; W_3: 50 % WW + 50 % DW; W_4: 75 % WW + 25 % DW
***WW: Wastewater; DW: Diluted wastewater
**** A: Alfalfa; CW: Crested Wheatgrass; OC: Orchard Grass

Table 4. Pearson's Correlation Coefficients (r) for diluted wastewater nutrients and metal concentration in crested wheatgrass, orchard grass and alfalfa *,**

*Çizelge 4. Arıtma suyunun otlak ayrığı, domuz ayrığı ve yoncadaki besin maddeleri ve metal konsantrasyonu için Pearson Korelasyon Katsayıları (r) *,***

Plants	P	Ca	Mg	Fe	Zn	Cu	Mo	Se	Mn	As	Pb
Agropyron cristatum	.959**	.918**	.948**	.896**	.956**	.988**	.602*	-.132**	.905**	.926**	.926**
Dactylis glomerata	.853**	.952**	.906**	.976**	.993**	.990**	.877**	.132**	.983**	.964**	.928**
Medicago sativa	.964**	.425	.800**	.943**	.943**	.995**	.680**	-.570	.886**	.825**	.883**

*Correlation is significant at the P<0.05 level, **Correlation is significant at the 0,01 level.

When the species are assessed together, the highest P, Ca, Mg, Zn, Se, and As concentrations were in alfalfa, with the highest Cu concentration in crested wheatgrass and the highest Mo and As in orchard grass. The highest Fe and Pb concentrations were identified in crested wheatgrass and orchard grass. Findings related to the content of calcium, magnesium, zinc, and selenium in alfalfa are consistent with results of the study by researchers reported that two-year wastewater irrigation of barley increased the macro and micronutrient element and heavy metal contents (Frame, 2005 & Rusan et al., 2007). Wastewater increases the nutrient and metal contents of alfalfa and grasses (Osman, 2017).

The insignificant correlation for different proportions of dilute wastewater with the Ca content of alfalfa, with high cation exchange capacity, is attributed to the high Na concentration of wastewater limiting the uptake of Ca (Siebe, 2017). There was a negative and insignificant relationship of wastewater with Se concentration (r=-0.507), but positive with Ca concentration (r=-0.425). However, the relationship of wastewater with other elements was significant for alfalfa (P<0.01). The relationship between dilute wastewater with crested wheatgrass Se concentration (r=-0.132) was negative and significant, but with orchard grass was positive and significant (P<0.01). However, for crested wheatgrass and orchard grass, the relationship between the concentration of other elements and wastewater was positive and very significant (P<0.01), whereas for crested wheatgrass Mo concentration was significant (P<0.05). It is estimated that the positive response of orchard grass to irrigation is higher than the other two plants, causing the relationship of the studied nutrient concentration with wastewater to be positive and very important (Table 4).

Some Physical and Chemical Features of Soil After Trial

In the potting soil after the experiment, the highest EC, organic matter, Fe, Cu, As was determined in 25%DW+ 75%WW irrigation, the highest Mn in three ratios of diluted wastewater, and the highest pH in the control pot. While the concentrations of Mo and Se could not be detected, the effect of irrigation on Pb concentration was insignificant.

Researchers reported that the use of wastewater for irrigation increased soil salinity (Gao et al., 2021; Yerli, 2023). It has been reported that soil pH changes towards neutral after 20 days of wastewater irrigation (Agrawal et al., 2014). Some researchers also found that wastewater reduced soil pH and increased EC and organic matter (Mojiri, 2011). The Fe, Cu, Zn, Mn, and As concentrations increased with the increasing wastewater proportions, while the Pb concentration was not affected (Table 5).

Table 5. Effect of different rates of wastewater on nutrients, heavy metals, pH, organic matter and EC concentration in pot soil after trial*.

Çizelge 5. Arıtma suyunun deneme sonrası saksı toprağının besin maddeleri, ağır metaller, pH, organik madde ve EC konsantrasyonu üzerine etkisi.*

Property	Control	75%DW+25%WW	50%DW+50%WW	25%DW+75%WW
pH	8.43[a]	8.36[b]	8.10[c]	8.07[c]
EC ((dS/m)	0.229[d]	0.362[c]	0.441[b]	0.505[a]
Organic Madde (%)	1.613[b]	1.62[b]	1.62[b]	1.64[a]
Iron (Fe)	88.76[d]	96.73[c]	102.16[b]	103.80[a]
Copper (Cu)	0.106[d]	0.113[c]	0.116[b]	0.121[a]
Zinc (Zn)	0.18[c]	0.21b[a]	0.22[a]	0.21[b]
Manganese (Mn)	2.24[b]	2.37[a]	2.36[a]	2.39[a]
Molybdenum (Mo)	nd**	nd**	nd**	nd**
Selenium (Se)	nd**	nd**	nd**	nd**
Arsenic (As)	0.10[c]	0.11b	0.11a	0.11[a]
Lead (Pb)	0.02	0.02	0.02	0.02

* Different letters in the same row are significantly different (P<0.05), ** nd: non detdected

CONCLUSION

Diluted biologically treated domestic wastewater caused an increase in the height of alfalfa and the two grasses. After the first mow, there was a reduction in the height of crested wheatgrass in the subsequent mows, which is due to the slow seedling development of crested wheatgrass. Having a higher plant height of orchard grass than the other two plants is since it is more resistant to frequent irrigation and rapid development after mowing. In addition, the significance of the wastewater relationship with all the examined elements can be attributed to the water consumption and rapid development of the orchard grass feature. The wastewater ratio increased the green and dry weight of alfalfa gradually compared to the grass. However, the increasing number of mows at all wastewater ratios and in control irrigation reduced the green and dry weights of crested wheatgrass. Based on the wastewater ratio, apart from selenium, there were increases in nutrients and heavy metals in all three plants. The exchangeable cation of Ca significantly increased in both grasses depending on the wastewater ratio, while it did not change in alfalfa. While wastewater provides a positive result by reducing alkaline soil pH and increasing its organic matter, it also causes an important risk by increasing salinity.

In conclusion, the development of treatment systems that remove or at least reduce will resolve salinity risk. If biologically treated domestic wastewater is used for irrigation purposes, it must be diluted by 75%. However, to obtain more reliable results, it is recommended to investigate dilute wastewater irrigation under field conditions for many years.

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Researchers' Contribution Rate Statement

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors declare no conflict of interest.

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