



## Original article

## Change in cotton plant quality in response to application of anaerobically digested sewage sludge



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## ABSTRACT

Treated municipal sewage sludge (TSS) was applied to the cotton plant at rates of 10, 20 and 30 t/ha per year. Seed cotton yield (71.4%), lint yield (67.7%) and cottonseed yield (74.1%) were increased significantly when sludge was applied at a rate of 30 t/ha (TSS3). The effects of TSS applications on seed yield, lint yield and cottonseed yield were listed as TSS3 > TSS2 > CF > TSS1 > C according to the applications. The increasing TSS levels had a positive effect and increased the total N concentration compared to the control. The highest N value was observed in TSS3 plots, while the lowest value was recorded in control (C) plots. The highest P value was found in control (C) at 0.80% and in 10 t/ha (TSS1) at 0.80%, while the lowest value was found in the TSS2 application (0.70%). The K concentration of cottonseed increased with the increasing TSS rates, from 1.56% in control plots to 2.20% in 20 t/ha application (TSS2). Corresponding to the TSS levels, the calcium of plant tissues was determined by a range of 0.12–0.13%. The treatments of TSS and mineral fertilizer had similar effects on the Mg content of cottonseed, which was in the range of 0.38–0.43%. Na content in plant tissue increased with increasing dose of sludge application compared to control soils. Increasing doses of TSS had no significant effect on the concentrations of iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron (B) in cottonseed. The order of the elements with respect to their amounts in cottonseed was as follows: Fe > Zn > Na > B > Mn > Cu. The concentrations of non-essential elements (Ni, Cd, Cr, Pb, Hg and As) in cottonseed were below the permissible limits.

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## 1. Introduction

Cotton (*Gossypium hirsutum* L.) is the most important cash crop, grown for the purpose of oil, seed, lint, fiber and animal feed all over the world. Mainly cultivated for its natural fiber (Constable and Bange, 2015), cotton also is the second largest potential source of plant protein and the fifth largest oil-producing plant in the world (Hu et al., 2017). Therefore, maintaining high quality fiber and cottonseed nutritional value is critical. There are four cultivated cotton species, *Gossypium hirsutum* (about 95% of the cultivated cotton), *G. barbadense*, *G. arboreum*, and *G. herbaceum*

together (the last three represent about 5%) (Bellaloui and Turley, 2013). Sewage sludge contains macro and micronutrients essential for plant growth and is a potentially valuable source of organic matter for most agricultural soils (Kominko et al., 2017), especially the soils under Mediterranean biodegradation conditions (Kayikcioglu et al., 2020). There are three main methods for sewage sludge disposal: soil application, landfilling and incineration. The use of sewage sludge as a fertilizer seems to be the best practicable option in most circumstances. Sewage sludge provides a valuable source of major nutrients required for plant growth. Approximately 50% of the solid fraction of sewage sludge is organic matter, which has a significant effect on physical, chemical and biological properties of the soil (Tsadilas et al., 2014; Kominko et al., 2017). Apart from the contribution to plant nutrition, land application of sewage sludge can increase soil organic matter content and improve soil physical, chemical and biological properties (Haynes et al., 2009). One of the potential use of treatment sludge is the agricultural lands applications e.g. slope stabilization, restoration of degraded soils, improvement of soil properties or facilitation

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of reforestation activities in order to improve both labile and stable soil organic carbon content (Kayikcioglu and Delibacak, 2018; Kayikcioglu et al., 2019). Many studies have investigated the effects of sewage sludge application on several arable crops, such as wheat (Tejada and Gonzalez, 2007), barley (*Hordeum vulgare* L.) (Antofin et al., 2005), maize (*Zea mays* L.) (Melo et al., 2018; Kayikcioglu et al., 2019), cotton (*Gossypium hirsutum* L.) (Samaras et al., 2008), bread wheat (*Triticum aestivum* L.) (Koutroubas et al., 2014) and sunflower (*Helianthus annuus* L.) (Koutroubas et al., 2020). Contamination is among the most important problems in cotton industry. Soil contamination is the leading parameter influencing food safety and reliability. Agricultural use of treatment sludges a) supports soil sustainability and fertility; b) allows the use of sink function of the soils in combat climate change; c) reduces greenhouse gas emissions of organic materials under wild storage conditions and d) generates an important source of organic matter from organic wastes. However, sufficient number of studies has not been conducted about transport of soil heavy metals coming from different sources into cotton plants and heavy metal concentrations of seedcotton. Cotton constitutes the primary raw material of textile products with direct contact to human body, thus such heavy metal contamination and concentrations should definitely be taken into consideration for food safety of cotton plants. Cottonseed nutritional qualities have been relatively neglected, narrowing the genetic variation of cotton for seed quality improvement through breeding. Therefore, identifying other cotton genotypes that influence the accumulation of micronutrients in seeds that determine cottonseed nutritional qualities is crucial (Bellaloui and Turley, 2013; Bellaloui et al., 2015).

The aim of the study was to evaluate the influence of sewage sludge applications obtained from municipal wastewater treatment plant to a Xerofluent soil on nutritional composition and heavy metal contents of cottonseed and quality parameters of cotton plants such as seed cotton yield, lint yield, cottonseed yield, and ginning out turn.

## 2. Material and method

The granulated and 90% dry municipal wastewater treatment sludge from Çiğli Wastewater Treatment Plant of İzmir Metropolitan Municipality, stabilized under anaerobic conditions, were applied at different doses to alluvial soils of Menemen district of İzmir province to grow cotton plants in 2015. Some physicochemical characteristics of treatment sludge and experimental soils are provided in Table 1 and Table 2, respectively.

The heavy metal contents of the treated sewage sludge (TSS) used were below the values permitted by the European directive 86/278/CEE (CEC, 1986) and the USA (EPA, 1993) and Turkish

**Table 1**  
Some physicochemical properties of TSS.

Properties	Values	Properties	Values
pH <sup>a</sup> 1:2.5 water	7.18	Zn <sup>e</sup> (mg/kg)	1377
EC <sup>b</sup> (dS/m)	1.95	Fe <sup>e</sup> (mg/kg)	12755
C:N ratio	9.90	Cu <sup>e</sup> (mg/kg)	177
C <sub>org</sub> <sup>c</sup> (%)	29.66	Mn <sup>e</sup> (mg/kg)	350
N <sub>kjeldahl</sub> <sup>d</sup> (%)	2.99	Ni <sup>e</sup> (mg/kg)	69.73
P <sup>e</sup> (%)	0.23	Pb <sup>e</sup> (mg/kg)	17.44
K <sup>e</sup> (%)	0.34	Cr <sup>e</sup> (mg/kg)	112.5
Ca <sup>e</sup> (%)	6.36	Cd <sup>e</sup> (mg/kg)	2.83
Mg <sup>e</sup> (%)	2.04	B <sup>f</sup> (mg/kg)	16.10
Na <sup>e</sup> (mg/kg)	1391		

a: 1:2.5 water extract, b: Electrical conductivity, w:v, 1:5 water, c: Organic carbon. d: kjeldahl, e: total (HNO<sub>3</sub> + HClO<sub>4</sub>) extract, f: ash were determined azometine-H methods. Each value is the mean of three replicates and on an oven-dry (105 °C) basis.

**Table 2**  
Some physicochemical properties of the experimental soil.

Properties	Values	Properties	Values
pH <sup>a</sup> 1:2.5 water	7.64	Ca <sup>f</sup> (mg/kg)	2162
EC <sup>b</sup> (dS/m)	0.44	Na <sup>f</sup> (mg/kg)	31.17
Texture	Sandy-loam	Fe <sup>g</sup> (mg/kg)	2.65
Sand (%)	55.84	Cu <sup>g</sup> (mg/kg)	0.89
Silt (%)	31.44	Zn <sup>g</sup> (mg/kg)	2.08
Clay (%)	12.72	Mn <sup>g</sup> (mg/kg)	1.83
CaCO <sub>3</sub> (%)	4.74	Ni <sup>h</sup> (mg/kg)	53.09
C <sub>org</sub> <sup>c</sup> (%)	0.876	Pb <sup>h</sup> (mg/kg)	13.34
N <sup>d</sup> (%)	0.081	Cr <sup>h</sup> (mg/kg)	25.57
P <sup>e</sup> (mg/kg)	14.66	Cd <sup>h</sup> (mg/kg)	0.75
K <sup>f</sup> (mg/kg)	237	B <sup>i</sup> hot water (mg/kg)	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 1 N NH<sub>4</sub>OAc extract, g: available DTPA extract, h: total HCl + HNO<sub>3</sub> extract, i: hot water extract were determined azometine-H methods. Each value is the mean of three replicates.

directives (RG, 2010), which regulate the use of treated sewage sludge in agricultural soil (Table 1). High value-added cotton (*Gossypium hirsutum* L. var. GSN 12), commonly grown in Aegean Region of Turkey, was selected as the plant material of the study. Field experiments had five different treatments; 1) control (C), 2) chemical fertilizer (CF), 3) 10 t TSS/ha (TSS1), 4) 20 t TSS/ha (TSS2) and 5) 30 t TSS/ha (TSS3). Experiments were conducted in randomized blocks design with 4 replications. Experimental plots were 3x3 m in size and 2 m spacing was provided between the plots. TSS was manually laid on the soil surface homogeneously and incorporated into the soil at 0–15 cm depth with a rototiller on 21 April 2015. Cotton seeds were sown with a drill as to have 70 cm × 18.3 cm (row × on-row plant) spacing on 29 April 2015. As the basic fertilization, 500 kg/ha composed fertilizer (15% N, 15% P<sub>2</sub>O<sub>5</sub>, 15% K<sub>2</sub>O) was applied only to chemical fertilizer treatment plots. Then, the first dressing fertilizer (150 kg/ha urea, 46% N) was applied to same plot in June 2015 and the second dressing fertilizer (270 kg/ha calcium ammonium nitrate (26% N) was applied to chemical fertilizer treatment plots at flowering period in July 2015. Irrigations were done through drip lines placed between cotton rows. Harvesting was practiced on 27 October 2015 and cotton bolls were collected manually. Then, plot yields were calculated and converted into yield per hectare (kg/ha).

### 2.1. Nutrient and heavy metal analyses

The cotton bolls harvested from each plot were separated into lint and seed. Seed samples were then dried at 65–70 °C and ground to make them ready for analyses. N analysis was conducted in accordance with modified Kjeldahl method (Bremner 1965). Acid-digestion (HNO<sub>3</sub>; HClO<sub>4</sub>; 4:1) was performed before nutrient analysis. Sample P contents were determined spectrophotometrically with the use of vanadomolibdo phosphoric yellow color method (Lott et al., 1956); K and Ca contents were determined in a flame photometer; Mg, Fe, Zn, Mn and Cu contents were determined in an Atomic Absorption Spectrophotometer (AAS) (Hanlon, 1992; Kacar and İnal 2008). Plant heavy metals (Cd, Pb, Cu, Cr and Ni) were determined with the use of an AAS (Lindsay and Norwell, 1978). Following dry-ashing, sample B contents were determined spectrophotometrically with the use of azometine-H method (Wolf, 1971). Results were calculated over dry matter.

### 2.2. Yield and quality parameters

Seed cotton yield (t/ha); all plants of a plot were harvested in a single hand and harvested bolls were weighted and resultant value

was converted into yield per hectare (t/ha). Total lint yield (t/ha); following the determination of boll yield, cotton bolls were separated into lint and seeds with the use of roller-gin-type ginning machine. Lint was weight to get lint yield. Ginning out turn (%); from each plot, 20 bolls were weighed, then bolls were separated into lint and seed with the use of a roller-gin ginning machine and they were weighed separately and ginning out turn was computed by using the following formula given by Singh (2004);

$$\text{Ginning out turn (\%)} = \frac{\text{Weight of lint in sample}}{\text{Weight of seed cotton in that sample}} \times 100$$

### 2.3. Statistical analysis

Two-way ANOVA (generalized linear model) was applied to experimental data to put forth the effects of sewage sludge treatment doses and the other treatments (TSS1, TSS2 and TSS3 stabilized treatment sludge; C, control and CF, chemical fertilizer) on analyzed dependent plant variables. Besides, effects of independent variables on each one of dependent variables were also put forth with two-way ANOVA. Statistical analyses were conducted with the use of IBM SPSS Statistics 20.0 software in accordance with randomized blocks design. Significant means were compared with the use of LSD multiple comparison test procedure of the same software.

## 3. Results

### 3.1. Seed cotton yield

There were significant differences in cotton seed yields of the experimental treatments ( $p < 0.01$ ). Seed cotton; raw cotton containing seed and lint that has been harvested from a field, but has not been ginned. Seed cotton yields varied between 2.86 and 4.93 t/ha with the greatest value (4.93 t/ha) was obtained from 30 t/ha TSS3 treatment and the lowest value (2.86 t/ha) from the control (C) treatment (Table 3 and Fig. 1).

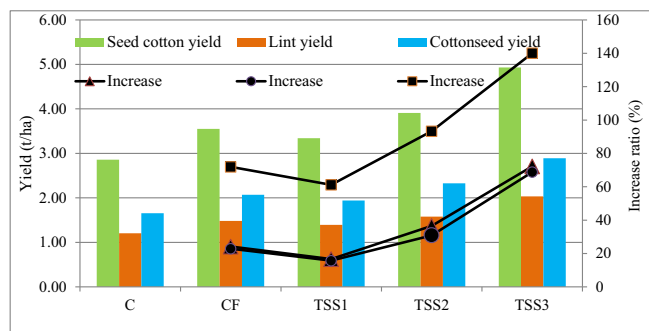
### 3.2. Lint yield

The effects of experimental treatments on lint yields were found to be significant ( $p < 0.01$ ). Lint yields varied between 1.21 and 2.03 t/ha with the greatest value in 30 t/ha TSS treatment (Table 2). The 20 t/ha TSS treatment and conventional fertilizer treatment were placed into the same group, in other words, these two treatments had similar effects on lint yields. Application of sewage sludge promoted lint yield, but effects of TSS on lint yields varied with the treatment doses. The lint yield of 1.21 t/ha in control treatment increased to 2.03 t/ha with 30 t/ha TSS treatment.

**Table 3**  
Effects of treatment sludge applications on cotton yield components.

Treatment	Seed cotton yield (t/ha)	Lint yield (%)	Cottonseed yield	Ginning out turn
C	2.87 <sup>d</sup>	1.21 <sup>c</sup>	1.66 <sup>d</sup>	42.26
CF	3.55 <sup>bc</sup>	1.48 <sup>b</sup>	2.07 <sup>bc</sup>	41.75
TSS1	3.34 <sup>cd</sup>	1.40 <sup>bc</sup>	1.94 <sup>cd</sup>	41.74
TSS2	3.91 <sup>b</sup>	1.58 <sup>b</sup>	2.33 <sup>b</sup>	40.43
TSS3	4.92 <sup>a</sup>	2.03 <sup>a</sup>	2.89 <sup>a</sup>	41.34
CV (%)	9.41	9.38	10.16	3.22
Significant	**	**	**	ns
LSD <sub>(0.01)</sub>	0.539	0.222	0.341	6.711

CV: Coefficient of variation \*\*;  $p < 0.01$  ns: not significant.



**Fig. 1.** Treated sewage sludge (TSS) applications effects of cotton yields parameters.

### 3.3. Cottonseed yield

Cottonseed is a source of oil for human consumption, cotton meal and minerals for livestock feed (He et al., 2013). The differences in cottonseed yields of the experimental treatments were found to be significant ( $p < 0.01$ ). Cottonseed yields varied between 1.66 and 2.89 t/ha with the greatest value (2.89 t/ha) from 30 t/ha sludge treatment and the lowest value (1.66 t/ha) from the control treatment.

### 3.4. Ginning out turn

Ginning out turn is an important parameter designating percentage of lint in seedcotton. The differences in ginning out turns of different treatment sludge applications were not found to be significant. Ginning out turns varied between 40.43 and 42.26 % with the greatest value from the control treatment and the lowest value from 20 t/ha sludge treatment.

### 3.5. Effects of experimental treatments on cottonseed nutrients

The effects of sludge treatments on macro- and micronutrients of cottonseed were shown in Tables 4 and 5. The increasing TSS levels had a positive effect and increased total N compared to the control. Total nitrogen (N) was determined in the range of 3.80–4.30%. The total N concentration changed in response to the applications were listed as TSS3 (4.28%) > TSS2 (4.24%) > TSS1 (3.93%) > CF (3.89%) and > C (3.70%). Nitrogen value of the cottonseed increased according to the sludge application dose and the highest and lowest N value was determined in TSS3 and TSS1, respectively in the amended soils. The differences in phosphorus (P) content of cottonseed were found statistically significant in response to the application doses. The P value of cottonseed was found to be in the range of 0.72–0.80%. The highest P value was obtained at 0.80% in both control (C) and 10 t/ha (TSS1) plots, while the lowest value was found in TSS2 plots (0.72%). Although increasing TSS

**Table 4**  
Effect of treated sewage sludge (TSS) on macronutrient of cottonseed.

Treatment	N (%)	P	K	Ca	Mg
C	3.70 <sup>b</sup>	0.80 <sup>a</sup>	1.56 <sup>b</sup>	0.12	0.43 <sup>a</sup>
CF	3.89 <sup>b</sup>	0.78 <sup>a</sup>	1.89 <sup>ab</sup>	0.13	0.42 <sup>ab</sup>
TSS1	3.93 <sup>b</sup>	0.80 <sup>a</sup>	1.78 <sup>b</sup>	0.12	0.42 <sup>ab</sup>
TSS2	4.24 <sup>a</sup>	0.72 <sup>b</sup>	2.20 <sup>a</sup>	0.13	0.38 <sup>b</sup>
TSS3	4.28 <sup>a</sup>	0.73 <sup>b</sup>	1.74 <sup>b</sup>	0.12	0.39 <sup>ab</sup>
CV (%)	7.12	4.65	17.94	8.32	8.28
Significant	*	*	*	ns	*
LSD (0.05)	0.312	0.030	0.371	0.015	0.046

CV: Coefficient of variation \*: p < 0.05 ns: not significant.

**Table 5**  
Effect of applications sewage sludge (TSS) on nutrient of cottonseed.

Treatment	Na (mg/kg)	Fe	Cu	Zn	Mn	B
C	36.86 <sup>c</sup>	134.53	8.40	83.26	14.60	15.69
CF	41.46 <sup>c</sup>	134.21	7.65	84.96	14.96	14.78
TSS1	42.13 <sup>c</sup>	132.94	8.80	83.40	14.14	14.73
TSS2	50.64 <sup>b</sup>	139.64	8.66	82.78	13.66	13.63
TSS3	65.48 <sup>a</sup>	136.77	8.51	87.47	13.52	16.08
CV	26.41	8.02	11.04	4.80	7.92	13.03
Significant	*	ns	ns	ns	ns	ns
LSD (0.05)	6.470	20.092	1.560	6.004	1.534	3.275

CV: Coefficient of variation \*: p < 0.05, ns: not significant.

application rates increased potassium (K) concentration of all the TSS plots compared to the control, only the increase provided by TSS2 was found to be statistically significant. K concentration of cottonseed increased from 1.56 % in the control plots to 2.20% in the 20 t/ha application plot (TSS2). It was found that there was no statistical relationship between TSS and calcium (Ca) contents. Depending on the TSS applications, calcium content was determined to be 0.12–0.13%. Addition of increasing TSS levels significantly (p < 0.05) decreased magnesium (Mg) levels of cottonseed compared to control application. TSS and mineral fertilizer treatments had similar effects on Mg contents of cottonseed that varied from 0.38 to 0.43%. The highest Mg value of 0.43% was obtained in control (C) while the lowest value was found in TSS2 plots (0.38%).

The effect of TSS applications on sodium concentration was shown in Table 5. The applications with increasing TSS rates significantly (p < 0.05) increased the Na concentration of cottonseed compared with the control plots. The highest Na value of cottonseed was found at the highest TSS3 application (65.48 mg/kg). This was followed by TSS2 (50.64 mg/kg), TSS1 (42.13 mg/kg), CF (41.46 mg/kg) and C (36.86 mg/kg) treatments. The experimental concentrations of iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron (B) in cottonseed showed no statistically significant

changes with increasing doses of TSS applications compared to the control. The highest Fe value of 132.94 mg/kg was obtained in TSS1 while the lowest value was 139.64 mg/kg in TSS2 plots. Cu contents varied between 7.65 and 8.80 mg/kg with the lowest and highest values in CF and TSS1 treatments, respectively. Zn content varied between 82.78 and 87.47 mg/kg with the lowest value in TSS2 and the highest value in TSS3 treatments. Application of TSS3 significantly increased the Zn content of cottonseed (87.47 mg/kg) as compared to the control (83.26 mg/kg). The highest manganese (Mn) content was obtained with TSS3 (13.52 mg/kg), while the lowest value was found with treatment CF (14.96 mg/kg). Boron (B) content varied between 13.63 and 16.08 mg/kg, with the lowest and highest values in treatments TSS2 and TSS3, respectively. The ranking of elements in terms of their amounts in cottonseed was as follows: Fe > Zn > Na > B > Mn > Cu.

### 3.6. Effects of sludge treatments on cottonseed heavy metal contents

Effects of mineral fertilizer and sludge treatments on cottonseed heavy metal contents are provided in Table 6. Accordingly, experimental treatments did not have significant effects on cotton-

**Table 6**  
Effect of applications sewage sludge (TSS) on heavy metal of cottonseed.

Treatment	Ni (mg/kg)	Cd	Cr	Pb	Hg (µg/kg)	As
C	3.22	0.22	bdl	bdl	13.24	69.10
CF	3.27	0.24	bdl	bdl	12.71	65.85
TSS1	2.95	0.21	bdl	bdl	11.44	66.28
TSS2	3.25	0.26	bdl	bdl	13.04	63.57
TSS3	3.07	0.23	bdl	bdl	12.50	66.49
CV	10.21	23.65			15.79	19.52
Significant	ns	ns			ns	ns
LSD (0.05)	0.520	0.098			3.040	24.113

CV: Coefficient of variation ns: not significant; bdl: below detection limit.



seed nickel (Ni), cadmium (Cd), chrome (Cr), lead (Pb), mercury (Hg) and arsenic (As) contents. Cottonseed Ni contents varied between 3.0 and 3.3 mg/kg, Cd contents between 0.21 and 0.26 mg/kg, Hg contents between 11 and 13 µg/kg and As contents between 64 and 69 µg/kg. Chrome and Pb were encountered at trace quantities (Table 6). A toxicity risk was not seen in terms of copper, zinc, nickel, cadmium, chrome, lead and mercury contents of cottonseed.

#### 4. Discussion

The values we obtained for seed cotton yield of 2.86–4.93 t/ha were slightly higher than the values for seed cotton yield of 2.471–4.290 t/ha reported by Yalçın et al. (2005) for the same region. Present high seed cotton yields with treatment sludge applications comply with the results of Samaras and Kallianou (2000). Similarly, Kayikcioglu et al. (2019) reported the greatest maize yields with 30 t/ha/yr TSS treatments. Researchers indicated that increased microbial activity with TSS treatments facilitated mineralization processes and thus increased yield levels. Evangelou et al. (2017) indicated that different treatment sludge doses had positive effects on cotton yields. Cotton yield increased with sludge and fertilizer application. Differences in yield between sludge and inorganic fertilizer treatments were evident indicating that sludge application, even at the lower rate of 20 t/ha, could successfully replace inorganic fertilizer needs. TSS application at the rate of 30 t/ha significantly increased seed cotton yield (t/ha), lint yield (t/ha) and cottonseed yield (t/ha) as compared to the control treatments. From the results, it is obvious that sewage sludge may replace inorganic fertilization without reducing in cotton yield. Similar results of the influence of TSS application on cotton yield were reported by Antoniadis et al. (2010) and Tsadilas et al. (2014). Previous researchers indicated that optimum plant density for lint yield varied based on environmental conditions and the cultivars to be grown (Mao et al., 2015). Yield and its components are influenced by genetic parameters and agronomic practices, where sowing density plays an important role (Bednarz et al., 2000). In cotton-farming, nitrogen (N) is the most essential production input, it controls the plant growth and prevents abscission of squares and bolls and constitutes the essential element of photosynthetic activity (Reddy et al., 1996). N also stimulates the mobilization and accumulation of metabolites in newly developed bolls, thus increases their number and weight. As expected, the greatest nitrogen input to soil is achieved with TSS3 treatments and these treatments had the greatest seed cotton and cottonseed yields. The heavy metals supplied to soil with TSS3 treatments also did not result in a toxicity in cotton plants. Moreover, as compared to CF treatments, greatest yields were achieved with organic matter treatments (Kayikcioglu et al., 2019). Greater seed cotton yields of the present study than the previous ones were attributed to differences in plant density (Siddiqui et al., 2007). Iqbal and Khan (2011) reported that seed cotton yields differed significantly among different plant spacing and genotypes. The highest values for seed cotton yield (4.92 t/ha), lint yield (2.03 t/ha) and cottonseed yield (2.89 t/ha) were determined in TSS3 plots, respectively. Seed cotton yield (71.4%), lint yield (67.7%) and cottonseed yield (74.1%) were significantly increased when phosphorus was applied at the highest rate of TSS. Several authors reported the improvements in cotton yield resulting from P application (Singh et al., 2006; Gebaly and El-Gabieri, 2012; Sawan, 2018). Phosphorus (P) is the second most limiting nutrient in cotton production after nitrogen (Girma et al., 2007; Sawan, 2016). As compared to CF treatments, TSS3 treatments have met phosphorus requirement of cotton plants. In present study, high experimental soil pH

(>7.6) and the moderate quantities of CaCO<sub>3</sub> resulted in precipitation of P in response to the chemical fertilizing, which reduced the soluble P supply. On the other hand, ambient pH decreases with the aid of low and high molecular weight organic acids released through microbial degradation of organic materials, then precipitation of phosphorus is prevented and plant use of phosphorus is sustained (Kayikcioglu et al., 2020). The seed yield of cotton significantly ( $p < 0.01$ ) increased (as much as 174.7%) by increasing TSS doses. Present findings on seed cotton yields comply with the findings of Sawan (2018). Yield and quality of cottonseed are very sensitive to changing environmental conditions (Chen et al., 2015). Cotton yield components (seedcotton yield, lint yield and cottonseed yield) increased with the application of TSS and CF. Differences in yields of sludge and inorganic fertilizer treatments were evident, indicating that sludge application rate of 20 t/ha could successfully replace inorganic fertilizer needs. Seed cotton weight significantly increased with high TSS doses. This may be due to increased photosynthetic activity that increases accumulation of metabolites, with direct impact on seed weight. It was reported that ginning out turn was an important parameter designating lint percentage and the cotton cultivars with ginning out turns of over 40 % is generally preferred by textile industry for high lint yields. Present findings complied with the results of Killi et al. (2016), who reported the ginning out turns as between 37.0 and 41.5 %, indicated insignificant effects of treated sludge doses on ginning out turn. We suppose that the climate and environmental conditions may be effective in ginning out turns. Additionally, the lint and seed cotton yield were affected significantly by the plant spacing and varieties (Siddiqui et al., 2007). Cottonseed quality is also determined by its content of mineral and non-mineral nutrients such as N, C, S, K, Ca, Zn, and Fe because of their direct or indirect contribution to biosynthesis in plants such as protein (e.g. N, K, S); oil (e.g. C and N); carbohydrates (e.g. C, K, B); metabolite (e.g. Cu, Zn, Fe, Mg); integrity of cell membrane and cell wall structure (e.g. Ca and B); cell membrane, lipid synthesis, energy transfer and phosphorylation reactions, carbohydrate metabolism, and nutrient active uptake processes (e.g. P); and osmoregulation, stomatal closure, carbohydrate movement, and nutrient mobility (e.g. K) (Bellaloui et al., 2015). The physiological and biochemical roles of these nutrients in plant growth and development were previously reported (Marschner, 2012).

Nutrient accumulation in seeds is controlled by several processes, including nutrient uptake, translocation, redistribution, and accumulation (White and Broadley, 2009). When the nutritional elements in seeds are compared to Bellaloui and Turley (2013), 2.8–3.6 % N, 0.3–0.4 % P, 0.6–1.5 % K, 0.1–0.2 % Ca, 0.2–0.3 % Mg, 140–245 mg/kg Na, 51–76.5 mg/kg Fe, 10.3–21.8 mg/kg Cu, 40.5–65.3 mg/kg Zn, 7.8–22.6 mg/kg Mn and 12.3–15.4 mg/kg B differences occurred between the elements K, P, Fe, Na and Zn. The nutrient content in seed (Bellaloui et al., 2019) ranged 2.50–4.47% for N (similar); 0.51–0.82% for P (similar); 1.04–1.2% for K (difference); 0.10–0.16% for Ca (similar); 0.34–0.43% for Mg (similar) 42.77–64.07 mg/kg for Fe (difference); 8.27–11.63 mg/kg for Cu (similar); 33.23–47.27 mg/kg for Zn (difference) 10.80–15.17 mg/kg for Mn (similar); 9.14–11.90 mg/kg for B (difference); and 2.13–4.03 mg/kg for Ni (similar). Although the Zn level of cottonseed in our study was different from the value of 40.2 mg/kg found by He et al. (2013), it was similar to the 56.8–92.5 mg/kg found by Bellaloui and Turley (2013) and 39.45–98.28 mg/kg found by Bellaloui et al. (2020). The Zn content in cottonseeds was significantly higher than the studies of other researchers. The Zn content (1377 mg/kg) of TSS treatment had a positive effect on the amount of Zn in cottonseed. It is thought that the differences between the values of the elements may be due to nutrients in cottonseed impacted by agronomic and environmental factors (Bellaloui

et al., 2015; Yunfei et al., 2016). Cottonseed also affected by fertilization management practices (He et al., 2020). It was reported that the concentrations of elements in grain are also influenced by complex genetics and environmental factors (Descalsota-Empleo et al., 2019). Corresponding to the TSS applications, Ni and Cd heavy metal concentrations of cottonseed in our study were determined to be 3.09 and 0.23 mg/kg, respectively, which is slightly higher than the concentration values of  $2.38 \pm 0.05$  mg/kg for Ni and  $0.02 \pm 0.00$  mg/kg for Cd determined in the previous study (Canikli et al., 2019), while the Pb concentration was below the detection limit, unlike that study in which the Pb was found to be  $0.13 \pm 0.06$  mg/kg. On the other hand, in accordance with the regulations on undesirable substances in animal feed, European Union and the Ministry of Agriculture and Forestry of Turkish Republic reported maximum values in mg/kg related to a feed with a moisture content (12%) of As (in feed materials and complete feeds), Cd (in feed materials and complete feeds for cattle, sheep, goat), Hg (in feed materials and complete feeds) and Pb (in feed materials and complementary feeds) for 2 mg/kg, 1 mg/kg, 0.1 mg/kg and 10 mg/kg, respectively (EC, 2002; TFC, 2005). Our results showed that the concentrations of some heavy metals or non-essential elements (Ni, Cd, Cr, Pb, Hg and As) in cottonseed were below these permissible limits. Moreover, the concentrations of the contaminants were also below the permissible limits for 0.1 mg/kg Pb, 0.1 mg/kg Hg which were given by the Codex Alimentarius Commission (1999) and 0.5 mg/kg Cd in food grade (Codex Stan, 1995). Heavy metals in cotton textile fibers were found to be 0.44–1.12 mg/kg for Cr and 0.18–6.00 mg/kg for Pb by Doğan et al. (2002) and 1.20–4.69 mg/kg for Ni by Tuzen et al. (2008). In our study, concentrations of Cr, Pb and Ni were found in cottonseed which less than all these values.

## 5. Conclusion

Treatment sludge applications increased total nitrogen, phosphorus and potassium contents of cottonseed. Sodium contents of cottonseed also increased with sludge treatments. The greatest seedcotton, lint and cottonseed yields were achieved with 30 t/ha sludge treatments. A toxicity risk was not seen in terms of sodium, iron, copper, zinc, manganese, boron, nickel, cadmium, chrome, lead, mercury and arsenic contents of cottonseed. Therefore, based on present findings, 30 t/ha treatment sludge application could be made on cotton fields. Amount of treatment sludge to be applied in agricultural practices could be calculated based on plant requirements and soil available nutrient quantities.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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