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Agricultural use of sewage sludge under sub-humid Mediterranean conditions: effect on growth, yield, and metal content of a forage plant

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Abstract

A field experiment was carried out in order to investigate the effects of sewage sludge application on the growth and yield components of triticale (*X Triticosecale Wittmack*). Five treatments were compared: a control (C) without application of sludge or nitrogen fertilization; a mineral fertilization treatment (MF) applied as ammonium nitrate; and three sewage sludge treatments (SS), 6, 12, and 18 t ha^{-1}, applied 15 days prior to triticale sowing. The main results showed that SS application improved plant growth by increasing leaf area index, tillering capacity, accumulated aboveground dry matter, and plant height of triticale. As a result, 18 t ha^{-1} of SS could be recommended the suitable dose for triticale, where dry matter production was more than twofold above the control value. No toxic effects arising from the heavy metals in triticale plants were observed. The Cu concentration was the only trace element that increased in the straw tissues with sludge application, although the values recorded were below critical environmental thresholds. Furthermore, growth and yield responses of triticale to all SS rates are comparable even sometimes more important than those for mineral fertilizer.

Keywords Triticale · Sewage sludge · Growth · Production · Heavy metals

Introduction

Triticale, the first successful human-made cereal grain, was deliberately produced in 1875 by crossing wheat with rye. Since then, the evolution of this crop has been the topic of keen interest for many plant scientists. According to the vision of early scientists, triticale should combine the best characteristics of both parents: wheat's qualities for making various food products with rye's robustness for adaptability to difficult soils, drought tolerance, cold hardiness, disease resistance, and low-input requirements (Mergoum and Gómez-Macpherson 2004). In Tunisia, triticale was introduced as cultivated specie for experimental purposes in the 1970s. Its production area jumped for 3 ha in 2006 to 15 m ha in 2016, accompanied by a consecutive increase in grain production (Tunisian Ministry of Agriculture 2016). While, its production cost also continued to increase attaining 48 TND/q in 2015 compared to 18 TND/q in 2006 (Tunisian Ministry of Agriculture 2016). The steady increase in triticale production cost may be attributed in part to the substantial increase in irrigation costs and fertilizer prices, especially N fertilizers. The use of drought resistant plant species could be an alternative to overcome such limiting factors, both by reducing watering, maintenance, fertilization, etc., costs and extending the lifespan of crops (Sevik and Cetin 2015; Yigit et al. 2016). Furthermore, it is well-known that the conciliation of performance improvement of cereal crops depends on maintaining the stock of nutrients in soil, which essential for plant growth and the use of conventional resources addressing the problem of water deficit (Huang et al. 2005). In this regard, several studies have shown that, by their richness in organic matter, organic residual products help improve the mineral and water statutes of soil and therefore increases crop production (Lobo et al. 2013).

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Indeed, a wide range of organic matter amendments, for example, green manuring, crop straw return, farmyard manure, and sewage sludge have been widely recommended as practices enhancing crop yield and soil fertility, while reducing fertilizer requirement (Alvarengaa et al. 2015). Sludge products represent a good source of nutrients for plant growth and a good soil conditioner to improve soil properties (Alcantara et al. 2009; Angın and Yağanoğlu 2009). The fertilizer effect enables a reduction in cost for nitrogen and phosphorus mineral fertilizers and may offer potential benefits in crop productions (Petersen et al. 2003).

Numerous publications described advantages of sewage sludge applications to agricultural soils, mainly its influence on yield of fertilized crops (Christie et al. 2001; Černý et al. 2012; Motta and Maggiore 2013; Bedada et al. 2014; Kchaou et al. 2018). Unwise sludge amendment may, however, disturb the soil properties, especially when it bears high concentrations of metals and toxic constituents. Heavy metals, as the limiting factor for the land use of sewage sludge, can have significant negative effects on groundwater, biological fertility, and crop quality, and furthermore, might pose a serious risk to human health (Nogueira et al. 2009).

As sludge researches are scarce in Tunisia, the study of the effect of this solid by-product on crop productivity and quality was found to be necessary. Therefore, the present work was carried out, under sub-humid conditions, to examine the response of a forage crop, triticale (*X Triticosecale Wittmack*) amended with different rates of sewage sludge, compared to mineral nitrogen fertilization.

Material and methods

Experimental site

The current study was performed as a field experiment on the Regional Crop Research Center, located at the governorate of Beja, Tunisia. The climate is sub-humid Mediterranean with an average annual rainfall of 563.1 mm, occurring mostly in March (115.6 mm). Table 1 illustrates the climatic data of the experimental region during the experimental period.

Field experiment

The trial was laid out in a split plot blocks design ((5 m × 5 m) each one) with four replications. Five treatments were considered: a control without application of sludge or nitrogen fertilization; a treatment fertilized with 100 kg N ha^{-1} applied in three fraction with 30 kg N ha^{-1} being applied at seedling development (third leaf at least 50% emerged, Zadocs 17, 40 N ha^{-1} at "1 cm ear" (Zadoks 30)); and 30 kg N ha^{-1} remainder being applied at "flag leaf visible" (Zadoks 37), three urban sewage sludge (SS) rates, 6, 12, and 18 t ha^{-1}, applied 15 days prior to sowing time. The respective rates of sewage sludge offered equivalents of 144, 288, 432 kg N ha^{-1} and 72, 144, 216 kg P ha^{-1}. The digested and dewatered sewage sludge was collected from sludge drying beds of an urban wastewater treatment plant located in Beja. The main physicochemical properties of soil and sewage sludge are presented in Table 2. Sewage sludge pH was found to be almost neutral, rich in organic matter, total nitrogen, and P contents. Heavy metal concentrations did not exceed Tunisian limits regulations (NT 106.2, 2002). The fecal coliform counts of the sewage sludge were below the Tunisian standards fecal coliform counts limits (≤ of 2.10^{6} CFU g^{-1}).

All recommended cultural practices were adopted uniformly according to standard crop requirements. Field plots were plowed, sown with triticale at 140 kg ha^{-1} seeding rate, with five rows per m^{2}, and 20-cm row spacing. During the cycle of triticale, hand-weeding and hoeing was done when necessity in all blocks. However, no irrigation was provided.

Plant sampling and analyses

To follow the effect of sewage sludge on triticale plants, tow harvests were conducted at 60 days after sowing

Table 1 Climatic data during the experimental period

Month	Rainfall (mm)	Temperature min (°C)	Temperature max (°C)	Moisture (%)
September	22.6	11.45	32.63	67.1
October	77.5	14.06	27.10	75.4
November	108.8	11.44	20.14	86.6
December	21.4	5.12	17.83	90.0
January	65	6.35	17.06	88.7
February	39.2	6.52	17.78	86.2
March	115.6	6.15	18.63	86.1
April	23.4	9.57	24.57	78.4
May	40.4	11.98	27.86	70.7

Table 2 The physicochemical characteristics of soil and sewage sludge used (based on dry matter DM)

Properties	Soil		Sewage sludge	*NT 106.20
	0–20 cm	20–40 cm		
Texture	Clayey loam	Clayey loam	Organic material	
pH	8.12	8.09	6.8	
Moisture			7.55	
DM (%)			92.45	
OM (%)	2.68	2.37	68.5	
TOC (%)	1.74	1.5	3.68	
Total nitrogen (%)	0.18	0.168	2.44	
Total phosphorus (%)			1.2	
Cd (mg/kg)			0.659	20
Cu (mg/kg)			120	1000
Pb (mg/kg)			38.6	800
Ni (mg/kg)			4.7	200
Zn (mg/kg)			470	2000
Cr (mg/kg)			70.5	500
Hg (mg/kg)			218	–
Fecal coliforms (CFU g^{-1})			5.410^4	$< 2.10^6$
Eggs of nematodes			–	

*Tunisian standards for sewage sludge reuse (NT 106.2, 2002)

(DAS) and 90 DAS corresponding to tillering and steam extension stages, respectively. Four linear meters were selected randomly from each treatment and hand harvested for biomass determination. For growth analyses, ten plants for each treatment were analyzed for shoot length, tiller and node numbers, and leaf area. The later parameter was measured by using digital images and ImageJ software according to the method described by Baker (2005). For biomass determination, plants were separated into roots and shoots and oven-dried at 70 °C until constant weight. The plant parts were then weighed separately and expressed in gram per square meter. The dry matter (DM) production was expressed in kg DM ha^{-1}. Plant samples were collected to determine plant heavy metal concentrations. Separately, the shoot and root samples were dried at 60 °C and ground. Metals (K, Mg, Fe, Mn, Cd, Cr, Ni, Cu, Pb, and Zn) in shoot parts were analyzed by using atomic absorption spectrophotometer (AAS).

Leaf chlorophyll contents (Chl a, Chl b, and total chlorophyll) of each treatment were also determined. The absorbance of fresh samples in acetone extracts were measured at 649 and 665 nm using a spectrophotometer, according to the method described by Torrecilas et al. (1984). The chlorophyll contents were calculated using the following equations:

$$\text{Chl a}(\mu g/mL) = 11.63(A665) - 2.39(A649)$$
$$\text{Chl b}(\mu g/mL) = 20.11(A649) - 5.18(A665)$$
$$\text{Total chlorophyll}(\mu g/mL) = 6.45(A665) + 17.72(A649)$$

Statistical analyses

The obtained results were assessed using ANOVA statistical analysis from the Statistical Analysis System (SPSS 20.0 for Windows). Means were compared by the Tukey test at the 5% level of significance. The mean values of each treatment are designed by letters (a, b, c...) which represent the significance degree of the difference between the means. The letter "a" means the highest average. Means represented by two letters in common indicated that the difference is not significant or weakly significant.

Results and discussion

Triticale growth response

Figure 1 represents some growth parameters of triticale grown in sewage sludge and mineral fertilizer amended soil. Data analysis revealed that both sewage sludge and chemical fertilizer showed positive responses in all measured parameters and for the two stages compared to control. The highest increment occurred with the highest SS rate, 18 t ha^{-1}, at 90 DAS reaching 20, 42, 27, and 179% for shoot length, number of tillers, leaf area, and node number, respectively, compared to control. These increases, significantly more important than that of conventional fertilizer (Fig. 1), could be due to the high concentrations of nitrogen (2.44%), phosphorus (1.2%), and also other several essential nutrient elements, including zinc,

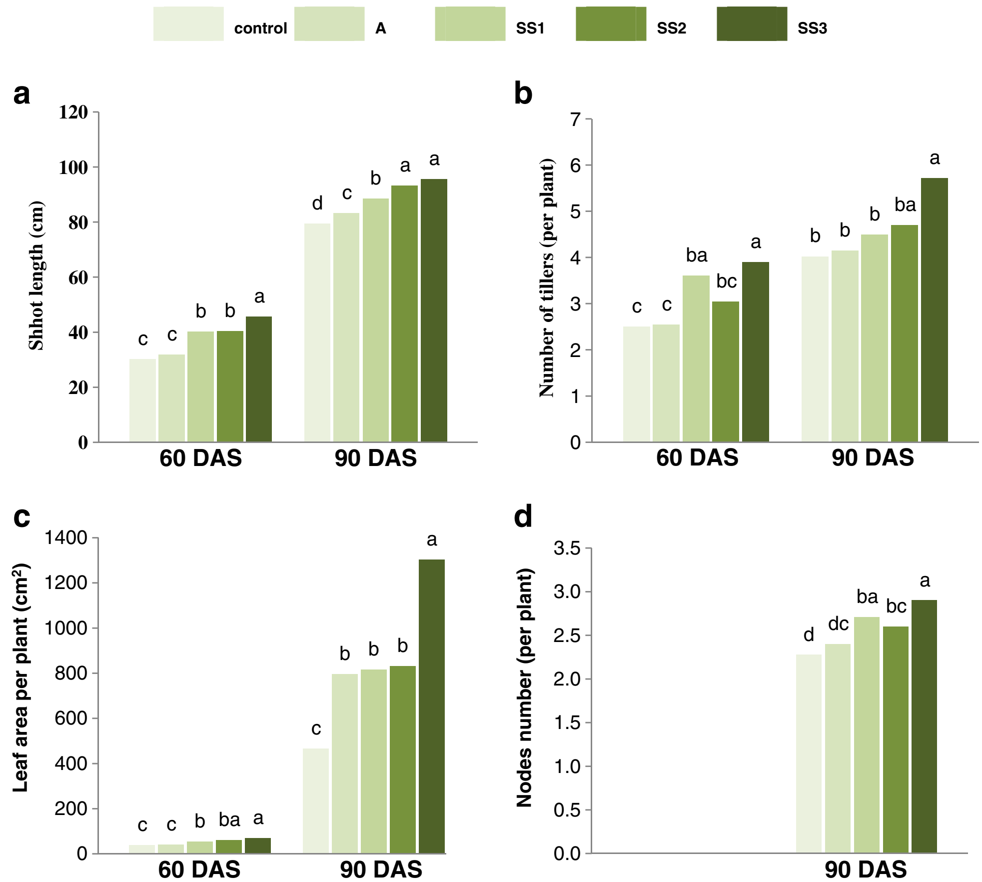

Fig. 1 Shoot length (**a**), number of tillers (**b**), leaf area (**c**), and number of nodes (**d**) of triticale plants grown at different SS rates. "DAS: Days after sowing," 60 DAS: tillering stage, 90 DAS: steam extension. (average values followed by the same letter are not significant different at $p < 0.05$)

copper, iron, and magnesium in the sewage sludge applied, which are lacking in the mineral fertilization. Similarly to these results, Bouzerzour et al. (2002) evidenced that sewage sludge increased leaves dimensions, leaf area index, tillering capacity, and plant height in barley (*Hordeum vulgare* L.) and in oat (*Avena sativa* L.). Akdeniz et al. (2006) reported also that sorghum plant increased in height during the growing season, after sewage biosolids soil addition. According to Yagmur et al. (2017), this effect could be explained as sewage sludge added plots contained sufficient moisture and nutrients for supporting triticale growth.

Triticale dry matter production

Similar trends were observed like for growth parameters. DM biomass production was significantly higher for plants amended with different SS rates compared with unamended soil (Fig. 2). Among the SS treatments, the highest increase was observed with 18 t ha^{-1} at 90 DAS. At this rate, we showed that DM production was more than twofold above the control value (17.34 vs. 7.8 t ha^{-1}). On the other hand, triticale production was tended to increase by conventional inorganic fertilizer, but not to as much as sewage sludge did

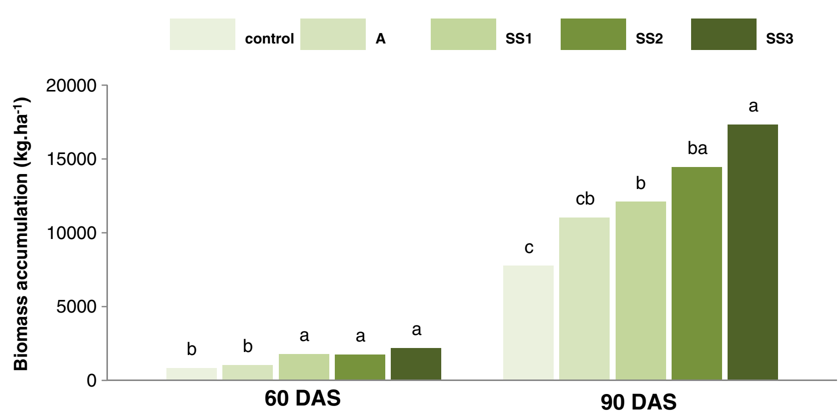

Fig. 2 Aboveground biomass (kg ha^{-1}) of triticale plants grown at different SS rates. "DAS: Days after sowing," 60 DAS: tillering stage, 90 DAS: steam extension (average values followed by the same letter are not significant different at $p < 0.05$)

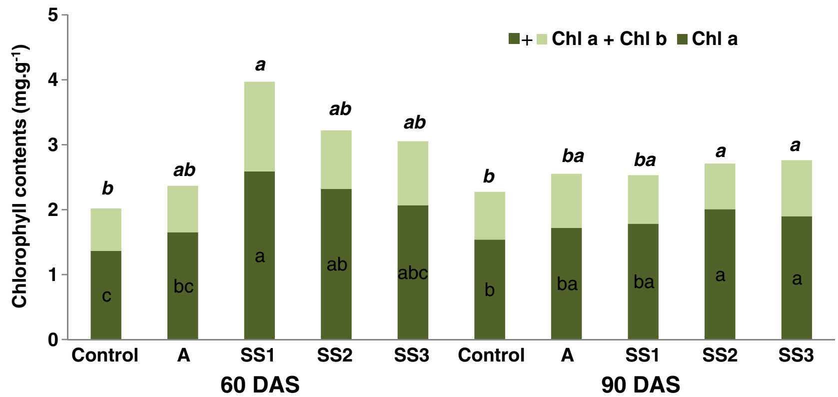

Fig. 3 Chlorophyll contents (Chl a, Chl b, and total chlorophyll) of triticale plants grown at different SS rates. "DAS: Days after sowing," 60 DAS: tillering stage, 90 DAS: steam extension, (a, b, c) and (*a*, *b*, *c*) indicate significance differences in Chl a and Chla+b contents at 60 and 90 DAS, respectively

(Fig. 2). These results confirm the higher effectiveness of nutrient elements, especially nitrogen from applied sewage sludge on the yield of crop plants compared with the N derived from the mineral fertilizer. According to Azam and Lodhi (2001), the benefits of sewage sludge amendment are derived mainly from a net release of nitrogen from decomposing organic matter with high nitrogen concentration and narrow C/N ratio, similar characteristics presented in the used sewage sludge.

Our results agreed with those of Yagmur et al. (2017) who applied different rates of sewage sludge (0, 5, 10, 15, 20, 25, 30 t ha^{-1}) to forage grasses, and those of Kchaou et al. (2018) who investigated in a pot trial with the impact of sewage sludge and green compost on winter triticale. Singh and Agrawal (2010) reported that yield of rice increased by 60%, 111%, 125%, 134%, and 137% at 3, 4.5, 6, 9, and 12 kg m^{-2} SS applications, respectively, as compared to those grown in unamended soil. Belhaj et al. (2016) revealed that amendment with different rates of SS (2.5, 5, and 7.5%) positively affected biomass of sunflower plants "Helianthus annus" (shoot and root fresh and dry weights) as compared to unamended soil. The beneficial effects of sewage sludge in yield production are usually attributed to an improvement in the soil structure by the supply of additional C deriving from sewage sludge application (Christie et al. 2001; Bipfubusa et al. 2008). In addition, sludge provides a short-term input of other plant available nutrients, particularly N (Kchaou et al. 2010), and it contributes to long-term maintenance of nutrients and organic matter pools (Korboulewsky et al. 2002). These beneficial effects were mainly due to the enhancement of the biological and enzymatic activity of the soil amended with sewage sludge (Lobo et al. 2013).

Chlorophyll content

All sewage sludge rates led to higher chlorophyll contents when compared with unamended soil by as much as 20 and 15% for chlorophyll a contents, at 60 and 90 days of sowing, respectively. These increases were comparable even sometimes more important than those for mineral fertilizer (Fig. 3).

According to Silva et al. (2010), the increase in chlorophyll content could be due to a high accumulation of essential metal ions required for the plant growth. Similar effects of sewage sludge have been referred by other researchers. Belhaj et al. (2016) observed an increase in chlorophyll contents in the sludge-grown plant of *H. annuus*. Singh and Agrawal

Table 3 Total heavy metal content (mg kg^{-1}) in shoot part of triticale plants grown at different SS rates "DAS: Days after sowing," 60 DAS: tillering stage, 90 DAS: steam extension (average values followed by the same letter are not significant different at $p < 0.05$)

Treatment	Stage	Zn	Ni	Cr	Pb	Cu	Cd
C	60 DAS	96.65a	4.26a	4.22a	9.25b	8.85b	0.97a
	90 DAS	54.82*a*	4.06*a*	4.99*a*	9.29*a*	5.31*b*	0.70*a*
A	60 DAS	102.21a	4.02a	3.71a	10.32ab	9.86ab	0.94a
	90 DAS	68.10*a*	5.55*a*	12.47*a*	12.36*a*	5.72*ab*	0.87*a*
SS1	60 DAS	88.97a	5.05a	7.78a	11.51ab	10.57ab	1.05a
	90 DAS	65.61*a*	5.17*a*	5.22*a*	10.39*a*	6.06*ab*	0.74*a*
SS2	60 DAS	95.72a	6.11a	6.15a	11.04ab	9.44b	0.95a
	90 DAS	71.72*a*	6.00*a*	6.00*a*	11.39*a*	6.84*a*	0.67*a*
SS3	60 DAS	98.66a	7.49a	6.91a	16.79a	11.98a	0.99a
	90 DAS	64.74*a*	5.64*a*	4.49*a*	10.54*a*	6.59*ab*	0.49*a*

(a, b, c) and (*a*, *b*, *c*) indicate significance differences in Zn, Ni, Cr, Pb, Cu, and Cd, Pb, Mn, Co, Cd, contents at 60 and 90 DAS, respectively

(2007) reported 27 and 89% increases in total chlorophyll content of *Beta vulgaris* plants grown at 20 and 40% sewage sludge amendment, respectively, at 40 DAS. However, total chlorophyll content decreased by 10 and 47%, in the same rates, at 60 DAS. This decrease was attributed to high accumulation of heavy metals in plants at later growing stage (Singh and Agrawal 2007).

Heavy metals accumulation in triticale straw

Shoot concentrations of heavy metals showed no significant differences among sewage sludge application rates and with mineral fertilizer (Table 3).

Compared to control, sewage sludge slightly increased plant Zn, Ni, and Cr concentrations, but this increase was not significant, in both tillering and steam extension stages. Although Zn presented the highest concentration, its content remained below the toxic concentration (230 mg kg^{-1}) in the aboveground part according to Borkert et al. (1998).

Conversely, the increase in the shoot Pb and Cu was found to be statistically significant, especially with SS3 (Table 3). Cu is an essential element in the growth of the plant. However, it can cause toxic effects in stem and leaf tissue if its concentration exceeds 20 mg/kg (Borkert et al. 1998). Our results showed that the highest value of Cu (11.98 mg kg^{-1}) was obtained in the presence of the highest sludge rate (18 t ha^{-1}) and which was found to be non-toxic. Indeed, such increment did not alter growth and development of plants. Moreover, it was previously showed that growth parameters and biomass production were positively affected by the application of sewage sludge, especially with the highest rate. According to Koupaie and Eskicioglu (2015) and Yagmur et al. (2017), only high dose and repeatedly applied sewage sludge into soil increased soil and plant tissue heavy metal concentrations, causing a decrease in the essential mineral concentrations, followed by growth retardation in plants. High heavy metal contents can also enter the photosynthetic apparatus by decreasing the photosynthetic pigment damaging chloroplast structure and inducing changes in the lipid and protein composition of the thylakoid membrane leading to lipid peroxidation related to the generation of toxic oxygen species (Wang et al. 2009). Commonly, this situation directly reduces all yield traits. Otherwise, heavy metal accumulations in any part of plant depended on sewage sludge origin and its heavy metal content. Mostly, the heavy metal content of sewage sludge was observed in high amounts in a heavy industry area (Reed et al. 1991).

Indeed, in the current study, the used sewage sludge was originated from an urban wastewater treatment plant, which explains its low heavy metal contents. As a consequence, no significant transfer to crops was observed, even if Cu and Pb increased in shoot parts.

Conclusion

The present study clearly showed that application of sewage sludge improved crop growth and accumulated aboveground biomass as well as providing a large part of the nitrogen, phosphorus, and others nutrient requirements of triticale plants. The highest rate 18 t ha^{-1} of sewage sludge increased straw yield more than 123% compared to control and nearly 57% compared to mineral fertilizer. Moreover, sewage sludge application did not cause negative effect on heavy metal content of plants. Thus, it could be recommended the suitable dose for triticale, to attain the maximum yield.

As a result, sewage sludge may be used as nitrogen source and could be a good substitute for synthetic N fertilizers, with no crop damage or toxic effects. Nevertheless, excessive accumulation of heavy metals must be taken into consideration under long-term application of sludge to obtain more accurate assessments of their fertilizing capacity and environmental impact. Thus, more continuous long-term field studies are recommended to improve the understanding of the effects of sewage sludge on triticale but also on other forage crops to contribute to the development of sustainable agricultural practices.

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