CHANGES IN SOIL HEALTH AND CROPS YIELD IN RESPONSE TO THE SHORT-TERM APPLICATION OF SEWAGE SLUDGE TO TYPIC XEROFLUVENT SOIL IN TURKEY

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Abstract. The aim of this study was to determine the possible effects on soil health and crop yield of the application of anaerobically digested sewage sludge (SS) at doses of 10 (SS_1), 20 (SS_2) and 30 (SS_3) t ha^{-1} yr^{-1} to degraded soils under maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) in the semi-arid Mediterranean ecosystem in Turkey. Application of SS at different doses did not show any disruptive effect on microbial biomass and activity in the soil. In the microbiochemical parameters analyzed in the soils under the two plant covers treated with SS, increases were seen of 29-30% compared with control, and of 28-30% in comparison with chemical fertilizer application. Yield increases secured by chemical fertilizers were statistically significantly higher than those from the SS_1. In maize grain yield, statistically significant increases were shown in comparison with the control of 90% with SS_3 and of 86% with SS_2. Similarly, seed cotton showed a statistically significant increase in yield of 72% with SS_3. Results obtained from the study show that for degraded soils in Mediterranean biodegradability conditions, application of SS at a rate of 30 t ha^{-1} yr^{-1} can be used both as a soil improver to maintain the soil and as an organic fertilizer to increase crop yield.
Keywords: *anaerobically digested sludge, soil enzymes, Mediterranean biodegradation condition, maize, cotton*

List of abbreviations: ALKPA – alkaline phosphatase enzyme activity; ArSA – aryl sulphatase enzyme activity; BSR – basal soil respiration; CF – chemical fertilizer treatment; DHG – dehydrogenase enzyme activity; GLU – ß-glucosidase enzyme activity; ha – hectare; MBC – soil microbial biomass carbon; N_{min} – N-mineralization; PRO – protease enzyme activity; SP_1 – first soil sampling period; SP_2 – second soil sampling period; SS – anaerobically digested sewage sludge; SS_0 – 0 t SS ha^{-1} yr^{-1} (control treatment); SS_1 – 10 t SS ha^{-1} yr^{-1} treatment; SS_2 – 20 t SS ha^{-1} yr^{-1} treatment; SS_3 – 30 t SS ha^{-1} yr^{-1} treatment; t – tonnes; UA – urease enzyme activity; yr – year

Introduction

The most important element necessary in ensuring sustainable soil fertility and productivity is soil organic matter. In conditions where soil organic carbon pool has not been well managed, long-term intensive agricultural activity inevitably results in a degradation of soil health. Examining soils affected by the Mediterranean climate, it is seen that the characteristics hindering fertility and crop development are high lime content and pH, and low organic material content. The soils of this region are also characterized by a progressive loss of fertility as a result of high degradation (Pascual et al., 1998). In order to increase soil fertility in a short time, it is necessary not only to add the plant nutrients which may be deficient in the soil by means of chemical fertilizers but also to rectify its characteristics in order to bring it into a sustainable condition. For this reason, in agricultural soils under the effects of the Mediterranean climate whose

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organic material content is low, it is of vital importance to apply organic wastes which have high potential for use.

The sludge varies in the amount and quality of the organic materials remaining according to the stabilization method used, and this determines the direction and strength of its effect on the soil-plant ecosystem. This is because sewage sludge of better quality and with a greater quantity of organic matter will have a greater positive effect on microbial activity and thus on crop yield than the same amount of sludge containing heavy metals. Thus it has been shown not only by researchers working with sewage sludges stabilized by different methods such as thermal drying, aerobic composting, anaerobic decomposition or calcification (Selivanovskaya et al., 2001; Fernández et al., 2009; Jamal et al., 2011), but also by researcher studying with sewage sludge from different wastewater treatment plants of the same municipality (Wong et al., 2001) that the effect of all of them on the soil and crop ecosystem will vary.

The nutrients and organic materials remaining in the sewage sludge at the end of the treatment processes provide for one of the most important methods of disposal of the sludge, that is its use on the soil as a fertilizer or soil improver. The application of sewage sludge to agricultural land is an economical and sustainable method both of recovering nutrients and of disposing of sewage sludge (Lundina et al., 2004; Laturnus et al., 2007; Ghazy et al., 2011). Enriching the soil in organic materials and providing nutrient or fertilizing materials such as nitrogen and phosphorus are the major advantages of this treatment, which can result in an increase in the productivity of the soil (Walia and Goyal, 2010; Saviozzi and Cardelli, 2014). Detailed studies have been conducted on this topic by many researchers in various countries (Gascó and Lobo, 2007; Laturnus et al., 2007).

It has been established in previous studies that the application of sewage sludge improved the physical (Griffiths et al., 2005) and chemical (Speir et al., 2004) characteristics of the soil, and generally supported microbial growth and activity (Debosz et al., 2002; García-Gil et al., 2004). In various other studies, it has been shown that the application of urban sewage sludge has developed the physical characteristics of the soil, such as bulk density, aggregate stability, water retention capacity, total porosity and saturated hydraulic permeability (Kahapanagiotis et al., 1991; Silveira et al., 2003; Rezig et al., 2013), increased the content of organic material, and also prevented erosion (Singh and Agrawal, 2008; Alcantara et al., 2009; Franco et al., 2010; Annabi et al., 2011). However, it has also been shown that the addition of sludge can cause unwanted changes such as a fall in pH values or a rise in salinity, or the concentration of heavy metals (Navas et al., 1998; Veeresh et al., 2003; Singh and Agrawal, 2008). Besides, the long-term repeated addition of sludge can potentially have harmful effects such as the accumulation of toxic metals or damage to microbial communities and their functions, thereby threatening the long-term vitality of the soil. However, observations by different researchers have shown variations in the long-term effects of sludge on the microbial biomass of the soil (Flieβbach et al., 1994; Defra, 2005). The ability to establish the biological characteristics of the soil which give the fastest and most accurate response to all soil management practices in the short term will form an important basis for creating a long-term perspective. Also, there has as yet been little research into the short term effects of the use of dried organic wastes left over after the extraction of energy by anaerobic decomposition on the chemical and biochemical characteristics of the soil and on the yield of annual plants.

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The aim of this study, designed from this starting point, was to research and compare the effect on cotton and maize yield of various doses of sewage sludge (SS) from the Çiğli Wastewater Treatment Plant of İzmir Metropolitan Municipality, stabilized in anaerobic conditions and converted to granules of 90% dryness, with the biochemical characteristics of a degraded soil in a semi-arid Mediterranean agro-ecosystem. In addition, variations in the heavy metal content of the soil were examined. Enzymes considered in the study were those involved in cycling in the soil: protease and urease of N, β-glucosidase of C, alkaline phosphatase of P, and aryl sulfatase of S. Different from the others, the enzyme dehydrogenase is an enzyme which functions in the mechanisms of intracellular respiration and shows the total oxidative capacity of the microbial biomass.

Materials and methods

Anaerobically digested sewage sludge (SS)

The sewage sludge used in the experiment was from the Çiğli Wastewater Treatment Plant of İzmir Metropolitan Municipality of Turkey, stabilized in anaerobic conditions and converted to granules of 90% dryness. Various characteristics of the SS are given in *Table 1*.

Table 1. *Mean values of the some physicochemical properties of anaerobically digested sewage sludge (SS) from Çiğli Wastewater Treatment Plant used in the experiment*

Parameter	Mean[a]	Turkish directive	European directive	USA (Part 503)
pH[c]	**7.18**			
EC[d] (dS m^{-1})	**1.95**			
Carbonates (g kg^{-1})	**53.50**			
$C_{(Org)}$ (g kg^{-1})	**296.98**			
C/N	**9.93**			
N[e] (Kjeldahl) (g kg^{-1})	**29.90**			
P[e] (g kg^{-1})	**2.28**			
K[e] (g kg^{-1})	**3.40**			
Ca[e] (g kg^{-1})	**63.60**			
Mg[e] (g kg^{-1})	**20.40**			
Na[e] (g kg^{-1})	**1.39**			
Fe[e] (g kg^{-1})	**12.76**			
Cu[e] (mg kg^{-1})	**176.50**	1000	1000-1750	4300
Zn[e] (mg kg^{-1})	**1376.59**	2500	2500-4000	7500
Mn[e] (mg kg^{-1})	**350.00**			
Cd[e] (mg kg^{-1})	**2.83**	10	20-40	85
Cr[e] (mg kg^{-1})	**112.53**	1000	-	3000
Pb[e] (mg kg^{-1})	**17.44**	750	750-1200	840
Ni[e] (mg kg^{-1})	**69.73**	300	300-400	420
Co[e] (mg kg^{-1})	**6.71**			

[a]Each value is the mean of three replicates and on an oven-dry (105 °C) basis; [b]Standard deviation; [c]pH of 1:2.5 water extract; [d]Electrical conductivity of 1:5 water extract; [e]Total

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The sewage treatment process was designed according to an advanced biological purification method, biologically removing phosphorus and nitrogen and able to produce water of a better quality, with a capacity of approximately 605 t day^{-1}. The facility has four digestion tanks, two biogas collection tanks and four dryers, each with the capacity to process 200 t day^{-1} of sludge cake. In 2015, the sludge digestion and drying unit produced a total of 11550 t of dried sewage sludge and 11755000 m^3 of biogas (İZSU, 2018). The heavy metal content of the SS used in the study was below the values allowed by Europe Directive 86/278/CEE (CEC, 1986), US (EPA, 1993) and Turkish regulations (RG, 2010) (*Table 1*).

Field experiments

Field experiments were conducted in 2015 at the Research, Application and Production Farm of Ege University Agriculture Faculty (longitude: 27°01'20"-27°01'22" E; latitude: 38°34'47"-38°34'45" N; average altitude 5 m), on sandy loamy soil (sand, silt and clay 558.4, 314.4 and 127.2 g kg^{-1} respectively). *Figure 1* shows the experimental area location and some photos of the experimental period. The soil is classified as Typic xerofluvent (Soil Survey Staff, 2010). The physicochemical properties of the experimental field at the beginning of the experiment are given in *Table 2*. The Menemen Plain, where the research area is located, has a Mediterranean climate, with hot dry summers and cool rainy winters. According to long-term (55 year) climate data, mean total annual precipitation is 525.3 mm. Approximately 50% of this precipitation occurs in winter, 25% in spring, 23% in autumn and 2% in summer. The mean temperature is 16.9 °C, mean relative humidity is 57.5%, and mean annual evaporation is 1532.1 mm (Anonymous, 2009).

Table 2. *Physicochemical characteristics of experimental soil at the beginning of the experiment*

Parameter	Mean[a]		Parameter	Mean[a]	
$pH_{(H_2O)}$	7.66	(0.1)[b]	Na^c (mg kg^{-1})	30.31	(4.2)
Salinity (mg kg^{-1})	286.7	(69.9)	Fe^d (mg kg^{-1})	2.21	(0.6)
Carbonates (g kg^{-1})	50.49	(3.5)	Cu^d (mg kg^{-1})	0.65	(0.2)
Sand (g kg^{-1})	558.4	(33.2)	Zn^d (mg kg^{-1})	1.54	(0.7)
Silt (g kg^{-1})	314.4	(21.2)	Mn^d (mg kg^{-1})	1.64	(0.4)
Clay (g kg^{-1})	127.2	(9.0)	Zn^e (mg kg^{-1})	58.29	(1.3)
Texture	Sandy loam		Cu^e (mg kg^{-1})	15.49	(0.6)
$C_{(Org)}$ (g kg^{-1})	7.73	(1.6)	Cr^e (mg kg^{-1})	23.22	(3.1)
C/N	9.72	(1.8)	Cd^e (mg kg^{-1})	0.82	(0.1)
$N_{(Kjeldahl)}$ (g kg^{-1})	0.79	(0.1)	Pb^e (mg kg^{-1})	11.66	(2.1)
$P_{(Olsen)}$ (mg kg^{-1})	18.88	(4.6)	Ni^e (mg kg^{-1})	55.49	(2.6)
K^c (mg kg^{-1})	231.3	(26.9)	Hg^e (mg kg^{-1})	61.52	(10.0)
Ca^c (mg kg^{-1})	2200	(119)	As^e (mg kg^{-1})	15.93	(2.8)
Mg^c (mg kg^{-1})	273.8	(23.2)	B^f (mg kg^{-1})	1.27	(0.4)

[a]Each value is the mean of four replicates of control soils; [b]Standard deviation; [c]NH_4OAc extract; [d]DTPA extract; [e]Total, HCl+ HNO_3 extract; [f]Total, hot water extract

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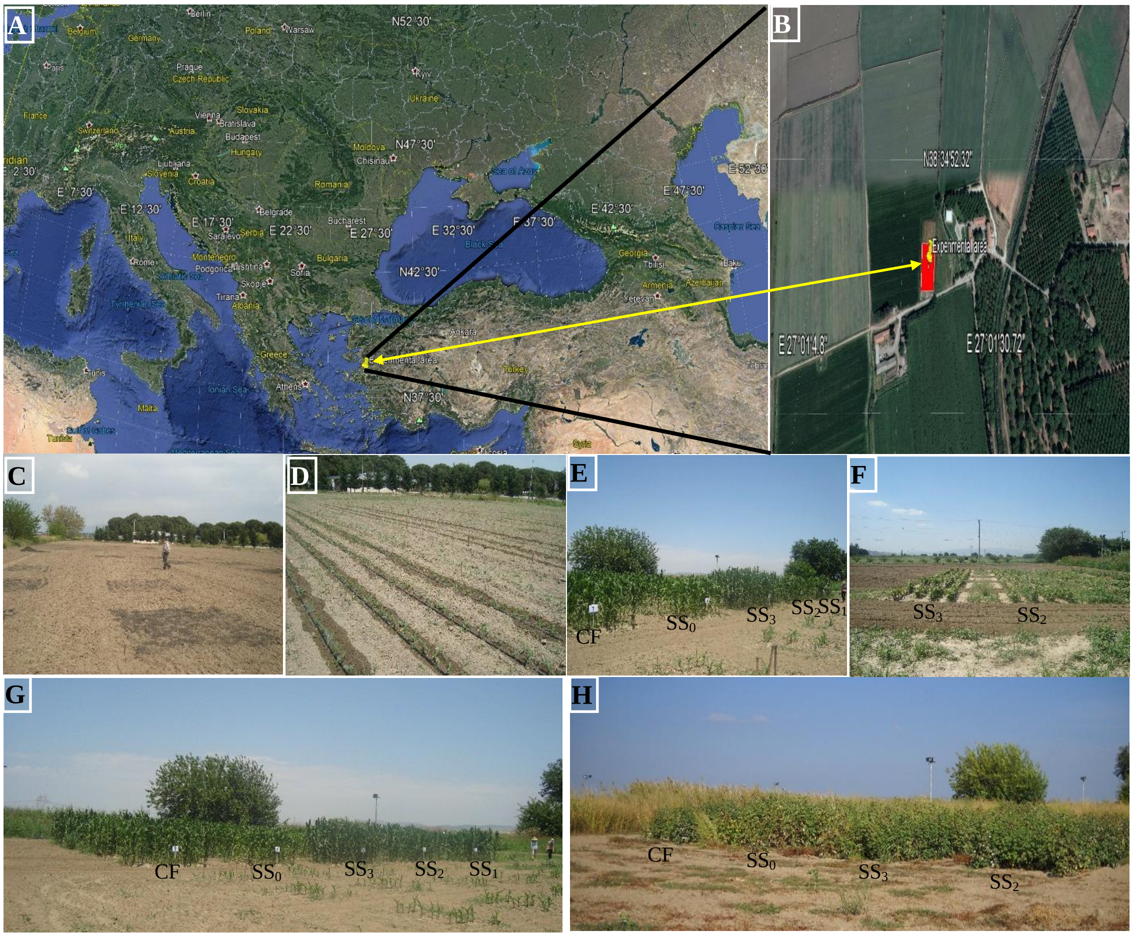

Figure 1. *Map showing (A) the location of the Research, Application and Production Farm of Ege University Agriculture Faculty experimental area in the İzmir Province, Turkey and (B) close-up view of the experimental field, (C) photo of sewage sludge applications, (D) photo of drip irrigation activity, (E) and (G) photos of the maize experiment, (F) and (H) photos of the cotton experiment*

The experiment was performed in four randomized blocks of soil plots (3 m × 3 m) with four replications cropped with maize (*Zea mays* L. var. ZP 737) and cotton (*Gossypium hirsutum* L. var. GSN 12), at the same time. Soil plots were either unamended (SS_0) or amended with SS at rates of 10, 20 and 30 t ha^{-1} on a dry weight basis (SS_1, SS_2 and SS_3, respectively). After application of the SS to the surface of the soil in the experiments, the soil was mixed to a depth of 15 cm using a rotary tiller (21 April 2015). 1 t ha^{-1} 15-15-15 composite fertilizer was used for the mineral fertilizer application in the maize experiment (CF). Maize sowing took place on the same day, 21.4.2015, on all experimental plots after the application of sewage sludge. Seeds were sown in rows 70 cm apart, at 18.3 cm intervals, using a seed drill. As mineral fertilizer, 150 kg ha^{-1} urea fertilizer (46% N) was applied as a top dressing on 4 June 2015. Cotton sowing took place on 29 April 2015. In the cotton experiment, 500 kg ha^{-1} of 15-15-15 composite fertilizer was applied as basic fertilizer to the mineral fertilizer plots (CF) on 21 April 2015. As a mineral fertilizer application in the cotton experiment, 150 kg ha^{-1} of urea fertilizer was applied to the plots as a top dressing on 4 June 2015. The second top dressing in the cotton experiment was applied during the flowering period (8 July

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2015), with only mineral fertilizer at a dose of 270 kg ha^{-1} of CAN (calcium ammonium nitrate). In both the maize and the cotton experiments, drip systems were set up for irrigation purposes. In order to meet the water requirement of the test plants, irrigation activities were carried out every 15 days from the sowing of seeds, taking into consideration the regional climatic conditions and producer practices. Irrigation water was given for maize plant in 6 times (total 600 mm) and for cotton plant in 8 times (800 mm in total) with 100 mm in each irrigation period.

The first soil samling period (SP_1) was taken fifteen days after the application of the SS. The second soil sampling period (SP_2) was taken 120 days later (19 August 2015) for maize and 189 days later (27 October 2015) for cotton. Soon after the plants were harvested individually, surface soil samples were collected randomly from the arable layer (Ap horizon, 0–15 cm depth) of each plot. Each soil sample consisted of a mixture of 10 soil cores, each 3 cm in diameter. Soil samples for biological and biochemical analyses were stored at 4 °C at field moisture. *Table 3* shows the results of the analysis of the soil samples (First period n = 40, second period n = 40) of the microbiological parameters microbial biomass carbon (MBC), basal soil respiration (BSR), N-mineralization (N_{min}), alkaline phosphatase enzyme activity (ALKPA), dehydrogenase enzyme activity (DHG), protease enzyme activity (PRO), urease enzyme activity (UA), ß-glucosidase enzyme activity (GLU) and aryl sulphatase enzyme activity (ArSA), while *Tables 4* and *5* show the Pearson correlation matrix for the chemical parameters analyzed with these parameters for maize and cotton vegetation respectively. When the maize plants came to harvesting maturity, the middle two rows of the four rows on each plot were used (19 August 2015). The cotton bolls were also harvested by hand (27 October 2015). At the end of harvest, plot yields were calculated and converted to t ha^{-1}.

Chemical analysis of soil and sewage sludge

Prior to analysis, soil samples were air-dried and passed through a 2 mm sieve. The principal chemical properties of soil and SS samples were determined by standard methods (Sparks et al., 1996). In particular, the pH was measured on satured soil and on mixtures of sludge:water = 1:2.5; the EC was measured on a 1:5 sample:water extract; and the TOC content was determined by dichromate oxidation of the sample and subsequent titration with ferrous sulfate heptahydrate. The total N content was obtained by the Kjeldahl method (Keeney and Nelson, 1982). Available P content was determined according to the Olsen method (Olsen and Sommers, 1982); and available K content was measured by Flame Photometer in 0.5 M ammonium acetate soil extracts using a ratio of soil to extractant of 1:10 (Sparks et al., 1996). For determination of heavy metals, the soils were extracted with 3 parts HCl + 1 part HNO_3. The concentrations of Pb, Ni, Cr, Co, Hg, As and Cd in the extracts were determined by atomic absorption spectrometry (AAS) (ISO, 1995, 1998). For determination of boron, soils were extracted with hot water for 5 min, centrifuged and filtered. Boron was determined using azomethine-H. Reagents formed using ammonium acetate and disodium ethylenediamine-tetra-acetate for this study are those used by Gupta (1979). The SS samples were dried at 70 °C for 72 h, and their dry weights were recorded. The samples were then ground to pass through a 2 mm sieve for subsequent analysis. After nitric and perchloric acid digestion (4:1), total Mg, Fe, Cu, Mn, Zn, Cd, Pb, Ni, Hg and Cr concentrations in the SS were determined using AAS, and Ca, K and Na concentrations were determined by flame photometry (Kacar and İnal, 2008). Total P content in the acid digest was determined using a spectrophotometer after developing the vanadomolybdophosphoric yellow color complex in a nitric acid medium (Kacar and İnal, 2008).

Soil biological and biochemical analyses

Basal soil respiration (BSR) was found using a 0.1 N NaOH solution after a 24 h incubation period at 25 °C (Isermeyer, 1952; Jäggy, 1976). In order to determine microbial biomass carbon (MBC), the moisture content of soil samples was determined, and after fumigation according to Jenkinson (1976), they were agitated with 0.5 M K_2SO_4 (Vance et al., 1987). After that, the amount of C in the filtrate was determined by wet digestion in the presence of strong acid (a mixture of H_2SO_4 and H_3PO_4) and 0.4 N $K_2Cr_2O_7$, after which the excess dichromate was titrated with a 25 mM 1.10-fenantrolin iron sulfate complex indicator solution (Kalembasa and Jenkinson, 1973; Vance et al., 1987). A kEC factor of 0.45 was used in the calculations (Jenkinson and Ladd, 1981). N-mineralization (N_{min}) was assayed according to the method of Keeney (1982). This method involves the incubation of a soil sample under waterlogged conditions at 50 °C. After seven days, NH_4-N released from the soil water mixture was determined with a modified Bertholet reaction. Dehydrogenase enzyme activity (DHG: EC 1.1) was determined after adding TTC (triphenyl tetrazolium chloride) solution at different concentrations according to the soil texture and the amount of organic matter to the soil samples and incubating for 16 h at 25 °C, by photometric measurement at a wavelength of 546 nm of the resulting TPF (triphenyl formazan) (Thalmann, 1968). The activity of urease, an enzyme of the hydrolase group (UA: EC 3.5.1.5), was determined by incubating the soil where urea was used as a substrate at 37 °C for 90 min, and determining the resulting ammonia with a modified Bertholet reaction after extracting it with 2 M KCl (Kandeler and Gerber, 1988). To determine alkaline phosphatase enzyme activity in the soil samples (ALKPA: EC 3.1.3.1), buffered p-nitrophenyl phosphate solution was added and p-nitrophenol, incubated for 1 h at 37 °C, and resulting by phosphomonoesterase activity, was colored with sodium hydroxide and measured photometrically at 400 nm (Eivazi and Tabatabai, 1977; Tabatabai and Bremner, 1970). For protease enzyme activity (PRO: EC 3.4), soils where casein was used as a substrate were incubated at 50 °C for two hours, and the resulting aromatic amino-acids were colored in an alkaline environment with folin-ciocalteu, and detected colorimetrically at 700 nm (Ladd and Butler, 1972). The soil samples were incubated for three hours at 37 °C with salicin, after which the resulting saligen was determined spectrophotometrically at 578 nm in order to determine β-glucosidase activity (GLU: EC 3.2.1.21) (Hoffman and Dedeken, 1965). In order to determine the activity of aryl sulfatase, which has a role in the sulfur cycle (ArSA: EC 3.1.6.1), a solution of p-nitrophenylsulfate was added to the soil samples as a substrate, and the resulting nitrophenol was measured photometrically at a wavelength of 430 nm (Tabatabai and Bremner, 1970).

Statistical analysis

All data were tested for normality and homogeneity of distribution, and were log-transformed if required prior to analyses. Multivariate analysis of variance (MANOVA) test was used to study the effects of treatment on soil chemical, biochemical and microbiological properties. Comparison of average values was performed with the Duncan multiple comparison test and with a significance level of $\alpha = 0.05$. Pearson

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correlation analyses were performed on all the chemical, microbiological and biochemical data. All statistical analyses were performed using the IBM SPSS 20.0. Standard deviation values showing distribution according to the average of data obtained in each period were also calculated by means of the same program.

Results and discussion

Maize and cotton yield

Figure 2 shows the effects on the yield of maize and cotton of SS applied at different doses. It was observed that SS applied in the experiment had no toxic effect on either of the crops. In fact, statistically significant increases in yield parameters of the crops were seen with increasing doses of SS in comparison with the SS_0. A yield increase of 90% was determined in maize grain yield with SS_3 compared to the control. Similarly, seed cotton yield increased by 72% with the same treatment. In a study conducted with maize in a hot, moist and semi-arid eco-subregion soils of India which climate is dominated by monsoons and strongly influenced by the Himalayas and the Thar Desert, a determination was made of the effect of the application of compost, stabilized sewage sludge and nitrogen fertilizer to the crop. According to the results of the field experiments, the two highest doses of stabilized sludge (80 and 160 t ha^{-1}) gave significant increases in yield, but at the same time increases were determined in the heavy metal contents of the crop. The researchers found that treatment at the level of 40-60 t ha^{-1} yr^{-1} of sewage sludge was the most suitable to achieve acceptable crop growth and a minimum of adverse effects on crop and soil quality (Begum, 2011). In a study, laboratory incubation and pot experiment were carried out to determine the effect of heavy metal concentration, adverse effects were shown in the growth of Indian mustard of applications of 40 and 80 t ha^{-1} of sewage sludge which had a heavy metal concentration of twice the limit set by the European Union (Walia and Goyal, 2010).

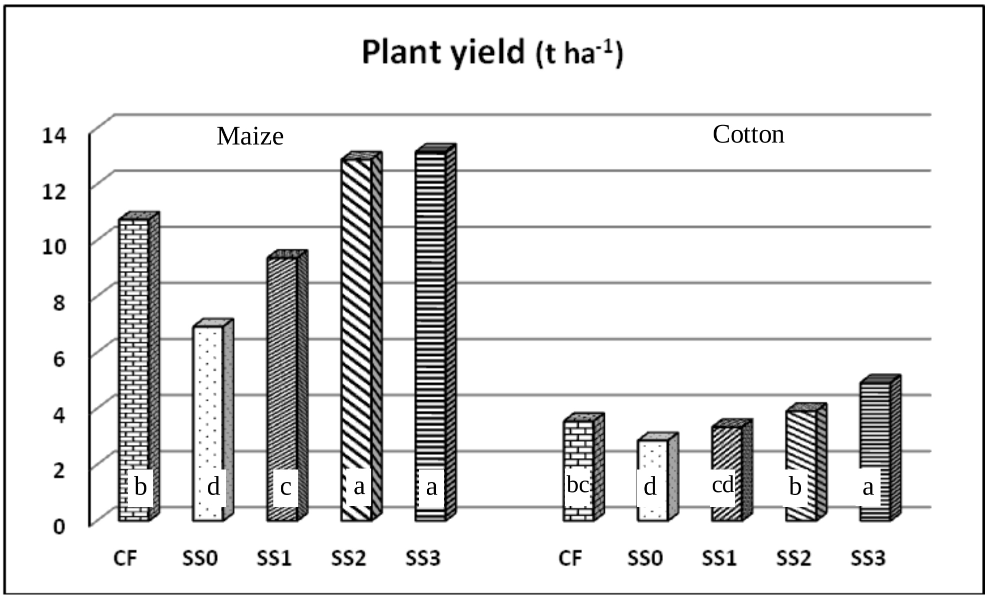

Figure 2. *The effect of sewage sludge on grain yield of maize and seed cotton yield. Each value is the mean of four replicates. Results within treatmentsof each plant capped with different letters are significantly different (Duncan's test: P < 0.05)*

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The response of the yield parameters of maize to the application of sewage sludge was clearer than that of cotton. In a study by Al Zoubi et al. (2008) on Syrian soils under the effect of a Mediterranean climate, an increase was shown in maize yield with increasing doses of sewage sludge compared with the application of chemical fertilizer. On the other hand, the same researchers found no difference between wheat yields with the application of sludge or inorganic fertilizer. Thus, the effects of the application of sewage sludge vary according to the type of crop. Examining the results obtained in the present study, because not only the increase of 86% in maize grain yield was obtained with the SS_2 but also there was statistically no difference between the SS_2 and SS_3 doses, application at 20 t SS ha^{-1} (SS_2) to Mediterranean soils where maize is grown may be recommended. Additionally, less heavy metal will be added to the soil with the SS_2 than with the SS_3 dose. However, a similar effect was not seen as per seed cotton yield. The statistically greatest yield was obtained with the highest sewage sludge application (SS_3), and so a dose of 30 t SS ha^{-1} may be recommended for soils where cotton is grown in Mediterranean climatic conditions. Of course, even when concentrations of nutrient elements in the sewage sludge are the same for the two crops, the differences in the morphological characteristics of the plants can cause differences to emerge in results in the same field. Similar results in terms of varieties of plant species have been obtained in studies of the effects of the application of sewage sludge on crop yield in field conditions. In a study by Tamrabet et al. (2009) examining the effect of the application of sewage sludge to the soil on the yield of durum wheat (*Triticum durum* Desf.), doses of 33 kg ha^{-1} of mineral fertilizer (urea) and doses of 20, 30 and 40 t SS ha^{-1} were applied to the soil under semi arid cropping conditions of Algeria. It was determined that the most effective application of sludge for wheat yield was 30 t ha^{-1}, and that in semi-moist conditions sewage sludge had a positive effect on the yield of that wheat variety, and could be used safely.

Another result emerging from our study was that the yield increase obtained with the application of chemical fertilizer was higher than that obtained with the 10 t SS ha^{-1} given with the SS_1, but lower than the yield increase provided by SS_2 and SS_3. It was seen that much more effective plant nutrition was achieved by the application of plant nutrient elements provided to the soil by sewage sludge together with the addition of organic materials, especially those which are not present in chemical fertilizers. Therefore, increasing the low level of organic carbon of approximately 7.73 g kg^{-1} in the soil may allow plants to benefit more effectively from plant nutrient elements either in the soil or mineralized in connection with SS applications. In addition to this, it must not be forgotten that organic soil improvers applied to sandy loamy soils can also support an increase in yield by improving the physico-chemical characteristics of the soil. Similar to our findings, a statistically significant values similar to that of a mineral fertilizer treatment were obtained in grain yield of maize at the end of 10 years due to increasing sewage sludge doses which accumulated in each treatment 50, 100 and 147.5 t ha^{-1} in total at the end of the experimental period in agricultural Oxisol under a tropical climate of Brazil (Melo et al., 2018). Researchers have suggested that purification sludge applications can meet all of the benefits of chemical phosphorus and microelement fertilization, while chemical nitrogen fertilization can be partially replaced without loss of yield in the maize plant.

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Soil microbiological and biochemical activities

A brief overview on soil health

All of the doses of SS applied to the soils affected the microbiological parameters analyzed significantly at a rate of 1%. The level of effect varied in connection with the type of crop, and it is thought that the greatest effect was the difference in the crop vegetation periods. Additionally, it can be said that when the root morphology and root secretion of the two test crops are considered, the differences in the rhizosphere zone also have an effect on managing microbial activity. It was observed that biological activity in maize plants with regard to the parameters examined was approximately 6.5% higher than that of cotton (*Table 3*). In maize soils, SS_3 more stimulated microbiological and biochemical parameters at a statistically significant level. The situation was somewhat different with soils under cotton, and it can be said that the effect of sewage sludge was almost the same on a dose basis. Thus, it can be seen from *Table 3* that an effect was shown at the same level of statistical significance on seven of the nine microbiological and biochemical parameters analyzed by both SS_3 and SS_2, and on three by SS_1. This is one of the important conclusions emerging from the results of the experiment: that is, that in soils under cotton, which has a longer growing period than maize, the differences between the microbiological and biochemical parameter values analyzed resulting from the treatments are reduced. The reason for this may be said to be that after the easily available carbon in SS is applied to the soil, it is used in a short time by heterotrophic microorganisms as a source of energy and carbon, leaving in the soil a valuable and stable humus which is resistant to breakdown. Looking at the overall picture, what emerges is that with crops with a short growing period, sewage sludge can be used as an organic fertilizer because of its capacity for mineralization.

Table 3. *Effects of stabilized sludge (SS) applications on some microbiological and biochemical properties of the soil under maize and cotton vegetation*

Parameters		1st SP [a]	2nd SP	Mean	1st SP	2nd SP	Mean
		Maize			Cotton		
BSR[h]	SS_0[b]	0.100 *b*[g]	0.091 *bc*	**0.096 *b***	0.097 *b*[f]	0.112	**0.104 *b***
	CF[c]	0.113 *b*	0.111 *a*	**0.112 *ab***	0.129 *ab*	0.119	**0.124 *ab***
	SS_1[d]	0.157 *a*	0.073 *c*	**0.115 *ab***	0.141 *a*	0.112	**0.127 *a***
	SS_2[e]	0.130 *ab*	0.102 *ab*	**0.116 *ab***	0.164 *a*	0.124	**0.144 *a***
	SS_3[f]	0.158 *a*	0.084 *bc*	**0.121 *a***	0.150 *a*	0.122	**0.136 *a***
MBC[i]	SS_0	221.7 *b*	160.1	**190.9 *b***	179.3 *b*	120.4	**149.9 *b***
	CF	209.6 *b*	186.7	**198.2 *b***	195.0 *ab*	160.5	**177.7 *ab***
	SS_1	223.0 *b*	199.0	**211.0 *b***	185.6 *b*	159.9	**172.7 *ab***
	SS_2	337.5 *a*	215.7	**276.6 *a***	246.6 *a*	202.4	**224.5 *a***
	SS_3	194.6 *b*	214.8	**204.7 *b***	220.2 *ab*	142.0	**181.1 ab**
DHG[j]	SS_0	147.6 *c*	124.8 *b*	**136.2 *c***	158.2 *b*	81.11 *b*	**119.67 *b***
	CF	130.8 *c*	113.1 *b*	**122.0 *c***	157.0 *b*	81.83 *b*	**119.44 *b***
	SS_1	228.9 *b*	161.5 *ab*	**195.2 *b***	230.8 *a*	110.6 *ab*	**170.7 *a***
	SS_2	327.6 *a*	193.8 *a*	**260.7 *a***	272.6 *a*	143.2 *a*	**207.9 *a***
	SS_3	307.7 *a*	207.0 *a*	**257.3 *a***	271.9 *a*	143.7 *a*	**207.8 *a***

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	SS_0	583.8 *d*	556.6 *b*	**570.2 *d***	518.0 *c*	529.4 *c*	**523.7 *c***
	CF	533.6 *d*	560.6 *b*	**547.1 *d***	523.1 *c*	547.8 *bc*	**535.4 *c***
ALKPA[k]	**SS_1**	728.9 *c*	727.5 *a*	**728.2 *c***	747.9 *b*	685.2 *ab*	**716.6 *b***
	SS_2	848.9 *b*	768.4 *a*	**808.6 *b***	758.3 *b*	787.4 *a*	**772.9 *b***
	SS_3	957.8 *a*	864.3 *a*	**911.0 *a***	1009.6 *a*	780.4 *a*	**895.0 *a***
	SS_0	151.1 *b*	108.3 *c*	**129.7 *c***	117.7 *d*	93.3 *c*	**105.5 *c***
	CF	128.6 *b*	90.3 *c*	**109.4 *c***	103.8 *d*	91.1 *c*	**97.45 *c***
PRO[l]	**SS_1**	267.2 *a*	226.1 *b*	**246.7 *b***	185.8 *c*	185.5 *b*	**185.6 *b***
	SS_2	270.9 *a*	256.7 *b*	**263.8 *b***	284.0 *b*	270.1 *a*	**277.0 *a***
	SS_3	360.9 *a*	385.1 *a*	**373.0 *a***	352.7 *a*	260.4 *a*	**306.5 *a***
	SS_0	60.19	54.33	**57.26 *ab***	52.31 *c*	61.31	**56.81 *b***
	CF	57.84	53.25	**55.55 *b***	52.84 *bc*	55.41	**54.13 *b***
UA[m]	**SS_1**	72.00	54.37	**63.19 *ab***	64.0 *abc*	55.22	**59.63 *ab***
	SS_2	68.23	61.46	**64.84 *a***	65.97 *ab*	69.08	**67.52 *a***
	SS_3	71.49	60.53	**66.01 *a***	74.15 *a*	61.41	**67.78 *a***
	SS_0	197.3 *b*	174.9 *b*	**186.1 *cd***	222.2 *ab*	173.5 b	**197.9 *b***
	CF	187.6 *b*	162.2 *b*	**174.9 *d***	204.7 *b*	168.8 *b*	**186.7 *b***
ArSA[n]	**SS_1**	235.6 *ab*	193.6 *ab*	**214.6 *bc***	239.6 *ab*	222.9 *a*	**231.2 *a***
	SS_2	262.3 *a*	206.1 *ab*	**234.2 *ab***	248.0 *a*	244.9 *a*	**246.5 *a***
	SS_3	273.4 *a*	223.1 *a*	**248.3 *a***	241.5 ab	223.8 *a*	**232.7 *a***
	SS_0	59.81 *b*	58.35 *c*	**59.08 *c***	58.07 *c*	60.71 *b*	**59.39 *c***
	CF	61.24 *b*	58.98 *c*	**60.11 *c***	63.87 *bc*	69.70 *ab*	**66.78 *bc***
GLU[o]	**SS_1**	80.11 *a*	65.73 *b*	**72.92 *b***	76.29 *ab*	73.59 *ab*	**74.94 *ab***
	SS_2	84.52 *a*	81.86 *a*	**83.19 *a***	84.29 *a*	85.86 *a*	**85.08 *a***
	SS_3	90.12 *a*	83.10 *a*	**86.61 *a***	77.86 *ab*	80.14 *ab*	**79.00 *b***
	SS_0	3.01 *bc*	2.97 *c*	**2.99 *c***	3.32 *d*	2.75 *ab*	**3.03 *c***
	CF	2.74 *c*	3.20 *c*	**2.97 *c***	3.43 *d*	1.62 *b*	**2.52 *c***
N_{min}[p]	**SS_1**	4.78 *ab*	3.83 *bc*	**4.31 *b***	5.20 *c*	3.35 *ab*	**4.28 *b***
	SS_2	5.93 *a*	4.61 *b*	**5.27 *ab***	6.62 *b*	3.29 *ab*	**4.95 *b***
	SS_3	6.18 *a*	5.66 *a*	**5.92 *a***	7.97 *a*	4.88 *a*	**6.42 *a***

[a]SP, sampling period; **[b]**SS_0, Unamended soil; **[c]**CF, Chemical fertilizer; **[d]**SS_1, 10 ton ha^{-1} SS; **[e]**SS_2, 20 ton ha^{-1} SS; **[f]**SS_3, 30 ton ha^{-1} SS; **[g]**same small letters in the same column (different treatments) do not differ by adjusted Duncan test ($P < 0.05$); **[h]**Basal soil respiration (mg CO_2-C g^{-1} 24 h^{-1}); **[i]**Microbial biomass carbon (µg C_{mic} g^{-1}); **[j]**Dehydrogenase enzyme activity (µg TPF g^{-1}); **[k]**Alkaline phosphatase enzyme activity (µg p-NP g^{-1} h^{-1}); **[l]**Protease enzyme activity (µg Tyrosin g^{-1} 2 h^{-1}); **[m]**Urease enzyme activity (µg N g^{-1} 2 h^{-1}); **[n]**Aryl sulphatase enzyme activity (µg p-NP g^{-1} h^{-1}); **[o]**β-glucosidase enzyme activity (µg Saligenin g^{-1} 3 h^{-1}); **[p]**Nitrogen mineralization (µg NH_4-N g^{-1} 24 h^{-1}). *****Each value was given on a dry matter basis as the average of four replicates

Even the heavy metal content of the 10, 20 and 30 t ha^{-1} doses of sewage sludge applied to the experimental soils in treatments SS_1, SS_2 and SS_3 respectively was not shown to have any statistically significant adverse biological activity on the soil. As can be seen from *Tables 4* and *5*, there was not even a negative correlation between the values of heavy metal in the sludge and microbiological activity in the soil. Many of the

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enzymes which are catalyzers of the microorganisms playing an active role in changes and cycles occurring in the soil contain various heavy metals. Examining the correlations of the analysis results, it was seen that DHG activity showed a greater number of positive correlations with the chemical parameter total Cu than with other parameters (*Tables 4* and *5*). A positive correlation of between 1% and 5% was found between all microbiological parameters and Hg and B content in cotton soils, while As content showed a significantly negative correlation with most parameters. In contrast to maize soils, these correlations found between B and As concentrations and microbiological parameters are thought to arise from the larger amounts of irrigation water supplied to the area where cotton is grown: the irrigation periods and thus the amount of irrigation water are greater than for maize.

Also, increases were seen of on average 29-30% compared with the SS_0 and 28-30% against the CF in the microbiological and biochemical parameters of the soils under each plant. Considering the average values obtained from soils under maize and cotton, all increases occurring with SS applications compared to SS_0 and CF treatments were found to be statistically significant ($\alpha = 0.05$). The greatest increase in the experimental area under each crop was related to PRO activity. According to the variance analysis of the maize experiment, sampling periods were statistically significant apart from ALKPA, PRO and Nmin, while treatments had a significant effect on all parameters except BSR. Period x treatment interaction was only significant on DHG and BSR. However, according to the variance analysis of the cotton experiment, periods were statistically significant apart from parameters other than ALKPA, GLU and UA, while treatments had a significant effect on all parameters other than MBC. Period x treatment interaction was found to be significant only on ALKPA. Examining the results obtained regarding the times of sampling of the soils in the experimental area, in relation to the soil samples taken 15 days after the application of sewage sludge (SP_1), the sampling in the harvest period (SP_2) was 120 days after the application of sewage sludge (19 August 2015) for maize, and 189 days after it (29 October 2015) for cotton growth, and it was seen that the values of the parameters analyzed in the soil samples were determined to be on average 13.5% lower. However, this reduction did not obstruct the difference in the effect on microbial activity which could occur in connection with the applications. It is thought that the slight reduction which occurred between sampling periods arose from the harvest times not providing suitable conditions for microbial activity in terms of the soil moisture in the soils under maize and the climatic conditions in the soils under cotton. In fact, this decline was expected to be more pronounced for the reasons given, but unexpectedly, only a slight decrease was determined in our study. The high enzyme activity determined in the soil samples taken in the first period after the application of SS shows the existence of large amounts of substrates which can be broken down biologically in these soils, which is consistent with high unstable-C content, stimulating microbial activity. However, the process of root development in the cotton rhizosphere especially can support biological activity in the soil up to the period of harvest sampling. Root secretion, which mostly stimulates microbial growth, is a side product which represents a part of growth and development, and apart from allowing the development of symbiotic relationships, in a simple way becomes the substrate of microorganisms (Uren, 2007). In addition, plant roots are accepted to be a source of extracellular enzymes in the soil (Egamberdieva et al., 2011). The low levels of difference in activity between the sampling times may also be an indicator of the continuation, even though slight, of the mineralization process of the SS, and this is of

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great importance from the point of view of sustainable management of organic matter in soils under the effect of a Mediterranean climate, especially those degraded by intensive agriculture.

Table 4. *Correlation between average values of heavy metals and microbiological parameters of maize soils at the end of the experiment*

Parameters[a]	**BSR**	**MBC**	**ALKPA**	**GLU**	**DHG**	**ArSA**	**PRO**	**UA**	**N_{min}**
BSR[b]	1			0.333*	0.540**	0.405**		0.597**	0.371*
MBC[c]		1		0.334*	0.458**	0.409**		0.331*	0.404**
ALKPA[d]			1	0.824**	0.862**	0.711**	0.866**	0.524**	0.830**
GLU[e]	0.333*	0.334*	0.824**	1	0.812**	0.850**	0.718**	0.727**	0.796**
DHG[f]	0.540**	0.458**	0.862**	0.812**	1	0.810**	0.744**	0.712**	0.771**
ArSA[g]	0.405**	0.409**	0.711**	0.850**	0.810**	1	0.602**	0.677**	0.657**
PRO[h]			0.866**	0.718**	0.744**	0.602**	1	0.488**	0.712**
UA[i]	0.597**	0.331*	0.524**	0.727**	0.712**	0.677**	0.488**	1	0.578**
N_{min}[j]	0.371*	0.404**	0.830**	0.796**	0.771**	0.657**	0.712**	0.578**	1
Total Zn		0.443**	0.758**	0.676**	0.742**	0.674**	0.700**	0.370*	0.528**
Total Cu	0.400*	0.336*	0.733**	0.764**	0.789**	0.742**	0.745**	0.530**	0.597**
Total Cr			0.625**	0.601**	0.465**	0.419**	0.577**	0.414**	0.616**
Total Cd		0.400*	0.499**	0.471**	0.460**	0.393*	0.532**		
Total Pb									0.355*
Total Ni	0.604**	0.348*			0.528**	0.508**		0.467**	
Total Hg		0.374*	0.590**	0.460**	0.551**	0.482**	0.479**	0.376*	0.451**
Total As									
B[k]					0.313*				

[a]The units of each variables are those given in *Table 2* and *3;* [b]Basal soil respiration; [c]Microbial biomass carbon; [d]Alkaline phosphatase; [e]ß -glucosidase; [f]Dehydrogenase; [g]Aryl sulphatase; [h]Protease; [i]Urease; [j]N-mineralization; [k]Hot water soluble boron. **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

Evaluating the experimental results, it is seen that one remarkable finding was the positive correlation between total Hg concentration in the soils and the analyzed microbiological and biochemical parameters. This correlation, found to be significant at a level of 1% to 5%, was determined in all parameters except BSR in the maize experiment (*Table 4*) and in all parameters in the cotton experiment (*Table 5*). Similar to this finding, it was shown in a study by Campos et al. (2018) in Spain that microbial biomass parameters and DHG activity were not adversely affected in the expected way by pollution levels caused by the toxic metal Hg.

Soil microbiological activities

Basal soil respiration (BSR)

Organic materials found in or applied to the soil are used as a carbon and energy sources by heterotrophic microorganisms. As a result of the growth and activities of them heat, CO_2, water vapor and humus are formed (Epstein, 1997) while the amount of CO_2 generated is defined as BSR. Taking into account the mean values of BSR obtained in the results of the experiment, there was a statistically significant increase in maize

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soils with SS_3, and in cotton soils with SS_1, SS_2 and SS_3 in comparison with SS_0 and CF. It is thought in studies conducted with sewage sludge that climatic conditions where the soils to which SS was applied are located were significant in the variation in the effect of the sludge. In a similar study in Russia, where climatic conditions are different, it was found that applications of sewage sludge stabilized by anaerobic decomposition did not have a statistically significant effect on MBC and BSR (Selivanovskaya et al., 2001). In our study, a significant high positive correlation was also found between BSR and the other biological parameters examined independent of crop type. The highest positive correlation with BSR in the experimental soils was with DHG (r = 0.668**) and the highest negative correlation was with total As (r = 0.372*) (*Table 5*).

Table 5. *Correlation between average values of some chemical and microbiological parameters of cotton soils at the end of the experiment*

Parameters[a]	BSR	MBC	ALKPA	GLU	DHG	ArSA	PRO	UA	N_{min}
BSR[b]	1	0.348^{*}	0.475^{**}	0.526^{**}	0.668^{**}	0.406^{**}	0.534^{**}	0.355^{*}	0.577^{**}
MBC[c]	0.348^{*}	1			0.506^{**}	0.377^{*}	0.373^{*}		
ALKPA[d]	0.475^{**}		1	0.678^{**}	0.652^{**}	0.663^{**}	0.849^{**}	0.491^{**}	0.684^{**}
GLU[e]	0.526^{**}		0.678^{**}	1	0.526^{**}	0.650^{**}	0.653^{**}	0.370^{*}	0.514^{**}
DHG[f]	0.668^{**}	0.506^{**}	0.652^{**}	0.526^{**}	1	0.705^{**}	0.670^{**}	0.446^{**}	0.806^{**}
ArSA[g]	0.406^{**}	0.377^{*}	0.663^{**}	0.650^{**}	0.705^{**}	1	0.645^{**}		0.575^{**}
PRO[h]	0.534^{**}	0.373^{*}	0.849^{**}	0.653^{**}	0.670^{**}	0.645^{**}	1	0.589^{**}	0.738^{**}
UA[i]	0.355^{*}		0.491^{**}	0.370^{*}	0.446^{**}		0.589^{**}	1	0.427^{**}
$\mathbf{N_{min}}$[j]	0.577^{**}		0.684^{**}	0.514^{**}	0.806^{**}	0.575^{**}	0.738^{**}	0.427^{**}	1
Total Zn			0.633^{**}	0.513^{**}	0.317^{*}		0.578^{**}	0.601^{**}	0.401^{*}
Total Cu	0.383^{*}		0.707^{**}	0.562^{**}	0.584^{**}	0.558^{**}	0.708^{**}	0.464^{**}	0.576^{**}
Total Cr			0.446^{**}		0.490^{**}	0.413^{**}	0.423^{**}		0.458^{**}
Total Cd			0.369^{*}	0.452^{**}			0.381^{*}	0.343^{*}	
Total Pb	0.373^{*}	0.408^{**}	0.343^{*}		0.763^{**}	0.427^{**}	0.339^{*}		0.623^{**}
Total Ni	0.380^{*}	0.350^{*}			0.724^{**}	0.471^{**}			0.565^{**}
Total Hg	0.384^{*}	0.326^{*}	0.619^{**}	0.346^{*}	0.578^{**}	0.344^{*}	0.686^{**}	0.453^{**}	0.509^{**}
Total As	-0.372^{*}	-0.349^{*}			-0.595^{**}	-0.414^{**}			-0.394^{*}
B[k]	0.394^{*}	0.383^{*}	0.464^{**}	0.384^{*}	0.700^{**}	0.543^{**}	0.448^{**}	0.350^{*}	0.506^{**}

[a]The units of each variables are those given in *Tables 2* and *3;* [b]Basal soil respiration; [c]Microbial biomass carbon; [d]Alkaline phosphatase; [e]ß -glucosidase; [f]Dehydrogenase; [g]Aryl sulphatase; [h]Protease; [i]Urease; [j]N-mineralization; [k]Hot water soluble boron. **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

Microbial biomass carbon (MBC)

Regarding the mean MBC values, the application of 20 t ha^{-1} of sewage sludge (SS_2) was found to provide an increase in both maize and cotton soils which was statistically significant (α = 0.05) in comparison with SS_0 and CF (*Table 3*). It was observed that the MBC values of soils under both crops were affected by the SS_3 dose, and showed the greatest activity with SS_2. A decrease was determined in the amount of MBC in connection with sewage sludge applied at 30 t ha^{-1} compared to the dose in SS_2 because of the concentration of heavy metals, of 26% in maize soils and of 19% in cotton soils. In spite of this decrease, the MBC values of soils under both crops were still analyzed to

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be at high levels compared to plots with SS_0 and CF treatments. Furthermore, according to some studies (Flieβbach et al., 1994; Filip and Bieleck, 2002), a decrease in MBC can be the result of metal pollution.

Microbial biomass is an available storage of nutrients in the soil, such as C, N, S and P, and an indicator of the cycle of organic materials in the soil (Jenkinson and Ladd, 1981). Although it represents a small amount of the total soil N, C and P, N and other plant nutrients make an important contribution to plant nutrition because of their rapid mineralization. The higher MBC value is thought to be caused by the organic C content of SS. In plots to which the SS_2 dose was applied it was found to be 47% higher than the control and 33% higher than the plot treated with chemical fertilizer. Alongside the heavy metal load of the sewage sludge, the stabilization method may have an effect on its mineralization levels. In a study conducted in field conditions over three years with two different sewage sludges stabilized by composting and thermal drying, microbial biomass increases at the end of three consecutive years of 20 t ha^{-1} yr^{-1} of SS were 3.5 times greater than the control in the case of the SS stabilized by composting and 1.8 times greater in the case of the SS stabilized by thermal drying (Fernández et al., 2009). As was shown in previous studies, when substrate C is added to soils, it stimulates growth in the autochthonous soil microbiota in relation to an increase in energy sources in the soil (Antolín et al., 2005; Pascual et al., 2007). However, in the same study, even though an increase was observed in MBC value with an application of the highest dose of sewage sludge (80 t ha^{-1} yr^{-1}) similar to that of our study, this increase was lower than that provided by 20 t ha^{-1} yr^{-1}. Therefore, microorganisms can to a certain extent tolerate potential toxic effects with both the C content and mineralization potential of SS, which are effective parameters on MBC. When the dose of SS increased beyond a certain point, toleration levels decrease, but MBC activity was still higher than that of the control or that of the chemical fertilizer treatment. In addition, the effects of two different crops on the MBC were not clearly revealed.

Although a significant correlation was found between MBC content and DHG and ArSA activity at the 1% level and GLU and UA enzyme activity at a level of 5%, no correlation was determined between ALKPA and PRO enzyme activities (*Table 4*). In cotton soils, DHG activity showed the highest correlation, while ArSA and PRO enzyme activities showed a slight correlation with MBC content. These results show that ArSA, GLU, UA and PRO activities, and especially DHG enzyme activity, were a good indicator of general microbiological activity in the soils examined in this study, and in addition to this, they can provide valuable information on plant nutrient cycle processes. In both maize and cotton soils, MBC gave the highest positive correlation with DHG activity, and the only negative correlation with total As.

Nitrogen mineralization (N_{min})

N-mineralization, the conversion of nitrogen in organic form to its inorganic form, is performed by microorganisms in the soil with various physiological characteristics. The realization of this process depends on the suitability of environmental conditions, the presence of the relevant microorganisms in the environment, and a supply of suitable organic material. An increase was observed statistically in N_{min} values in relation to SS_0 and CF soils with all three doses of sewage sludge applied to the experimental soils. Also, from among the three doses, SS_3 had the greatest effect on N_{min}. It is thought that the observed increase in nitrogen mineralization during the growing period of each of the crops is an indicator that this waste has a high potential to increase crop

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productivity. Similarly, in a study in which SS was applied to sandy soil at a rate of 20-320 g kg^{-1} for 100 days, it was shown that N mineralization took place although at a low rate, and for this reason N was added to the soil by mineralization throughout the production season for plants growing in a single year (Alva et al., 2006). Also, the fact that no decline was determined in the levels of N-mineralization with increasing SS doses. This showed that the SS applications up to 30 t ha^{-1} did not have a restrictive effect on ammonification and nitrification bacteria in the same year. The N_{min} value shows a high correlation with the other microbiological and biochemical parameters analyzed in the soils under both crops (*Tables 4* and *5*). N_{min} showed the highest positive correlation in maize soils with ALKPA (r = 0.830**) and on cotton soils with DHG (r = 0.806**).

Soil biological activities

Dehydrogenase enzyme activity (DHG)

Dehydrogenase is an intracellular enzyme which functions inside living cells. Different from the other enzymes examined, which function both inside and outside cells, DHG gives more reliable information on the size and activity of the living microbial population (Bergstrom et al., 1998). Applications of sewage sludge, and especially treatments SS_2 and SS_3, increased DHG by between 42 and 53% in relation to treatments SS_0 and CF, with a 5% level of significance. Similar studies are also to be found in which SS applied to soils increased DHG activity (Fernández et al., 2009; Mondal et al., 2015). On the other hand, some researchers have reported that DHG activity was inhibited by the toxic effects of heavy metals with the addition of organic wastes rich in Pb (Marzadori et al., 1996) and Cu (Chander and Brookes, 1991). Thus, the lack of a statistically significant difference between DHG activity analyzed in relation to SS_2 and SS_3 treatments in maize soils and all three applications in cotton soils is an indicator of the lack of a clear increase in the number of living microbial cells. The observed trend in MBC values supported this situation. However, even though an increase in the amount of organic C added to the soil provided by the increased doses did not seem to support a numerical increase in living cells, positive changes observed in other microbiological and biochemical parameters may be an indication that various species of microorganisms with different functions in the ecosystem are supported. High positive correlations were found between DHG and all the microbiological parameters analyzed (*Tables 4* and *5*). With the result that there is a positive correlation between the heavy metal content of the soil and DHG, it may be concluded that soils to which sewage sludge is applied for a single year are not negatively affected in terms of microbial activity.

Alkaline phosphatase enzyme activity (ALKPA)

Phosphatase enzymes hydrolyze organic phosphorus compounds to ortho-phosphate, a form which plants can uptake (Amador et al., 1997). Because they are only produced by microorganisms, alkaline phosphatases are directly related to microorganism activity in the soil (Cayuela et al., 2008). A statistically significant increase was seen in ALKPA activity at an average of 37-41% compared to SS_0 and CF treatments with SS_3, and this was found to be 911.04 μg p-NP g^{-1} h^{-1} for maize soils and 894.97 μg p-NP g^{-1} h^{-1} for cotton soils. In another study, conducted with soils in a Mediterranean climate, aerobically stabilized sewage sludge was applied to soils in proportions varying

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between 6.2 and 10 g SS kg^{-1} soil (equivalent to 0.5 g P_2O_5 kg^{-1} soil). After incubation periods of 25 and 87 days following the incorporating of sewage sludge to the soil, an increase was found in phosphatase enzyme activities which decreased with a lengthening of the incubation period (Criquet et al., 2007). Other studies have shown similar patterns in soil enzyme activities following the application of SS (Pascual et al., 1998; Antolín et al., 2005; Criquet et al., 2007). Considering all these similar results, it is seen that sewage sludge has a short-term increasing effect on soil phosphatase activities, followed by a rapid decrease. Although we determined a decrease in ALKPA activity, it cannot be said that this decrease was on a large scale. Presumably, just as the quantity of living microorganisms in the sewage sludge and later their activity in the soil to which it was applied may result in an increase in enzyme activity (Dick and Tabatabai, 1984), at the same time the content of the existing substrates in the soil is increased due to SS has various organic substrates, and in this way the activity of microorganisms is supported (Kizilkaya and Bayrakli, 2005). These existing substrates include acid and alkaline phosphomonoesterase substrates (García et al., 1993) and phosphodiesterases (Turner and Haygarth, 2005) in generally large amounts in the organic matter in sewage sludge. Thus, when the amount of available P in the soil is a limiting factor for microbial growth, microorganisms can increase phosphatase production in order to be able to mineralize available P from organic P substrates added to the soil with SS (Criquet et al., 2007). Another result emerging from our study concerned correlations. The highest positive correlation with ALKPA was with PRO for maize and cotton vegetations (r = 0.866**and 0.849**, respectively).

Protease enzyme activity (PRO)

Protease hydrolyzes proteins into polypeptides, oligopeptides and amino acids. Because most N compounds are in an organic-related form in mineral soils, organic nitrogen must be converted to an inorganic form in order for N to be taken up by plants. Organic wastes applied to the soil stimulate protease (Rezende et al., 2004), but the breakdown products of these wastes can hinder this enzyme (Dilly and Nannipieri, 2001). In the present study, higher protease activity was determined in the soils to which the three doses of sewage sludge were applied than in SS_0 and CF soils; breakdown products of the sewage sludge did not hinder this enzyme, but on the contrary increased the amount of substrate necessary for the enzymatic reaction. Among the treatment doses, SS_3 resulted in PRO activity at the highest and most significant levels for maize soils, while with cotton soils, SS_2 and SS_3 showed PRO activity at a significant level. The highest correlations with the PRO value were shown by ALKPA both in maize soils (r = 0.866**) and in cotton soils (r = 0.849**).

Urease enzyme activity (UA)

Urease is an enzyme catalyzing the hydrolysis of urea to ammonia or ammonium, depending on soil pH (Tripathi et al., 2007). According to García et al. (2000), the presence of substrates or the demand of plants and microorganisms for nutrients increases the activity of this enzyme involved in the N cycle. Indeed, as Fernández et al. (2009) determined previously, the highest UA levels in connection with SS applications coincide with the highest crop yield parameters determined (*Fig. 2*). Statistically significant increases of 12-20% were determined with SS_2 and SS_3 compared to treatments SS_0 and CF. Nickel is necessary as a co-factor for the urease enzyme. In

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other words, in order for the urease enzyme to become active, it must join with two nickel ions for each sub-unit. The positive correlation (r = 0.467**) between UA and total Ni determined in maize soils may be an indication of this.

Aryl sulfatase enzyme activity (ArSA)

Aryl sulfatase enzyme which is responsible for the hydrolysis of aryl sulfate esters by the fusion of oxygen-sulfur bonds (Tabatabai, 1994). Ester sulfate, the substrate of aryl sulfate, is only found in fungi, and therefore it can also be a direct indicator of the presence of fungi in the soil (Bandick and Dick, 1999). ArSA was determined at approximately the same levels with all SS treatments, showing that there was a suitable substrate of the enzyme at approximately the same amounts in all sewage sludges. Renella et al. (2005) indicated that the coarse sandy soils from long-term field experiments under maize contaminated with high Cd–Ni-containing sludge reduced arylsulfatase and β-glucosidase activities and were significantly inhibited them by Cd and Ni at concentrations of 13.1 and 52.3 mg kg^{-1}, respectively. On the other hand in our study, the highest ArSA activity analyzed in maize soils was 248.25 μg p-NP g^{-1} h^{-1} with SS_3, and this value was achieved in cotton soils at a level of 246.47 μg p-NP g^{-1} h^{-1} with SS_2. ArSA activity showed an increase of 13-30% with SS applications relative to SS_0 and CF. Positive correlations were determined between ArSA and all microbiological and biochemical parameters. The highest correlation for maize soils was with GLU activity (r = 0.850**), and for cotton soils with DHG activity (r = 0.705**).

β-glucosidase enzyme activity (GLU)

Sugars with low molecular weight, which are hydrolysis products of glucosidases, are an important source of energy for soil microorganisms. One of the most important glucosidases in the soil is β-glucosidase which catalyzes the hydrolysis of cellobiose and contributes to the mineralization of the main organic carbon compound in nature, cellulose (Landgraf et al., 2003). The highest GLU activity in soils was shown with SS_3 in maize soils (86.61 μg Saligenin g^{-1} 3 h^{-1}), and in cotton soils with SS_2 (85.08 μg Saligenin g^{-1} 3 h^{-1}). An average increase of 22-32% was achieved in relation to SS_0 and CF. Hattori (1988) and Dick et al. (1988) determined higher GLU in applications of stable organic material containing cellulose. The highest positive correlation with GLU activity for maize soils was with ArSA (r = 0.850**), and for cotton soils with ALKPA (r = 0.678**).

Conclusions

Increases were seen in all microbiological parameters analyzed in relation to sewage sludge treatments and were found to be 22% for SS_1, 35.5% for SS_2, and 33% for SS_3 in relation to SS_0 and CF. In particular, it was observed that the SS applications of 20 and 30 t ha^{-1} increased microbiological parameters more. Correlation matrices showed that the SS doses of 10, 20 and 30 t ha^{-1} yr^{-1} applied did not result in a negative correlation between heavy metals and microbiological parameters, but rather showed positive correlations. This is because the result of the application to the soil of sewage sludge containing organic matter of a quality and amount to encourage microbial activity in the soil is to mask the negative effect created by the heavy metals it contains either on

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microorganisms or on the plant. Even though there was a decrease of 13.5% in the biological parameters analyzed in the second period compared with the first period, this decrease was not at a level to eliminate the differences between SS applications. Moreover, this is important for the management of sustainable organic matter in agricultural soils, and it brings the sewage sludge stabilization method to the fore. Biological activity in soils under different vegetation was also different. Microbial activity in the rhizosphere area of maize plants was found to be 6.5% greater than that of cotton. The highest dose of SS gave an increase in maize grain yield of 90% and in seed cotton yield of 72% over CF soils, and these increases were both much greater than the increases secured by chemical fertilizer applications and also different at a statistically significant level. The potential of organic matter to improve the physicochemical characteristics of a sandy loamy soil may have made a positive contribution to this. Even with the highest dose of sewage sludge applied in the experiment (SS_3), no toxic effect was observed in either maize or cotton plants. Therefore, in soils with a poor light-textured and low organic matter content in a Mediterranean climate and with one-year applications of sewage sludge, applications of stabilized sewage sludge at a level of 30 t ha^{-1} whether for the purpose of sustainable management of the soil's organic matter or to increase the crop yield can be used without ecotoxicological effects. This study, however, was conducted in a representative area of the Mediterranean Basin where arid and semi-arid climate conditions are dominant, so the results obtained can also be applied to other Mediterranean countries where SS incorporating to agricultural soil at a rate up to 30 t ha-1 yr-1 can be a cheap valuable solution to manage and utilize this waste as both fertilizer and soil conditioner. Our results suggest that attention should be paid to reducing the environmental risks of heavy metals in sludge utilization as soil improvement, not only to control the sewage sludge application dosage, but also to cultivate appropriate plant species. In the future, more field experiment is needed to evaluate the long-term residual effects of sewage sludge in the soil in terms of microbiological and biochemical properties of soils under different climate conditions and crop patterns

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APPENDIX

Table A1. *Multivariate hypothesis tests (MANOVA) for maize experiment*

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.998	962.414[b]	9.000	19.000	.000
	Wilks' Lambda	.002	962.414[b]	9.000	19.000	.000
	Hotelling's Trace	455.880	962.414[b]	9.000	19.000	.000
	Roy's Largest Root	455.880	962.414[b]	9.000	19.000	.000
Sampling Periods	Pillai's Trace	.780	7.467[b]	9.000	19.000	.000
	Wilks' Lambda	.220	7.467[b]	9.000	19.000	.000
	Hotelling's Trace	3.537	7.467[b]	9.000	19.000	.000
	Roy's Largest Root	3.537	7.467[b]	9.000	19.000	.000
Treatments	Pillai's Trace	1.917	2.250	36.000	88.000	.001
	Wilks' Lambda	.019	3.806	36.000	72.939	.000
	Hotelling's Trace	13.314	6.472	36.000	70.000	.000
	Roy's Largest Root	10.861	26.548[c]	9.000	22.000	.000
Replications	Pillai's Trace	1.116	1.383	27.000	63.000	.146
	Wilks' Lambda	.205	1.498	27.000	56.132	.101
	Hotelling's Trace	2.471	1.617	27.000	53.000	.067
	Roy's Largest Root	1.845	4.304[c]	9.000	21.000	.003
Sampling Periods * Treatments	Pillai's Trace	1.668	1.748	36.000	88.000	.018
	Wilks' Lambda	.075	2.019	36.000	72.939	.006
	Hotelling's Trace	4.496	2.185	36.000	70.000	.003
	Roy's Largest Root	2.110	5.158[c]	9.000	22.000	.001

a. Design: Intercept + Sampling Periods + Treatments + Replications + Sampling Periods * Treatments
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level

Table A2. *Multivariate hypothesis tests (MANOVA) for cotton experiment*

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.997	832.912[b]	9.000	19.000	.000
	Wilks' Lambda	.003	832.912[b]	9.000	19.000	.000
	Hotelling's Trace	394.537	832.912[b]	9.000	19.000	.000
	Roy's Largest Root	394.537	832.912[b]	9.000	19.000	.000
Sampling Periods	Pillai's Trace	.917	23.172[b]	9.000	19.000	.000
	Wilks' Lambda	.083	23.172[b]	9.000	19.000	.000
	Hotelling's Trace	10.976	23.172[b]	9.000	19.000	.000
	Roy's Largest Root	10.976	23.172[b]	9.000	19.000	.000
Treatments S	Pillai's Trace	1.898	2.207	36.000	88.000	.001
	Wilks' Lambda	.017	3.983	36.000	72.939	.000
	Hotelling's Trace	18.014	8.757	36.000	70.000	.000
	Roy's Largest Root	16.405	40.100[c]	9.000	22.000	.000
Replications	Pillai's Trace	.837	.903	27.000	63.000	.605
	Wilks' Lambda	.318	.998	27.000	56.132	.487
	Hotelling's Trace	1.680	1.099	27.000	53.000	.375
	Roy's Largest Root	1.375	3.207[c]	9.000	21.000	.013
Sampling Periods * Treatments	Pillai's Trace	1.504	1.473	36.000	88.000	.073
	Wilks' Lambda	.108	1.643	36.000	72.939	.037
	Hotelling's Trace	3.705	1.801	36.000	70.000	.018
	Roy's Largest Root	2.435	5.951[c]	9.000	22.000	.000

a. Design: Intercept + Sampling Periods + Treatments + Replications + Sampling Periods * Treatments
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level

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