

Article

The Use of Aerobic Urban Sewage Sludge in Agriculture: Potential Benefits and Contaminating Effects in Semi-Arid Zones

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Abstract: In this work, three wheat crops were planted successively under semi-arid climatic conditions; we wished to evaluate the positive and negative effects of the addition of sewage sludge (SS) on plants and soils under the conditions encountered during conventional agricultural management. SS was added to the first two crops at doses equivalent to 170 kg N/ha, and the third crop was left untreated. The soils were sampled initially and at the end of each cultivation period. At the end of the third crop's cultivation period, the heavy metal and organic pollutant contents of grain and straw were analyzed, as well as the presence of *Escherichia coli* and *Salmonella*. The amended soils showed a higher N content, greater microbial respiration, and greater dehydrogenase and phosphatase activity than the control. The amended plants showed higher N, Ca, and K contents than the control. Yields were 11% and 16% higher in the SS-amended soils than in the control in the experiments involving the second and third crop, respectively. No problems related to salinity or the heavy metal content were observed in both the soil and plant. However, nitrate content increased in the amended soils compared to the control. Among the persistent organic compounds, only linear alkyl benzene sulphonates and polycyclic aromatic hydrocarbons increased with the addition of SS, but such differences from the control disappeared gradually. No problematic coliform content or presence of *Salmonella* spp. was detected in the soil or plant. We can thus conclude that SS of adequate quality can be recycled in agricultural soils, but adequate monitoring of the receiving soil is crucial.

Keywords: inorganic and organic contaminant; pathogens; semi-arid ecosystems; sewage sludge; soil microbial activity; wheat crop



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1. Introduction

The increasing amount of sewage sludge (SS) being generated warrants environmentally safe, socially acceptable, and economically viable methods for its disposal [1,2]. In countries like Spain and other European countries (particularly Southern European countries, which suffer from significant agricultural and soil organic matter (OM) scarcity), a possible alternative to SS valorization is the agricultural recovery of sludge through its direct recycling in soil as an amendment or organic fertilizer [3–5]. This form of recovery turns such waste into a valuable resource, and it is currently the most economical and popular method of valorizing sewage sludge. Through this method, exogenous OM is introduced into the soil, and if it is of sufficient quality, it contributes to the maintenance of soil fertility and productivity, thereby reducing the need for chemical fertilizers [1,6]. It is true that SS can be recovered energetically through the processes of combustion, biomethanization, pyrolysis, and hydrothermal carbonization; however, such methods are not economically viable at present. In the pursuit of environmental sustainability, increasing the organic matter in our soils by adding a sludge of sufficient quality should be considered

a priority if we want to avoid the inevitable desertification processes being initiated in the soils lacking in organic matter [3,7].

The application of sludge improves the state of nutrients in soil, serving as a source of macro- and micro-elements and improving soil's physical properties by increasing porosity, water retention, and the content of "humic-like" substances known as polycondensed carbonated macrostructures, which have direct and indirect positive effects on soil fertility and plant growth [8,9]. Soil's enzymes can be protected in these "humic-like" organic compounds for a long time and are thus preserved from possible denaturation by proteolysis. Enzymes play a fundamental role in the cycling of important elements such as nitrogen (urease and proteases), phosphorus (phosphatases), and carbon (β -glucosidase); they are considered useful for monitoring changes in microbial activity [10–12].

However, the recycling of SS in the soil may not always be beneficial, particularly when the SS is high in salinity, heavy metal content, organic pollutants, and pathogenic microorganisms [13,14]. Lipophilic non-biodegradable organic pollutants tend to bioaccumulate within the food chain [15]. Some of the most frequently detected organic pollutants in SS are polycyclic aromatic hydrocarbon (PAH), linear alkyl benzene sulphonates (LAS), di-2-ethylhexyl phthalate (DEHP), nonylphenol (NP) and nonylphenol ethoxylates (NPE), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins and furans (PCDDs/Fs) [16,17]. The European Commission [18], in the third draft of the "Working document on sludge", has proposed limiting the content of these organic pollutants in SS.

An important factor to consider in the use of sludge in agriculture is the instability of the pollutant content, particularly the variation in heavy metal pollution, which can differ based on the local living and production conditions. This variability necessitates a cautious approach to promoting agricultural policies involving sludge. To address the issue of pollutant content, it is essential to implement stringent monitoring and regulation practices to ensure that the sludge applied to agricultural fields meets safety standards. Regular testing for heavy metals and other contaminants can help mitigate the risks associated with the use of sludge. Additionally, the implementation of best practices for the treatment and application of sludge will further enhance its safety and effectiveness as a soil amendment.

Organic pollutants can undergo biological, chemical, and photochemical degradation reactions in soil. In addition, these organic pollutants can be fixed to various components of soil and are able to temporarily inactivate; this may have repercussions for the degradation of the soil structure due to the alteration of its fixing components (clays and organic matter).

Consequently, it is very important to carry out a prior characterization of the SS to be used as an organic amendment for soils, thus avoiding any risk derived from its use. It is similarly necessary to carry out the analytical monitoring of soils and crops in the area in which the SS will be used.

The use of SS varies across Europe. Some countries, as a precaution to reduce potential contamination and risks to human health, have adopted policies that try to prevent its application in agriculture. On the contrary, other countries are clearly committed to its use in agriculture, considering it a very valuable raw material when managed properly and within safe limits [19].

Many works have shown the positive effect of sludge and its compost both in agricultural soils [20–22] and in the recovery of degraded soils [23–25]. However, fewer studies have been carried out on the behavior of potential organic pollutants and pathogens present in sludge, particularly organic contaminants, as it pertains to soil [10,26–28]. Therefore, the aim of this work was to evaluate (in three successive wheat crops and under the conditions of conventional management and a semi-arid climate) both the positive and negative effects of the addition of SS as a soil amendment on soil characteristics.

2. Materials and Methods

2.1. Experimental Design

The wheat crop experiment took place at a farm with sandy clay loam soil, situated in the highlands of the Region of Murcia (SE Spain), under semi-arid climate conditions.

At this farm, four 1000 m² control plots (K) and four 1000 m² SS-treated plots (SS) were delimited. SS was added in October (at the beginning of the first and second crops' cultivation) at a dose equivalent to 170 kg of N/ha, and no organic amendment was implemented in the third crop. Wheat was harvested each year in July. No mineral fertilization or irrigation was applied to the soils. This form of management is typical of farmers in the area (SE Spain). A dose of 170 Kg N/ha was selected because it is the maximum amount of N that can be added annually per hectare to agricultural soils in vulnerable areas according to the European Union stipulations [29]. The SS used in this assay came from an urban aerobic wastewater treatment plant (WWTP) situated in Murcia, SE Spain; their characteristics are shown in Tables 1 and 2.

Table 1. Characteristics of the sewage sludge (SS) applied to the soil.

Parameters	SS First Crop	SS Second Crop	EU Limit for Heavy Metals in SS [30]
Moisture (%)	81.00 (1.24)	84.90 (0.99)	
pH	6.75 (0.04)	6.49 (0.03)	
Electrical conductivity (dS/m)	2.63 (0.06)	2.18 (0.08)	
Volatile organic matter (%)	94.47 (0.47)	98.30 (0.63)	
Organic C (g/100 g)	41.09 (0.12)	40.24 (0.20)	
N (g/100 g)	6.36 (0.03)	7.06 (0.02)	
P (g/100 g)	1.11 (0.01)	0.82 (0.01)	
K (g/100 g)	0.68 (0.02)	0.31 (0.01)	
Fe (g/100 g)	0.21 (0.04)	0.22 (0.06)	
Mg (g/100 g)	0.49 (0.01)	0.43 (0.03)	
Mn (mg/kg)	92.83 (4.82)	63.60 (3.97)	
Ca (g/100 g)	2.38 (0.14)	1.36 (0.06)	
Na (g/100 g)	0.16 (0.02)	0.18 (0.02)	
Mo (mg/kg)	5.00 (0.48)	3.17 (0.38)	
Chlorides (mg/L)	546.80 (13.2)	244.20 (9.47)	
Sulphates (mg/L)	590.00 (12.48)	127.40 (11.36)	
Phosphates (mg/L)	639.50 (11.05)	804.40 (9.32)	
Bromides (mg/L)	<0.1	<0.1	
Ni (mg/kg)	10.60 (0.44)	18.53 (0.69)	400
Cu (mg/kg)	105.81 (1.69)	62.79 (0.93)	1750
Cd (mg/kg)	0.50 (0.10)	0.14 (0.07)	40
Zn (mg/kg)	326.80 (2.24)	292.10 (7.83)	4000
Hg (mg/kg)	0.33 (0.01)	<0.10	10
As (mg/kg)	1.35 (0.01)	1.60 (0.03)	40
Cr (mg/kg)	22.05 (2.05)	40.95 (2.33)	1500
Pb (mg/kg)	21.99 (1.05)	11.07 (0.99)	1200
<i>Salmonella</i> (in 25 g)	Absence	Absence	
<i>Escherichia coli</i> (CFU/g)	169.000	17.000	

This SS was selected as a representative of the aerobic urban SS produced in the Region of Murcia (Spain) based on the results obtained in a doctoral thesis [31] in which a wide range of analyses were performed (at three different times of the year) on a total of 30 WWTP sludges produced in a group of urban WWTPs located in the Region of Murcia. These WWTPs generate about 90% of the total sludge produced in the region. The flocculant used in the wastewater treatment plants in the Region of Murcia is (SO₄)₃Fe₂. This type of flocculant does not negatively affect the properties of the soil.

Table 2. Organic contaminants in the sewage sludges applied to the soil.

Parameters		SS First Year	SS Second Year	Proposed Limits [18]
PAH (µg/kg)	Naftalene	1.20 (0.01)	<LC	
	Acenaphthylene	142.50 (2.27)	<LC	
	Acenaphthene + Fluorene	170.00 (1.79)	57.09 (2.01)	
	Fenantrene	1.10 (0.05)	105.00 (1.03)	
	Anthracene	20.25 (1.18)	2.66 (0.89)	
	Fluorantene	34.95 (2.26)	<LC	
	Pyrenean	86.45 (3.50)	128.00 (6.47)	
	Benzo[a]anthracene	3.17 (0.06)	16.50 (0.93)	
	Crisene	2.48 (0.48)	3.92 (0.19)	
	Benzo[b]fluorantheon	0.81 (0.02)	1.39 (0.03)	
	Benzo[k]fluorantheon	1.25 (0.04)	0.42 (0.01)	
	Benzo[a]pyrenean	3.71 (0.02)	1.00 (0.02)	
	Dibenzo[ah]anthracene	2.05 (0.06)	<LC	
	Benzo[ghi]perilene	<LC	4.08 (0.24)	
	Indeno[123cd]pyrenean	3.17 (0.29)	<LC	
ΣPAH (µg/kg)		469.50 (5.17)	320.00 (3.20)	6000 µg/kg
LAS (mg/kg)	C10	21.65 (1.03)	0.46 (0.03)	
	C11	133.00 (1.22)	2.24 (0.19)	
	C12	214.00 (2.56)	1.80 (0.08)	
	C13	259.00 (2.83)	1.40 (0.08)	
ΣLAS (mg/kg)		627.00 (3.27)	5.91 (0.72)	2600 mg/kg
NPE (µg/kg)	NP2E0	0.04 (0.01)	<LC	
	NP1E0	0.24 (0.01)	0.01 (0.00)	
	NP	<LC	0.18 (0.01)	
ΣNPE (µg/kg)		1.66 (0.03)	0.19 (0.01)	50.000 µg/kg
DEHP (mg/kg)		0.81 (0.01)	0.02 (0.00)	100 mg/kg
	PCB 101	15.65 (1.06)	<LC	
PCB (µg/kg)	PCB 118	<LC	<LC	
	PCB 138	<LC	<LC	
	PCB 153	<LC	2.36 (0.61)	
	PCB 18	<LC	<LC	
	PCB 180	17.60 (1.02)	<LC	
	PCB 28	<LC	<LC	
	PCB 52	11.90 (0.93)	<LC	
ΣPCB (µg/kg)		<LC	2.36 (0.06)	800 µg/kg

2.2. Sampling of Vegetal Material and Soil

The soils were sampled for analysis at the beginning (after each SS addition) and end (just before harvesting) of each crop's cultivation period and stored at 4 °C until analysis.

At the end of the second and third crop's cultivation, a central 1.20 m wide band was mechanically harvested in each plot, and yields were calculated in kg ha^{−1}. The ears and straw were separately weighed both fresh and after being dried in an oven at 65 °C. The ears were threshed, and the grain weight was calculated both fresh and having been dried at 65 °C. The representative straw and grain samples were ground and analyzed for macro- and micro-nutrient and heavy metal contents and for the determination of the presence of *Escherichia coli* and *Salmonella* spp. Organic pollutant content was determined in the grain and straw of the third crop.

2.3. Analytical Methods

2.3.1. Physicochemical and Chemical Parameters

Electrical conductivity (EC) and pH were measured in a 1/5 (*w/v*) aqueous solution using a conductivity meter (Crison CM 2200; Crison Hach Lange, Alella, Spain) and a pH meter (Crison GLP21), respectively. To determine soil bulk density, a cylindrical container of known volume (V) and weight was used to collect an undisturbed soil sample. The container and soil were weighed after drying (P), and the bulk density values were obtained

via the ratio P/V [32]. The water-holding capacity (WHC) of the soil was determined by weighing the amount of water retained in a saturated paste of the soil after submitting it to 1/3 atm of pressure [32]. Organic C and N were determined using a LECO TruSpec C/N/S automatic elemental analyzer (St. Joseph, MI, USA) [33]. The nutrient and heavy metal contents in the vegetal material and soil were analyzed after microwave digestion in 65% HNO₃ using inductively coupled plasma optical emission spectrometry (ICP-OES, model ICAP 6500 DUO THERMO, Thermo Scientific, Wilmington, DE, USA) [33].

2.3.2. Soil Respiration and Biochemical Parameters

For soil respiration, 15 g of the moistened soil was incubated for 21 days, during which time any CO₂ produced was assessed with an infrared gas analyzer (Toray PG-100, Toray Engineering Co., Ltd., Tokyo, Japan) [34]. Basal respiration indicates the amount of CO₂ that was produced per day during the incubation period, and it is expressed as mg C-CO₂/kg soil day. Dehydrogenase activity was measured using p-iodonitrotetrazolium chloride (INT) as a substrate [35]. For β -glucosidase and alkaline phosphatase activities, modified universal buffer (MUB; pH 6) and 0.025 M p-nitrophenyl- β -D-glucopyranoside or MUB (pH 11) and 0.025 M p-nitrophenyl-phosphate were used, respectively [36,37]. Soil urease was quantified colorimetrically as the NH₄⁺ produced after incubating (37 °C, 2 h) 1 g of the soil in 4 mL of borate buffer (pH 10) and 0.5 mL of 0.48% urea [38]. The glycine-aminopeptidase activity was determined by the addition of 2 mL of 50 mM tris-HCl buffer at pH 7 and 2 mL of 50 mM glycine p-nitroaniline substrate to 0.5 g of the soil and by the colorimetric measurement of the p-nitroaniline formed after incubating the suspension for 2 h at 40 °C [39].

2.3.3. Pathogenic Microorganisms

For the determination of *Escherichia coli*, a suspension of the samples in 100 mM phosphate buffer (pH = 7.2) (1:10 w:v) was homogenized for 10 min in a rotary shaker; then, the fecal Streptococci was analyzed via the membrane filtration technique (in Chromocult growth medium for coliforms). For the detection of *Salmonella* sp., the pre-treated sample was plated in Rappaport Vassiliadis broth (Merck 7700, Darmstadt, Germany). Three series of five tubes were prepared: 10 mL of the sample was sown in the first, 1 mL in the second, and 0.1 mL in the third. After 24 h of incubation at 45 °C, 100 μ L of each positive tube was sown on modified brilliant green agar (OXOID, CM 329, Waltham, MA, USA), and the plates were incubated at 37 °C for 24 h. In this medium, *Salmonella* colonies were colored red. Those colonies that were red in color indicated *Salmonella* and were sown on iron-lysine agar (OXID; 381); the only known groups of Enterobacteriaceae that regularly decarboxylate lysine rapidly and produce large amounts of hydrogen sulfide are the members of the *Salmonella* and Arizona groups. Finally, and when the above qualities had been confirmed, an agglutination test was performed using the A-S polyvalent serum (Wellcome, London, UK). If agglutination occurred, we were able to confirm the presence of a species of the genus *Salmonella*.

2.3.4. Organic Contaminants

DEHP, NPE, and PCB were simultaneously extracted from the sewage sludge, soil, or plants with n-hexane via sonication-assisted extraction and were then simultaneously determined by gas chromatography–mass spectrometry [40]. LAS and PAH were simultaneously extracted from the samples with methanol via sonication-assisted extraction and cleaned up by solid-phase extraction [41]; subsequently, they were separately determined through high-performance liquid chromatography (HPLC) with a diode array (DAD) and fluorescence (FI) detectors [41].

2.4. Statistical Analysis

The data were analyzed using the SPSS 19.0 software (IBM SPSS statistics, Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was used to identify the effects of the

treatments on soil characteristics. The normality (Shapiro–Wilk test) and homoscedasticity (Levene’s test) of the data were previously tested, and the ANOVA post hoc analyses were based on Tukey’s honestly significant difference (HSD) test ($p \leq 0.05$). The significance of the differences between the control and amended soil in grain yields, soil density, and water-holding capacity was tested using Student’s *t*-test.

3. Results

3.1. Effects on Soil Characteristics

3.1.1. Agronomic, Microbiological, and Biochemical Soil Parameters

Soil density and soil water-holding capacity (WHC) were analyzed only at the end of the third crop’s cultivation since changes in soil’s physical parameters occur more slowly than in microbiological and biochemical parameters. At the end of the third crop’s cultivation, the amended soil showed a significantly ($p \leq 0.05$) lower density (1.12 g/cm^3) than the control soil (1.21 g/cm^3). The WHC was higher in the amended soil (55.14%) than in the control soil (53.17%), although the difference was not statistically significant. The pH values varied little throughout the three years of wheat cultivation and were not affected by the addition of the SS to the soil (Table 3), ranging from 8.5 to 9.0.

Regarding the nutritional parameters of agronomic value, the soil N content increased in the three crops to which the SS was added; differences from the control soil were more noticeable in the second and third crops (Figure 1). It is noteworthy that despite no sludge being added to the third wheat crop, soils that received SS exhibited higher N contents than the control. Such a result highlights the positive residual effect of the successive additions of the SS to the soil. Soil nitrate content was also higher in the amended soils than in the control (Table 3).

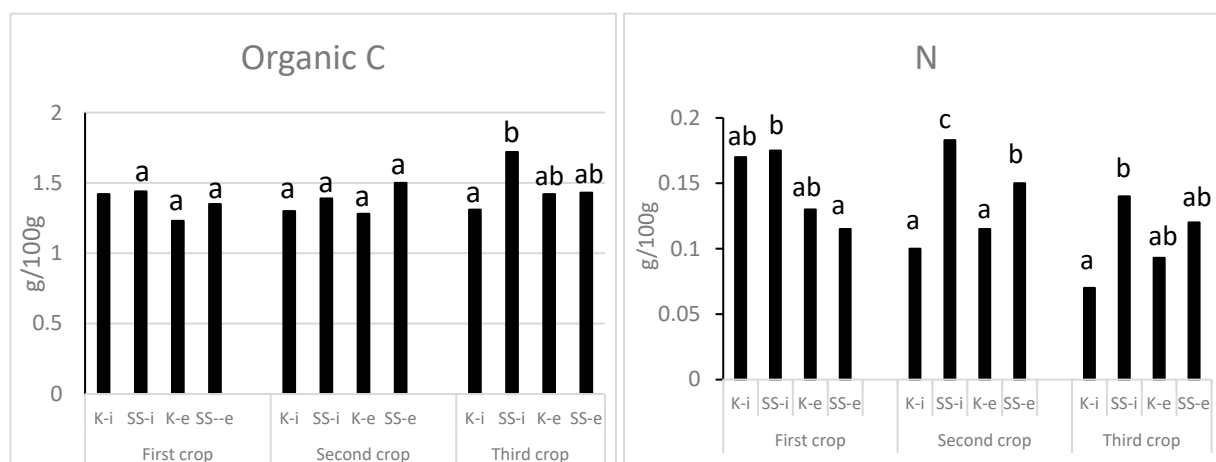


Figure 1. Changes in the soil organic carbon and nitrogen contents throughout cultivation. For each crop, the bars with the same letter are not statistically different according to Tukey’s test ($p \leq 0.05$). K-i and K-e: the control soil at the start and end of the crop’s cultivation, respectively. SS-i and SS-e: the amended soil at the start and end of the crop’s cultivation, respectively.

The organic C content increased slightly with the addition of the SS; however, this content only was significantly ($p \leq 0.05$) higher than the control at the start of the third crop’s cultivation period (Figure 1). This may be due to the fact that the applied dose of SS was not very high, so the amount of organic C provided (along with the spatial variability of the soil) was insufficient for the increases to be statistically significant.

In general, the amended soils showed, in the three crops, a higher content of sulfates and nitrates than the control (Table 3). The contents of the rest of the analyzed macro- and micro-nutrients were quite similar in the amended and control soils (Table 3).

Table 3. Changes in soil characteristics throughout wheat cultivation (with standard deviations in parentheses).

FIRST CROP	Control-Initio	SS-Initio	Control-End	SS-End
pH	8.99 (0.04)	9.08 (0.05)	9.18 (0.05)	9.12 (0.03)
Electrical conductivity ($\mu\text{S}/\text{cm}$)	118.03 (5.32)	119.33 (6.12)	114.3 (6.27)	122.17 (5.28)
Ammonium ($\text{mg N-NH}_4/\text{kg}$)	<2.5	<2.5	<2.5	<2.5
Total P ($\text{g}/100\text{ g}$)	0.03 (0.01)	0.03 (0.00)	0.02 (0.00)	0.03 (0.01)
Total K ($\text{meq}/100\text{ g}$)	0.69 (0.03)	0.70 (0.02)	0.55 (0.05)	0.55 (0.06)
Fe (mg/kg)	16,677 (450)	16,709 (448)	14,028 (515)	17,384 (715)
Mg ($\text{g}/100\text{ g}$)	1.29 (0.27)	1.34 (0.27)	1.65 (0.18)	1.57(0.17)
Mn (mg/kg)	286 (12.42)	288 (13.29)	182 (10.15)	199 (11.59)
Ca ($\text{g}/100\text{ g}$)	10.80 (0.71)	11.65 (0.81)	14.0 (0.80)	16.63 (0.67)
Chlorides (mg/kg)	71.43 (9.32)	57.78 (7.43)	91.57 (11.35)	76.00 (10.51)
Nitrates (mg/kg)	529.36 (120)	561.41 (134)	82.31 (23)	250.11 (69)
Phosphates (mg/kg)	<1.0	<1.0	<1.0	<1.0
Sulphates (mg/kg)	121.0 (49)	89.93 (44)	50.25 (15)	74.66 (18)
SECOND CROP				
pH	8.85 (0.06)	8.61 (0.07)	8.63 (0.07)	8.58 (0.05)
Electrical conductivity ($\mu\text{S}/\text{cm}$)	134.20 (20.02)	174.48 (16.89)	182.6 (19.36)	293.42 (11.03)
Ammonium ($\text{mg N-NH}_4/\text{kg}$)	<2.5	16.10 (0.05)	<2.5	<2.5
Total P ($\text{g}/100\text{ g}$)	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.03 (0.01)
Total K ($\text{meq}/100\text{ g}$)	0.66 (0.07)	0.63 (0.08)	0.65 (0.06)	0.56 (0.07)
Fe (mg/kg)	11,200 (716)	10,625 (1332)	17,000 (1308)	12,674 (2308)
Mg ($\text{g}/100\text{ g}$)	1.38 (0.06)	1.26 (0.09)	1.39 (0.22)	1.39 (0.23)
Mn (mg/kg)	287.5 (27.15)	286.88 (31.48)	266.6 (30.27)	242.63 (28.96)
Ca ($\text{g}/100\text{ g}$)	8.6 (0.56)	9.13 (0.75)	10.7 (1.02)	13.53 (2.77)
Chlorides (mg/kg)	17.1 (1.05)	17.2 (2.93)	7.99 (0.96)	7.49 (0.65)
Nitrates (mg/kg)	23.3 (3.27)	97.9 (22.55)	6.21 (2.12)	9.69 (2.87)
Phosphates (mg/kg)	<0.1	<0.1	<0.5	<0.5
Sulphates (mg/kg)	15.28 (2.17)	25.63 (5.32)	4.65 (1.10)	5.88 (1.21)
THIRD CROP				
pH	8.61 (0.04)	8.65 (0.05)	8.50 (0.04)	8.54 (0.03)
Electrical conductivity ($\mu\text{S}/\text{cm}$)	116.3 (9.22)	117.11 (10.73)	127.5 (8.54)	134.53 (5.91)
Ammonium ($\text{mg N-NH}_4/\text{kg}$)	8.79 (0.05)	8.53 (0.05)	<2.5	<2.5
Total P ($\text{g}/100\text{ g}$)	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)
Total K ($\text{meq}/100\text{ g}$)	0.61 (0.04)	0.55 (0.06)	0.65 (0.05)	0.69 (0.06)
Fe (mg/kg)	14,375 (1.302)	13,626 (1405)	16,377 (1505)	17,273 (2700)
Mg ($\text{g}/100\text{ g}$)	1.00 (0.02)	0.88 (0.03)	1.47 (0.10)	1.25 (0.11)
Mn (mg/kg)	254.7(24.03)	241.53(20.28)	279.43 (23.17)	276.73 (25.10)
Ca ($\text{g}/100\text{ g}$)	10.1 (0.98)	8.9 (1.05)	13.2 (1.02)	13.45 (0.97)
Chlorides (mg/kg)	15.96 (1.32)	15.40 (1.52)	7.71 (1.29)	9.54 (2.6)
Nitrates (mg/kg)	19.25 (6.54)	28.69 (9.17)	13.01 (2.01)	23.31 (1.92)
Phosphates (mg/kg)	<0.6	6.42 (0.43)	<0.1	<0.1
Sulphates (mg/kg)	17.77 (2.53)	22.71 (2.16)	9.8 (0.06)	16.65 (2.79)

Microbiological and biochemical parameters are much more sensitive than physical or chemical parameters to any changes occurring in the soil. Thus, the amended soils showed significantly ($p \leq 0.05$) higher microbial respiration values than those of the control at all the sampling times (Figure 2), indicating the stimulation of microbial activity in these soils. Dehydrogenase activity, which is also an indicator of the overall metabolic activity of microorganisms [42,43], also tended to be higher in the amended soils, particularly in the second and third crops (Figure 2).

The B-glucosidase activity was not affected by the incorporation of the sludge in the first crop; meanwhile, at the start of the second crop's cultivation (after the addition of the sludge) and at the end of the third crop's cultivation, the amended soils showed higher β -glucosidase activity than the respective controls. Likewise, the amended soils tended to show higher phosphatase activity than the controls, particularly in the second and third crops, with differences from the control being statistically significant ($p \leq 0.05$) in

most cases. In contrast, a significant ($p \leq 0.05$) decrease in urease activity was observed with the incorporation of the SS at all the sampling points (except at the end of the third crop's cultivation). However, the addition of SS did not appear to have any effect on the glycine-aminopeptidase activity (Figure 2).

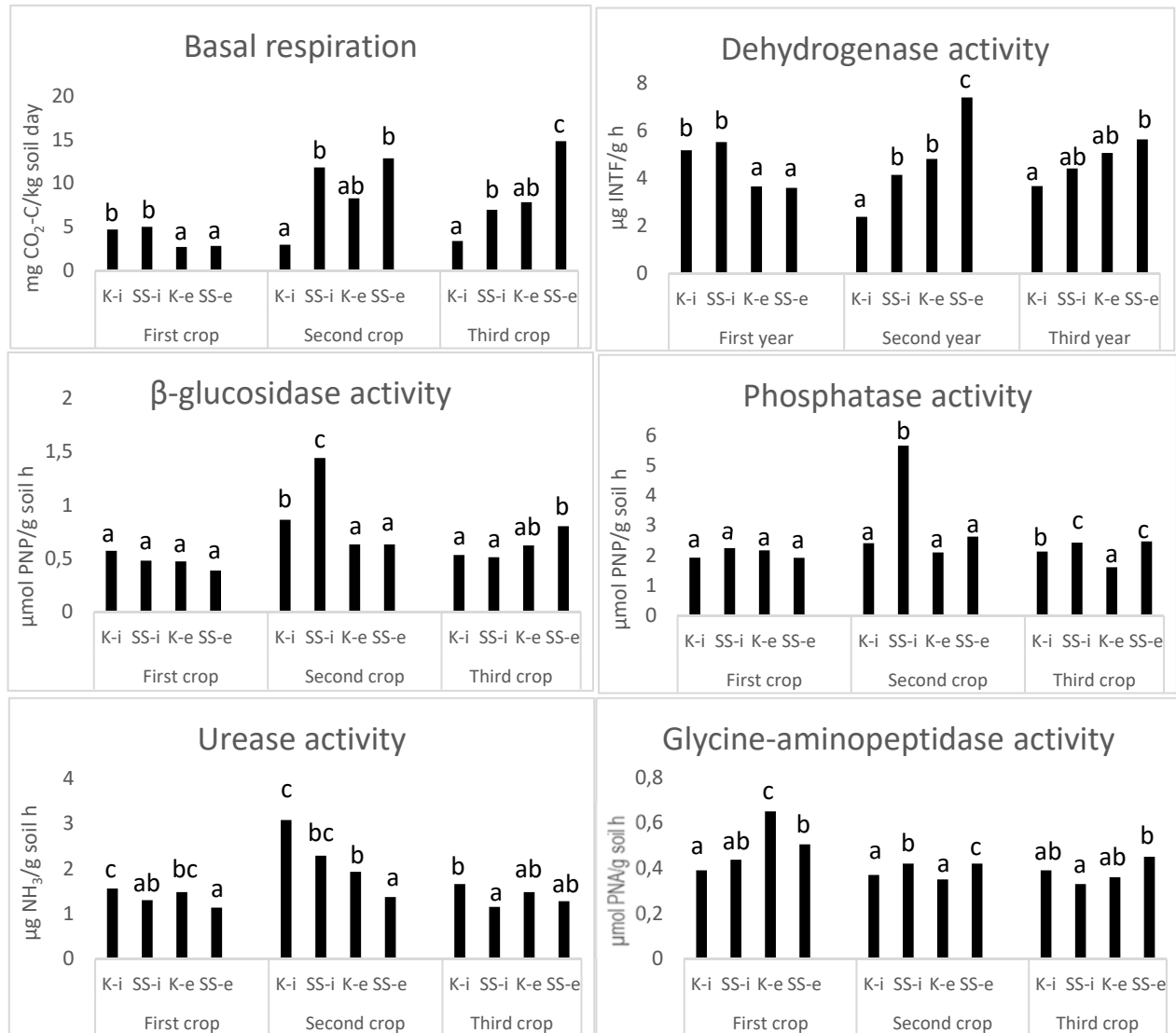


Figure 2. Changes in the soil basal respiration and enzyme activity throughout cultivation. For each year, the bars with the same letter are not statistically different according to Tukey's test ($p \leq 0.05$). K-i and K-e: the control soil at the start and end of the crop's cultivation, respectively. SS-i and SS-e: the amended soil at the start and end of the crop's cultivation, respectively.

3.1.2. Potential Hazardous Parameters

As shown in Table 4, the incorporation of the SS at the dose used in this assay did not cause the salinization of the soil; the treated and control soils showed similar EC values. Similarly, the heavy metal content did not increase with the addition of the SS, and there were no differences between the control and the treated soil at the different sampling points established (Table 4).

No presence of *Salmonella* was observed in the different soils analyzed, while in the first crop, the amended soils exhibited contamination with *Escherichia coli* at the start of the crop's cultivation (just after the addition of the SS), which had disappeared by the end of the crop's cultivation (Table 4).

Table 4. Variation in the heavy metal content and pathogens in the soils throughout crop cultivation.

FIRST CROP	Control-Initio	SS-Initio	Control-End	SS-End
Ni (mg/kg)	11.46 (2.95)	13.255 (3.00)	8.90 (0.63)	9.54 (0.56)
Cu (mg/kg)	39.08 (1.02)	39.24 (1.19)	4.99 (0.53)	5.93 (0.77)
Zn (mg/kg)	28.16 (1.23)	27.84 (1.39)	19.27 (1.45)	20.98 (1.82)
As (mg/kg)	0.74 (0.11)	0.67 (0.12)	4.32 (0.22)	3.31 (0.44)
Cr (mg/kg)	28.04 (0.99)	28.23 (1.07)	13.19 (1.82)	15.86 (1.02)
Pb (mg/kg)	22.13 (1.13)	20.37 (1.36)	11.00 (0.43)	9.58 (0.41)
<i>E. coli</i> (UFC/g)	<10	<40	<10	<10
<i>Salmonella</i> in 25 g	Absence	Absence	Absence	Absence
SECOND CROP				
Ni (mg/kg)	10.95 (1.03)	10.60 (1.29)	11.49 (0.83)	11.01 (0.99)
Cu (mg/kg)	5.96 (0.65)	6.16 (0.68)	7.72 (0.53)	7.64 (0.86)
Zn (mg/kg)	19.23 (1.27)	19.92 (2.15)	28.98 (4.32)	26.41 (5.86)
As (mg/kg)	1.30 (0.15)	1.07 (0.18)	0.42 (0.33)	0.81 (0.56)
Cr (mg/kg)	25.40 (2.53)	24.47 (3.11)	32.89 (2.63)	29.03 (4.09)
Pb (mg/kg)	14.90 (0.99)	12.90 (1.10)	20.84 (1.80)	16.50 (2.30)
<i>E. coli</i> (UFC/g)	<10	<10	<10	<10
<i>Salmonella</i> in 25 g	Absence	Absence	Absence	Absence
THIRD CROP				
Ni (mg/kg)	10.45 (0.99)	9.75 (1.07)	12.82 (1.03)	12.59 (1.12)
Cu (mg/kg)	7.89 (0.76)	7.93 (0.82)	8.78 (0.63)	9.32 (0.76)
Zn (mg/kg)	19.20 (1.53)	20.00 (1.78)	22.51 (1.42)	24.29 (1.99)
As (mg/kg)	0.76 (0.19)	0.50 (0.20)	0.73 (0.15)	0.55 (0.19)
Cr (mg/kg)	28.17 (1.86)	26.36 (2.28)	29.39 (1.36)	29.77 (2.59)
Pb (mg/kg)	17.22 (0.47)	15.06 (0.57)	19.25 (0.72)	18.03 (0.84)
<i>Escherichia coli</i> (CFU/g)	<10	<10	<10	<10
<i>Salmonella</i> in 25 g	Absence	Absence	Absence	Absence

In all the samples, Hg < 0.1 mg/kg and Cd < 0.5.

Persistent organic pollutants (LAS, PAH, NPE, DEHP, and PCB), identified by the EU as possible contaminants that should be controlled in the future use of SS [17], were analyzed in the soils at the start of the first and second crops' cultivation and at the end of the third crop's cultivation.

As shown in Table 5, the LAS contents increased with the addition of the SS at the start of the first crop, whereas the PAH contents increased with the addition of the SS at the start of the second crop's cultivation. However, these values were very low and decreased with time, with the amended soil showing similar or even lower values than the control by the end of the third crop's cultivation. In both the control and amended soils, the rest of the organic pollutants analyzed (NPE, DEHP, and Σ PCB) were found in negligible quantities or amounts beneath the limits of quantification.

Table 5. Evolution of the organic contaminant content in the soils throughout wheat cultivation.

Organic Contaminant		Initio First Crop		Initio Second Crop		End Third Crop	
		Control	SS	Control	SS	Control	SS
PAH (ng/kg)	Naftalene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	Acenaphthylene	395 (7.89)	122 (5.93)	<LQ	<LQ	<LQ	<LQ
	Acenaphthene + Fluorene	429 (8.32)	637 (10.24)	<LQ	<LQ	<LQ	<LQ
	Fenanthrene	34 (2.25)	36.8 (3.26)	879 (22.32)	1150 (91.24)	19.38 (1.67)	6.60 (0.86)
	Anthracene	368 (7.23)	436.8 (4.82)	<LQ	<LQ	2.68 (0.27)	0.69 (0.06)
	Fluorantene	378 (9.47)	440 (10.50)	769 (9.24)	1035 (0.76)	<LQ	<LQ
	Pyrenean	233 (9.43)	443 (10.22)	5063 (115.24)	11,239 (187.89)	5.98 (0.89)	4.48 (1.22)

Table 5. Cont.

Organic Contaminant		Initio First Crop		Initio Second Crop		End Third Crop	
		Control	SS	Control	SS	Control	SS
	Benzo[a]anthracene	357 (7.28)	467 (9.53)	736 (11.04)	238 (7.31)	<LQ	<LQ
	Crisene	181 (2.25)	178 (3.32)	234 (5.24)	511 (7.32)	10.83 (0.89)	0.37 (0.03)
	Benzo[b]fluoranthene	112 (5.09)	101 (2.17)	493 (5.76)	331 (4.28)	6.70 (0.99)	<LQ
	Benzo[k]fluoranthene	162 (3.27)	<LQ	56 (1.43)	246 (3.89)	9.73 (1.14)	<LQ
	Benzo[a]pyrene	<LQ	244 (2.12)	<LQ	<LQ	8.70 (0.57)	<LQ
	Dibenzo[ah]anthracene	390 (5.23)	321 (6.09)	<LQ	<LQ	<LQ	<LQ
	Benzo[ghi]perilene	<LQ	<LQ	335	744	1.76	<LQ
	Indeno[123cd]pyrene	<LQ	<LQ	<LQ	<LQ	4.78	<LQ
	ΣPAH (ng/kg)	3039 (18.22)	3427 (15.77)	8565 (96.84)	15,494 (117.32)	70.54 (8.93)	12.14 (1.12)
LAS (µg/kg)	Q10	0.83 (0.03)	1191 (4.86)	25 (1.16)	22 (1.93)	4.34 (0.78)	0.63 (0.76)
	Q11	5.18 (0.57)	6195 (5.92)	128 (2.18)	61 (2.45)	6.32 (0.89)	<LQ
	Q12	7.32 (0.86)	8888 (3.97)	187 (3.91)	126 (3.27)	11.86 (1.65)	0.82 (0.06)
	Q13	4.85 (0.06)	6213 (5.16)	148 (7.42)	41 (4.18)	5.49 (1.18)	<LQ
	ΣLAS (µg/kg)	18.18 (1.27)	25,914 (12.04)	488 (3.23)	250 (3.82)	28.01 (2.26)	1.45 (0.09)
NPE (µg/kg)	NP2EO	0.38 (0.01)	0.37 (0.06)	<LQ	<LQ	0.14 (0.01)	<LQ
	NP1EO	1.25 (0.19)	1.12 (0.06)	<LQ	<LQ	<LQ	<LQ
	NP	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	ΣNPE (µg/kg)	1.63 (0.09)	1.49 (0.19)	<LQ	<LQ	0.14 (0.01)	<LQ
DEHP (µg/kg)	DEHP	11.3 (1.11)	12.63 (1.02)	12.9 (1.05)	2.38 (0.10)	0.17 (0.03)	<LQ
PQB (µg/kg)	PQB 101	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 118	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 138	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 153	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 18	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 180	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 28	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	PQB 52	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	ΣPQB (µg/kg)	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

PAH: aromatic hydrocarbons; LASs: linear alkylbenzene sulphonates; NP: nonylphenol; NPE monoethoxylated nonylphenol; NPE: diethyloxyated nonylphenol; DEHP: di-(2-ethyl-hexil) ftalate; PQBs: polychlorinated biphenyls; <LQ: below quantification limit.

3.2. Effects on Plants

The grain and straw production were evaluated in the second and third wheat crops. As shown in Figure 3, the grain yields in the amended soils were 11% and 16% higher than those of the control for the second and third crops, respectively. In both the crops, the N contents in the grain and straw were higher in the plants from the amended soils than in the control plants (Table 6). The grain of the amended soils also had higher K and Ca contents than that of the control soil. The addition of the SS did not increase the contents of heavy metals in the plants, with heavy metal concentrations being similar in the amended and control plants (Table 6). The *Escherichia coli* content in both the straw and grain was <10 CFU in both the crops, and no presence of *Salmonella* was observed in 25 g of plant material.

The concentration of organic pollutants in the plant material of the third crop was then determined; we observed that both the grain and the straw contained small amounts of PAH and LAS. Their values in the plants of the amended soils were similar and even somewhat lower than those in the control plants (Table 7). The rest of the organic pollutants analyzed were generally present in amounts beneath the limit of quantification.

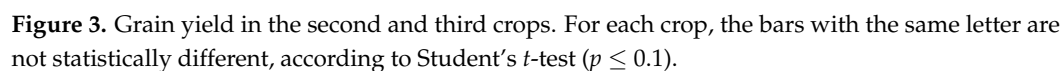


Table 6. Contents of macro- and micro-nutrients and heavy metals in the straw and grain of the second and third wheat crops (dwt.) (with standard deviations in parentheses).

[illegible]

Table 7. Organic contaminants in the grain and straw (dwt) of the third wheat crop (with standard deviations in parentheses).

		Grain		Straw	
Organic Contaminants		Control	Amended Soil	Control	Amended Soil
PAH ($\mu\text{g/kg}$)	ΣNPE ($\mu\text{g/kg}$)	<LQ	<LQ	<LQ	14.17 (1.76)
	Naftalene	<LQ	<LQ	<LQ	<LQ
	Acenaphthylene	<LQ	<LQ	<LQ	<LQ
	Acenaphthene + Fluorene	<LQ	18.9	<LQ	<LQ
	Fenantrene	<LQ	LQ	<LQ	<LQ
	Anthracene	107 (3.5)	<LQ	61.0 (2.95)	<LQ
	Fluorantene	<LQ	<LQ	<LQ	38.5 (1.24)
	Pyrenean	<LQ	<LQ	<LQ	<LQ
	Benzo[a]anthracene	<LQ	<LQ	<LQ	<LQ
	Crisene	<LQ	25.3	<LQ	<LQ
	Benzo[b]fluorantheon	48 (2.80)	90.0 (3.47)	4.8 (0.28)	24.8 (0.99)
	Benzo[k]fluorantheon	8.4 (0.19)	<LQ	<LQ	<LQ
	Benzo[a]pyrenean	1.8 (0.09)	<LQ	<LQ	<LQ
	Dibenzo[ah]anthracene	<LQ	<LQ	<LQ	3.7 (0.22)
	Benzo[ghi]perilene	2.1 (0.03)	<LQ	<LQ	<LQ
	Indeno[123cd]pyrenean	<LQ	<LQ	<LQ	<LQ
	Indeno[123cd]pireno	<LQ	<LQ	<LQ	<LQ
	ΣPAH ($\mu\text{g/kg}$)	167.3 (10.7)	134.2 (8.47)	66.8 (5.32)	67.0 (4.16)
	C10	4.2 (0.59)	2.6 (0.43)	22.3 (2.57)	17.0 (3.83)
LAS ($\mu\text{g/kg}$)	C11	10.5 (1.05)	4.0 (0.23)	75.7 (8.79)	61.8 (9.52)
	C12	23.5 (2.19)	11.8 (1.43)	139.5 (5.43)	133.0 (4.86)
	C13	4.8 (0.25)	1.2 (0.06)	30.7 (5.12)	40.3 (4.32)
	ΣLAS ($\mu\text{g/kg}$)	43.0 (3.24)	19.6 (2.32)	268.2 (9.84)	252.1 (11.02)
NPE ($\mu\text{g/kg}$)	NP	<LQ	<LQ	<LQ	9.02
	NP1EO	<LQ	<LQ	<LQ	2.32
	NP2EO	<LQ	<LQ	<LQ	2.83
DEHP ($\mu\text{g/kg}$)		<LQ	<LQ	<LQ	<LQ
	PCB 101	<LQ	<LQ	<LQ	<LQ
	PCB 118	<LQ	<LQ	<LQ	<LQ
	PCB 138	<LQ	<LQ	<LQ	<LQ
PCB ($\mu\text{g/kg}$)	PCB 153	<LQ	<LQ	<LQ	<LQ
	PCB 18	<LQ	<LQ	<LQ	<LQ
	PCB 180	<LQ	<LQ	<LQ	<LQ
	PCB 28	<LQ	<LQ	<LQ	<LQ
	PCB 52	<LQ	<LQ	<LQ	<LQ
	ΣPCB ($\mu\text{g/kg}$)	<LQ	<LQ	<LQ	<LQ

PAH: aromatic hydrocarbons; LASs: linear alkylbenzene sulphonates; NP: nonylphenol; NP1EO: monoethoxylated nonylphenol; NP2EO: diethyloxylated nonylphenol; DEHP: di-(2-ethyl-hexil) ftalate; PQBs: polychlorinated biphenyls; <LQ: below quantification limit.

4. Discussion

4.1. Agronomic, Microbiological, and Biochemical Soil Parameters

One of the main advantages of the application of organic amendments to soil is the resulting improvements in its physical properties [44]. In this study, we observed a positive effect of the addition of sludge on soil's physical properties, leading to a significant reduction ($p \leq 0.05$) in soil density and a slight increase in WHC. These effects, clearly positive for the fertility and productivity of soil, are strongly related to the exogenous contribution of organic matter, and its influence will last in the soil as long as the said organic matter is protected from microbial attack that is associated either with the surface of the clays or located inside the micropores. Improved soil structure and water retention can lead to better crop performance, particularly in environments with scarce water. Diacono and Montemurro [45] reviewed long-term experiments (3–60 years) on the effects of organic

amendments and concluded that the regular addition of organic waste increased the soil's physical fertility by improving aggregate stability and decreasing soil bulk density.

Likewise, the amended soils were observed to have higher N contents than the control at both the start and end of the second and third crop's cultivation periods; such higher N contents result in greater fertility (Figure 1) and are consistent with the higher N content and resultant protein content observed in the grain of the plants from the amended soils (Table 6). The higher N content in the second and third crops is a result of the combined effects of the residual N from the initial application of the SS, slow nutrient release, enhanced microbial activity, and gradual cumulative improvements in soil fertility.

The higher contents of some macro-nutrients such as K and Ca in the plant material suggest that sludge mobilizes nutrients in the soil, thus favoring their uptake by plants [22,46]. K is essential for various physiological functions in plants, including enzyme activation, photosynthesis, protein synthesis, and osmoregulation. In turn, Ca is important for cell wall structure and stability, root development, and nutrient uptake. It is involved in signal transduction and helps to mitigate the effects of abiotic stress [47].

It should be noted that although many researchers have described an increase in the content of organic C (and therefore of organic matter) with the addition of sludge as an amendment [7,48], in our case, the increase in organic C was only significant ($p < 0.05$) at the start of the third crop's cultivation period (Figure 1). This may be attributed to several factors. Firstly, only two applications of the sludge were made, and further successive applications may be necessary to induce a significant increase in soil organic C. Additionally, the dose of SS used was relatively low (14 t/ha in the first crop and 16 t/ha in the second crop) and may have been insufficient to cause a detectable increase in organic C content. Furthermore, the increase in organic C was only significant at the start of the third crop's cultivation, suggesting that more time and repeated applications are required to bring about notable changes in the soil's organic C content. Parat et al. [49] carried out a long-term experiment (20 years) in which sewage sludge was added to fluvisol soil at the doses of 10 and 100 t/ha every two years; the authors observed that the highest SS dose produced a stronger effect on the organic C content and microbial diversity than the lowest dose. The increase in organic C observed at the start of the third crop's cultivation (despite sludge not being applied to this third crop) can be attributed to the plant remains provided by the previous crops, which were more abundant in the amended soils than in the control.

The increased microbial respiration and dehydrogenase activity detected in the amended soils (Figure 2) shows that the addition of the SS stimulates the microbial activity of the soil to which it is applied. This results in the increased potential of microorganisms to synthesize enzymes such as the hydrolases determined in this study. In agreement with our results, Dhanker et al. [50] observed a dose-dependent increase in dehydrogenase activity with the application of sludge in their 120-day incubation trial using soil amended with different doses of sludge. They attributed this increase to the low-molecular-weight proteins and nutrients added to the soil with the SS, which stimulate microorganism growth.

Urease is an extracellular enzyme that catalyzes the hydrolysis of urea or urea-like substrates to produce CO_2 and NH_3 as the products of the hydrolytic reaction. The synthesis of this enzyme can result in large losses of nitrogen in soils in the form of ammonia, with consequent economic repercussions. The fact that the amended soils had a lower urease activity than the control (Figure 2) is of great agronomic interest because urease activity causes the more gradual release of ammonium, thereby preventing its loss by volatilization and favoring the utilization of N by the crop. Likewise, the higher phosphatase activity detected in the amended soils at the start and end of the second and third crop's cultivation (Figure 2) is of significant agronomic relevance since this enzyme catalyzes the transformation of the organic forms of P (the esters and anhydrides of phosphoric acid) to inorganic P, thus making it available to plant roots. In agreement with our results, Roy et al. [51] observed an increase in alkaline phosphatase and dehydrogenase activity in soils amended with 58 t/ha of SS.

β -Glucosidase is an enzyme that plays a critical role in the carbon cycle by breaking down β -glucosides into glucose, which is an energy source readily available to soil microorganisms. This process is essential for the decomposition of organic matter and the release of nutrients. The increased β -glucosidase activity in the SS-amended soils indicates enhanced microbial activity and the decomposition of organic matter, thus contributing to improved soil fertility. The absence of significant differences in the β -glucosidase activity between the control and the SS-amended soils during the first crop cycle could be due to the initial microbial community's structure and the time required for the microbes to adapt to the new organic inputs from the SS. The increased β -glucosidase activity in the subsequent crop cycles would suggest that the microbial community had adapted to the SS amendments, leading to enhanced enzyme production and the breakdown of organic matter [47].

The enzyme glycine-aminopeptidase catalyzes the hydrolysis of proteins, and its increased presence at the start of the first and second crop's cultivation in the amended soils indicates that the SS provides protein compounds that can act as substrates for this enzyme, thus stimulating the N cycle in the soil and favoring the availability of this nutrient to the plant.

Overall, the enzyme activities along with other soil health indicators suggest that the SS amendments positively impact the soil quality, particularly over successive crop cycles. The increased microbial and enzyme activities could indicate the better availability of nutrients and soil fertility, both of which bolster sustainable agricultural practices.

4.2. Potential Hazardous Parameters

Another observation of interest is that there was no increase in soil salinization with the addition of the SS; the EC values did not exceed 400 $\mu\text{S}/\text{cm}$ (Table 3) in any case, and this finding is of significant interest from an environmental perspective. Likewise, the contents of heavy metals in the soil were always below the limitations stipulated for agricultural soils, and no differences in the heavy metal contents in the soil or plants (grain or straw) were observed between the amended soils and the control soil at the different sampling times (Tables 4 and 6). This suggests that the use of high-quality sludge (i.e., that with low heavy metal content) at low doses does not introduce the risk of metal contamination. However, this does not mean that there should be no "control" of this parameter, since heavy metals accumulate in soils and therefore should be continuously monitored.

In all three crops, the addition of the sludge increased the nitrate content in the soil with respect to the control (Table 3), thus favoring the absorption of this nutrient by the plants. That said, an increase in the soil nitrate content may increase the risk of environmental contamination via nitrate leaching. However, the nitrate contents in the soils of the second and third crops were very low. In any case, this parameter must be controlled in order to avoid contamination problems arising from nitrate leaching.

We can further conclude that the application of the sludge does not introduce any problems associated with the pathogenic microorganisms *Salmonella* and *Escherichia coli* (Table 4). The dilution of these microorganisms in the soil, the time elapsed, the soil temperature, and the survival rate of these microorganisms sufficiently justify their non-persistence in the soil. As indicated with the heavy metals, the fact that we observed no problems does not mean that their existence should not be controlled. In addition, perhaps particular control should be exerted over the time at which the sludge is applied to the soil by handlers; such specific timing is crucial to prevent the aforementioned problems.

Some of the pollutant organic compounds analyzed, such as LAS and PAH, were present in the control soil and increased in abundance after the addition of the sludge (Table 5). However, the ΣPAH content (3.04–15.5 $\mu\text{g}/\text{kg}$ soil) and ΣLAS content (0.01–25.9 mg/kg soil) were very low, and these compounds were degraded throughout the experimental period. In agreement with this result, Ekmekyapar and Çeltikli [52] observed a decrease in LAS concentrations ranging from 41 to 63% within their 30-day incubation experiment using soil contaminated with commercial LAS at four different levels. Jensen

et al. [53] also indicated the rapid degradation of LAS in soil, concluding that normal SS amendment does not pose a significant risk to agricultural soils.

It should be noted that high-quality SS was used in this experiment, with the LAS and PAH contents under the limits of 2600 mg/kg and 6 mg/kg for Σ LAS and Σ PAH, respectively, as established in the EU draft [18].

5. Conclusions

The results obtained in this study show that in semi-arid zones, the recycling of sludge in agricultural soils as an organic amendment or fertilizer is acceptable as long as the sludge complies with the current European legislation and its dose and handling are well controlled. It can be concluded that SS favorably influences soil fertility, stimulating microbial activity and enzyme synthesis. We deduced this from the greater microbial respiration and dehydrogenase, phosphatase, β -glucosidase, and glycine-aminopeptidase activity in the three tested soils than in the control soils, as well as the greater N content and lower urease activity, which served to prevent ammonium losses. This greater activity is due to the fact that substrates are provided with these sludges and are capable of activating enzyme synthesis (which favors the provision of nutrients to plants and microorganisms). The addition of sludge enhances the grain yield and absorption of N, Ca, and K by plants.

Regarding the potential negative effects of sludge on soil quality and the environment, we observed that although SS has salinity due to its own composition, salinity should not be a major problem if the dose of the sludge to be added is controlled and high-quality sludge is used. The same can be said for nitrates, heavy metals, organic contaminants, and pathogens. Organic pollutants are considered persistent contaminants; however, in our study, LAS and PAH, which were the most abundant organic contaminants in our SS, were found to be within safe limits and to degrade over time. This aligns with other studies showing the rapid degradation of these compounds in the soil, indicating that normal sludge amendments do not pose a significant risk to agricultural soils. It is important that we expand our collective scientific and technical knowledge of organic pollutants (concerning their degradability when they reach the soil from sludge or their degradability when such sludge's organic matter content undergoes transformation). In general, special attention should be paid to the contents of LAS and PAH, whose monitoring in sludge is of great interest.

The data from this study provide robust evidence supporting the positive impacts of high-quality SS amendments on soil health and crop productivity. By improving soil structure, increasing nutrient availability, and stimulating microbial activity, SS can play a crucial role in sustainable agriculture. These findings, contextualized within the broader literature, highlight the potential of SS as a valuable soil amendment while also underscoring the importance of careful management in maximizing benefits and minimizing risks.

Based on the findings of this study, several areas warrant further research to expand our understanding of the use of sewage sludge (SS) in agriculture and optimize its benefits while mitigating potential risks. Such areas include the long-term effects of SS on soil health; the optimization of application rates and timing; and the degradation of organic pollutants. Future research in these areas will build on the current body of evidence, providing a deeper understanding of the benefits and challenges associated with the use of sewage sludge in agriculture. By addressing these research gaps, we can develop more effective, sustainable, and safe practices involving SS application, ultimately enhancing soil health, crop productivity, and the quality of our environment.

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References

1. Yagmur, M.; Arpah, D.; Gulser, F. The effects of sewage sludge treatment on triticale straw yield and its chemical contents in rainfed condition. *J. Anim. Plant Sci.* **2017**, *27*, 971–977.
2. Melo, W.; Delarica, D.; Guedes, A.; Lavezzo, L.; Donha, R.; de Araújo, A.; de Melo, G.; Macedo, F. Ten years of application of sewage sludge on tropical soil: A balance sheet on agricultural crops and environmental quality. *Sci. Total Environ.* **2018**, *643*, 1493–1501. [[CrossRef](#)] [[PubMed](#)]
3. Silva, J.D.; Leal, T.T.; Araújo, A.S.; Araújo, R.M.; Gomes, R.L.; Melo, W.J.; Singh, R.P. Effect of different tannery sludge compost amendment rates on growth, biomass accumulation, and yield responses of Capsicum plants. *Waste Manag.* **2010**, *30*, 1976–1980. [[CrossRef](#)] [[PubMed](#)]
4. Plaza, C.; Giannetta, B.; Fernández, J.M.; López-de-Sá, E.G.; Polo, A.; Gascó, G.; Méndez, A.; Zacccone, C. Response of different soil organic matter pools to biochar and organic fertilizers. *Agric. Ecosyst. Environ.* **2016**, *225*, 150–159. [[CrossRef](#)]
5. Ding, Z.; Kheir, A.M.S.; Ali, M.G.M.; Ali, O.A.M.; Abdelaal, A.I.N.; Lin, X.; Zhou, Z.; Wang, B.; Liu, B.; He, Z. The integrated effect of salinity, organic amendments, phosphorus fertilizers, and deficit irrigation on soil properties, phosphorus fractionation, and wheat productivity. *Sci. Rep.* **2020**, *10*, 2736. [[CrossRef](#)] [[PubMed](#)]
6. Hernandez, T.; Berlanga, J.G.; Tormos, I.; Garcia, C. Organic versus inorganic fertilizers: Response of soil properties and crop yield. *AIMS Geosci.* **2021**, *7*, 415–439. [[CrossRef](#)]
7. Suhadolc, M.; Schroll, R.; Hagn, A.; Dörfler, U.; Schlöter, M.; Lobnik, F. Single application of sewage sludge: Impact on the quality of an alluvial agricultural soil. *Chemosphere* **2010**, *81*, 1536–1543. [[CrossRef](#)] [[PubMed](#)]
8. Cherfouh, R.; Lucas, Y.; Derridj, A.; Merdy, P. Long-term, low technicality sewage sludge amendment and irrigation with treated wastewater under Mediterranean climate: Impact on agronomical soil quality. *Environ. Sci. Pollut. Res.* **2018**, *25*, 35571–35581. [[CrossRef](#)] [[PubMed](#)]
9. Dhanker, R.; Chaudhary, S.; Goyal, S.; Garg, V.K. Influence of urban sewage sludge amendment on agricultural soil parameters. *Environ. Technol. Innov.* **2021**, *23*, 101642. [[CrossRef](#)]
10. Hamdi, H.; Hechmi, S.; Khelil, M.N.; Zoghalmi, I.R.; Benzarti, S.; Mokni-Tlili, S.; Hassen, A.; Jedidi, N. Repetitive land application of urban sewage sludge: Effect of amendment rates and soil texture on fertility and degradation parameters. *Catena* **2019**, *172*, 11–20. [[CrossRef](#)]
11. Wolna-Maruwka, A.; Sulewska, H.; Niewiadomska, A.; Panasiewicz, K.; Borowiak, K.; Ratajczak, K. The influence of sewage sludge and a consortium of aerobic microorganisms added to the soil under a willow plantation on the biological indicators of transformation of organic nitrogen compounds. *Pol. J. Environ. Stud.* **2018**, *27*, 403–412. [[CrossRef](#)] [[PubMed](#)]
12. Panasiewicz, K.; Niewiadomska, A.; Sulewska, H.; Wolna-Maruwka, A.; Borowiak, K.; Budka, A.; Ratajczak, K. The effect of sewage sludge and BAF inoculant on plant condition and yield as well as biochemical and microbial activity of soil in willow (*Salix viminalis* L.) culture as an energy crop. *PeerJ* **2019**, *7*, e6434. [[CrossRef](#)] [[PubMed](#)]
13. Pourcher, A.M.; Françoise, P.B.; Virginie, F.; Agnieszka, G.; Vasilica, S.; Gérard, M. Survival of faecal indicators and enteroviruses in soil after land-spreading of municipal sewage sludge. *Appl. Soil Ecol.* **2007**, *35*, 473–479. [[CrossRef](#)]
14. Clarke, B.O.; Smith, S.R. Review of ‘emerging’ organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ. Int.* **2011**, *37*, 226–247. [[CrossRef](#)]
15. Smith, S.R. Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. *Philos. Trans. R. Soc. A* **2009**, *367*, 4005–4041. [[CrossRef](#)] [[PubMed](#)]
16. Gibson, R.; Wang, M.J.; Padgett, E.; Beck, A.J. Analysis of 4-nonylphenols, phthalates, and polychlorinated biphenyls in soils and biosolids. *Chemosphere* **2005**, *61*, 1336–1344. [[CrossRef](#)] [[PubMed](#)]
17. Oleszczuk, P. Changes of polycyclic aromatic hydrocarbons during composting of sewage sludges with chosen physico-chemical properties and PAHs content. *Chemosphere* **2007**, *67*, 582–591. [[CrossRef](#)] [[PubMed](#)]
18. European Commission. *Working Document on Sludge 3rd Draft*; Document 27th April 2000; European Commission: Brussels, Belgium, 2000.

19. European Commission. Ex-Post Evaluation of Certain Waste Stream Directives. *European Commission*. 2014. Available online: https://ec.europa.eu/environment/waste/pdf/target_review/Final%20Report%20Ex-Post.pdf (accessed on 26 February 2022).
20. Scotti, R.; Bonanomi, G.; Scelza, R.; Zoina, A.; Rao, M.A. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. *J. Soil Sci. Plant Nutr.* **2015**, *15*, 333–352. [[CrossRef](#)]
21. Hernandez, T.; Chocano, C.; Moreno, J.L.; García, C. Use of compost as an alternative to conventional inorganic fertilizers in intensive lettuce (*Lactuca sativa* L.) crops—Effects on soil and plant. *Soil Tillage Res.* **2016**, *160*, 14–22. [[CrossRef](#)]
22. Tontti, T.; Poutiainen, H.; Heinonen-Tanski, H. Efficiently treated sewage sludge supplemented with nitrogen and potassium is a good fertilizer for cereals. *Land Degrad. Dev.* **2017**, *28*, 742–751. [[CrossRef](#)]
23. Tejada, M. Application of different organic wastes in a soil polluted by cadmium: Effects on soil biological properties. *Geoderma* **2009**, *153*, 254–268. [[CrossRef](#)]
24. Larney, F.J.; Angers, D.A. The role of organic amendments in soil reclamation: A review. *Can. J. Soil Sci.* **2012**, *92*, 19–38. [[CrossRef](#)]
25. Nkoa, R. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. *Agron. Sustain. Dev.* **2014**, *34*, 473–492. [[CrossRef](#)]
26. Laternus, F.; von Arnold, K.; Grøn, C. Organic contaminants from sewage sludge applied to agricultural soils. False alarm regarding possible problems for food safety? *Environ. Sci. Pollut. Res.* **2007**, *14*, 53–60. [[CrossRef](#)]
27. Sieciechowicz, A.; Sadecka, Z.; Myszograj, S.; Włodarczyk-Makula, M.; Wiśniowska, E.; Turek, A. Occurrence of heavy metals and PAHs in soil and plants after application of sewage sludge to soil. *Desalin. Water Treat.* **2014**, *52*, 4014–4026. [[CrossRef](#)]
28. Koyuncu, S. Occurrence of organic micropollutants and heavy metals in the soil after the application of stabilized sewage sludge. *J. Environ. Health Sci. Eng.* **2022**, *20*, 385–394. [[CrossRef](#)]
29. The Council of the European Communities. EU Council Directive (91/676/EEC) of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Off. J. Eur. Union* **1991**, *L 375*, 1–8.
30. The Council of the European Communities. European Directive 86/278/EEC, of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. *Off. J. Eur. Union* **1986**, *L 181*, 6–12.
31. Murcia, F.J. Lodos de Depuradora: Una Visión Integral para su Posible Aplicación a Suelos Desde una Perspectiva Agrícola (Sewage Sludge: A Comprehensive Vision for Its Possible Application to Soils from an Agricultural Perspective). Ph D. Thesis, University of Murcia, Murcia, Spain, 2013.
32. Ondoño, S.; Martínez Sánchez, J.J.; Moreno, J.L. The inorganic components of Green roof substrates impacts the growth of Mediterranean plant species as well as the C and N sequestration potential. *Ecol. Indic.* **2016**, *61*, 739–752. [[CrossRef](#)]
33. CEBAS-CSIC Ionomic Laboratory Service. Available online: http://www.cebas.csic.es/general_spain/ionomica.html (accessed on 26 February 2022).
34. Hernandez, T.; García, C. Estimación de respiración microbiana del suelo (Stimulation of soil respiration). In *Técnicas de Análisis de Parámetros Bioquímicos en Suelos. Actividades Enzimáticas y Biomasa Microbiana (Techniques of Analysis of Biochemical Parameters in Soils. Enzymatic Activities and Microbial Biomass)*; García, C., Gil-Soitres, F., Hernandez, T., Trasar-Cepeda, C., Eds.; Mundi-Prensa: Madrid, Spain, 2003; pp. 311–346.
35. García, C.; Hernandez, M.T.; Costa, F. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. *Commun. Soil Sci. Plant Anal.* **1997**, *28*, 123–134. [[CrossRef](#)]
36. Tabatabai, M.A.; Bremner, J.M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *1*, 301–307. [[CrossRef](#)]
37. Eivazi, F.; Tabatabai, M.A. Glucosidases and galactosidases in soils. *Soil Biol. Biochem.* **1988**, *20*, 601–606. [[CrossRef](#)]
38. Kandeler, E.; Gerber, H. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fertil. Soils* **1988**, *6*, 68–72. [[CrossRef](#)]
39. Sinsabaugh, R.L.; Antibus, R.K.; Linkins, A.E.; Mcclaugherty, C.A.; Rayburn, L.; Repert, D.; Weiland, T. Wood decomposition: Nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology* **1993**, *74*, 1586–1593. [[CrossRef](#)]
40. Aparicio, I.; Santos, J.L.; Alonso, E. Simultaneous sonication-assisted extraction, and determination by gas chromatography–Mass spectrometry, of di-(2-ethylhexyl)phthalate, nonylphenol, nonylphenol ethoxylates and polychlorinated biphenyls in sludge from wastewater treatment plants. *Anal. Chim. Acta* **2007**, *584*, 455–461. [[CrossRef](#)] [[PubMed](#)]
41. Santos, J.L.; Aparicio, I.; Alonso, E. A new method for the routine analysis of LAS and PAH in sewage sludge by simultaneous sonication-assisted extraction prior to liquid chromatographic determination. *Anal. Chim. Acta* **2007**, *605*, 102–109. [[CrossRef](#)] [[PubMed](#)]
42. Beyer, L.; Wachendorf, C.; Elsner, D.C.; Knabe, R. Suitability of dehydrogenase activity assay as an index of soil biological activity. *Biol. Fertil. Soils* **1993**, *16*, 52–56. [[CrossRef](#)]
43. Masciandaro, G.; Ceccanti, B.; Ronchi, V.; Bauer, C. Kinetic parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilisers. *Biol. Fertil. Soils* **2000**, *32*, 479–483. [[CrossRef](#)]
44. Ramulu, U.S. *Reuse of Municipal Sewage Sludge in Agriculture*; Scientific Publisher: Jodhpur, India, 2001.
45. Diacono, M.; Montemurro, F. Long-term effects of organic amendments on soil fertility. A review. *Agron. Sustain. Dev.* **2010**, *30*, 401–422. [[CrossRef](#)]
46. Lee, C.G.; Alvarez, P.J.J.; Kim, H.G.; Jeong, S.; Lee, S.; Lee, K.B.; Lee, S.H.; Choi, J.W. Phosphorous recovery from sewage sludge using calcium silicate Hydrates. *Chemosphere* **2018**, *193*, 1087–1093. [[CrossRef](#)]

47. Smith, J.; Johnson, L.; Davis, R. Effects of sewage sludge amendments on soil properties and crop performance. *J. Environ. Agric.* **2023**, *12*, 456–472.
48. García, C.; Hernandez, T.; Coll, M.D.; Ondoño, S. Organic amendments for soil restoration in arid and semiarid areas: A review. *AIMS Environ. Sci.* **2017**, *4*, 640–676. [[CrossRef](#)]
49. Parat, C.; Chaussod, R.; Lévêque, J.; Andreux, F. Long-term effects of metal-containing farmyard manure and sewage sludge on soil organic matter in a fluvisol. *Soil Biol. Biochem.* **2005**, *37*, 673–679. [[CrossRef](#)]
50. Dhanker, R.; Chaudhary, S.; Goyal, S.; Kumar, R. Soil microbial properties and functional diversity in response to sewage sludge amendments. *Arch. Agron. Soil Sci.* **2020**, *68*, 809–822. [[CrossRef](#)]
51. Roy, T.; Biswas, D.R.; Ghosh, A.; Patra, A.K.; Singh, R.D.; Sarkar, A.; Biswas, S.S. Dynamics of culturable microbial fraction in an inceptisol under short-term amendment with municipal sludge from different sources. *Appl. Soil Ecol.* **2019**, *136*, 116–121. [[CrossRef](#)]
52. Ekmekyapar, F.; Çeltikli, D.O. Effects of linear alkylbenzene sulfonate on agricultural soil and its degradation. *Fresenius Environ. Bull.* **2014**, *23*, 3188–3192.
53. Jensen, J.; Hans, L.; Holmstrup, M.; Krog, P.H.; Elsgaard, L. Effects and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 5. Probabilistic risk assessment of linear alkylbenzene sulfonates in sludge-amended soils. *Environ. Toxicol. Chem.* **2009**, *20*, 1690–1697.

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