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Impact of Organic Soil Amendments on Phytochemicals and Microbial Quality of Rocket Leaves (*Eruca sativa*)

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The effect of soil amendments prepared from organic wastes (sewage sludge and urban solid waste) at two concentrations (45 t ha^{-1} as D1 and 135 t ha^{-1} as D2) was evaluated on phytochemicals and microbial quality of rocket, a highly valuable vegetable. The addition of sewage sludge to the soil increased rocket yield 5.5 times compared to control and urban solid waste. Organic amendments increased the water content and the maturity stage of the leaves, which contributed to a reduction in the content of total and individual glucosinolates as well as flavonols and anthocyanins. However, higher content of vitamin C was observed after cultivation with sewage sludge at D2 compared to control leaves (204.6 and 177.4 mg 100 g^{-1} of fw, respectively). This study shows that sewage sludge at optimum doses can be considered a suitable amendment because of increased crop yield without detrimental effects on phytochemicals, including vitamin C content, when the leaves reached the commercial maturity stage.

KEYWORDS: Glucosinolates; flavonoids; vitamin C; sewage sludge; urban solid waste; coliforms; *Listeria*; agricultural practices; phytonutrients

INTRODUCTION

Global trends in modern agriculture are aimed at maximum short-term profit, based on the most efficient use of resources and maximization of labor and technological efficiencies. To increase crop yield, large amounts of chemical fertilizers, pesticides, and herbicides are applied. Consequently, reduction of biodiversity, irreversible soil erosion, and potential negative effect on human health derived from pesticide use occur (*1*). In this context, adoption of the most suitable agricultural practices is essential to improve produce safety and soil preservation. On the other hand, there is a trend toward sustainable, fresh(like) healthy convenience foods and organic produce (*2*). As a consequence, organic food global sales have risen about 20% in the past five years because of the consumer perception that these are healthier foods and also have environmental benefits (*3*). In fact, it is assumed that organic foods contain higher nutritional and antioxidant properties, although the results of research studies are controversial (*4*, *5*).

The use of organic amendments as fertilizers is one of the main characteristics of organic farming practices (*6*). Several studies have demonstrated that a wide variety of stabilized organic wastes can be used to amend soils, such as animal manure, vegetable waste, urban solid waste, and sewage sludge (*7*, *8*). According to those results, adding organic wastes to soil may represent the best alternative to prevent rapid degradation of soil and improve its physical properties to achieve high productivity levels. Furthermore, the production of on- and off-farm wastes has increased at a very high rate, making it urgent to find an environmentally friendly alternative use. Therefore, the addition of composted organic wastes to the soil could represent an economical and environmentally safe way to recover value from a wide variety of organic wastes.

Despite the potential soil benefits related to the use of different composted organic wastes, few studies have investigated their influence on the bioactive constituents of the edible plant material (*9*, *10*). Furthermore, depending on the organic waste origin and composting process, the amendments could represent a pathogen contamination source of the crops. This contamination risk is especially high with regard to horticultural plants that are in direct contact with the soil and are consumed raw, as is the case of leafy vegetables including spinach, lettuce, and baby leaves such as rocket (*11*). Recent foodborne illness outbreaks have been increasingly linked to the consumption of raw produce (*12*). The presence of pathogenic microorganims in soil amendments can be solved using stabilized organic residues (wastes) instead of fresh organic wastes, ensuring proper composting (*13*). Indeed, the progressive implementation of treatments of organic wastes, such as sewage sludge thermal treatments, has changed European Commission opinion. This body now considers that the use of sewage sludge on agricultural soils as fertilizer is normally the best environmental option provided that the sludge does not pose any threat to the environment or to animal and human health. This is included in a new proposal for a directive on spreading sludge on land. Thus, the preservation of soil and environmental health must be one of the objectives of global trends in modern agriculture,

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Table 1. Physicochemical Characteristics of Soil and Organic Soil Amendments[a]

	soil	urban	sewage 1	sewage 2	sewage 3	sewage 4	pig	veggie
pH	8.0 ± 0.1	7.2 ± 0.2	6.6 ± 0.2	8.1 ± 0.2	7.2 ± 0.3	7.2 ± 0.3	9.5 ± 0.2	8.0 ± 0.2
EC (dS m^{-1})	0.5 ± 0.1	6.4 ± 0.4	6.8 ± 0.2	2.5 ± 0.5	2.5 ± 0.3	3.7 ± 0.2	9.3 ± 0.2	8.7 ± 0.4
TOC (g kg^{-1})	28.0 ± 2.5		226.1 ± 3.2	216.1 ± 3.0	210.0 ± 3.0	185.2 ± 3.2	196.1 ± 3.0	338.2 ± 4.0
organic matter (g kg^{-1})		524.0 ± 3.2	432.0 ± 5.6	584.2 ± 4.9	641.1 ± 4.0	619.2 ± 3.9	338.1 ± 3.5	476.2 ± 4.0
N_{kj} (g kg^{-1}dm)	2.5 ± 0.3	24.3 ± 2.0	75.1 ± 2.0	45.2 ± 1.8	59.6 ± 1.7	46.9 ± 1.5	14.1 ± 1.0	21.3 ± 1.5
N-NH_4 (mg kg^{-1} of dm)	<2.5	275.6 ± 2.8	1126.2 ± 1.5	767.0 ± 4.0	913.1 ± 4.2	445.2 ± 3.0	368.1 ± 2.5	300.4 ± 3.8
N-NO_3 (mg kg^{-1} of dm)	436.1 ± 5.0	58.4 ± 2.5	75.2 ± 2.0	41.9 ± 1.9	18.8 ± 3.1	78.1 ± 2.9	2140.0 ± 5.0	3416.6 ± 7.0
P_2O_5 (g kg^{-1} of dm)	0.8 ± 0.1	18.1 ± 1.0	13.1 ± 1.5	17.0 ± 2.2	15.0 ± 1.0	13.0 ± 1.6	10.1 ± 1.5	12.1 ± 1.2
K (g kg^{-1} of dm)	3.8 ± 0.2	6.6 ± 0.1	6.9 ± 0.1	2.9 ± 2.0	5.0 ± 0.1	4.3 ± 0.1	15.7 ± 0.2	10.6 ± 0.2
Cu (mg kg^{-1} of dm)		395.6 ± 2.9	176.2 ± 3.0	266.1 ± 4.8	174.0 ± 2.6	500.2 ± 5.0	26.4 ± 0.35	26.7 ± 0.50
Ni (mg kg^{-1} of dm)		59.1 ± 2.5	80.1 ± 2.0	29.0 ± 2.1	22.1 ± 1.5	36.0 ± 2.0	13.9 ± 0.6	8.8 ± 0.5
Zn (mg kg^{-1} of dm)		3083.1 ± 7.0	389.0 ± 4.1	1096.2 ± 4.0	333.1 ± 3.8	606.0 ± 2.9	35.1 ± 1.0	105.8 ± 1.9
Cd (mg kg^{-1} of dm)		<2.5	<3.0	4.0 ± 0.1	<2.5	6.0 ± 0.1	<2.5	<2.5
Cr (mg kg^{-1} of dm)		113.7 ± 3.1	79.0 ± 5.0	1474.1 ± 4.5	52.4 ± 3.0	88.2 ± 2.1	39.2 ± 1.5	<5.0
Pb (mg kg^{-1} of dm)		83.8 ± 2.2	24.0 ± 2.0	83.6 ± 1.5	41.3 ± 2.0	140.5 ± 4.0	15.3 ± 0.4	11.6 ± 0.1

[a] Urban, urban solid waste; sewage, sewage sludge; pig, pig slurry; veggie, vegetable waste; dm, dry matter.

and the use of composted organic waste can be a useful tool. Therefore, it is essential to consider the microbial safety and health properties of vegetable foods cultivated using these amendments.

Thus, the purpose of this study was to evaluate the effect of different soil amendments prepared from organic wastes on the phytochemical content, such as vitamin C (measured as ascorbic acid plus dehydroascorbic acid) and individual and total glucosinolates and flavonoids, as well as the microflora of rocket leaves (*Eruca sativa* Mill.). Rocket leaves were selected for this study as they are a highly valuable leafy product with increasing economic potential mainly due to their short biological cycle (30−60 days).

MATERIALS AND METHODS

Plant Material. Pot experiments were carried out in triplicate under controlled conditions. The soil was obtained from an experimental farm in southeastern Spain (Murcia) (38° 1′ N, 1° 3′ W), with a particle size distribution of 76% smooth sand (0.25−0.05 mm), 6.4% coarse sand (2−0.25 mm), 7.2% silt (0.05−0.002 mm), and 10.1% clay (< 0.002 mm). Soil was air-dried at room temperature and sieved to pass a 2 mm mesh. Different organic wastes were previously assayed to evaluate if they had any inhibitory effect using ryegrass (*Lolium perenne* L. cv. Billion) in a pot experiment. This preliminary selection of the organic soil amendments was carried out on the basis of plant yield production and microbiological counts. Seven types of organic wastes were tested: four sewage sludges from different treatment plants (aerobic digestion) located in southeastern Spain (sewage 1, sewage 2, sewage 3, and sewage 4); an urban solid waste (urban); one pig slurry (pig); and a vegetable waste (veggie). The organic wastes, except pig slurry, were stabilized, ground to pass a 0.75 mm sieve, and individually mixed with the soil at two doses: 45 t ha^{-1} as D1 and 225 t ha^{-1} as D2. Pig slurry was also mixed at two doses: D1 (150 m^3 ha^{-1}) and D2 (600 m^3 ha^{-1}). Soil was kept at 60% water-holding capacity. Physicochemical characteristics of the soil and organic amendments are shown in **Table 1**. The grass was cultivated for approximately 2 months in 0.06 m^2 surface trays and harvested 4 times by cutting to 2 cm above the soil surface. Plants were also grown in nonamended soil as control. Rocket leaves (*E. sativa* Mill.) were cultivated using the same experimental design with the selected organic soil amendments: sewage sludge (sludge) and urban solid waste (urban) at two doses: 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2. Rocket was grown in 0.06 m^2 surface trays and harvested 35 days after planting. Plant yield, phytochemicals such as vitamin C, glucosinolates, and phenolic compounds, and microbiota were evaluated.

Soil Characteristics. Values of pH and electrical conductivity (EC) were measured in 1:2.5 and 1:5 (w/v) soil aqueous extracts, respectively. Total organic carbon (TOC) content was determined by oxidation with potassium dichromate in an acid medium. Measurement of the excess of dichromate was carried out using Mohr's salt as previously described (*14*). Nitrogen content was determined according to the Kjeldahl method. Total phosphate (P) and potassium (K) were determined in the nitric−perchloric digestion extract (*15*) and flame photometry, respectively. Micronutrients and heavy metal content were determined by atomic absorption spectrometry (Perkin-Elmer 1100B, Shelton, CT) of the extracts obtained from the soil nitric/perchloric 1:1 digestion.

Plant Yield and Water Content. Plant yield was determined by harvesting four randomly placed quadrates in each treatment. Fresh weight was measured at harvesting, and this material was oven-dried at 60 °C until a constant mass was obtained.

Analysis of Vitamin C. Fresh sampled leaves, cut into pieces (5−10 g of fw), were used for the extraction. The HPLC system, standard solutions, column conditioning, and derivatization procedures were used as previously described (*16*, *17*). The vitamin C content was calculated as ascorbic acid content plus dehydroascorbic acid content, and the results were expressed as milligrams per 100 g of fresh weight (fw).

Extraction and Analysis of Glucosinolates. Total glucosinolate content was determined according to the methodology reported by Bennett et al. (*18*) with some modifications (*19*). Samples of freeze-dried rocket leaves (40 mg) were mixed with 950 μL of MeOH/H_2O (7:3, v/v) and 50 μL of 12 mM sinigrin (internal standard). Sample extraction was carried out in a thermostated bath at 70 °C for 30 min followed by centrifugation at 13000*g* for 30 min at 4 °C. The methanol was completely removed using a rotary evaporator, and the pellets were resuspended in ultrapure water and filtered through 0.45 μm pore filter (Millex HV13, Millipore, Bedford, MA). Before filtration, 5 mg of tris(2-carboxyethyl)phosphine hydrochloride (Fluka, Glossop, U.K.) was added (*20*). The HPLC system consisted of a W600E multisolvent delivery system, an in-line degasser, a W717plus autosampler, and a W2996 photodiode array detector at 227 nm (Waters Cromatografía S.A., Barcelona, Spain). The standard solutions, column conditioning, separation procedures, identification, and quantification were as previously described (*19*, *21*). The recovery of internal standard was > 90%, and the results were expressed in milligrams of GLS K^+ per 100 g of fw.

Extraction and Analysis of Phenolic Compounds. The same extract and HPLC system and conditions used for the analysis of glucosinolate content were used for the analysis of phenolic compounds. However, in this case, chromatograms were recorded at 335 nm. Flavonol derivatives and anthocyanins were characterized according to UV spectra and quantified by comparison with external standards of rutin and cyanidin-3-glucoside (Sigma, St. Louis, MO) (*22*). Results are expressed in milligrams per 100 g of fw.

Microbial Quality of Rocket Leaves. Samples of 25 g of soil, amendments, and rocket leaves were homogenized in a 1:10 dilution of sterile 0.1% buffered peptone water (BPW, AES Laboratoire, Combourg, France) using sterile filter stomacher bags (Seward Limited, London, U.K.) and a stomacher (IUL Instrument, Barcelona, Spain) for 90 s and plated on appropriate media. Total aerobic mesophilic bacteria were enumerated using plate count agar (PCA) (Scharlau Chemie S.A., Barcelona, Spain) after incubation at 30 °C for 48 h. Total and fecal coliforms were isolated using chromocult agar (Oxoid, Basingstoke, Hampshire, U.K.) after incubation for 24 h at 37 and 44.5 °C, respectively. *Clostridium* spp. counts

Table 2. Plant Yield (Grams of Fresh Weight per Square Meter) of Ryegrass Cultivated with Different Organic Soil Amendments at Two Doses, 45 t ha^{-1} as D1 and 225 t ha^{-1} as D2[a]

	control	urban	sewage 1	sewage 2	sewage 3	sewage 4	pig	veggie
D1	538.4 ± 74.4 b	613.9 ± 70.7 ab	553.7 ± 49.3 ab	607.2 ± 13.6 ab	545.7 ± 30.1 b	508.9 ± 3.4 b	557.6 ± 23.3 ab	669.8 ± 16.7 a
D2	538.4 ± 74.4 cd	720.1 ± 6.2 a	537.6 ± 66.6 cd	653.1 ± 29.6 ab	504.6 ± 20.6 d	614.8 ± 23.6 b	584.2 ± 2.6 bcd	683.6 ± 46.3 ab

[a] Urban, urban solid waste; sewage, sewage sludge; pig, pig slurry; veggie, vegetable waste. Results are mean ± standard deviation of three replicates. For each compound, means within a row followed by the same letter are not significantly different at $P \leq 0.001$.

were carried out using sulfite−polymyxin−sulfadiazin-A (SPSS) agar (Scharlau Chemie S.A.) incubated at 37 °C for 24−36 h in anaerobic jars with an atmosphere generation system (Oxoid). Suspected *Clostridium botulinum* and *Clostridium perfringens* colonies were confirmed using API 20 A strips (bioMérieux, Marcy L'Etoile, France). Colonies of *Listeria* spp. were enumerated on plates of *Listeria* selective agar (Oxford formulation) incubated 48 h at 37 °C. All samples were analyzed in duplicate. Microbial counts were expressed as log colony-formin units (CFU) per gram or milliliter.

Statistical Analysis. Analysis of variance (ANOVA), followed by Tukey's multiple-range test with a significance level of $P \leq 0.05$, was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Physicochemical Characteristics of the Organic Soil Amendments. The different organic amendments added to the soil showed values of pH ranging from 6.6 to 9.5 (**Table 1**). Soil pH decreased after the addition of sewage sludge and urban solid waste but increased with pig slurry, whereas it was not modified by vegetable waste. The initial EC value of the soil was 0.55 dS m^{-1} and increased after the addition of the amendments, reaching values of 1.00, 0.85, and 0.82 dS m^{-1} with vegetable waste, urban solid waste, and pig slurry, respectively (data not shown). Organic amendments showed suitable values for crop cultivation regarding organic matter and macro- and micronutrients (**Table 1**). The ecotoxicity of the soil and the organic amendments was established by the inhibition test of luminescent bacteria (*Photobacterium phosporeum*) and the 50% effective concentration (EC_{50}) determined. The percentage of inhibition ranged from 35 to 55% among the organic amendments. However, the EC_{50} values were always higher than the limit established for a material to be considered toxic for the ecosystem (EC_{50} < 3.00 g L^{-1}).

Selection of Organic Soil Amendments. The selection of the most suitable organic soil amendments was done in a preliminary study using ryegrass cultivation, based on plant yield (**Table 2**) and microbiological counts of the soil (**Figure 1**) or the plant tissue (**Figure 1**). Most of the organic soil amendments did not affect ryegrass yield. However, vegetable waste at doses 1 and 2, urban solid waste, and sewage sludges 2 and 4 at dose 2 significantly increased the ryegrass yield compared to the control (**Table 2**).

The addition of the organic soil amendments did not generally affect the microbiota of the soil including total coliforms, fecal coliforms, and *Listeria* spp. However, pig slurry significantly increased microbial loads of the soil regarding total coliforms, fecal coliforms, and *Listeria* spp. counts, whereas vegetable waste increased only *Listeria* spp. counts (**Figure 1**). On the other hand, the addition of all the tested organic soil amendments significantly increased the total coliforms of ryegrass except sewage 1, which maintained similar counts to control samples (**Figure 1**). The highest total coliform counts among the organic soil amendments were found when pig slurry and vegetable waste were added. No differences were observed in fecal coliforms and *Listeria* spp. when ryegrass was grown in nonamended and amended soils. Thus, taking into account yield production and microbial counts, urban solid waste and sewage sludge 2 were selected for cultivation of rocket leaves. *Clostridium* spp. including *C. perfringens* and *C. botulinum* were not detected in any of the soil and ryegrass samples.

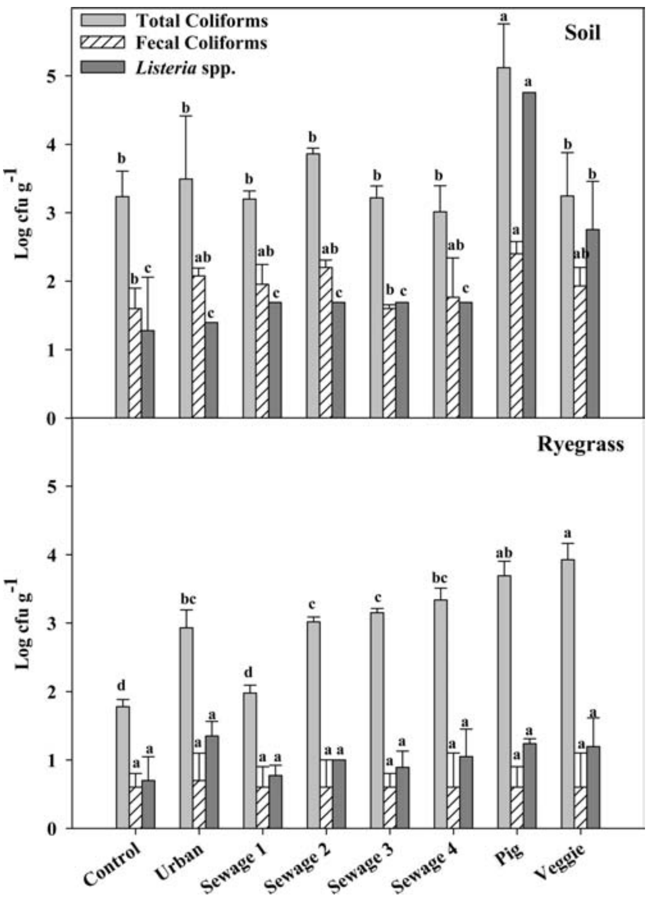

Figure 1. Total and fecal coliforms and *Listeria* spp. counts in nonamended (Control) and amended soils and ryegrass cultivated at a dose of 45 t ha^{-1}. Error bars represent the standard deviation. Bars followed by the same letter within the same microbial group are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; Sewage, sewage sludge; Pig, pig slurry; Veggie, vegetable waste. See Material and Methods for organic soil identification.

Influence of Organic Soil Amendments on Plant Yield and Water Content of Rocket Leaves. After 35 days of cultivation, the addition of sewage sludge to the soil significantly increased rocket yield, reaching 3 and 5.5 times increases at doses 1 and 2, respectively, compared to the control and urban solid waste (**Figure 2**). On the other hand, the addition of urban solid waste and sewage sludge at both doses significantly increased the water content of rocket leaves, changing from 82.5% in control leaves to 88.6 and 89.5% in urban solid waste and sewage sludge leaves, respectively (**Figure 2**). No differences were found in the water content of rocket leaves between the different doses. Rocket leaves grown in amended soils showed a similar maturity stage determined by the leaf size (8−10 cm), whereas control leaves showed a lower size (< 8 cm) associated with a less mature stage.

Influence of Organic Soil Amendments on Vitamin C Content of Rocket Leaves. Total vitamin C content of rocket leaves was not significantly affected by the organic soil amendments when compared to control leaves (**Figure 3**). This content increased, although not significantly, when sewage sludge was added at low dose, whereas it decreased when organic amendments were added at high dose. In all cases, ascorbic acid (AA) was the principal form of vitamin C, but the proportion of AA and dehydroascorbic acid (AA/DHA) changed depending on the organic soil amendments. This proportion was similar in rocket leaves cultivated with sewage sludge and control soil, whereas it was higher in those leaves cultivated with urban solid waste (**Figure 3**).

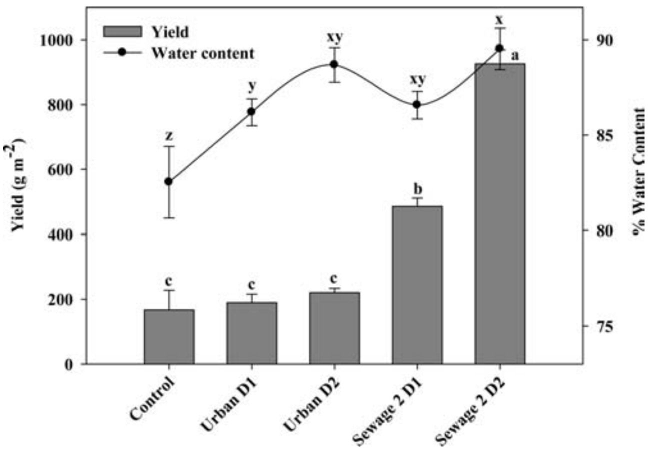

Figure 2. Crop yield and water content of rocket leaves grown in nonamended and amended soils with organic wastes at two doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2. Error bars represent the standard deviation. Bars followed by the same letter are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; Sewage, sewage sludge.

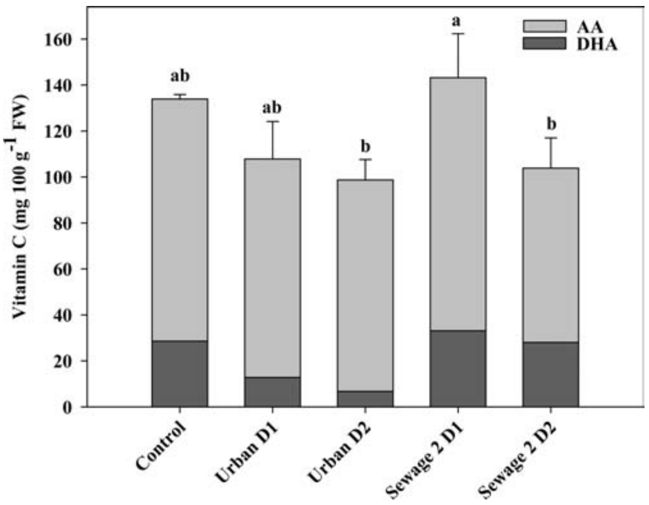

Figure 3. Vitamin C content as the sum of ascorbic acid (AA) and dehydroascorbic acid (DHA) of rocket leaves grown in nonamended and amended soils with organic wastes at two doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2. Error bars represent the standard deviation. Bars followed by the same letter are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; Sewage, sewage sludge.

Influence of Organic Soil Amendments on Phytonutrients of Rocket Leaves. The total and individual contents of glucosinolates and phenolic compounds were analyzed in rocket leaves cultivated with different organic soil amendments.

Glucosinolates. Three main glucosinolates (glucosativin, glucoraphanin, and glucoerucin) were identified and quantified in rocket leaves. The most abundant was glucosativin, which represented about 56% of the total content. Rocket leaves grown in nonamended soil showed a significantly higher content of the individual glucosinolates, including glucosativin, glucoraphanin, and glucoerucin, than leaves cultivated in amended soils (**Table 3**). Sewage sludge at dose D1 reduced significantly the content of glucosativin compared to the other amended soils tested. However, glucoraphanin was better preserved in rocket leaves cultivated with sewage sludge than with urban solid waste, whereas no differences were observed in the content of glucoerucin among amended soils. The contents of the total glucosinolates were significantly reduced by about 60% in those leaves cultivated with organic soil amendments compared to control leaves without significant differences among them (**Figure 4**).

Flavonoids. The phenolic content of rocket leaves was mainly composed of flavonoids. The main flavonoids consisted of kaempferol derivatives, followed by isorhamnetin and, in minor proportion, quercetin-derived flavonoids such as glycosides or acylatedglycosides. Six main flavonols were identified: kaempferol 3,4′-diglucoside; kaempferol 3-(2-sinapoylglucoside)-4′-glucoside; isorhamnetin 3,4′-diglucoside; isorhamnetin 3-glucoside; quercetin 3-glucoside; and kaempferol 3-glucoside (**Table 4**). The content of total flavonoids of rocket leaves cultivated

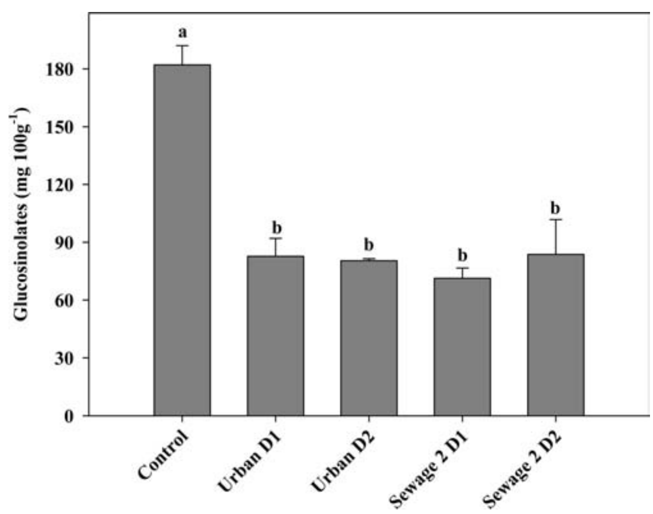

Figure 4. Total glucosinolates of rocket leaves grown in nonamended and amended soils with organic wastes at two doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2. Error bars represent the standard deviation. Bars followed by the same letter are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; Sewage, sewage sludge.

Table 3. Glucosativin, Glucoraphanin, and Glucoerucin Contents of Rocket Leaves Grown in Nonamended and Amended Soils with Organic Wastes at Two Doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2[a]

glucosinolate compound	control	urban D1	urban D1	sewage 2 D1	sewage 2 D2
glucosativin	107.8 ± 6.3 a	57.5 ± 5.4 b	63.9 ± 1.2 b	38.3 ± 3.4 c	51.1 ± 11.3 bc
glucoraphanin	45.6 ± 4.8 a	14.3 ± 2.7 c	8.9 ± 0.8 c	26.1 ± 2.9 b	26.6 ± 4.9 b
glucoerucin	28.6 ± 3.3 a	10.9 ± 3.3 b	7.7 ± 0.2 b	6.9 ± 0.4 b	5.6 ± 2.3 b

[a] Values are the mean ± standard deviation. Values in the same row followed by the same letter are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; sewage, sewage sludge.

Table 4. Contents of Kaempferol, Isorhamnetin, Quercetin, and Anthocyanin Derivatives of Rocket Leaves Grown in Nonamended and Amended Soils with Organic Wastes at Two Doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2[a]

flavonoid	control	urban D1	urban D2	sewage 2 D1	sewage 2 D2
kaempferol 3,4′-diglucoside	179.8 ± 8.1 a	131.3 ± 9.7 b	106.1 ± 18.2 b	112.5 ± 2.7 b	54.4 ± 8.0 c
kaempferol 3-(2-sinapoylglucoside)-4′-glucoside	59.3 ± 1.3 a	37.2 ± 0.9 ab	28.7 ± 3.4 b	27.5 ± 19.4 b	18.1 ± 3.1 b
kaempferol 3-glucoside	11.3 ± 0.5 a	6.3 ± 1.0 b	2.3 ± 0.6 d	4.9 ± 0.7 bc	2.7 ± 1.3 cd
isorhamnetin 3,4′-diglucoside	59.2 ± 4.6 a	32.8 ± 3.2 b	27.0 ± 2.4 b	26.0 ± 0.5 b	10.1 ± 0.9 c
isorhamnetin 3-glucoside	50.4 ± 1.4 a	19.4 ± 0.8 b	12.5 ± 2.0 c	14.8 ± 2.6 c	4.1 ± 0.6 d
quercetin 3-glucoside	15.5 ± 4.9 a	7.6 ± 2.5 ab	3.6 ± 1.5 b	7.5 ± 2.6 ab	4.3 ± 1.7 b
anthocyanins	24.6 ± 1.3 a	12.1 ± 2.6 b	4.2 ± 1.5 c	8.7 ± 1.8 bc	1.0 ± 0.3 d

[a] Values are the mean ± standard deviation. Values in the same row followed by the same letter are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; sewage, sewage sludge.

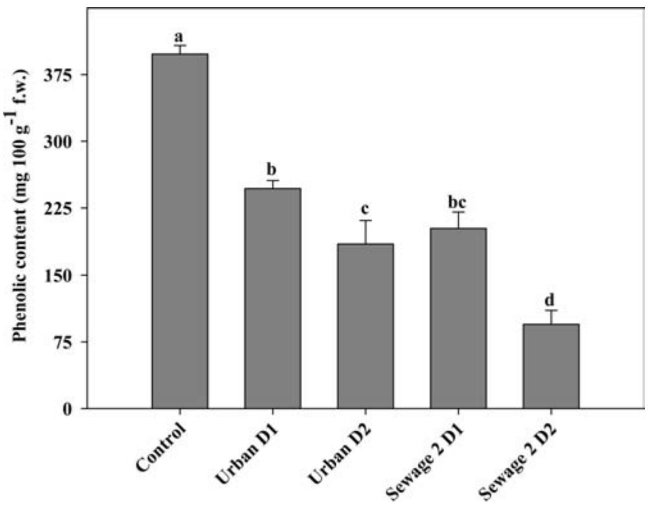

Figure 5. Total phenolic compounds of rocket leaves grown in nonamended and amended soils with organic wastes at two doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2. Error bars represent the standard deviation. Bars followed by the same letter are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; Sewage, sewage sludge.

without amendments was 398 mg 100 g^{-1} of fw and decreased significantly when leaves were cultivated with amendments (**Figure 5**). Among doses, the content of total flavonoids in amended soil leaves was significantly higher at the lowest dose compared to the highest dose (**Figure 5**).

Anthocyanins represented a minor percentage of the total phenolic content (1.1−6.1%). The content of total anthocyanins was higher in control leaves than in those leaves cultivated with amended soils, as occurred with the other groups of flavonoids (**Table 4**). In addition, the use of soil amendments at the highest dose reduced the anthocyanin content of rocket leaves when compared to the lowest dose. Thus, the content of anthocyanins in control leaves was 24.6 mg 100 g^{-1} of fw, whereas it was not higher than 1 mg 100 g^{-1} of fw when soil was amended with sewage sludge at dose D2.

Influence of Organic Soil Amendments on the Microbial Quality of Rocket Leaves. The addition of urban solid waste did not affect the microbial load of soil including total and fecal coliforms as well as *Listeria* spp. counts (**Figure 6**). However, sewage sludge significantly increased the fecal coliform counts of soil samples. When the microbial load of rocket leaves was evaluated, an increase in fecal coliforms was observed when leaves were cultivated with sewage sludge at the highest dose (**Figure 6**).

DISCUSSION

The composition of the organic soil amendments strongly depends on the substrate used to elaborate them and also varies

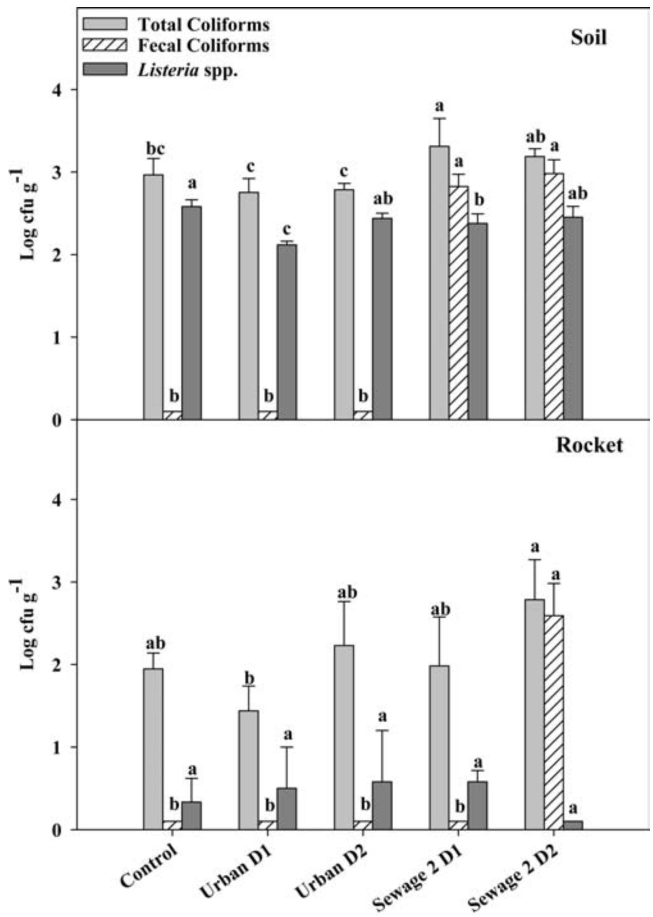

Figure 6. Total and fecal coliforms and *Listeria* spp. counts in nonamended and amended soils and rocket leaves cultivated with different organic amendments at two doses, 45 t ha^{-1} as D1 and 135 t ha^{-1} as D2, as well as rocket leaves cultivated on nonamended and amended soils. Error bars represent the standard deviation. Bars followed by the same letter within the same microbial group are not significantly different at ($P \leq 0.05$). Urban, urban solid waste; Sewage, sewage sludge.

with the processing methodology and the elapsed time of composting (*23*). In addition to this, the different sources of the organic amendments affect the main physicochemical properties of soil. The organic amendments tested in this study did not represent any toxicity risk of heavy metals as the content was always lower than the limit established by the European Union Normative for agricultural soils (*24*) and that proposed by the European Parliament and the Council for spreading sludge on land (*25*). This guaranteed the soil safety after the addition of the organic amendments at rates 1 and 2. However, the presence of heavy metals in this kind of organic materials always makes their analyses necessary to ensure the toxicological safety of the soil.

The addition of organic amendments increased soil salinity, but EC values were lower than the established limit for soil salinization risk (>2 dS m^{-1}). However, it is well recognized that salt injury to sensitive vegetable crops may result unless the organic matter is subjected to, at least, a period of composting (*26*). Saline conditions are known to suppress plant growth because sodium and chlorine ions reduce water availability due to the high osmotic pressure and restrict the mobility and transport of the potassium and calcium ions to the growing parts of plants, reducing the yield and quality of the crops (*27*). In our study, we observed that the supplementation of the soil with the different types of organic soil amendments caused differences in the plant yield. The reason why sewage sludges 1 and 3 did not increase plant yield could be due to the high concentration of phytotoxic compounds, such as ammonium and other salts, found in these sewage sludges, which reduced vegetable growth as described previously (*28*).

The phytonutrient composition is highly dependent on climate, soil conditions, agricultural practices, light intensity, and pesticides. The content of vitamin C in rocket leaves cultivated in nonamended soil was in the same range as previously described (134 mg 100 g^{-1} of fw) (*29*). This content was maintained when the different organic soil amendments were added, and even statistically higher levels of AA have been described in organically and sustainably grown crops as compared to those produced by conventional agricultural practices (*4*). The total glucosinolate content of control leaves was slightly higher than that previously found (*19*). Several authors reported that the addition of different nutrients to the soil can reduce the glucosinolate content (*30*−*32*) as confirmed in our study. Kim et al. (*30*) found that glucosinolate content from the edible parts of *Brassica rapa* was strongly affected by N and S applications. In general, *Brassica* crops require more S than most other crops due to its role in the synthesis of glucosinolates. Nitrogen is also a constituent of the glucosinolate molecule, but high doses of N decrease glucosinolate levels as it increases plant growth (*31*). Rosen et al. (*32*) showed that total glucosinolate content, mainly glucobrassicin, was maximized in cabbage cultivars grown at low N and high S application doses. According to this, the high N content of urban solid waste and sewage sludge could explain the lower glucosinolate and phenolic contents of rocket leaves when compared to control. Coria-Cayupán et al. (*23*) found that the N presence in the soil adversely affects the synthesis of phenolic compounds in lettuce. In addition, it was observed that the use of organic soil amendments increased the water content and the maturity stage of the leaves, which contribute to a reduction in the content of phytonutrients. Koukounaras et al. (*33*) found that rocket leaves showed a high phenolic content at a lower maturity stage compared to a more mature one. The total flavonoid content of rocket leaves cultivated in nonamended soil was 398 mg 100 g^{-1} of fw. This content was higher than that found in previous studies (55−132 mg 100 g^{-1} of fw) (*21*, *34*, *35*).

The microbial counts of amended soils confirm that organic amendments could be potential transmission sources of fecal microorganisms to the crops, especially for leafy greens that are in direct contact with the soil (*26*). Good agricultural practices are indispensable to prevent plant food contamination by foodborne pathogens. Among the tested soil amendments, noncomposted materials such as pig slurry showed the highest microbial risks. In fact, it is assumed that proper composting of fresh organic amendments via thermal treatment reduces the risk of potential human pathogen survival (*11*). In this respect, composting plays an important role in enhancing the availability of nutrients essential to plant growth and reducing the presence of human pathogens in amendments. Thus, organic wastes properly composted such as urban solid waste and sewage sludge at optimum doses can be considered a suitable alternative to other soil amendments for the production of baby leaves such as rocket. This study demonstrated that the use of sewage sludge at a low dose (45 t ha^{-1}) increased rocket yield without detrimental effects on phytochemicals including vitamin C content when the leaves reached the commercial maturity stage.

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