10122 *J. Agric. Food Chem.* **2009,** *57,* 10122–10129
DOI:10.1021/jf903019d

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY
ARTICLE

Variations in Bioactive Substance Contents and Crop Yields of Lettuce (*Lactuca sativa* L.) Cultivated in Soils with Different Fertilization Treatments

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Changes in crop yields and bioactive substance contents were studied in lettuce crop concerning the influence of soil nutritional status as a result of compost and vermicompost additions obtained from different organic substrates. Plant productions and main pigment contents in lettuce were higher in all the fertilized soils than in the untreated soil, with the exception of the one treated with urban solid waste compost. These positive effects correlate with nitrogen level increase in soil. However, the high saline input of this compost prepared from food home wastes interferes in lettuce growth and prevents it from being higher than the control. Marked decreases in lettuce phenolic contents and antiradical activity were found in most of the treatments. Composts and vermicomposts produced through the processing of cattle manures, agro-industrial organic wastes significantly increased lettuce crop yield enriching its pigment contents, although, in some cases, antioxidant value and phenolic levels were reduced.

KEYWORDS: *Lactuca sativa*; antioxidants; vitamins; pigments; micronutrients; soil effect; organic waste compost; soil amendment; compost; vermicompost

INTRODUCTION

Several epidemiological studies indicate that diets rich in fruit and vegetables are related to lower incidences of some types of cancer and cardiovascular diseases. These health-promoting effects have been associated with the action of some compounds, such as carotenoids and polyphenols present in those natural foods. Carotenoid presence in food is important not only for their antioxidant properties but also for giving it color. Besides, some of them are vitamin A precursors. In leafy vegetables, the yellow or orange color due to carotenoid presence is masked by the green one of chlorophylls. Color is one of the most important external food qualities and it determines its acceptance by the consumers. Another important group of bioactive compounds is that of phenolic compounds. These are widely distributed in fruits, herbs and vegetables and their antioxidant activity has been extensively reported. Lettuce (*Lactuca sativa* L.) is cultivated worldwide and is one of the green leafy vegetables most consumed in raw form for its good taste, low price, and high nutritive value, especially due to its vitamin, fiber, and mineral input to a diet. It has been reported that bioactive substances content in plants strongly depends on the cultivar characteristics, climate conditions, seasonal changes, maturity, post harvest treatment, and storage conditions. However, the soil effect has not been completely understood.

As a consequence of the growing global demand for food, the need to increase crop production through specific agricultural fertilization practices arises. A method of sustainable soil management also implies the maintaining or restoring of organic matter levels by adding some amendments and organic fertilizers that allow the recovery of its structure and maintenance of its fertility. Soils with low organic matter content may lead to reduced crop yields and productivity, even when sufficient nutrients are supplied by inorganic fertilizers (*1*). Organic amendment additions to soils decrease soil bulk density while raising their cumulative water infiltration rates, soil moisture content, and aggregate stability. These positive factors lead to reduced environmental and agronomic limitations of soils with inherently low organic matter content (*2*). Other research results have indicated that soil organic matter, macronutrient, and micronutrient levels go up by manure compost addition according to the rate of compost application (*3*). Furthermore, animal manures, crop residues, and composts have increased organic matter up to 57% over a two-year period (*4*). On the other hand, it has been reported that soil organic matter level is significantly higher after the first amendment application of vermicompost from household solid waste (*5*). Besides, concerning lettuce growth, food waste compost has been used as a good alternative to chemical fertilizer to raise the levels of soil microbial population and enzyme activities as well as to promote soil nutrients (*6*). Concerning the influence of fertilizer treatment on plant nutrient levels, compost treatments have stimulated sesame plant growth and have enhanced its pigment, carbohydrate, and mineral contents (*7*).

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However, there are few reports about the variation in bioactive substance contents and antioxidant activity of crops such as lettuce in relation to different organic fertilization treatments and their nutrient input to soil. The main aim of this work was to study the variation of pigment and antioxidant levels in lettuce plants as a consequence of compost and vermicompost additions to soil, taking as reference urea fertilization and soil without treatment. These materials were obtained from different solid wastes as cattle manures and agro-industrial and solid urban wastes and were subsequently characterized. Carotenoid, chlorophyll, and phenolic composition, as well as crop yield and antiradical activity of lettuce plants were determined and related to the main characteristics for each soil treatment.

MATERIALS AND METHODS

Compost and Vermicompost Production. Composts and vermicomposts were prepared in an experimental waste-treatment plant at the University of Santiago del Estero campus. Organic urban solid wastes were collected in a small neighborhood in Santiago del Estero, Argentina. The separate organic fraction was constituted mainly of residues of fruits and green leafy vegetables, cooked foods, red meat, fish, chicken, and grass. Fruit and vegetable solid wastes were provided by the main fruit and vegetable market from Santiago del Estero. Cattle manure was obtained from the dairy farm of the Agriculture, Livestock, and Farm School belonging to the University of Santiago del Estero. Organic wastes generated from cattle slaughterhouses were collected. They are mainly constituted of ruminal content and manure semisolids placed in an open-air pool. Mentioned organic wastes were processed to elaborate both composts or vermicomposts: fruit and vegetable solid waste compost (FVSW-C), cow manure compost (CM-C), cattle slaughterhouse waste compost (CSW-C), cattle slaughterhouse waste vermicompost (CSW-V), urban solid waste compost (USW-C), and fruit and vegetable solid waste vermicompost (FVSW-V). Triplicate samples formed by three subsamples of each pile were randomly collected after 180 days of composting (USW-C, FVSW-C, CM-C), after 60 days of composting (CSW-C), after 100 days composting + 80 days vermicomposting (FVSW-V), and after 60 days composting + 120 days vermicomposting (CSW-V).

Analysis of Soil, Compost, and Vermicompost Samples. The main soil parameters were determined in samples taken 0−30 cm from the top. Soil, compost, and vermicompost samples were manually mixed to ensure their homogeneity: dried under forced air, crushed, and sieved through a 2 mm sieve. Electrical conductivity (EC) and pH of the samples were determined from water extracts in a 1:10 and 1:2.5 w/w solid/liquid ratio, respectively (*8*). Total organic carbon (TOC) content (g of C/100 g sample) was determined by oxidation with $K_2Cr_2O_7/H_2SO_4$ at 120 °C and spectrophotometric determination of Cr(III) at 590 nm by using potassium tartrate as the calibration compound (*8*). Cation exchange capacity (CEC) was determined as suggested in the literature (*9*). Total organic nitrogen content (TON) was measured by the Kjeldahl method (*8*). Total Na, K, and Ca contents were determined by flame photometry (Crudo Caamaño, Buenos Aires, Argentina), and total metal contents of Mg, Fe, Mn, Zn, Cu, Cd, Cr, Ni, and Pb were determined by atomic absorption flame spectrophotometry after nitric/perchloric acid digestion of the samples. Water-soluble carbon (WSC) percentage was determined as suggested by Charest et al. (*10*). Biological germination test was carried out and germination index (GI) was determined by employing *Allium cepa* L. seeds and by measuring the average length of roots and percentage of germination after 5 days (*11*).

Experimental Design for Plant Growth. The experimental site was located in Santiago del Estero (Argentina; 27°50′7″S, 64°13′46″W). Lettuce plants (*Lactuca sativa* L. var. Capitata) were grown in 5.0 × 1.0 m plots. They were harvested in July corresponding to the winter season in the Southern hemisphere. The experimental field design was done in completely randomized blocks with nine treatments and labeled as follows: control, without fertilizer addition (T1), mineral nitrogen adding of urea in a 300 kg/ha rate (T2), FVSW-C in 10Tn/ha (T3), CM-C in 10Tn/ha (T4), CSW-C in 10Tn/ha (T5), CSW-V in 10Tn/ha (T6), 150 kg/ha urea) combined with 5 Tn/ha FVSW-C (T7), USW-C in 10Tn/ha (T8), and FVSW-V in 10 Tn/ha (T9). All determinations were performed considering three plots per treatment and two replicates. Fertilizer rates (expressed in dry weight) were chosen based on lettuce crop requirements and according to the practices of local producers.

Plant Material. For yield determination, all the plants of the plots were harvested after 8 weeks and weighed. A total of 10 lettuce plants were taken from each plot. Vegetable samples were stored at 8 °C until immediate chemical analysis. Plant leaves were washed, dried, and separated to extract a 5 or 10 g representative portion of their edible part. Samples of 30 g were placed in paper bags and dried in a hood at 100 °C to constant weight to determine dry matter.

Extract Preparation. Representative samples of fresh leaves of lettuce (10 g) were extracted with 30 mL aliquots of acetone using a blender and filtered under vacuum. This procedure was repeated twice and these three extracts were combined and then transferred to 100 mL of petroleum ether and diethyl ether (1:1) mixture by adding small portions of the acetone extract and large amounts of water in a separatory funnel. Ethereal extract was separated and the remaining traces of water were removed by addition of anhydrous Na_2SO_4. The extract was filtered, concentrated in a rotary evaporator, and dried finally under N_2. Extracts were prepared in triplicate.

Determination of Chlorophyll and Carotenoid Contents. Determinations were performed by spectrophotometry using a UV/vis spectrophotometer (UNICAM, Cambridge, U.K.). Dried extract was redissolved in 5 mL of ethyl acetate. An aliquot of 50 μL diluted in 3 mL of ethyl ether. UV/vis spectra were recorded for pigment analysis and absorbance measured at 430, 642, and 660 nm. Carotenoid absorption coefficients were used for total content calculations (*12*). Chlorophylls *a* and *b* and total contents were calculated according to Comar and Zscheile equations reported in the literature (*12*).

Analysis of Carotenoid Composition. Carotenoid analyses of lettuce extracts were performed by high performance liquid chromatography (HPLC) using a chromatograph (Lab Alliance, Alvarado, TX) equipped with a 20 μL Rheodyne 7125 injector and a detector operating at 430 nm (Konik, Barcelona, Spain) commanded by a data processor software. The column used was a 250 × 4.6 mm i.d., 5 μm, Spherisorb S5 CN (Phase Sep, Clwyd, U.K.) and held at 29 ± 1 °C. A 10 × 4.6 mm i.d., 5 μm, guard column of the same material was fitted to protect the main column. A mixture of ethyl acetate−hexane was used as mobile phase at 1 mL/min in gradient elution from 25% ethyl acetate increasing up to 65% in the first 12 min and held isocratically for 1 min. The extract was redissolved in 5 mL of ethyl acetate and diluted 1/5 before injection. The quantitative analysis was carried out by the external standard method using calibration curves, constructed with a minimum of five concentrations for each standard, determined by UV/vis spectroscopy using the absorption coefficients (*12*).

Standard Compound Purification for Quantitative Analysis. The standards of β-carotene, violaxanthin, neoxanthin, lactucaxanthin, and lutein were obtained from natural sources and purified by open column, preparative HPLC and thin layer chromatography (TLC) (*13*). Purifications of crude extracts were performed using a 310 × 25 mm i.d., 40−63 μm, Lobar C18 preparative column (Merck, Damstadt, Germany). Carotenoid separation was achieved by a gradient elution at 1 mL/min with methanol and increasing amounts of chloroform. Eluted fractions were dried under N_2 and redissolved in ethanol or petroleum ether according to the solubility to determine the UV/vis spectra. Fractions were loaded on (1:1) MgO−Hyflosupercel TLC plates when further purification was necessary. Elution was carried out using (20:80) ethyl acetate−petroleum ether or (15:85) acetone−petroleum ether mixtures. Purity of the standards was verified by HPLC.

Identification of Carotenoids in Lettuce Samples. The identification of the carotenoids present in lettuce samples was based on (a) their chromatographic parameters compared to those of standard compounds obtained from recognized sources (*13*), (b) the chemical reactions of functional groups, and (c) their UV/vis spectra by considering the wavelength of maximum absorption and the spectrum fine structure which is characteristic of the chromophore (*12*).

Analysis of Phenolic Compounds. *Methanolic Extract Preparation*. Portions of 5 g of lettuce samples were extracted using a blender with 30 mL aliquots of methanol. This procedure was repeated twice; the extracts were filtered under reduced pressure, combined, and constituted to a total volume of 100 mL. The extracts were prepared in triplicate.

Table 1. Main Physicochemical Properties of Soil, Compost, and Vermicompost[a]

soil parameters	soil	CM-C	CSW-C	CSW-V	FVSW-C	FVSW-V	USW- C
pH	8.2 d,e	7.9 c	6.2 a	6.5 b	8.3 ef	8.0 cd	8.5 f
EC (mS/cm)	0.11 a	1.8 d	1.6 c	0.5 b	2.0 e	0.4 b	2.1 e
TOC (g/100 g)	0.62 a	13.5 d	15.9 e	19.4 f	10.0 c	7.8 b	11.0 c
TON (g/100 g)	0.23 a	1.2 d	1.5 e	1.6 e	0.64 c	0.46 b	1.2 d
TOC/TON	2.70	11.3	10.6	12.1	15.6	16.9	9.2
WSC (%)	<0.1 a	0.5 b	2.3 e	0.9 d	0.44 b	<0.1 a	0.74 c
WSC/TON		0.42	1.53	0.56	0.69		0.62
CEC (meq/100 g)	12.8 a	63.2 d	42.6 b	55.8 c	64.3 e	67.9 f	68.2 f
CEC/TOC	20.6	3.97	2.84	2.88	6.43	8.70	6.20
GI		93.6 c	50.8 a	94.8 c	90.1 b	95.2 c	99.6 d
				Mineral Contents (g/100 g)			
Na	0.05 a	0.13 c	0.06 a	0.08 b	0.12 c	0.27 d	1.12 e
K	0.92 b	0.96 b	0.58 a	0.50 a	1.83 c	0.82 b	4.80 d
Ca	0.47 a	1.57 bc	1.14 b	1.82 c	1.39 bc	1.41 bc	8.07 d
Mg	0.88 c	0.72 b	0.48 a	0.48 a	0.46 a	0.49 a	0.49 a
P	0.08 a	0.56 d	0.40 c	0.56 d	0.15 b	0.20 b	0.75 e
				Heavy Metal Contents (mg/kg)			
Fe	21410 g	7500 b	13250 e	10202 c	12000 d	13750 f	7300 a
Mn	435 e	253 a	348 b	536 f	366 c	392 d	1245 g
Zn	66 a	74 b	88 c	238 f	90 d	92 e	1205 g
Cu	15.2 a	20.4 c	16.6 b	28.4 d	35.6 e	40.9 f	78.8 g
Cd	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Cr	10.4 f	2.7 a	8.1 e	7.5 d	3.5 b	5.9 c	3.5 b
Ni	16.9 f	4.3 a	9.1 d	10.5 e	4.8 b	5.2 c	4.8 b
Pb	<0.2 a	<0.2 a	<0.2 a	<0.2 a	14.9 d	12.3 c	1.6 b

[a] Values followed by the same letter indicate no significant differences ($P < 0.05$) and belong to a homogeneous group.

The quantitative analysis of phenolic compounds was carried out using a liquid chromatograph (Konik, Barcelona, Spain) coupled with a UV detector operating at 330 nm (Konik, Barcelona, Spain) and a 250 × 4.6 mm i.d., 5 μm, reverse phase SS WAKOSIL C18RS column (SGE, Byron Bay, Australia). A 10 × 4.6 mm i.d., 5 μm, guard column of the same material was fitted to protect the main column. The mobile phase was an acetonitrile−water−acetic acid mixture (25:74.5:0.5), with a flow rate of 1 mL/min at 30.0 ± 0.5 °C, and the injection volume was 20 μL. For the identification of phenolic compounds, analytical grade authentical samples (Sigma and Aldrich, Buenos Aires, Argentina) were used for calibration purposes.

Determination of Antiradical Activity. Free radical scavenging activity of lettuce samples was measured by using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^•$) method (*14*). An aliquot of the methanolic lettuce extract was added to a DPPH$^•$ methanolic solution and spectrophotometrically monitored at 515 nm. Antiradical activity (ARA) was calculated according to the following equation: $\text{ARA\%} = 100(1 - A_{ss}/A_0)$, where A_{SS} is the absorbance of the solution at the steady state and A_0 is the absorbance of DPPH$^•$ solution before antioxidant addition. A_{SS} was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software. ARA was also expressed as vitamin C equivalent antioxidant capacity (VCEAC) in mg of vitamin C/g of lettuce FW (*15*). All determinations were performed in duplicate for each extract.

Statistical Analysis. The data were statistically analyzed using Stat Graphics plus version 3.0 computing program (Statistical Graphics, Rockville, MD). The analysis includes the measures of central tendency, arithmetic mean and standard deviation. Multiple comparison procedure using least significant difference (LSD) was applied to determine which means were significantly different at $P < 0.05$ confidence level. The correlations were established using simple regression and analysis of variance models (ANOVA).

RESULTS AND DISCUSSION

Analysis of Soil, Compost, and Vermicompost Samples. Physicochemical and biological analyses were carried out to characterize the initial soil and all different composts and vermicomposts prepared. **Table 1** shows their main nutrient contents and physicochemical parameters measured. The compost and vermicompost quality strongly depend on the substrate used to elaborate them and also vary with the processing methodology and the elapsed time of composting. As **Table 1** shows, pH value for all the products ranged between 6.2 and 8.5 being acceptable for a good plant development. In all the cases, EC was lower than the maximum recommended limit of 4 mS/cm (*16*). The composts presented higher EC values (1.6−2.1 mS/cm) than the vermicomposts (0.4−0.5 mS/cm). Concerning mineral nutrients, USW-C presented higher contents of Na, K, and Ca than the other organic fertilizers, as well as high contents of Mn and Zn. Moreover, the compost and vermicompost maturity is very important for their utilization in agricultural purposes, therefore, a series of parameters must be considered to estimate their maturity state. When a compost has reached a mature status, it meets most of the following conditions: TOC/TON ratio must be closer to 15 and lower than 20; CEC higher than 60 meq/100 g (*17*); CEC/TOC ratio higher than 3.2 (*18*); WSC lower than 0.5% and a WSC/TON ratio lower than 0.7 (*19*); besides, in biological seed germination assay, GI must be higher than 50% compared to control (*16*). Physicochemical and biological parameters shown in **Table 1** indicate that all the composts and vermicomposts have reached a mature state with exception of CSW-C because this was collected after a short composting time of 60 days.

Effect of Soil Treatment on Lettuce Production Yield. Lettuce crop yields were determined for all the treatments comparing them with untreated soil (T1). **Figure 1** compares plant fresh mass production for all treatments and it shows that the crop yields in fertilized and amended soils were higher than T1 except with soil treated with USW-C (T8). The highest lettuce productions were obtained for urea fertilization (T2) and FVSW-C addition (T3) with yield increases of 121 and 105% in respect of T1,

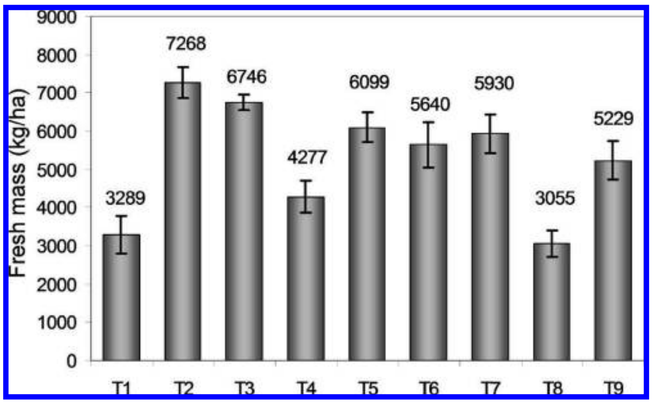

Figure 1. Variations in lettuce crop yield in relation to soil fertilization treatments. T1: control, T2: mineral nitrogen adding of urea, T3: fruit and vegetable solid waste compost (FVSW-C), T4: cow manure compost (CM-C), T5: cattle slaughterhouse waste compost (CSW-C), T6: cattle slaughterhouse waste vermicompost (CSW-V), T7: urea combined with FVSW-C, T8: urban solid waste compost (USW-C), T9: fruit and vegetable solid waste vermicompost (FVSW-V).

respectively. Combined addition of both of them (T7) produced an increment of 80%.

As expected, mineral fertilization by urea addition provides readily available inorganic nitrogen for the plant, leading to a higher production yield. Compost and vermicompost treatments to soil raised its organic matter content and nutrients favoring plant growth. The beneficial action of sheep manure and food waste composts on production and yield of lettuce crop has also been previously reported (*20*) as a consequence of increases in the organic matter and nutrient levels improving the soil aggregation state and stimulating microbial activity and diversity (*6*).

The remarkable yield enhancement observed for T3 would be mainly due to a good potassium input to soil. T8 decreased fresh mass production in a 7% in respect of T1 being the only case where a compost addition did not increase the crop yield. Despite the nutrient input to soil, the high sodium content of this USW-C (1.12 g/100 g compost), which results mainly from home food wastes cooked with added salt, prevent plant production from improving. Lettuce is a highly sensitive crop toward salinity, as indicated by the decline observed in romaine lettuce yield when they were irrigated with concentrated salt solution for long periods of time (*21*). Saline conditions are known to suppress plant growth because sodium and chloride ions reduce the water availability due to the high osmotic pressure of the external medium and restrict the availability, mobility, and transport of potassium and calcium ions to the growing parts of plants affecting the quality of both vegetative and reproductive organs and consequently reduce the yield and quality of the crop (*6*). Moreover, the sodium accumulation in plant tissues limiting essential nutrient uptake was proposed to explain the reductions in the growth of the leaf and the fresh and dry weight of the roots and leaves in five varieties of sugar beet after irrigation with sodium chloride solution (*22*). Potassium availability also plays a key role in plant water stress tolerance. Despite the positive impact of the potassium contribution to soil due to USW-C addition, the negative effect of its high saline input prevailed and the crop yield remained similar to the untreated soil.

Moisture. Lettuce moisture contents were determined from plant leaves grown in treated soils. The mean value obtained was 92.2 ± 0.7% and no significant differences were observed among samples.

Pigment Analyses of Lettuce Samples. The total pigment contents were analyzed in lettuce plants cultivated in fertilized soils and compared with those obtained for the untreated soil. A typical UV/vis spectrum of lettuce extracts shows two main absorbance bands characteristic of chlorophylls and carotenoids at $\lambda_{\max} = 662$ and 430 nm, respectively. No qualitative differences were observed among sample spectrum profiles.

Chlorophylls. Plants have two types of chlorophylls, named *a* and *b*. Chlorophyll *a* is the major one, although it is the most easily degraded (*12*). The absorption band at 662 nm corresponds to both chlorophylls *a* and *b*, which allows the measurement of the total pigment content. Ethyl ether was found to be more effective than methanol as an extractive solvent because the total mean chlorophyll content, considering all the samples, was of 1226 ± 188 μg/g for an ethereal extraction, while for the methanolic extraction was only of 507 ± 55 μg/g.

Quantitative results for chlorophyll determinations in lettuce leaf samples for all soil fertilization treatments are presented in **Table 2**. In all the cases, pigment levels were higher or at least not significantly different than control. The highest total chlorophyll content was obtained for T7 with a 56.1% of increase in relation to T1. Intermediate values were observed for T2, T6, T8, and T3 with marked rises of 35.3, 35.0, 20.1, and 15.6%, respectively, with respect to the control, whereas T5, T4, and T9 produced levels at least similar to T1 with differences of 7.3, 4.7, and 0.6%, respectively, compared with the control. Both chlorophylls *a* and *b* presented individually quite similar trends, although, in T7, chlorophyll *a* increased 62.2% while chlorophyll *b* increased 39.6%.

Urea fertilization produced a significant rise in chlorophyll level due basically to its available nitrogen contribution to soil, an important constituent of the chlorophyll molecular structure. However, the remarkable increase observed in T7 is ascribed to the combined nutrient input of mineral nitrogen given by urea and the different elements involved in the chlorophyll biosynthetic steps such as Mn, Mg, Cu, Zn, and Ca.

Mn presence in soil affects the level of chlorophyll in the plant because it is required for the maintenance of chloroplast structure as it was reported in the literature (*23*). This is the case of T6, which has a good supply of this mineral with the greatest contribution of nitrogen among the composts and a remarkable level of calcium. T3 presents a good input of Mn and Ca, although its level of TON is lower than other composts. USW-C has the highest level of Mn and Ca, but its high sodium addition to the soil would inhibit their positive effect in chlorophyll biosynthesis.

Carotenoid Analysis. Carotenoid composition as well as total content was determined for the lettuce samples. Mean values obtained were 155 ± 25 μg/g of total carotenoid content by UV/vis spectrophotometry and 174 ± 25 μg/g by HPLC. Five carotenoids were isolated from lettuce and characterized afterward as follows: β-carotene, lutein, violaxanthin, lactucaxanthin, and neoxanthin. Determination of carotenoid composition carried out by HPLC gave individual content means of 17 ± 2 μg/g for β-carotene, 92 ± 15 μg/g of lactucaxanthin, 30 ± 4 μg/g for lutein, 30 ± 3 μg/g violaxanthin, and 5 ± 2 μg/g for neoxanthin.

Carotenoid composition in green leaves is quite constant, different from what occurs in mature fruits where noticeable qualitative and quantitative variability is observed. Pigments occur in green leafy vegetables as a regular pattern constituted by β-carotene, lutein, violaxanthin, and neoxanthin (*13*). Lactucaxanthin is an additional carotenoid observed specifically in lettuce plants and is the major constituent of the carotenoid fraction in this type of lettuce. The nutritional role of this carotenoid has not been established at present, although lactucaxanthin has been detected in low concentrations in human serum and retina. Its chemical structure is very similar to that of lutein because they differ in only one double-bond location at the

Table 2. Chlorophyll and Carotenoid Contents in Lettuce Leaves Grown in Treated Soils[a]

	treatments								
pigments	T1	T2	T3	T4	T5	T6	T7	T8	T9
					Chlorophylls (μg/g)				
chlorophyll *a*	751 a	1019 c	866 b	797 a	801 a	1032 c	1218 c	914 b	749 a
chlorophyll *b*	275 a	369 d	320 c	277 a	300 b	353 c	384 e	318 c	283 a
total content	1026 a	1388 b	1186 b	1074 a	1101 a	1385 b	1602 c	1232 b	1032 a
					Carotenoids (μg/g)				
total content[b]	124 a	164 d	145 c	173 c	129 b	188 d	194 e	156 d	125 a
total content[c]	147 a	188 d	159 b	174 c	154 b	200 d	226 e	175 c	148 a
β-carotene	15 a	18 c	16 b	17 c	15 a	19 d	22 e	18 d	15 a
lactucaxanthin	78 a	104 b	83 a	89 b	83 a	110 b	124 c	86 a	75 a
lutein	26 a	31 b	28 b	30 b	25 a	34 b	37 c	32 b	28 b
violaxanthin	26 a	30 d	29 c	32 f	27 a	31 e	33 g	33 g	28 b
neoxanthin	2.0 a	5.1 d	2.8 b	4.6 c	4.0 c	6.4 e	9.9 f	5.4 d	2.1 a
vit A value (RE/ g)	2.46 a	2.98 c	2.64 b	2.90 c	2.50 a	3.12 d	3.63 e	3.04 d	2.48 a

[a] Experimental details in Materials and Methods. Values followed by the same letter indicate no significant differences ($P < 0.05$) and belong to a homogeneous group. [b] Determined by UV−vis spectrophotometry. [c] Determined by HPLC.

end of the long polyenic system and, therefore, their maximum absorption wavelengths are quite similar. The value measured for lactucaxanthin content of 92 ± 15 μg/g is considerable higher than that reported for Boston and Curly lettuces of 7.5 and 6.7 μg/g, respectively, indicating the great variability due to the cultivar or variety studied (*24*). Furthermore, lactucaxanthin and lutein contents of 1.48 and 1.70 μg/g were measured in romaine lettuce, respectively (*25*). A strong dependence of the leaf maturity state on the carotenoid levels were also reported, indicating that lactucaxanthin contents varies from 9.3 to 13.6 and those of lutein from 14.8 to 30.9 when changes from young to mature leaves of Boston lettuce (*26*). Besides, β-carotene content mean is included in the concentration range reported for different varieties, from 9.9 μg/g for Freelice type to 29 μg/g for Boston lettuce (*27*). Seasonal differences in carotenoid contents were also informed in the literature in leafy vegetables.

Because all the lettuce samples were equally grown in this experimental design, differences due to these factors are neglected and the relative values show changes ascribed exclusively to soil treatments. Among all carotenoids determined in lettuce samples, only β-carotene has provitamin A activity. The mean vitamin A value calculated according to FAO documents was 2.86 ± 0.36 RE/g. According to this measurement, approximately 750 μg necessary for the daily vitamin A intake are contained in 265 g of fresh lettuce leaves. This vitamin A value of lettuce is lower than those calculated from carotenoid determinations in other green leaves as spinach of 8.33 RE/g (*26*), chicory, rucula, and cress of 5.9, 3.01, and 2.95 RE/g, respectively (*24*).

The quantitative results for carotenoid contents in lettuce leaf samples for all soil treatments are presented in **Table 2**. Total carotenoid contents were determined by two different techniques. Those results estimated by spectrophotometry were lower values than those obtained by HPLC as the equivalent to the individual content sums. However, similar trends are observed for the results measured by both techniques. All the treatments produced higher carotenoid contents in lettuce samples than control, except T9 where no significant difference was found. The highest level of carotenoid content was obtained for T7 with a marked increase of more than 50% with respect to T1. In the case of individual contents, T7 raised β-carotene level in a 46.7% compared to that of control. Furthermore, the increment was of 20.0% for mineral fertilization T2 in respect of the untreated soil, while the rise was of 6.7% for T3. Besides, T6 and T8 produced increases of 26.7 and 20.0%, respectively, while no changes were found for T5 and T9. In the case of lactucaxanthin, the major component of the carotenoid fraction, the highest level was obtained for T7. Lutein, violaxanthin, and neoxanthin presented similar trends for all the treatments, the highest contents being for T7, while T9 and T5 contents were similar to the control. All the other treatments produced intermediate behaviors.

Because β-carotene is the only provitaminic carotenoid among those identified in lettuce, vitamin A values correlate directly with its contents, and the highest level corresponds to T7, while no significant differences were observed among T5, T9, and T1 as the lowest values. Urea fertilization T2 and T3 produced both intermediate levels with increases of 21 and 7%, respectively, compared to control assay. Thus, T7 of urea application combined with FVSW-C addition was found to be remarkably more effective raising the lettuce vitamin A value by 47%.

The combination of urea and FVSW-C was the most effective to increase the carotenoid content in lettuce, as **Table 2** shows, followed by CSW-V and urea. Carotenoid biosynthesis depends basically on the mineral nitrogen supply (*28*). TON level of CSW-V is the highest among these compost and vermicompost and its adequate TOC/TON ratio favors the mineralization rate of the organic nitrogen in the soil. The low T9 carotenoid level, similar to the control, also corresponds to this dependence.

Carotenoids are precursors of abscisic acid, a phytohormone involved in the regulation of plant development under salt and water stress (*21*). Accumulation of secondary metabolites in lettuce under stress was proposed as linked to the induction of phytohormones; thus, an increment in β-carotene level is expected under stress conditions. They observed that the total carotenoid content increased in lettuce with NaCl irrigation, the long-term treatment effect being larger than that of the short-term treatment. This is consistent with the high carotene level found in lettuce cultivated in soil treated with a saline compost as the USW-C in T8.

Phenolic Compound Analyses. Several phenolic compounds are found by HPLC analysis in a typical methanolic extract of lettuce leaves. Individual contents of caffeic and coumaric acids were determined as major constituents. Mean values obtained were 46 ± 5 μg/g for caffeic acid and 811 ± 122 μg/g for coumaric acid. Phenolic fraction composition is highly dependent on climate, soil conditions, agricultural practices, light irradiation intensity, and pesticides. Phenolic compound presence in lettuce has been

Table 3. Phenolic Contents and Antiradical Activity in Lettuce Leaves Cultivated in Soils with Different Treatments[a]

	treatments								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
				Phenolic Compounds (μg/g FW)					
caffeic acid	49 e	53 e	44 d	54 e	26 b	43 c	46 e	75 e	23 a
coumaric acid	952 g	634 b	730 c	889 f	830 e	934 g	609 a	929 g	794 d
total	1001 e	687 a	774 b	943 d	856 c	977 d	655 a	1004 e	817 c
				Antiradical Activity					
ARA%/ mg	20.5 c	14.7 b	17.8 c	21.2 c	12.7 a	17.3 c	12.8 a	16.3 b	17.9 c
VCEAC (mg/g)	4.83 c	3.46 b	4.20 c	5.00 c	3.00 a	4.08 c	3.00 a	3.85 b	4.22 c

[a] Means followed by the same letter do not differ ($P < 0.05$) and belong to a homogeneous group.

previously reported identifying caffeic acid derivatives as well as ferulic acid traces (*29*). Caffeic acid and two derivatives, chlorogenic and dicaffeoyl tartaric acids, were also found in six lettuce cultivars (*30*).

Variations in phenolic contents of lettuce cultivated with different soil fertilization treatments were evaluated and the results are shown in **Table 3**. Coumaric acid, the major constituent in the phenolic fraction, significantly reduced its concentration in the lettuce samples by 36 and 33.4% in T7 and T2, in respect to T1, respectively. Then, urea addition, both alone and combined with FVSW-C, had a negative effect on coumaric acid levels. Among other composts, FVSW-C produced the more important decrease with respect to the control. Almost all compost and vermicompost additions reduce the total phenolic contents, although no significant differences were observed between T8 and T1.

These results found in treated soils indicate that there are some factors present in the compost and vermicomposts that negatively interfere in the phenolic compound synthesis produced through the phenylpropanoid pathway. On the contrary, the activity of such a pathway was found to be increased under stressful conditions, so the phenolic compounds are synthesized and accumulated (*31*). According to results presented in **Table 3**, it can be stated that the nitrogen presence in the soil adversely affects the synthesis of phenolic compounds. This trend is in agreement with a study performed in tomato leaves (*32*), which indicates that N deficiency stimulates the biosynthesis of these secondary compounds in response to its low availability. The level of P would negatively affect the NO_3^- absorption, as indicated in the literature (*33*).

Moreover, the effect of salt irrigation treatment on the phenolic content was recently studied in romaine lettuce (*21*). Compounds such as chlorogenic and caffeic acids significantly decreased their levels after a short-term salt irrigation treatment, whereas no effect was observed for a long-term treatment with low salt concentration when compared with the control. These results do not correlate well with phenolic compound levels measured in lettuce grown in a soil treated with salt-rich compost. The results obtained for T8, as an example of a salt-rich compost addition, showed that phenolic contents did not go down, indicating that the positive action of P outweighs the high saline content of USW-C.

Antiradical Activity. The spectrophotometric method of DPPH$^\bullet$ consumption measures the ability of pure substances or extracts for trapping free radicals, producing in consequence the bleaching of the colored solution (*14*). ARA values were determined and ranged between 12.7 and 21.2%/mg FW. The mean VCEAC value obtained was of 4.0 ± 0.7 mg of ascorbic acid per g of fresh leaves.

Coumaric acid, as the major constituent of lettuce phenolic extracts, is one of the possible compounds responsible for the antiradical activity, although caffeic acid present in much lower levels is more than 24 times stronger as a radical scavenger. However, relatively weak correlations ($R < 0.60$) were observed between this antiradical ability and the individual contents of caffeic and coumaric acids and also with the total phenolic content, indicating the contribution of other antioxidant compounds in such activity. Kinetic profile for the radical disappearance by addition of a lettuce extract presented a two-stage differential behavior when the radical interacts with the plant methanolic extract. An initial step presents the fast action of certain antioxidants producing an instantaneous solution bleaching and a second one corresponding to the scavenging reaction of those having lower reactivity. Thus, the radical consumption process continues but at a lower rate. In the case of fresh fruit and vegetables, the compounds responsible for this initial fast action toward DPPH$^\bullet$ are mainly ascorbic acid and polyphenols such as caffeic acid, but this one is present in these lettuce samples in a very low content. Ascorbic acid produces an instantaneous absorbance drop by a fast reaction with the radical. Therefore, the analysis of radical consumption in the rapid stage allows the estimation of the ascorbic acid content as a maximum value. Fast-antioxidant action accounts for between 57 and 63% of the total antiradical activity in these lettuce extracts, as shown in **Table 3**, and it is ascribed mainly to the ascorbic acid presence. Previous reports about six lettuce cultivars ascribed to dicaffeoyl tartaric acid presence accounted for more than 50% of the DPPH$^\bullet$ scavenging activity, while ascorbic acid action accounted for up to 24.5% (*29*). Anyway, this particularly active polyphenol was not detected in our lettuce cultivar. A recent study about 10 genotypes of green leafy vegetables reported a good correlation between the antiradical activity and polyphenol content for only five lettuce varieties, although, when all greens were taken into account, no correlation was found, indicating that compounds and antiradical mechanisms depend on the different species. This absence of correlation was also ascribed by the authors to interferences by substances such as ascorbic acid (*34*).

Free radical scavenging activity in lettuce leaf samples for all soil treatments are presented in **Table 3**. The lowest antiradical values were observed in T7 and T5, with declines of 38% in both cases with respect to the control. Reductions in antioxidant activity of 28 and 20% were found for T2 and T8, respectively, while T3, T4, T6, and T9 produced similar activities to T1. The results obtained for T2 allow the distinguishing of the effect of the nitrogen adding on the biosynthesis of antioxidant substances, excluding the influence of other nutrients. This treatment resulted in a decline in the antiradical activity of 28% compared with the control. The high mineral nitrogen content in soil has been previously reported as responsible for the decrease in the biosynthesis of ascorbic acid in green leafy vegetables (*35*). The lack of a direct correlation of lettuce ARA with a single soil nutrient content demonstrates the complexity of a system where numerous factors are closely involved in a soil−plant nutritional interrelation. Some soil nutrients promote lettuce growth, although they may reduce certain metabolite product levels by interfering in the biosynthesis pathway or stimulating the degradation.

Results obtained in this work show that there are significant differences between the control and the fertilization treatments not only in crop yield but also in the bioactive substances concentration and antioxidant activity of lettuce plants. Organic and mineral soil treatments improved the crop yield except in the case of T8 high saline conditions. On the contrary, total phenolic levels were significantly lower than the control, except for T8. Moreover, all chlorophyll contents were at least similar to T1. Total carotenoid levels were in most of the cases significantly higher than the control, except for T9. The biosynthesis by the lettuce plant of the different bioactive compound groups responds differently to certain nutrient additions to soil. On the one hand, plant pigment formation (carotenoids and chlorophylls) is mainly stimulated by the nitrogen level increase in soil. On the other hand, water-soluble antioxidant substances present in lettuce decrease as a function of the high nitrogen supply.

Compost and vermicompost treatments of urban, agricultural, and cattle organic wastes reduce the environmental pollution that results when they are not properly treated. The use of compost and vermicompost restores soil organic matter depletion associated with soil degradation. Besides, the agricultural applications of these products as an alternative to chemical fertilizers enhance soil nutritional status; hence, lettuce crop yields are positively affected without significant loss of the food nutritional value and even, in some cases, enriching the content of health-promoting substances.

ABBREVIATIONS USED

FVSW-C, fruit and vegetable solid waste compost; CM-C, cow manure compost; CSW-C, cattle slaughterhouse waste compost; CSW-V, cattle slaughterhouse waste vermicompost; USW-C, urban solid waste compost; FVSW-V, fruit and vegetable solid waste vermicompost; EC, electrical conductivity; TOC, total organic carbon content; CEC, cation exchange capacity; TON, total organic nitrogen content; GI, germination index; UV/vis, ultraviolet−visible; $\lambda_{\max}$, maximum absorption wavelength; FW, fresh weight; HPLC, high performance liquid chromatography; TLC, thin layer chromatography; DPPH$^{\bullet}$, 1,1-diphenyl-2-picrylhydrazyl radical; ARA, antiradical activity; LSD, least significant difference; WSC, water-soluble carbon percentage; HAC, humic acid content; FAC, fulvic acid content; RE, retinol equivalent; VCEAC, vitamin C equivalent antioxidant activity.

ACKNOWLEDGMENT

We are especially grateful to Dr. Lucrecia Chaillou for the statistical analysis.

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Received for review April 20, 2009. Revised manuscript received September 14, 2009. Accepted September 15, 2009. This work was financially supported by CICYT-UNSE. Y.C.C. acknowledges her fellowship granted by CONICET.