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Changes in Carbon Fractions during Composting of Plant Wastes and the Influence of a Humic Extract on Soil Microorganism Growth

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ABSTRACT

A composting experiment was carried out to assess the best parameters as indicators for degree of compost maturity and to determine humification indices for practical use. The effect of humic acids extracted on soil microorganisms growth was also simultaneously investigated *in vitro* under axenic conditions. The parameters used were oxidizable carbon (C_o), alkaline-extractable carbon (C_{ex}), humic and fulvic-like carbon (C_{ha}, C_{fa}), humification ratio (HR), humification index (HI), percent humic acid (P_{ha}) and C_{ha}/C_{fa} ratio. Results indicated that HR, HI, P_{ha} and C_{ha}/C_{fa} ratio could be used to describe the evolution of organic matter during the composting of horticultural residues as well as the humification degree of compost. Our microorganism incubation study showed that the addition of humates from lignocellulosic waste based compost at 1, 5 and 10% to the culturing medium enhanced bacterial growth while the above effect was not clearly observed when this product was added to fungi and actinomycetes.

Keywords: agricultural biostimulants, compost-stabilized waste, organic carbon fractions, humification indices, soil microbial populations

INTRODUCTION

The aim of the present study was to quantify selected humic substances produced during the composting of several plant wastes which had been inoculated with different lignocellulolytic microorganisms and to evaluate the different maturation parameters during the process. This work also studied the effect of humic acids extracted from compost on the *in vitro* growth of several soil microorganisms.

Soil organic matter plays a crucial role in soil fertility of horticultural systems (Allison 1973; Kononova 1973; Vaughan and Ord 1985; Marinari *et al*. 2000). However, the continuous use of large quantities of chemical fertilizers has led to reduced levels of organic matter in most agricultural soils (Mann 1986; Schlesinger 1986; Ayuso *et al.* 1997).

The humic fraction, as the more stable component of soil organic matter, is involved in many processes such as water retention, improvement of soil structure and aggregation and buffer capacity as well as supply of nutrients (Brady 1974; Bohn *et al.* 1985; Unsal and Sozudogru 2001; Sánchez-Monedero *et al.* 2002; Vargas-García *et al*. 2006). This fraction not only influences soil chemical and physical properties but also microbial growth and metabolism. Numerous investigations have shown that many soil microorganisms react positively to the presence of humic substances in cultivation media (Visser 1985a, 1985b). In an experiment carried out in soil with humified organic amendment, Schisler and Linderman (1989) demonstrated that humic substances could stimulate the growth of soil microbial populations. These stimulatory effects may be explained by the modification of cell membrane permeability to nutrients (Visser 1985b; Müller-Wegener 1988; Samson and Visser 1989; Schisler and Linderman 1989; Valdrighi *et al.* 1996). In addition, the humus-related increase in soil microorganisms may inhibit the growth of pathogenic fungi which infect plant roots (Scheuerell and Mahaffee 2004, 2006).

Composts are increasingly being used in agriculture since they contribute to the disposal of waste materials and thus the preservation of the environment. Even though the incorporation of composted waste with high organic matter content could improve soil quality and fertility and reduce waste materials (García *et al.* 1991; Requena *et al.* 1996; Marinari *et al*. 2000; Elorrieta *et al*. 2002), it could also produces toxicity problems that inhibit seed germination and plant development if inadequately mature composts are used (Zucconi *et al*. 1981; Mitsuyo *et al*. 1986; García *et al*. 1990, 1991; Marambe and Ando 1992). For this reason several parameters have been established to determine the compost maturity (Iglesias-Jiménez and Pérez-García 1992; Smith and Hughes 2001; Pietro and Paola 2004).

The composting process is a bio-oxidative process in which microorganisms transform the most easily biodegradable organic matter into CO_2, H_2O and minerals (mineralization process). With time, the remaining organic matter is converted into a stabilized end product through mineralization and humification, due to several biotic and abiotic factors (Gray *et al.* 1971; Viel *et al*. 1987). The weight loss observed in the product during composting is mainly due to the mineralization of the organic matter. The greatest changes in organic carbon fractions take place when microorganisms are more active (Levi-Minzi *et al.* 1986; García *et al.* 1991). The bio-oxidative phase of the composting process should be followed by a maturation process in order to obtain a product with stable organic matter which, when incorporated into soil, will increase the soil's humic fraction content while avoiding the risks observed when fresh organic waste is applied to agricultural crops (Roletto *et al*. 1985; Levi-Minzi *et al.* 1986).

Currently, there is an increasing need to find new sources of organic substrates and humic acids due to dwindling supplies of manures, peats and leonardites. In this sense, the intensive agriculture practiced during the last decades in the southeast of Spain has favored both losses in soil quality and fertility and the accumulation of huge amounts of plant

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wastes. In addition, the scarcity and poor quality of water in this area are limiting factors with regard to the intensive agriculture practiced. However the use of fertilisers added in liquid form does not help to solve the low soil organic matter content (Ayuso *et al.* 1997).

At present, there is a growing tendency to use humic acids as liquid organic amendment (Ayuso *et al.* 1997). Many products are available for agricultural use falling into the broad category of biological soil conditioners, stimulants or additives. They enhance crop growth and yield through several mechanisms, such as microbial inoculation of soil, stimulation of soil microbial activity, increasing the activity of critical enzymes, production of plant growth hormones in the soil, or supplementation of micronutrients (Chen *et al.* 2002). The addition of humic acids to soil contributes to the aggregation of soil particles and the soil's cation exchange capacity (Piccolo and Mbagwu 1990). Humic acids also have a positive effect on phosphorus and nitrogen metabolism (Figliolia *et al.* 1990; Biondi *et al.* 1994) and on the assimilation of other nutrients by plants and microorganisms.

MATERIALS AND METHODS

Experiment to determine changes in organic carbon fractions during composting

Composting experiment

Melon plant waste (*Cucumis melo* L. cv. 'Galia') was mixed with olive-oil mill waste, pruning waste, rice straw or almond shells and the different mixtures were arranged in 1.5 m^3 piles (**Table 1**). With the exception of one windrow which was used as uninoculated control, each compost pile was initially inoculated with different lignocellulolytic microorganisms from our own strain collection to assess the effect of microbial inoculation on the properties of the final compost product. Therefore, each compost mixture was performed in four replicates (three inoculated windrows and another non inoculated control) (**Table 1**). No attempt was made to identify and classify these strains. A total of three isolates from horticultural wastes were used as inoculum. Each windrow was inoculated with its respective bacterial suspension to reach a concentration between 10^7-10^8 cfu/g of waste. All composting conditions were performed in two replicates.

Table 1 Raw materials and microbial inoculant used in the composting experiment.

Raw material		Inoculant	
Mixture	**Percentage[a]**	**Code**	**Class**
M: O	75:25	162	Thermophile bacteria
M: A	75:25	671	Thermophile bacteria
M: Pr	75:25	252	Thermophile actinomycetes
M: R	75: 25	Control	

A: almond shells, M: melon plant waste, O: olive-oil mill waste, Pr: pruning waste, R: rice straw
[a]: by volume

Sampling and sample preparation

During the composting process samples were collected at the beginning of the experiment (day 0) and, coinciding with the turning of the compost piles, at days 14, 28, 45 (bio-oxidative phase), 90 and 180 (maturation phase). Each sample was composed of nine subsamples. Three replications were taken at each sampling time.

The preparation of the samples was similar to that described by other authors who analysed soluble and extractable carbon in compost samples (González-Vila and Martín 1985). In this case, samples were air dried (105°C, 24 h) and grinded in a hammer mill (mesh size 1 mm).

Analytical determinations of carbon fractions and humification indices

Total oxidizable carbon (C_o) was determined by dichromate oxidation at 150°C ($K_2Cr_2O_7$ + H_2SO_4 mixture) (Kononova 1973). Extractable carbon (C_{ex}), corresponding to fulvic and humic acid-like carbon, was determined with 0.1 M $Na_4P_2O_7$ + 0.1 N NaOH solution (1:1). The fulvic acid-like carbon (C_{fa}) was determined in the supernatant after acidification to pH 1 with sulphuric acid. The precipitated humic acid-like carbon (C_{ha}) was calculated from the difference between C_{ex} and C_{fa} (Sugahara and Inoko 1981; Iglesias-Jiménez and Pérez-García 1992). To follow the mineralization and maturation process quantitatively, all carbon fractions were expressed on ash-free basis. The following humification indices were calculated based on the works by Iglesias-Jiménez and Pérez-García (1989, 1992): C_{ha}/C_{fa} ratio, humification ratio ($HR=C_{ex}/C_o$ X 100), humification index ($HI=C_{ha}/C_o$ X 100) and percent humic acids ($P_{ha}=C_{ha}/C_{ex}$ X 100).

In vitro experiment to investigate the effect of humic extract on the growth of soil microorganisms

Extraction of humic substances

The humic extraction process was achieved using a compost mixture from the composting experiment previously indicated. This sample was taken before the maturation phase, around 30 days from the first of the process. The optimum extraction conditions were applied as follows: oven-dried composted vegetable residues were digested with 0.35% KOH at a ratio of 1/10 (w/v) for 2 h at 121°C. The soluble fraction was then separated from the undigested residue by sedimentation and decanting and concentrated 10 times by evaporation.

KOH was used as the extractant instead of NaOH, since it seems to be more suitable for the industrial production of humic amendment for agricultural applications.

Microbial strains

The microbial strains used were supplied by the Spanish Type Culture Collection (CECT). Bacterial cultures of *Pseudomonas putida* CECT 324, *Streptomyces badius* CECT 3275, *Arthrobacter globiformis* CECT 388 and *Nocardia asteroides* CECT 3051T, were kept on nutrient agar (NA) slants while fungal cultures of *Aspergillus niger* CECT 2807, *Penicillium burgense* CECT 2889 and *Trichoderma harzianum* CECT 2413 were kept on potato-dextrose-agar (PDA) at 4°C. *Azotobacter vinelandii* CECT 204 was kept on Janshekar salts-glucose-agar at 4°C.

Microbial growth experiment

Fungal and bacterial cultures were grown in 250-ml Erlenmeyer flasks containing 25 ml of potato dextrose broth (PDB) and nutrient broth (NB), respectively. *A. vinelandii* was grown in Janshekar salts-glucose broth. All cultures were incubated under aerobic conditions at 28°C on a rotary shaker for the duration of 48 to 120 h.

The effect of humic substances on the growth of soil microorganisms was studied in 250-ml Erlenmeyer flasks containing 25 ml of Janshekar-salts to which the humic extract obtained from the composted vegetable residue was added at 0, 1, 5 and 10%. After sterilization (121°C, 20 min) the flasks' contents were inoculated with a 1% cell suspension of the microbial strains described above and incubated at 28°C on a rotary shaker. Each treatment consisted of three replicates. Microbial growth was determined in aliquots taken at 0, 24, 72, 120 and 168 h. The total number of bacteria, actinomycetes and fungi was determined by colony count on NA and PDA plates.

Statistical analysis of data

All values were expressed as the mean of three measurements for each treatment. Data were subjected to one multifactorial analysis of variance (ANOVA). With respect to the parameters investigated during composting process (C_o, C_{ex}, C_{ha}, C_{fa}, C_{ha}/C_{fa}, HI, HR and P_{ha}), mean values were compared for the different levels of raw materials, sampling time and inoculation pattern. On the other hand, relating to microbial experiment growth, mean bacterial and

fungal counts were compared for the different levels of humic extract application rate and sampling time. In order to determine which means were significantly different from which others ($p < 0.05$), multiple comparison tests (Fisher's least significant difference) were used (Dowdy and Wearden 1991).

RESULTS AND DISCUSSION

Changes in carbon fractions during the composting process

In the present work an increase in C_{ha} values (expressed as a percentage of C_o) was in general recorded until day 30 of the composting experiment (except in the case of Olive-oil mill waste), followed by a decrease until day 180 whereas, a global decrease in C_o, C_{ex} and C_{fa} values (expressed as a percentage of C_o) was in general observed during the process. After 30 days approximately, C_{ha}/C_{fa} ratio was surprisingly similar at values observed at the end of the process but in all cases, this ratio was higher at the end of the composting process than at the beginning (**Table 2**). Das (1988) found a gradual increase in the polymerization rate (HA/HF) during composting. In our work, this parameter must not be considered a good maturation indicator although it may constitute a valid parameter to establish the evolutional grade of the organic matter during horticultural waste composting. This behavior was similar to observed in the case of P_{ha} values (**Table 2**). On the other hand, except in the case of almond-melon piles, significantly highest values of the HR and HI parameters were reached and the end of the composting (maturation phase).

Parallel to the global increasing C_{ha}/C_{fa}, HR, HI and P_{ha} values, a constant loss of carbon during the bio-oxidative phase of composting was therefore observed (**Table 2**). The calculation of a carbon loss factor may serve as an indirect indicator for the degree of compost maturity of and, therefore, the minimum period of composting (Iglesias-Jiménez and Pérez-García 1992). Under our experimental conditions the parameters of maturity reached optimal values when the material approximately contained 2 times less total carbon than the original material in the case of olive mill waste piles, while this value was around 1.5-1-7 in the rest of the mixtures.

The influence of the compost raw material on these parameters is shown in **Table 3**. Data showed in this table are relating to all analysed treatment as a whole as well as at the end of the process (180 days). In this case, when data were analysed irrespective of time, as an average of all treatments (microbial inoculants), compost made from melon and olive-oil mill waste showed the highest HR value

Table 2 Changes in carbon fractions and maturity and humification indices during plant waste composting. Means with the same letter are not significantly different ($p < 0.05$) by Fisher's LSD test.

Material	Parameter	Days					
		0	**15**	**30**	**45**	**90**	**180**
O[9]	C_o[1]	37.1104 d	30.9834 c	28.3564 b	28.8134 b	18.3143 a	18.233 a
	C_{ex}[2]	5.9268 e	5.1677 cd	5.0544 c	5.5431 de	4.3777 b	3.7235 a
	C_{ha}[3]	1.5422 b	1.4870 b	1.4074 b	1.4503 b	1.0824 a	1.1074 a
	C_{fa}[4]	4.3843 c	3.6806 b	3.6469 b	4.0927 c	3.2952 b	2.6141 a
	C_{ha}/C_{fa}[5]	0.3543 ab	0.4127 b	0.3957 ab	0.3628 ab	0.3337 a	0.4845 c
	HR[6]	17.166 a	16.9441 a	18.1057 ab	19.979 bc	24.2783 d	21.0197 c
	HI[7]	4.5646 a	4.8848 a	5.0095 a	5.2256 a	5.9774 b	6.2394 b
	P_{ha}[8]	25.884 ab	28.9663 bc	28.1588 bc	26.1134 ab	24.8401 a	31.1808 c
Pr[10]	C_o[1]	28.277 b	32.2376 c	26.148 b	26.4951 b	16.9022 a	18.1363 a
	C_{ex}[2]	5.1807c	4.5118 b	4.8397 bc	4.5706 b	3.8976 a	3.7889 a
	C_{ha}[3]	1.3299 b	1.2660 b	1.4631 c	1.3414 b	1.1247 a	1.0776 a
	C_{fa}[4]	3.8512 c	3.2459 b	3.3767 b	3.2292 b	2.7730 a	2.7104 a
	C_{ha}/C_{fa}[5]	0.3557 a	0.3976 ab	0.4541 b	0.4305 ab	0.4386 ab	0.4484 b
	HR[6]	18.6244 bc	14.6179 a	18.7776 bc	17.5462 b	23.549 d	20.9829 c
	HI[7]	4.8151 ab	4.0840 a	5.6143 cd	5.1399 bc	6.7126 e	6.0321 d
	P_{ha}[8]	25.9013 a	28.2395 ab	30.6181 b	29.6692 ab	29.7565 ab	29.8128 ab
R[11]	C_o[1]	27.7957 b	26.2141 b	27.0071 b	26.7846 b	16.1666 a	17.1629 a
	C_{ex}[2]	5.1726 e	4.3881 cd	4.1044 bc	4.6348 d	3.7860 ab	3.6823 a
	C_{ha}[3]	1.1793 ab	1.3248 c	1.2306 bc	1.1877 b	1.0548 a	1.0619 a
	C_{fa}[4]	3.9935 d	3.0633 b	2.8738 ab	3.4471 c	2.7313 a	2.6197 a
	C_{ha}/C_{fa}[5]	0.3027 a	0.4489 c	0.4364 c	0.3587 ab	0.3936 bc	0.4500 c
	HR[6]	19.2313 bc	16.8467 ab	15.4698 a	18.1808 b	24.2525 d	21.8227 c
	HI[7]	4.4759 a	5.0763 a	4.6385 a	4.7333 a	6.8105 b	6.2436 b
	P_{ha}[8]	22.7716 a	30.4416 c	30.0848 c	25.9205 ab	28.0277 bc	29.8076 c
A[12]	C_o[1]	36.1076 d	30.3759 c	27.8042 b	27.9609 b	20.13 a	20.1996 a
	C_{ex}[2]	4.2938 b	4.4032 b	4.3503 b	4.5361 b	3.587 a	3.6815 a
	C_{ha}[3]	1.0819 ab	1.2406 c	1.4156 d	1.2031 bc	1.1336 abc	1.0715 a
	C_{fa}[4]	3.2117 c	3.1625 c	2.9346 bc	3.3329 c	2.4533 a	2.6095 ab
	C_{ha}/C_{fa}[5]	0.3407 a	0.4136 ab	0.4965 b	0.4226 ab	0.4887 b	0.4377 ab
	HR[6]	11.9032 a	14.576 b	16.9039 c	16.7267 bc	18.3982 c	18.6659 c
	HI[7]	3.0484 a	4.1550 b	5.554 c	4.4864 b	5.8251 c	5.5647 c
	P_{ha}[8]	25.4526 a	28.8883 ab	32.8276 b	27.6121 a	32.19 b	29.9079 ab

[1] Oxidizable carbon (g/100 g of dry matter)
[2] Alkaline-extractable carbon (g/100 g of dry matter, ash-free basis)
[3] Humic acid-like carbon (g/100 g of dry matter, ash-free basis)
[4] Fulvic acid-like carbon (g/100 g of dry matter, ash-free basis)
[5] Cha/Cfa ratio
[6] Humification ratio (Cex/Co X 100)
[7] Humification index (Cha/Co X 100)
[8] Percent humic acid (Cha/Cex X 100)
[9] Olive-oil mill waste-melon waste compost
[10] Pruning waste-melon waste compost
[11] Rice straw-melon waste compost
[12] Almond shell-melon waste compost
* Each raw material mixture was inoculated with all different inoculants and a control windrow was besides achieved in all cases. Data showed in this table have been analysed irrespective of microbial inoculants. All analyses were conducted in triplicate and data subjected to a multifactorial analysis of variance (ANOVA)

Table 3 Effect of compost raw materials on carbon fractions and maturity and humification indices of plant waste compost. Data were analysed both as a whole (i) and on the end product (ii). Means with the same letter are not significantly different ($p < 0.05$) by Fisher's LSD test.

Parameter	Analyses irrespective of time (i)				End product (ii)				Increase factor (180/0 days)			
	O[9]	Pr[10]	R[11]	A[12]	O[9]	Pr[10]	R[11]	A[12]	O[9]	Pr[10]	R[11]	A[12]
C_o[1]	26.9358 b	24.6047 a	23.7240 a	27.0216 b	18.314 a	18.0342 a	17.264 a	20.1282 b	-	-	-	-
C_{ex}[2]	4.4817 b	4.2666 a	4.1301 a	4.9887 c	3.7364 a	3.7910 a	3.6527 a	3.6656 a	-	-	-	-
C_{ha}[3]	1.2771 b	1.1971 a	1.1527 a	1.3507 c	1.1147 a	1.0826 a	1.0488 a	1.0801 a	-	-	-	-
C_{fa}[4]	3.6377 c	3.2044 b	3.1138 a	2.9329 a	2.6198 a	2.7075 a	2.6031 a	2.5851 a	-	-	-	-
C_{ha}/C_{fa}[5]	0.3899 a	0.4216 ab	0.3927 a	0.4390 b	0.4877 a	0.4505 a	0.4490 a	0.4455 a	1.3674	1.26	1.4866	1.2847
HR[6]	19.5922 b	19.1694 b	19.0316 b	16.3016 a	21.1633 ab	21.1874 ab	21.3839 b	18.8775 a	1.2244	1.1266	1.1347	1.56
HI[7]	5.3197 b	5.4768 b	5.1935 b	4.8261 a	6.2707 a	6.1074 a	6.1876 a	5.6530 a	1.3669	1.2527	1.3949	1.8254
P_{ha}[8]	27.5236 a	29.0899 bc	27.5742 ab	29.6579 c	31.3448 a	29.9304 a	29.7491 a	30.2188 a	1.2046	1.151	1.3089	1.175

[1] Oxidizable carbon (g/100 g of dry matter)
[2] Alkaline-extractable carbon (g/100 g of dry matter, ash-free basis)
[3] Humic acid-like carbon (g/100 g of dry matter, ash-free basis)
[4] Fulvic acid-like carbon (g/100 g of dry matter, ash-free basis)
[5] Cha/Cfa ratio
[6] Humification ratio (Cex/Co X 100)
[7] Humification index (Cha/Co X 100)
[8] Percent humic acid (Cha/Cex X 100)
[9] Olive-oil mill waste-melon waste compost
[10] Pruning waste-melon waste compost
[11] Rice straw-melon waste compost
[12] Almond shell-melon waste compost

and a high HI value. These values did, however, not differ significantly from those determined in composts made from melon waste with pruning waste or rice straw. The lowest C_{ha}/C_{fa} ratio and percent humic acid values were detected in the melon and olive-oil mill waste compost but they were only significantly different from those detected in compost made from melon waste with either pruning waste (P_{ha}) or almond shells (P_{ha}, C_{ha}/C_{fa}). Compost made with almond shells showed the significantly lowest HR and HI values and had the significantly highest C_{ha}/C_{fa} ratio. These results agree with the observation made by García *et al.* (1991), Veeken *et al*. (2000), and Wu *et al.* (2000), that stability and degree of humification of the compost raw material affect its transformation during composting. However, though several differences are detected from a global point of view, there were no statistical differences in the different final products (**Table 3**). Significant highest and lowest values of C_o and HR respectively, were detected just in the case of almond shell waste (**Table 3**).

The different values for C_{ha}, C_{fa}, HR, HI and P_{ha} obtained in these experiments when compared to those described by other authors (Iglesias-Jiménez and Pérez-García 1992; Bernal *et al.* 1997; Vargas-García *et al.* 2006) may be due to the different nature of raw material. However, the increase factor (**Table 3**) for C_{ha}/C_{fa}, HI and P_{ha} (quotient between final and initial values) was in agreement in most cases with those considered optimal for maturity (1.7 for C_{ha}/C_{fa}, 1.34 for HI and 1.27 for P_{ha} according to Iglesias-Jiménez and Pérez-García (1992). Thus, the values obtained for this increase factor for C_{ha}/C_{fa} were 1.36 (O-piles), 1.26 (Pr-piles), 1.48 (R-piles) and 1.28 (A-piles). Values of 1.36 (O-piles), 1.25 (Pr-piles), 1.39 (R-piles) and 1.82 (A-piles) were observed for the HI ratio, while values of 1.20 (O-piles), 1.15 (Pr-piles), 1.30 (R-piles) and 1.17 (A-piles) were observed for the P_{ha}.

On the other hand, microbial inoculation of the composting piles had no significant effect on the carbon fractions and humification indices observed during the composting process (data not shown). These results are contradictory when they are compared to those described by others authors. In this sense, Vargas-García *et al.* (2006) corroborated that different microbial inoculants significantly affected the composting process evolution. However, this affirmation has been just occasionally supported and contradictory results have been described by different authors (Golueke *et al*. 1954; Elorrieta *et al.* 2002). This confusion is not surprising bearing in mind the different chemical and biological events that occurs during a composting process. Therefore, raw materials, composting conditions (size of piles, aeration rate, temperature evolution) and nature of microbial inoculants must be carefully studied to improve a composting process and to obtain a better final compost.

Effect of humic extract on microbial growth

Microbial growth was affected by the rate at which the humates had been added to the culture media, the microbial group and the incubation time (**Table 4**). Multifactorial analysis of variance showed the significant influence of all three factors. In addition, significant interactions between microbial group and rate of humic extract addition, microbial group and incubation time and rate of humic extract addition and incubation time were observed (**Table 4, Fig. 1**).

The microorganisms assayed could be divided into three homogeneous groups (fungi, actinomycetes and bacteria), The LSD test (**Table 5**) indicated significantly greater microbial count values for the bacterial group. The fungi

Table 4 Effect of microbial group, humic extract addition rate, incubation time and replication on microbial growth

Factor	Sums of squares	Degrees of freedom	p[a]
A: Microbial group	934.0140 *	2	0.0000
B: Humic extract addition rate	29.4227 *	3	0.0000
C: Incubation time	7.6594 *	4	0.0390
D: Replication	0.3321 *	2	0.8019
A x B	51.9224 *	6	0.0000
A x C	15.5996 *	8	0.0089
B x C	50.3125 *	12	0.0000

[a] Significant differences are observed at 95% confidence level ($p < 0.05$)

Table 5 Effect of microbial group, humic extract addition rate and incubation time on microbial growth. Means with the same letter are not significantly different ($p < 0.05$) according to Fisher's protected LSD test.

Factor	Mean
Microbial group	
Fungi	4.07 A
Actinomycetes	6.75 B
Bacteria	7.27 C
Humic extract addition rate (%)	
0	6.04 B
1	6.30 C
5	6.15 BC
10	5.62 A
Incubation time (hours)	
0	5.79 A
24	6.03 AB
72	6.18 B
120	6.10 B
168	6.04 B

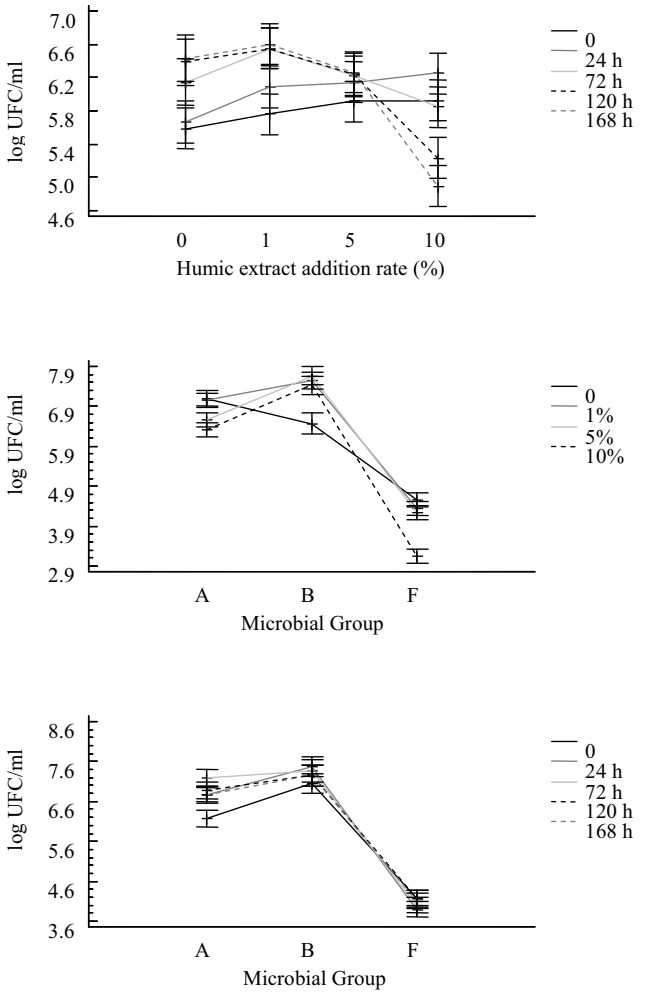

Fig. 1 Effect of the interactions between: Humic extract addition rate and Incubation time (top), Humic extract addition rate and Microbial group (A: *Actinomycetes*, B: Bacteria, F: Fungi; center) and Incubation time and Microbial group on microbial counts (bottom). The ANOVA test decomposes the variability of microbial counts into contributions due to the interactions between various factors. Since P-values are less than 0.05, these interactions have a statistically significant effect on microbial counts at the 95.0% confidence level.

showed the significantly lowest propagule count values. The aspect in relating to the behavior of the different microbial groups was beside corroborated by graphics showing interactions between the analysed factors (**Fig. 1**, centre and bottom).

In general, increasing concentrations of humic extract in the growth media resulted in a progressive decrease relating to microbial counts (**Table 5**). Humates added at 1% had a significantly positive effect on microbial growth and showed the highest microbial counts. However, results obtained when the extract was added at 5% were not significantly different from those obtained at 1% (**Table 5**). We need to emphasize that no significant differences were observed when the bacterial group was treated with the extract at 1, 5 and 10% (**Fig. 1**, center). This evidence is fully consistent with previous observations in which humic acids from either leonardite or green compost were added on *in vitro* axenic cultures of soil autotrophic nitrifying bacteria (Vallini *et al.* 1997). Therefore, the different results observed in these assays when compared to those described by other authors (Vallini *et al.* 1997) may be due to the different type of microorganisms.

All microbial groups were detected at the different sampling times (**Fig. 1**, bottom). Microbial counts were achieved even when the humic extract was added at 10% (**Fig. 1**, center). However, in this case, microbial counts significantly decreased after 72 h (**Fig. 1**, top).

With regard to the incubation time, microbial counts in general remained constant from 24 to 168 h after the beginning of the assay (**Table 5, Fig. 1**, bottom). In this sense, this behavior was consistent with previous observations described by Vallini *et al.* (1997).

Similar work carried out *in vivo* by Valdrighi *et al.* (1995) showed that the addition of humates to the soil at different rates influenced soil microbial populations depending on the origin of the amendment used. Unlike in our experiment, Valdrighi *et al.* (1995) observed that increasing concentrations of different humic substances generally resulted in a progressive stimulation of heterotrophic aerobic bacteria, soil cellulolytic microorganisms and autotrophic ammonia and nitrite oxidizers.

Several authors suggest that humates are not used as a source of carbon and nutrients by different microbial groups (Visser 1985b; Samson and Visser 1989) and that the effect of these compounds may depend on their surfactant characteristics, which may increase cell membrane permeability, allowing a better absorption of both, mineral nutrients and oxidizable energy-yielding substrates. This hypothesis agrees with the latest findings by Valdrighi *et al.* (1996), who demonstrated that humates behave similarly to synthetic surfactants such as Tween 80 in improving microbial uptake of mineral nutrients from the soil solution. The stimulation of microbial growth by humic acids may also be related to other direct influences on either physiological or biochemical levels (Müller-Wegener 1988). Differences in the characteristics of the humic extract used by us compared to those used by other authors might explain the different effects on microbial growth observed.

CONCLUSIONS

From the results obtained in our work, it can be concluded that changes and behavior observed in organic matter during stabilization of different inoculated plant waste are similar. In this sense, compost parameters at the end of the process were in general statistically not different, irrespective of raw materials and microbial inoculants used. Only in the case of almond shell piles, several differences were observed in the mature compost in relating to HI and HR values. These differences may be possibly due to the almond shell complex nature.

On the other hand, from the results obtained in the second part of this work and by other authors, depending on the application rate and organic matter sources, humic acids from organic matrices that have undergone (partial or total) stabilization through a composting process behave as stimulatory or inhibitory substances depending on microorganism nature. Our *in vitro* experiment showed that lignocellulosic waste based compost can be used for the extraction of humic substances, which could offer interesting new perspectives for the compost market. Differences in the characteristics of the humic extract used by us compared to those used by other authors (composition and concentration just as maturation degree of extracted material and type of microorganisms) might explain the different effects on microbial growth observed.

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