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# Elucidation of functional chemical groups responsible of compost phytotoxicity using solid-state $^{13}\text{C}$ NMR spectroscopy under different initial C/N ratios

Khalid Azim<sup>1,2</sup>  · Youssef Faissal<sup>3</sup> · Brahim Soudi<sup>2</sup> · Claude Perissol<sup>4</sup> · Sevastianos Roussos<sup>4</sup> · Imane Thami Alami<sup>5</sup>

## Abstract

More than 1 million tons of fresh organic wastes is produced in the Souss-Massa region in Morocco. Tomato organic residues represent more than 25% of the total organic wastes and are deposited in uncontrolled landfills. Thus, composting can represent a valuable and pertinent solution to this environmental problem. The objectives of this experiment are to identify the potential functional groups responsible for compost phytotoxicity and to determine the optimum initial carbon to nitrogen ratio (C/N) for maximum recovery of tomato residues. The experiment consisted of the variation of the initial C/N ratios (25, 30, 35, and 40) using mixtures of different raw materials (tomato residues, melon residues, olive mill pomace, and sheep manure). Physicochemical parameters (pH, electrical conductivity, C/N ratio, and humic acid/fulvic acid ratio) were determined and spectroscopic analyses (UV-vis and NMR- $^{13}\text{C}$ ) were performed during the composting process along with quality parameters (germination and phytotoxicity tests) at the end. The results showed that the compost with the initial C/N ratio of 35 is the most humified with the least phytotoxic effect. The germination and phytotoxicity tests were negatively correlated with the methoxyl/N-alkyl-C ratio and O-alkyl-C. These two functional groups are probably the origin of phytotoxicity expression in compost quality tests. Thus, a simple and precise quality test could be performed to evaluate directly the phytotoxicity and maturity of compost.

**Keywords** NMR- $^{13}\text{C}$  · Tomato residue · Compost · Phytotoxicity · C/N ratio

Khalid Azim  
azim.khalid@yahoo.fr

Research Unit of Integrated Crop Production, Regional Center of Agronomy Research, INRA-CRRA, B.P. 124, Inzegane, CP: 80350 Agadir, Morocco

Department of Environment and Natural Resources, IAV Hassan II, B.P. 6202. Madinat Al Irfane, Rabat, Morocco

Department of Biology, Faculty of Science, University of Mohamed I, Bd Mohammed VI. B.P. 717, CP: 60000 Oujda, Morocco

Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE) Aix-Marseille Université, CNRS, IRD, Avignon Université, Campus de l'Etoile, Centre Saint-Jérôme, Avenue Escadrille Normandie-Niemen, 13397 Marseille cedex 20, France

Laboratory of Microbiology, Regional Center of Agronomy Research, INRA-CRRA, Avenue Mohamed Belarbi Alaoui B.P. 6356, CPP: 10101 Rabat, Morocco

## Introduction

Agriculture is by far the largest sector of economic and social activity in Morocco, generating about 15% of the national gross domestic product (GDP), and is one of the main sources of income for nearly 40.5% of the Moroccan population (El Bilali et al. 2012). For such relative economic weight, the nature and quantity of the residues that it generates, the Moroccan agricultural sector is one of the largest organic waste producers. In the Souss-Massa region, the production of crop by-products has reached 1,307,465 tons/year (Azim et al. 2017). The constant increase in production volumes is causing a significant problem today in the destination of organic waste generated, which is almost adapted to the composting process. This biological process allows the conversion and valorization of organic matter into a stabilized and hygienic product, which is rich in humic compounds and thus can be used as an organic amendment. The problem associated with the application of immature compost is the presence of

phytotoxic compounds during the composting process (Gopinathan and Thirumurthy 2012). Phytotoxicity refers to the “intoxication of living plants by substances present in the growth medium, when these substances are taken up and accumulated in plant tissue” (Araujo and Monteiro 2005). Previous research has demonstrated that the application of immature compost onto the soil has a negative effect on seed germination, as well as plant growth (especially vegetables) and development. Mathur et al. (1993) stated that if unstable or immature compost is applied, it can induce anaerobic conditions, in the same way as the microorganisms utilize O<sub>2</sub> in the soil pores to break down the material. Immature compost can also contain phytotoxic compounds such as phenolic substances; introduce heavy metals, ethylene, and ammonia; and cause excess accumulation of salts and organic acids that could delay seed germination and plant growth (Selim et al. 2012; Tiquia and Tam 1998; Manios et al. 1989). Compost maturity can be assessed using physical, chemical, or biological technique (Asgharipour and Sirousmehr, 2012). Phytotoxicity is one of the most important criteria for evaluating the suitability of compost as a soil amendment and for avoiding possible environmental risks (Brewer and Sullivan 2003). There is no universal method for measuring compost phytotoxicity (Goyal et al. 2005). The use of several indicators such as the C/N ratio, germination tests, and spectral analysis is necessary (Said-Pullicino et al. 2007). In general, the optimal C/N ratios in the composting of most materials have been reported to vary from 25 to 30 (Choi 1999). In addition, Aslam et al. (2008) have found that mineralized carbon estimated using the first-order model was significantly correlated to the probability of phytotoxicity in compost-amended soil. Leifeld et al. (2002) stated that the compost phytotoxicity was also correlated to the alkyl-CO/alkyl-C ratio (humification index). In fact, the ratio alkyl-CO/alkyl-C was correlated to a low degree of humification of the organic matter of the soil after use of green waste compost. Larre-larrouy and Thuries (2006) also have revealed that methoxyl groups (–OCH<sub>3</sub>) appeared to be a good tracer of compost phytotoxicity. The objectives of this study are to identify the potential functional group(s) responsible for compost phytotoxicity and to determine the optimum initial C/N ratio for maximum tomato residue recovery.

## Materials and methods

### Trial location of the experimental design and setup

This study was carried out in the Souss-Massa region in the Technology Transfer Center (APEFEL/ORMVA SM). Four mixtures of agricultural residues, with four basic raw materials, have been the subject of this experiment: melon residues, sheep manure, olive mill pomace, and tomato residues. The incorporation of different raw materials was done in different

C/N ratios. With several raw materials (Soudi 2005), this formula was developed to determine the initial C/N ratio:

$$C/N(\text{mixture}) = \frac{\sum_{n=1}^{\infty} \left( Q_n \left[ C_n \left( 100 - M_n \right) \right] \right)}{\sum_{n=1}^{\infty} \left( Q_n \left[ N_n \left( 100 - M_n \right) \right] \right)}$$

with:

- C/N as the ratio of the mixture of materials to be composted
- Q<sub>n</sub> as the percentage of material in the total mass (in % weight)
- M<sub>n</sub> as the humidity of the material
- C<sub>n</sub> as the carbon content of matter (percentage of dry weight)
- N<sub>n</sub> as the nitrogen content of matter (percentage of dry weight)

### Characterization of the mixtures

The characteristics of the different raw materials are summarized in Table 1.

These raw materials were mixed in different proportions to obtain different C/N ratios (Table 2). Standard pile composting with open windrows of approximately 27.5 m<sup>3</sup> (3 m widths and 1.6 m height) was conducted with dynamic aeration using a backhoe loader. Aeration is performed every 10–15 days when the temperature dropped under 45 °C. The pile is restructured again with the same shape as mentioned above to maintain optimal composting conditions. Since the overall moisture content of different studied organic wastes (Table 1) was below the optimal range (50–65%), no bulking agent was added and the moisture content of the mixture was adjusted with an onward sprinkler until the optimal value was reached. The moisture content was monitored with the same method and adjusted after every aeration if needed. Composting is done on a waterproof surface with 3% slope to collect compost leachates to be filtered and reinjected to the system of windrows sprinkling.

### Evolution of the composting process

The composting process took place over a period of 169 days. During this period, triplicate samples were collected in 70 cm depth at the shaded pile of the compost at the 1st, 11th, 21st, 53rd, 73rd, 109th, and 127th day after windrowing (DAW). The samples were weighed for moisture content measurement after 48 h oven-drying at 105 °C (Petard 1993) prior to laboratory analysis. Temperature and moisture content inside the pile were measured daily using multi-parametric probes

**Table 1** Physical and chemical characteristics of the raw materials

Organic wastes	Moisture content (%) when mixing	pH	Electrical conductivity (dS/m)	Bulk density (kg/m <sup>3</sup> )	Total organic matter (%)	C/N ratio	Total nitrogen (%)	Phosphorus content (%)
Melon residues	17.00 ± 1125	6.14 ± 0.410	6.13 ± 0.190	164.00 ± 2.000	60.92 ± 2.851	20.72 ± 4.652	1.47 ± 0.179	3.11 ± 0.200
Olive mill pomace	36.00 ± 3971	9.40 ± 0.276	5.86 ± 0.078	832.00 ± 35.000	69.25 ± 1414	39.80 ± 9595	0.87 ± 0.191	2.50 ± 0.438
Tomato residues	25.75 ± 2671	7.69 ± 1047	2.98 ± 0.511	116.00 ± 18.000	78.37 ± 3300	50.24 ± 11.651	0.78 ± 0.184	2.00 ± 0.099
Sheep manure	32.00 ± 3231	8.35 ± 0.129	3.36 ± 0.204	368.00 ± 8.000	76.00 ± 3143	19.00 ± 5563	2.00 ± 0.215	1.06 ± 0.105

(Green Mountain Technologies, USA) at 1 m depth in three points of each treatment pile.

### Physicochemical analysis

Oven-dried samples were subjected to pH analysis in 1:10 soil/water (*w/v*) suspensions using a glass electrode pH meter (BioBlock pH-meter Microprocessor 99621) at room temperature (Rhoades 1982). Electrical conductivity (EC) ( $\mu\text{S}/\text{cm}$ ) was measured using an electrical conductivity meter (WTW Inolab-Cond Level-2P, Germany) in 1:5 soil/water (*w/w*) solutions (Rhoades 1982). Total organic matter (TOM) content was determined by loss of ignition of a dry sample at 550 °C for 24 h (Lee 1995). Total nitrogen content (NTK) analysis was performed using standard Kjeldahl procedure (Gerhardt: Kjeldatherm-Vapodest 20, Germany) as described by Jackson (1967). The C/N ratio was calculated by dividing total carbon (issued from TOM) by total nitrogen. Humic and fulvic acid (HA, FA) content were analyzed following the method described by Calderoni and Schnitzer (1984). The HA and FA are extracted by stirring (2500 rpm) for 2 h 10 g of dried compost added to 100 mL of 0.1 M NaOH in 250-mL Erlenmeyer flasks. This alkaline-soluble fraction (HA + FA) is recovered by centrifugation at 2500 rpm for 25 min. The solution is then acidified to pH 1 by addition of 6 M

hydrochloric acid (HCl). After one night at +4 °C, the acid-insoluble fraction (FA) of fulvic acids is separated from the insoluble fraction of humic acids (HA) by centrifugation at 10,000 rpm for 10 min. The two fractions are weighed after being dried in an oven at 105 °C.

### Spectroscopic analysis

#### UV-vis spectroscopy

UV-vis spectroscopy was performed according to Zbytniewski and Buszewski (2005) using an Optizen 3220UV double-beam UV-vis spectrophotometer. It consists of the addition of 50 mL of 0.5 M NaOH to 1 g of dried compost followed by 2 h of agitation. All solutions were first centrifuged (25 min, 3000 rpm) and the supernatant filtered using a 0.45- $\mu\text{m}$  Millipore-syringe filter to remove insoluble particulate materials. The optical length of the quartz cell was 10 mm, and the absorption spectra recorded were from 200 to 800 nm. Analysis by UV-visible spectroscopy of the studied mixtures allowed having the specific spectra (280 nm [2], 472 nm [4], and 664 nm [6]). The calculation of ratios Q2/4, Q2/6, and Q4/6 aims to evaluate the degree of humification of the studied mixtures.

**Table 2** Proportion (% *w/w* of fresh matter) and quantities (kg) of raw material used to constitute the treatments

Treatments (initial C/N ratio)		Melon residues	Sheep manure	Olive mill pomace	Tomato residues
C/N25	%	10	55	5	30
	Quantity (kg)	558.9	2683.8	289.4	1736.6
C/N30	%	10	30	20	40
	Quantity (kg)	547.8	1413.3	1157.6	2351.1
C/N35	%	5	20	20	55
	Quantity (kg)	285.8	943.4	1157.4	3182.3
C/N40	%	5	10	15	70
	Quantity (kg)	282.5	474.9	868.4	4055.5

## NMR-<sup>13</sup>C analysis

NMR-<sup>13</sup>C analysis was performed for each sample sieved with a 2-mm sieve and crushed to powder in a mortar. Spectra were obtained using a spectrophotometer (Bruker DSX 400 MHz) operating at 100.7 MHz. Samples (400 mg) were spun at 10 kHz at the magic angle, and the deconvolution of spectra was made using Dmfit 2011 Software to determine each chemical shift region intensity (Massiot et al. 2001). The method used previously to characterize the processes of mineralization and humification of organic matter provides valuable information about their state of evolution. It is a powerful technique for more in-depth investigation of the state of organic matter (Kögel-Knabner 2002). The characterization of the carbon structure of the studied mixtures was conducted in order to increase knowledge of the mechanisms involved in the composting process.

## Bioassay tests

**Cress test** It aims at assessing the maturity of the compost through its effect on the cress seeds' germination. The test consists of sprouting cress seeds on a mixture of tested compost (1/3; v/v) with peat moss (2/3; v/v) and counting the number of seedlings emerged at 3 and 7 days. Each treatment was represented by four replicates in addition to the control (peat moss only). This test is performed in a growth chamber under controlled conditions.

**Phytotoxicity test** A modified phytotoxicity test according to Zucconi et al. (1981) was used. This assay consisted of the germination of seeds of *Lepidium sativum* in petri dishes, using the water extract of compost (1/1; v/v in

distilled water considered as 100% water extract), and the observation of the effect of phytotoxicity on the seeds' germination. For each compost, there were three concentrations (50, 75, and 100%) with four replicates in addition to the control (distilled water only). The petri dishes were put in the germination chamber for 7 days at a temperature of 25 °C and a relative humidity between 85 and 90%. Counting germinated seeds per dish after 3 and 7 days was performed.

## Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 21. The multiple comparisons of means were confirmed by Tukey's test at  $P < 0.05$ .

## Results and discussions

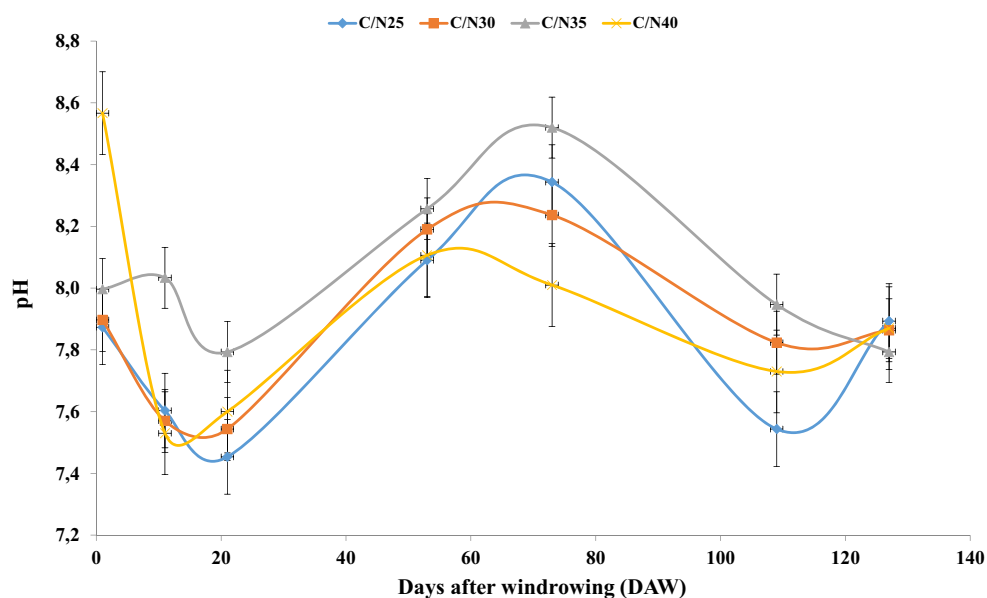
### Chemical parameters

The following figures show the main chemical indicators studied, pH, EC, C/N ratio, and HA/FA ratio.

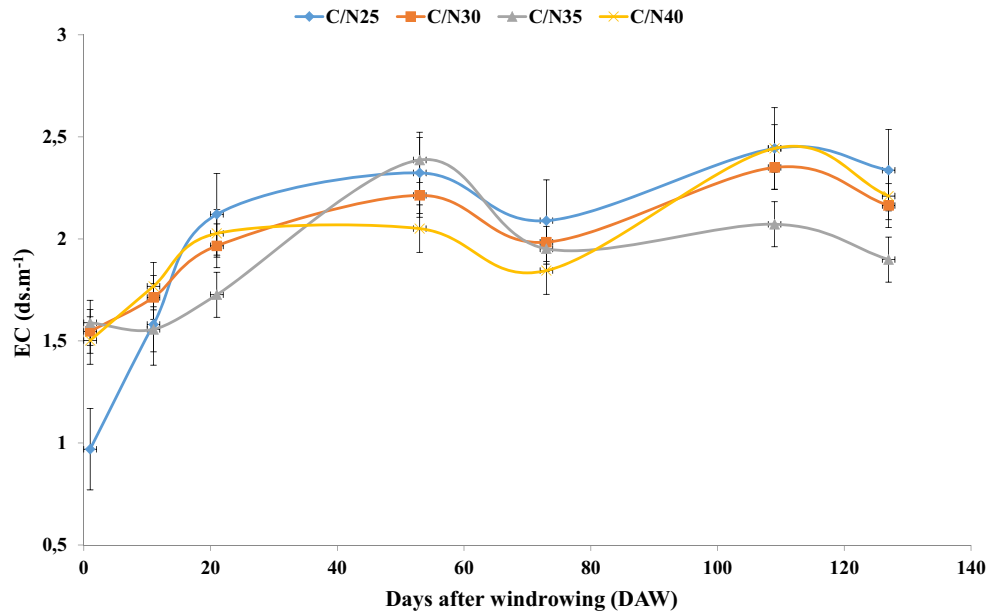
### pH

The pH evolution during composting shows the same trend (Fig. 1). During the first composting phase up to 21 days (mesophilic phase), the pH decreases in all treatments. This acidogenic phase occurs following an intense production of carbon dioxide and organic acids due to the volatilization of ammonia (Rynk 1992). During the second phase

Fig. 1 Evolution of pH during composting



**Fig. 2** Evolution of the electrical conductivity during composting

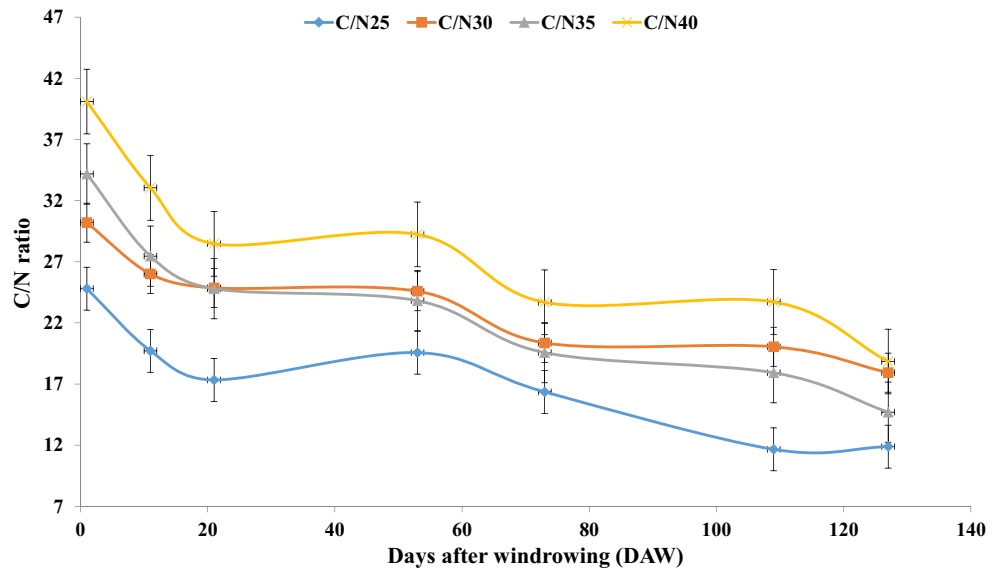


(21st to 73rd day), the different treatments show a gradual increase attributed to the development of thermophilic flora responsible for protein and organic nitrogen hydrolysis, which generates an increase of ammonia concentration causing the rise of pH (alkalization stage) (Jordan and Mullen 2007). During the third phase (70th to 108th day), the pH decreases gradually because of the decrease of the *C/N* ratio which slows down after the loss of ammonia through volatilization since microorganisms require nitrogen to achieve biosynthesis of humic materials. It was noted that the pH value tends towards neutrality regardless of the treatment, which is in conformity with the work of Soclo et al. (1999).

### Electrical conductivity

Evolution in the electric conductivity of different treatments follows the same trend (Fig. 2), the same as pH. Treatment *C/N30*, *C/N35*, and *C/N40* ratios start from a value between 1.50 and 1.59 dS m<sup>-1</sup>. This relatively high value can be explained by the high salinity of olive mill pomace. Treatment *C/N25* that contains only 5% (by weight) of olive mill pomace starts from 0.97 dS m<sup>-1</sup>. Between the 20th and 53rd day, there has been a significant increase in EC that can be attributed to the optimal temperature and moisture content that are results of microbial action in decomposition and mineralization causing an increase in salt concentration. From the 53rd day, there is a

**Fig. 3** Evolution of the *C/N* ratio during composting





**Table 3** Mean values of the C/N ratio at the end of the experiment

Treatments	Mean	Standard deviation
C/N25	12.41 a	0.272
C/N30	16.74 c	0.349
C/N35	14.90 b	0.609
C/N40	19.10 d	0.591

Means with the same letter in the same column indicate no significant difference among treatments at  $P < 0.0001$

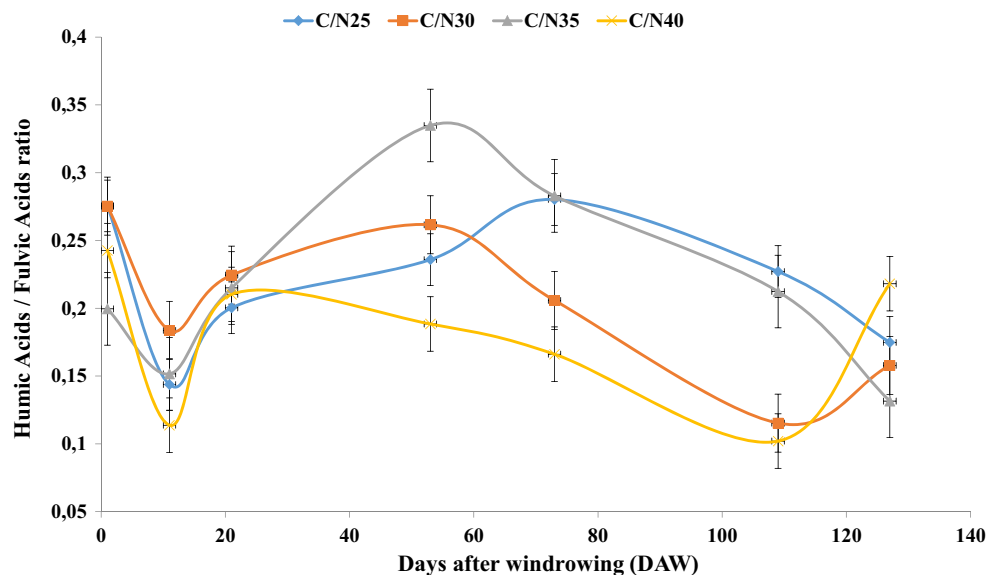
decrease in EC for treatment C/N35; this indicates the end of its thermophilic phase.

### C/N ratio

In Fig. 3, the high ratio of treatment C/N40 can be explained by the excessive presence of carbon-rich tomato stems (70% w/w). On the other hand, the low value of C/N25 is due to the richness of this pile with sheep manure (50% w/w). During the composting process, the C/N ratios decrease following the release of carbon as CO<sub>2</sub> because of mineralization (Mustin 1987).

Concerning carbon loss, treatments C/N25, C/N30, C/N35, and C/N40 have respectively lost 43.65, 29.14, 44.61, and 42.43% of their carbon as CO<sub>2</sub>. At the end of the composting process, the final C/N ratios of the different treatments are around 12 and 20, which are in the optimal range (between 12 and 28) according to N'Dayegamiye et al. (1997). Treatment C/N40 had the highest final C/N ratio while treatment C/N25 had the lowest one. Statistical analysis revealed that there was a very highly significant difference between the treatments with  $P < 0.0001$  (Table 3).

**Fig. 4** Evolution of the HA/FA ratio during composting



### HA/FA ratio

Figure 4 shows the evolution of HA/FA ratios during composting. In fact, treatment C/N25 with a high percentage (by weight) of sheep manure has higher rates of the HA/FA ratio. This can be attributed to the partial previous degradation of sheep manure during its storage prior to the trial use. The evolution of HA/FA is dominated by HA rather than FA which was steady during the experiment. During the first 10 days of composting, all HA/FA ratios have recorded a significant decrease probably due to their instability which confirms the results given by Smidt et al. (2008). For treatment C/N35, it was greatly increased between the 20th and 50th day after windrowing. It has to be noted that the higher organic matter loss (44.61%) due to carbon mineralization during the thermophile phase of treatment C/N35 affects the results of humic acid contents, and consequently increases the HA/FA ratio through humic substance formation (Wichuk and McCartney 2013).

### Bioassays

#### Cress test

Figure 5 shows the evolution of the percentage of germination of cress seeds, 3 and 7 days after sowing in the petri dishes. The germination during composting has an increasing trend for the different types of compost. After 21 days of composting, treatments C/N30 and C/N35 have a germination of 62%. The substrates from treatments C/N35 and C/N30 gave the highest germination percentages of 97 and 76% respectively after 127 days followed by C/N25 (72%). However, the germination for C/N40 did not exceed 40% at the end of the test. It has been reported that a germination percentage

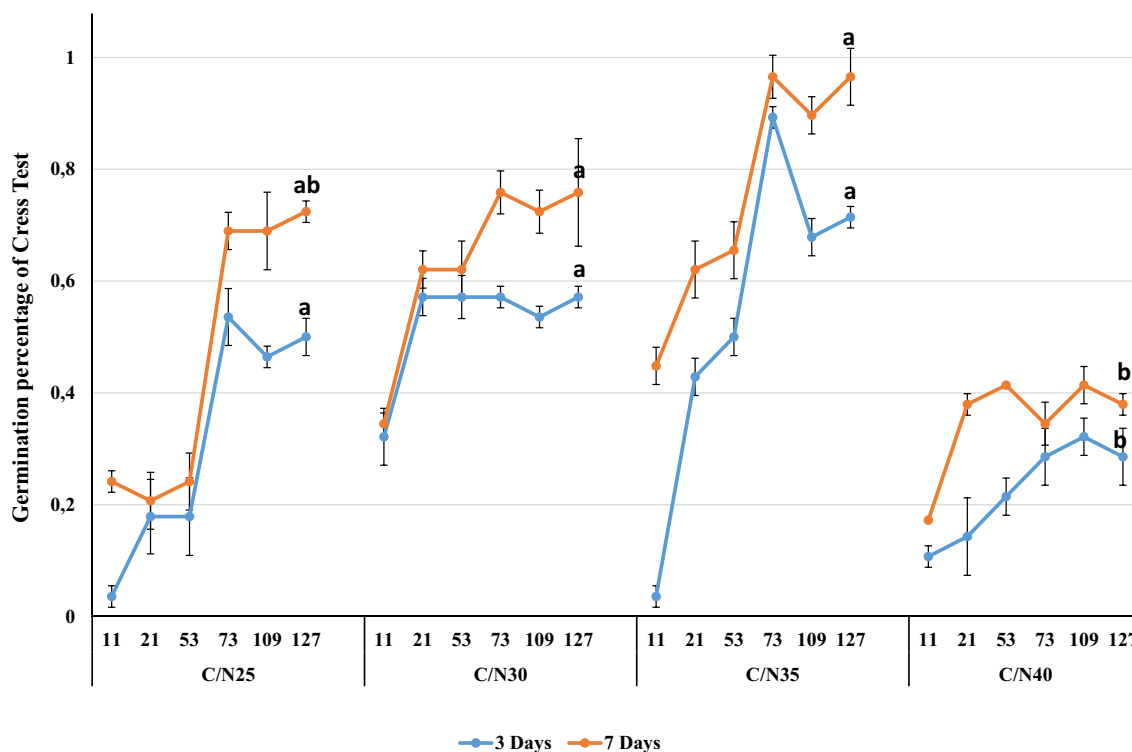


Fig. 5 Evolution of germination percentage during composting (numbers indicate days after windrowing—letters indicate Tukey means test ( $\alpha = 5\%$ ) for the same day of dishes reading (3 and 7 days))

value of more than 80% is an indication of phytotoxic-free and mature compost (Zucconi et al. 1981; Tiquia and Tam 1998).

Thus, treatments *C/N35* and *C/N30* are mature and can be amended to agricultural soil.

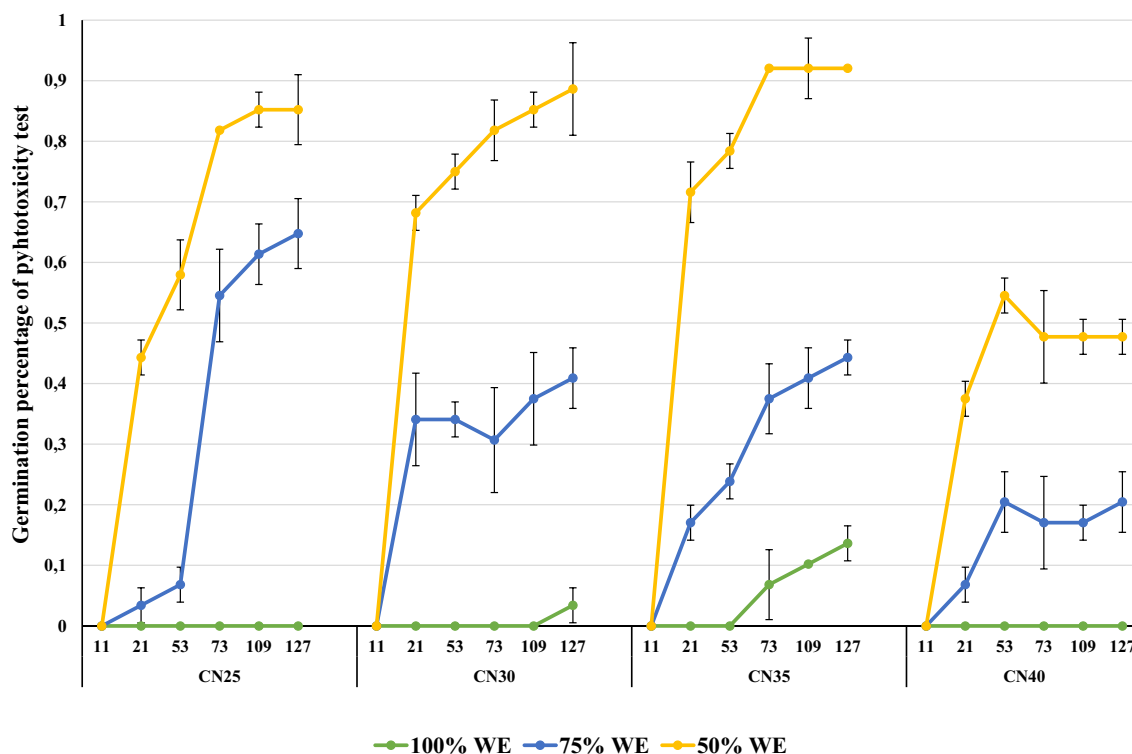
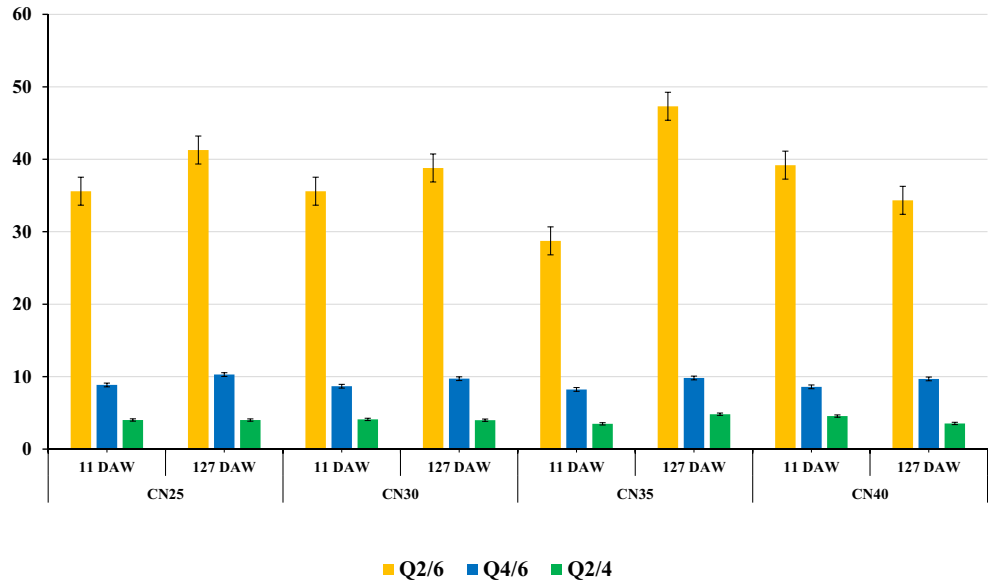


Fig. 6 Evolution of percentage of germination seeds of the phytotoxicity test after 3 days for the three different concentrations of aqueous extract (water extract = WE) (numbers indicate days after windrowing)



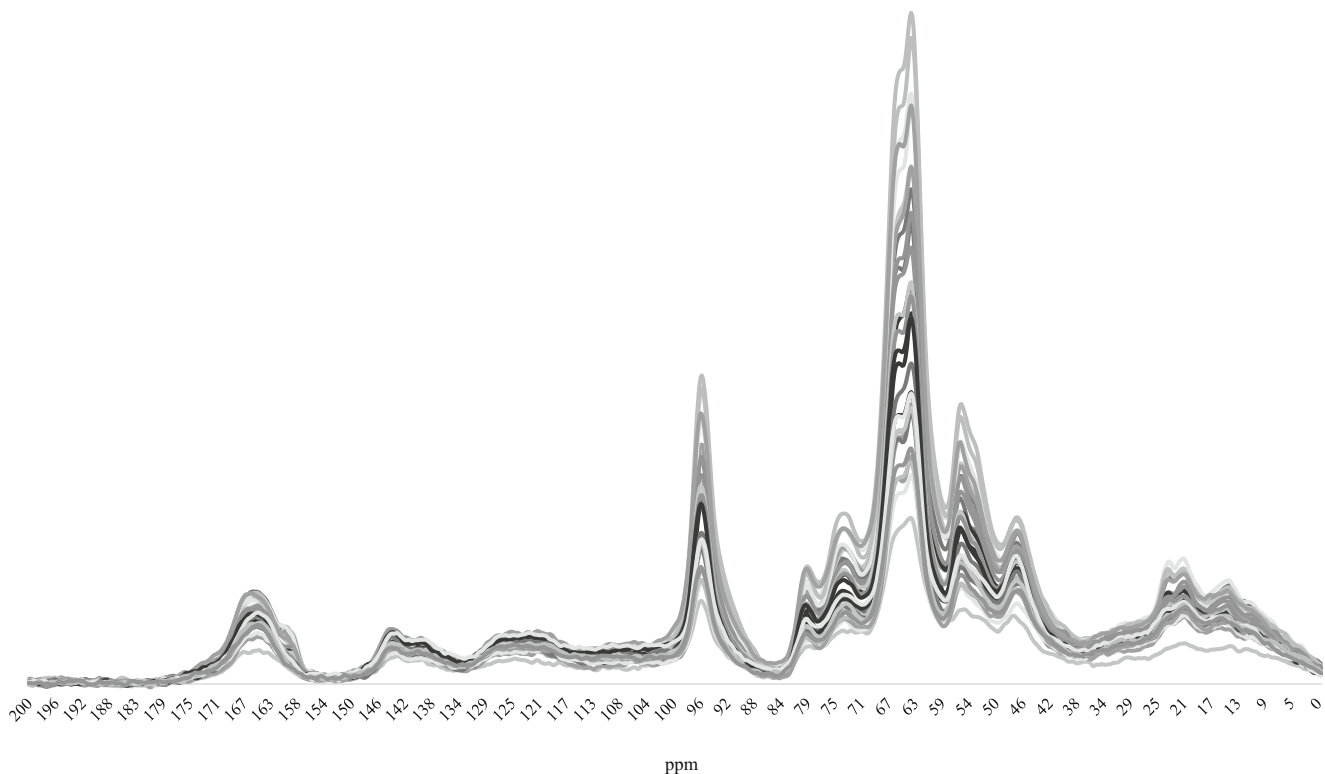
**Fig. 7** Evolution of ratios: Q2/6, Q2/4, and Q4/6 between the 11th day and the 127th day of composting



### Phytotoxicity test

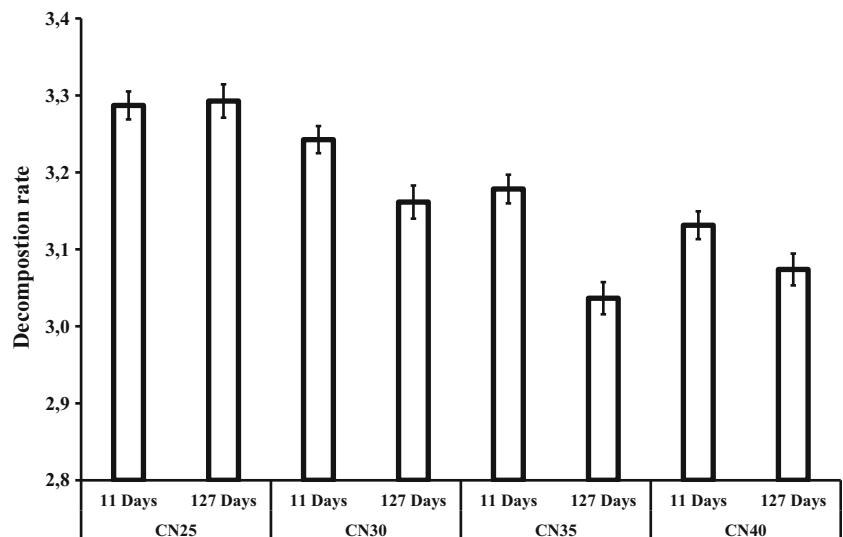
Figure 6 illustrates the change in percentage of seed germination after 3 days watered by the three concentrations (50, 75, and 100%) of aqueous extract of different treatments. Samples from each treatment were taken according to six sampling dates to assess the evolution of the phytotoxicity of the organic material.

**Concentration of 50%** The highest germination percentage was obtained with the treatment of initial *C/N35*. The germination reached 92%. The treatments with initial *C/N30* ranks second with 89%, followed by *C/N25* with 85%. The treatments with initial *C/N40* had an average germination percentage of 48%.



**Fig. 8**  $^{13}\text{C}$  NMR spectra of the different treatments sampled during the composting process (baseline corrected)

**Fig. 9** Evolution of the decomposition ratio between the 11th and the 127th day of composting



**Concentration of 75%** The highest percentages were obtained with treatment *C/N25* with 65%, followed by treatments *C/N35* (44%) and *C/N30* (41%). The treatments with initial *C/N40* could not exceed 20% germination for the second concentration.

**Concentration of 100%** The concentration of 100% is generally characterized by low percentages of germination. Treatment *C/N35* has shown the highest percentage of 13%. Germination did not exceed 3% for the treatments with initial *C/N30*, while no seed has germinated for treatments *C/N25* and *C/N40*.

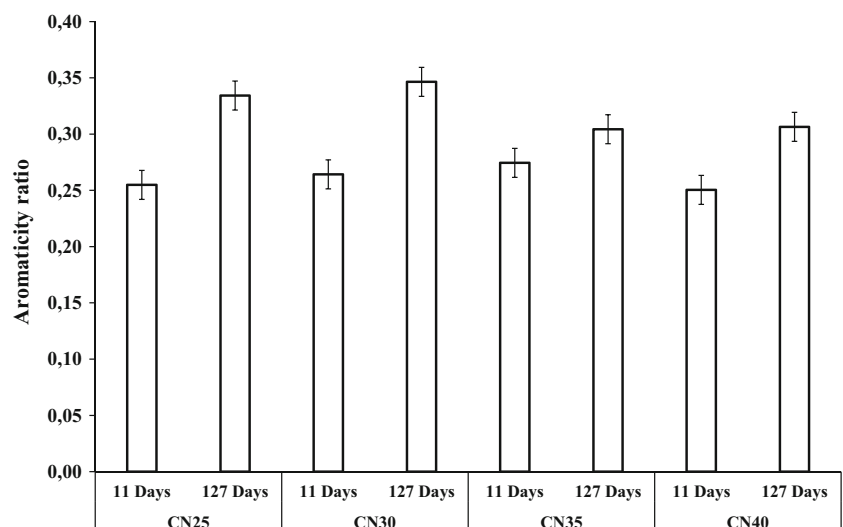
Tiquia and Tam (1998) suggested that a stable compost is not necessarily mature as it can still have an inhibitory effect or phytotoxicity on germination and plant growth. In the test of phytotoxicity, Soudi (2005) stated that germination percentage less than 60% of the control indicates a fresh compost that should be applied 90 days prior to plantation, whereas germination percentage less than 50% suggests an immature

compost. The phytotoxicity test on compost extract confirmed the results of the germination test. Indeed, three different concentrations are usually used to evaluate whether the compost has a phytotoxic or a stimulant effect. In our case, all the mixtures have a phytotoxic effect, and the treatments with initial *C/N35* have the least phytotoxic effect. Generally, the phytotoxic effect of immature compost is due, among other causes, to the ammonia emission, the synthesis of ethylene oxide during the decomposition of the compost, and also to the presence of organic acids (Manios et al. 1989; Tang 2010).

### UV-visible spectroscopy

Zbytniewski and Buszewski (2005) have reported three main spectrum regions in the alkaline compost extract: 260–280 nm corresponds to lignin and quinone, products of organic matter at the beginning of transformation; a region of 460–480 nm corresponds to organic matter at the beginning of

**Fig. 10** Evolution of the aromaticity ratio between the 11th and the 127th day of composting



**Table 4** Decomposition and aromaticity ratios and their respective variation between the initial situation (27 DAW) and the final situation (127 DAW)

Treatments	Decomposition ratio			Aromaticity		
	11 DAW	127 DAW	Variation (%)	11 DAW	127 DAW	Variation (%)
<i>C/N25</i>	3.287 a	3.293 a	+0.17	0.255 c	0.334 b	31.16
<i>C/N30</i>	3.243 a	3.161 b	-2.50	0.264 b	0.346 a	31.11
<i>C/N35</i>	3.178 b	3.037 c	-4.46	0.274 a	0.304 d	10.90
<i>C/N40</i>	3.131 c	3.074 c	-1.84	0.250 d	0.306 c	22.38

Means with the same letter in the same column indicate no significant difference among treatments at  $P < 0.0001$

humification; and a region of 600–670 nm matches highly humified organic matter with abundant aromatic functional groups. Zbytowski and Buszewski (2005) have applied the Q4/6 ratio as the humification index with values below five that reveal humic acid dominance and above five for fulvic acid dominance. Further, Ammari et al. (2012) have reported ratios of Q2/6 and Q2/4 that reflect respectively aromatic condensation and organic matter humification.

Figure 7 illustrates the calculated ratios between 11 and 127 days after windrowing as initial and final sampling. Ratio Q4/6 follows an identical trend for the four treatments. Ratio Q2/4 did not have important changes for treatments *C/N25* and *C/N30*, while treatment *C/N35* has increased. Contrary to this development, treatment *C/N40* has decreased during composting. As for the Q2/6 ratio, moderate growth was observed for treatments *C/N25* and *C/N35*, whereas treatment *C/N35* has highly increased. On the other hand, Q2/6 decreased for treatment *C/N40*. Considering the Q2/6 ratio, treatment *C/N35* has ended with values 37.79, 21.95, and 14.63% higher respectively than those of *C/N40*, *C/N30*, and *C/N25*. Thus, treatment *C/N35* ended with highly condensed aromatic molecules. Concerning the Q2/4 ratio, treatment *C/N35* has ended with values 36.02, 20.75, and 20.12% higher respectively than those of *C/N40*, *C/N30*, and *C/N25*. Consequently, treatment *C/*

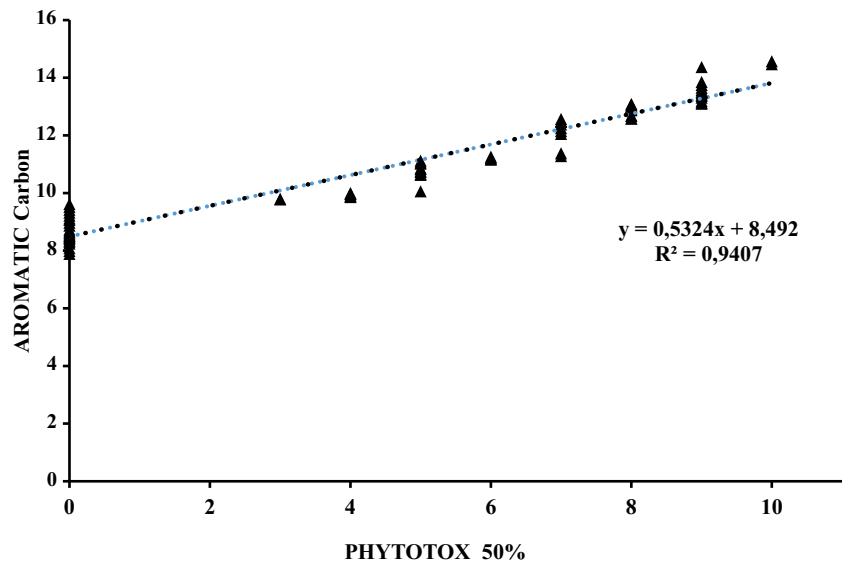
*N35* ended with more humified organic matter than the other treatments. At the end of the composting process, the Q4/6 ratio exceeded five for all treatments. This means that these compost samples are rich in fulvic acid (Larbi 2006) which is in line with the results of the HA/AF ratio not exceeding 1 as seen in Fig. 4. This may suggest that humification is not complete (Zbytowski and Buszewski 2005).

### Solid-state $^{13}\text{C}$ nuclear magnetic resonance spectroscopy

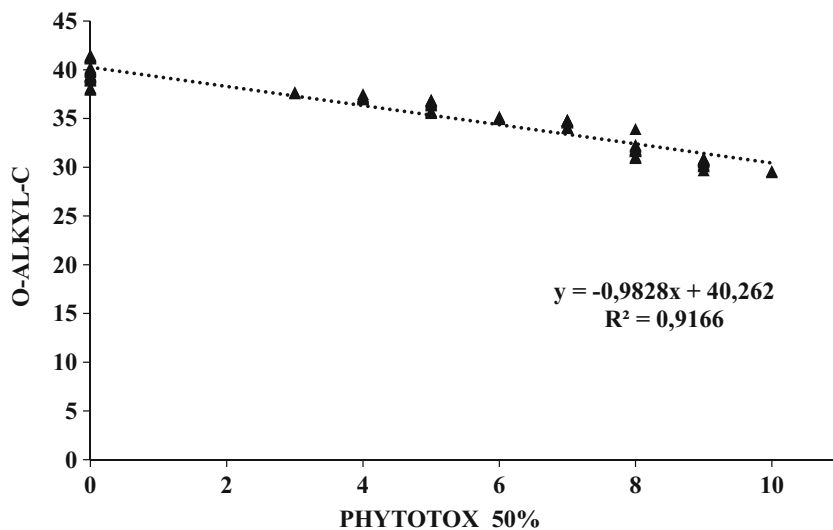
The deconvolution of the  $^{13}\text{C}$  NMR spectra of mixtures has generated peaks corresponding to different conformations of carbon (Fig. 8). The integration of these peaks was used to calculate the contribution of each chemical group. The overall chemical shift range is usually divided into the following main resonance regions:

- Alkyl-C (0–45 ppm)
- Methoxyl-C + N-alkyl-C (45–64 ppm)
- O-Alkyl-C (65–90 ppm)
- O-di-Alkyl-C (95–107 ppm)
- Aromatic-C (107–140 ppm)
- Carboxyl- and carbonyl-C (160–190 ppm)

**Fig. 11** Correlation between the aromatic carbon chemical group and the phytotoxicity test at 50% of the WE



**Fig. 12** Correlation between the O-alkyl-C chemical group and the phytotoxicity test at 50% of the WE



The relative areas obtained from the integration of the above-cited regions were used for elaboration of two indicators:

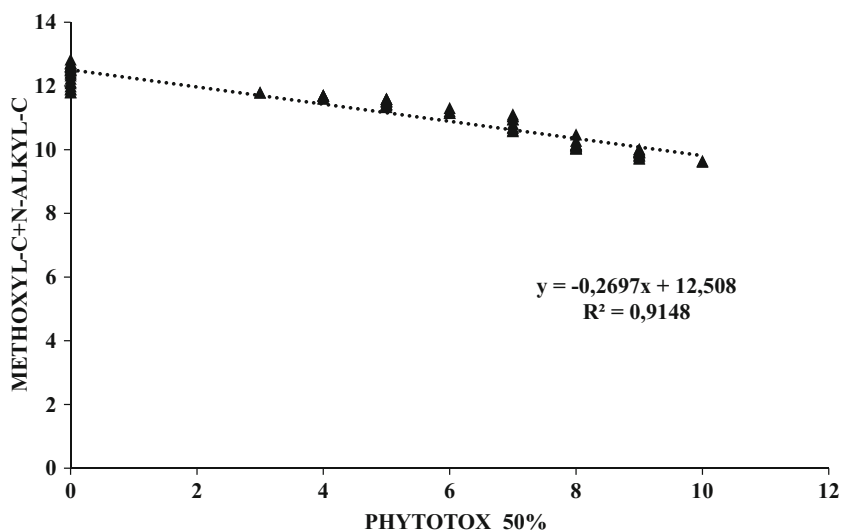
- The aromaticity ratio, an index of humification commonly used in the literature providing a global vision of the evolution of both aromatic compounds and humification. It corresponds to the ratio aromatic-C/(alkyl-C + aromatic-C) as reported by Vincelas-Akpa and Loquet (1997).
- The decomposition ratio, which gives an idea about the decay of the raw material during composting. It corresponds to the ratio O-alkyl-C/(methoxyl-C + N-alkyl-C) (Mathers et al. 2007).

Figures 9 and 10 illustrate respectively the evolution of the decomposition and the aromaticity ratios during composting.

The decomposition ratio decreased during composting for treatments C/N30, C/N35, and C/N40, respectively, by -2.50,

-4.46, and -1.84% (Table 4). This reduction was very significant for treatment C/N35. In contrast, this ratio was relatively stable for treatment C/N25 (0.17%). A high amplitude indicates a tendency for faster decomposition; however, a low amplitude refers to a material recalcitrant to decomposition (Blumfield et al. 2004; Mathers et al. 2007). Therefore, results revealed that treatment C/N35 has been significantly decomposed rather than the other treatments. In terms of the aromaticity ratio, all the treatments had the same tendency for evolution. Treatment C/N30 ended with the highest value (0.346), with 31.11% of variation. Treatment C/N35 marked the lowest value (0.304), with 10.9% of variation. The increase in the concentration of aromatic carbon (aromatic-C) and the decrease in the concentration of alkyl-C and O-alkyl-C generate a significant increase in the aromaticity in the mixtures as cited by Guénon (2010).

**Fig. 13** Correlation between the methoxyl-C + N-alkyl-C chemical group and the phytotoxicity test at 50% of the WE



## Correlation between the results of the <sup>13</sup>C NMR solid and of bioassays

The correlations between variables are performed using SPSS 21 software. The correlation measures the relationship between two or more variables. The correlation data set (Tables 5 and 6) shows three main correlations between bioassays and aromatic-C, O-alkyl-C, and methoxyl/N-alkyl-C ratio. Figures 11, 12, and 13 show the correlations found between the results of bioassays (phytotoxicity tests at 50% of water extract) and the main chemical groups revealed by the <sup>13</sup>C NMR analysis.

Concerning aromatic-C, there is an important positive correlation with the germination index after 3 days ( $r = 0.836$ ) and after 7 days ( $r = 0.781$ ). Additionally, there is a strong negative correlation between the phytotoxicity test at 50% of WE and the two groups O-alkyl-C ( $r = -0.957$ ) and methoxyl-C + N-alkyl-C ( $r = -0.956$ ). Therefore, they can act negatively on the germination given that a high concentration of these chemical groups has been associated with a reduction of the germination rate in the experiments of Leifeld et al. (2002) and Larre-larrouy and Thuries (2006). The <sup>13</sup>C NMR analysis showed a decrease in the levels of both groups during composting which is inversely proportional to the percentage of germination. These results are confirmed for treatment C/N35, in which the Pearson correlation is more highlighted (Table 6). Thus, the phytotoxicity test with 50% of water compost extract could be a reliable quality test for compost maturity. The two chemical groups (O-alkyl-C and methoxyl-C + N-Alkyl-C) could be responsible for phytotoxicity for seeds and seedlings in compost bioassays.

## Conclusion

Bioassay and UV-vis spectroscopy results showed a significant quality performance for treatment C/N35. In terms of the <sup>13</sup>C NMR, the calculated indicators revealed that the treatment with initial C/N ratio 35 (5% melon residues + 20% sheep manure + 20% olive mill pomace + 55% tomato residues [ $w/w$ ]) is the most mature. Statistical tests have revealed that the phytotoxicity test with 50% of compost water extract could be a reliable quality test for compost maturity. The two chemical groups (O-alkyl-C and methoxyl-C + N-alkyl-C) may well be responsible for the phytotoxicity of seed germination in compost bioassays. Thus, a precise quality test of compost could be performed using only the methoxyl/N-alkyl-C ratio and the O-alkyl-C ratio.

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## Appendix

**Table 5** Pearson correlation between bioassays and chemical groups

	Germination_1 days	Germination_3 days	Germination_7 days	Phytotox_100%	Phytotox_75%	Phytotox_50%	Carboxyl- and carbonyl-C	Aromatic-C	di-O-Alkyl-C	O-Alkyl-C	Methoxyl-C + N-alkyl-C
Germination_3 days	Pearson correlation 1										
Germination_7 days	Significance (P) –	0.803**	1								
Phytotox_100%	Pearson correlation 0.339	Significance (P) 0.000	0.430*	1							
Phytotox_75%	Pearson correlation 0.679	Significance (P) 0.105	0.036	–	1						
Phytotox_50%	Pearson correlation 0.781	Significance (P) 0.000	0.751**	0.401	–	1					
Carboxyl- and carbonyl-C	Pearson correlation 0.567**	Significance (P) 0.000	0.829**	0.052	0.922**	1					
		Significance (P) 0.000	0.000	0.005	0.000	–	0.628**				
			0.424*	0.538**	0.720**	1					

**Table 5** (continued)

	Significance (P)	Germination_3 days	Germination_7 days	Phytotox_100%	Phytotox_75%	Phytotox_50%	Carboxyl- and carbonyl-C	Aromatic-C	di-O-Alkyl-C	O-Alkyl-C	Methoxyl-C + N-alkyl-C
Aromatic-C	0.004	0.039	0.007	0.001	0.000	0.000	–	–	–	–	–
	Pearson correlation	0.781***	0.440*	0.793***	0.969***	0.733***	0.733***	1	–	–	–
di-O-Alkyl-C	0.000	0.000	0.031	0.000	0.000	0.000	0.000	–	–	–	–
	Pearson correlation	0.561***	–0.204	0.585***	0.522**	0.061	0.061	0.538**	1	–	–
O-Alkyl-C	0.004	0.001	0.339	0.003	0.009	0.778	0.778	0.007	–	–	–
	Pearson correlation	–0.727**	–0.580**	–0.538**	–0.937**	–0.853**	–0.853**	–0.930**	–0.220	1	–
Methoxyl-C + N-alkyl-C	0.000	0.003	0.007	0.000	0.000	0.000	0.000	0.000	0.303	–	–
	Pearson correlation	–0.770**	–0.650**	–0.501*	–0.956**	–0.825**	–0.825**	–0.966**	–0.351	0.983	1
Alkyl-C	0.000	0.001	0.013	0.000	0.000	0.000	0.000	0.000	0.092	0.000	–
	Pearson correlation	0.722**	0.576**	0.537**	0.755**	0.841**	0.841**	0.901**	0.185	–0.982**	–0.941**
	Significance (P)	0.000	0.003	0.007	0.000	0.000	0.000	0.000	0.388	0.000	0.000

\*Correlation is significant at 0.05 (bilateral), \*\*correlation is highly significant at 0.01 (bilateral)

**Table 6** Pearson correlation between bioassays and chemical groups in compost C/N35

	Significance (P)	Germination_3 days	Germination_7 days	Phytotox_100%	Phytotox_75%	Phytotox_50%	Carboxyl- and carbonyl-C	Aromatic-C	di-O-Alkyl-C	O-Alkyl-C	Methoxyl-C + N-alkyl-C
Germination_3 days	Pearson correlation	1	–	–	–	–	–	–	–	–	–
	Significance (P)	0.757	–	–	–	–	–	–	–	–	–
Germination_7 days	Pearson correlation	0.082	1	–	–	–	–	–	–	–	–
	Significance (P)	0.474	0.834	1	–	–	–	–	–	–	–
Phytotox_100%	Pearson correlation	0.342	0.039	–	–	–	–	–	–	–	–
	Significance (P)	0.717	0.987	0.850	1	–	–	–	–	–	–
Phytotox_75%	Pearson correlation	0.109	0.000	0.032	–	–	–	–	–	–	–
	Significance (P)	0.742	0.973	0.894	0.988	1	–	–	–	–	–
Phytotox_50%	Pearson correlation	0.091	0.001	0.016	0.000	–	–	–	–	–	–
	Significance (P)	0.753	0.939	0.879	0.955	0.983	1	–	–	–	–
Carboxyl- and carbonyl-C	Pearson correlation	0.084	0.005	0.021	0.003	0.000	–	–	–	–	–
	Significance (P)	0.747	0.968	0.893	0.983	0.999	0.991	1	–	–	–
aromatic-C	Pearson correlation	0.747	0.968	0.893	0.983	0.999	0.991	1	–	–	–
	Significance (P)	0.747	0.968	0.893	0.983	0.999	0.991	1	–	–	–

**Table 6** (continued)

	Germination_3 days	Germination_7 days	Phytotox_100%	Phytotox_75%	Phytotox_50%	Carboxyl- carbonyl-C	Aromatic- C	di-O- Alkyl- C	O-Alkyl-C	Methoxy]-C + N-alkyl-C
	Significance (P)	0.088	0.016	0.000	0.000	0.000	-	-	-	-
di-O-Alkyl-C	Pearson correlation	-0.656	-0.843	-0.963	-0.942	-0.866	-0.926	1	1	1
	Significance (P)	0.157	0.035	0.002	0.005	0.026	0.008	-	-	-
O-Alkyl-C	Pearson correlation	-0.739	-0.894	-0.990	-1.000	-0.979	-0.998	0.949	1	1
	Significance (P)	0.093	0.016	0.000	0.000	0.001	0.000	0.004	-	-
Methoxy]-C + N-alkyl-C	Pearson correlation	-0.729	-0.891	-0.993	-0.997	-0.965	-0.992	0.967	0.998	1
	Significance (P)	0.101	0.017	0.000	0.000	0.002	0.000	0.002	0.000	-
Alkyl-C	Pearson correlation	0.752	0.889	0.972	0.994	0.998	0.998	-0.899	-0.991	-0.981
	Significance (P)	0.085	0.018	0.001	0.000	0.000	0.000	0.015	0.000	0.001

\*Correlation is significant at 0.05 (bilateral), \*\*correlation is highly significant at 0.01 (bilateral)

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