

Microbiological parameters and maturity degree during composting of *Posidonia oceanica* residues mixed with vegetable wastes in semi-arid pedo-climatic condition

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Abstract

The aim of this study was to characterize the biological stability and maturity degree of compost during a controlled pile-composting trial of mixed vegetable residues (VR) collected from markets of Tunis City with residues of *Posidonia oceanica* (PoR), collected from Tunis beaches. The accumulation in beaches (as well as their removal) constitutes a serious environmental problem in all Mediterranean countries particularly in Tunisia. Aerobic-thermophilic composting is the most reasonable way to profit highly-valuable content of organic matter in these wastes for agricultural purposes. The physical, chemical, and biological parameters were monitored during composting over 150 d. The most appropriate parameters were selected to establish the maturity degree. The main result of this research was the deduction of the following maturity criterion: (a) C/N ratio < 15; (b) $\text{NH}_4^+\text{-N}$ < 400 mg/kg; (c) $\text{CO}_2\text{-C}$ < 2000 mg $\text{CO}_2\text{-C/kg}$; (d) dehydrogenase activity < 1 mg TPF/g dry matter; (e) germination index (GI) > 80%. These five parameters, considered jointly are indicative of a high maturity degree and thus of a high-quality organic amendment which employed in a rational way, may improve soil fertility and soil quality. The mature compost was relatively rich in N (13.0 g/kg), P (4.74 g/kg) and MgO (15.80 g/kg). Thus composting definitively constitutes the most optimal option to exploit these wastes.

Key words: soil degradation; recycling; composting; organic amendment; maturity degree

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Introduction

The overuse of chemical fertilisers and excessive disturbance often leads to soils low in soil organic matter (SOM). The levels of SOM in Tunisian soils are declining strongly in last decade, which increase the soil degradation. Municipal solid compost could bring some pollutants like heavy metals into soil (Jedidi *et al.*, 2003). As alternative to municipal solid compost, we propose another waste like vegetable wastes or *Posidonia* residues.

Posidonia oceanica is the main sea grass in Mediterranean countries, as Tunisia, and provides substrates to a species-rich epiphytic community, which achieves maximum biomass between the end of spring and the end of summer (Terrados and Medina-Pons, 2008), covering over 50000 km² (Bethoux and Copin-Monte, 1986). As Castaldi and Melis (2002) indicated, the disposal of the annual accumulation of *P. oceanica* on the beaches of the Mediterranean causes a series of economic and environmental problems. In this particular situation, the leafy deposits of *P. oceanica* on the beach can be considered

refuse. At present they are dumped as waste, which result in the loss of enormous mass of organic material.

The agronomic reuse of *P. oceanica* refuse may be an interesting way to provide high-quality organic matter to soils. The dead sheets of *P. oceanica* were traditionally used during a long time as compost by the farmers of the coasts Mediterranean (Saidane *et al.*, 1979). This sea plant is known by its high content in C, N and P. The desalination of this plant did not present a technical problem since *P. oceanica* was a plant with a smooth surface, impermeable to salt existing in its natural environment and a simple rinsing eliminates the quasi totality of chlorides.

It is estimated that the quantity of composted vegetable residues and market wastes produced by Tunis City is greater than 17 tons/d, which is a sizable volume of substrate for a biological treatment (ANPE, 2007). Nevertheless, more than 90% of vegetable residues (VR) are dense material with low water content that requires mixing with other wastes or bulking materials.

As well as in other Mediterranean countries, mixing of these biodegradable wastes (VR and PoR) and composting in piles or windrows can produce mature, highly-quality

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organic products which are very useful to increase the SOM content of Tunisian soils.

Compost stability/maturity is critical to the successful use of biosolids composts for agricultural purposes (Iannotti *et al.*, 1994). Application of unstable compost may slow plant growth and damage crops by competing for oxygen or causing phytotoxicity to plants due to the insufficient biodegradation of organic matter (Brodie *et al.*, 1994). Stability and maturity are both commonly used to define the degree of OM decomposition during the composting process even they are conceptually different. Compost maturity refers to the level of stability of most criterion maturities (Wu *et al.*, 2000; Tiquia, 2005) and many criterions were established to characterize the compost stability and maturity. Microbial criteria include CO₂ trends (Wu *et al.*, 2000) and enzyme activity (Tiquia, 2005). Seed germination indexes in compost are a common biological method to evaluate the degree of maturity of the composting material (Gomez-Brandon *et al.*, 2008). Some chemical parameters such as C/N ratio (Iglieas-Jimenez and Perez Garcia, 1992), NH₄⁺-N and NO₃⁻-N contents (Gomez-Brandon *et al.*, 2008) have also been studied to characterize the compost stability. Nevertheless, there is a lack of information regarding these parameters during VR and PoR composting.

The objective of this study was to evaluate physical, chemical, and microbial maturity criteria to determine the quality of compost made from the substrate mixed vegetable and *P. oceanica* residues.

1 Materials and methods

1.1 Composting materials

The initial compost material consisted of 70% of vegetable residue (VR) and 30% of *P. oceanica* residues (PoR). VR consisted of branches, leaves, weeds, and grass cuttings collected from the Greater Tunis area. PoR collected from Tunis beaches was washed several times to eliminate chlorides. The materials were stacked in an uncovered pile. The pile had a trapezoidal cross section (2 m × 1.5 m and 1.5 m high) and an initial mass of approximately 30 tons. Pile temperature was measured daily using a digital thermometer at the depth of 0.5 and 1 m, in the pile. Piles were turned when the inner temperatures reached 70°C, and then watered to 45%–50% moisture content. Three windrows were envisaged. The composting began in April 2004. After five months, the product was sieved to pass a 10-mm sieve, and the residual was grind at the beginning of the process.

1.2 On site sampling

The windrow was sampled during each turning. Four samples were taken at the start of the composting process and samples were collected every 5 for 150 d. Samples 5 kg were taken from various parts of the pile according to the method introduced by De Guardia (1998). The sample was subdivided into three subsamples (1 kg each). The first subsample was stored at -20°C for enzyme analysis; the

second was used for the physicochemical analyses, and the third was used for microbiological analyses. The subsample for physicochemical analysis was dried at 70°C for 2 d and crushed.

1.3 Microbial analysis

Ten grams of compost were mixed with 90 mL of sterile distilled water and mechanically stirred for 2 h. Microorganisms were enumerated by serial dilutions on trypticase-soy-agar (TSA) plates after 5 d incubation at 22°C for mesophiles and 45°C for thermophiles. *Salmonella* was determined according to the standard methods of American Public Health Association (1998).

DHA was measured according to the method described by Tabatabai (1994) using triphenyl tetrazolium chloride (TTC) as the artificial electron acceptor, which was reduced to the red-coloured triphenylformazan (TPF).

1.4 Chemical analysis

Each fraction obtained was characterised by measuring the following parameters: CO₂ released, pH, Kjeldahl-N and inorganic N concentrations. pH and electric conductivity (EC) were measured in a 1:5 (W/V) compost-water suspension. Oxidable-C was determined by dichromate oxidation according to the procedure described in norm NF T 90-101 (Oct., 1998). Total organic N was measured using the Kjeldahl procedure and the inorganic N content was determined in a 1 mol/L KCl extract (1:10, W/V) by steam distillation in the presence of MgO (NH₄⁺-N) or MgO + Devarda's alloy (NH₄⁺-N + NO₂⁻-N + NO₃⁻-N) (Keeney and Nelson, 1982).

The CO₂ evolution was measured according to the incubation method of Wu *et al.* (2000). Previously screened sample (25 g) at 60% (W/W) moisture content was sealed in 0.5 L respirometer flasks along with a beaker containing 5 mL of 0.5 mol/L NaOH solution. The samples were incubated at room temperature ((25 ± 2)°C). During the incubation, the released CO₂ was captured by the NaOH solution, which was then analyzed titrimetrically with 0.2 mol/L. HCl in an excess of BaCl₂ at regular intervals.

1.5 Evaluation of the compost toxicity using seed germination and root elongation (GI index)

Germination tests were performed with wheat (Karim, var) provided by the gene bank of National Agronomic Institute Tunisia. Eight seeds, three replicates for each sample of the compost, were left to germinate in the water extract of the compost at 25°C for 72 h. The germination index (GI) was computed by the formula (Zucconi *et al.*, 1981)

$$GI = \frac{n_{VSS} \times RL_S}{n_{VSC} \times RL_C} \times 100\%$$

where, n_{VSS} and n_{VSC} express the number of viable seeds in the sample and in the control, respectively (extract compost was replaced by distilled water); RL_S and RL_C expressed the root length in the sample and in the control, respectively.

Compost was considered to be immature if GI was less than 50% compared to the control. High compost maturity was assumed by GI values which are greater than 80% (Iglesias-Jiménez and Pérez-García, 1992).

1.6 Statistical analysis

All measurements of parameters (temperature, pH, N, C/N, CO₂, DHA, GI and microbiological enumeration) were performed in triplicates for the same sample and analysed by the SPSS statistical program (SPSS for Windows, SPSS Inc. Version 10). The values presented are the average of three replicates and the means of the three piles were separated by the least significant difference (LSD test). Statistical analysis of Pearson's correlation was conducted using the SPSS statistical program.

2 Results and discussion

2.1 Physiochemical properties of compost

The thermophilic phase was characterized by temperatures greater than 60°C for 70 d (Fig. 1a). The pile temperature reached 55°C only 12 d after initiating the composting. The biooxidative phase (mesophilic + thermophilic periods) of the pile was 105 d, which was sufficient to destroy thermo sensible pathogens. The pH values increased with compost curing time. These results are consistent with the findings of Iannotti *et al.* (1994)

where municipal solid waste compost was used. Avnim-elech *et al.* (1996) also reported similar results and further explained that pH changes were caused by decomposition of organic acids, suggesting that simple parameters such as pH might be good indicators of compost stability. Eklin (1998) studied the effects of six different amendments on the composting processes and found in all cases that the compost pH increased from 6 to > 8 during the early stage of composting (< 35 d) and then decreased slowly but steadily after that point.

The C/N ratio can be used to characterize compost maturity. It was set at 30 in the present experiment and decreased afterwards, according to the progress of the composting process, and it stabilized around 12 (Fig. 1b) which is the established value for highly mature compost (Iglesias-Jiménez and Pérez-García, 1992). According to Jedidi *et al.* (1991), this decrease corresponded to a stable form of the organic matter. Indeed the practical coincidence in the stability of temperature and C/N ratio is in agreement with compost literature (Iglesias-Jiménez and Pérez-García, 1992). Hachicha and Ghouli (1991) showed that municipal solid waste (MSW) compost reached maturity only after 70–75 d according to the stabilisation of the C/N ratio. This difference in duration could be attributed essentially to the nature of the substrate and experimental conditions. Therefore, the C/N ratio alone is not a sufficient criterion to determine the compost maturity as was found in the case of sewage sludge compost which presented C/N values lower than 15 sometimes, despite the instability of the product due to the high N-richness of the waste (Iglesias-Jiménez and Pérez-García, 1992). It is necessary to associate it with some others physicochemical and biochemical parameters as well as a phytotoxicity test to establish compost maturity (Goyal *et al.*, 2005).

The NH₄⁺-N concentration decreased notably during the composting process and notably an increase of NO₃⁻-N was observed (Fig. 1c). The decrease of “apparent” ammonification all along the thermophilic period was due to the progressive mineralization of the most labile organic N-compounds (mainly proteins) and depends on the rate of oxygenation (Veeken *et al.*, 1999). In addition, important N-losses (ammonia volatilisation) may occur due to the high temperature reached and the alkaline pH (Iglesias-Jiménez and Pérez-García, 1992). Thus, the “real” ammonification extent during composting was greater than indicated in Fig. 1c. Nevertheless, at the end of composting (temperature stabilisation), the NH₄⁺-N content in compost was lower than the value (400 mg/kg) recognized for mature compost as set by Bernal *et al.* (1998). As observed in Fig. 1c, NO₃⁻-N appeared during the biooxidative phase which was apparently in contradiction with general idea that nitrification not occurred under thermophilic conditions since high temperatures (above 40°C) inhibited the activity of nitrifying bacteria (Bernal *et al.*, 1998). However, appreciable amounts of NO₃⁻-N were observed only at the end of biooxidative phase and ammonification was the predominant process during composting (biooxidative phase).

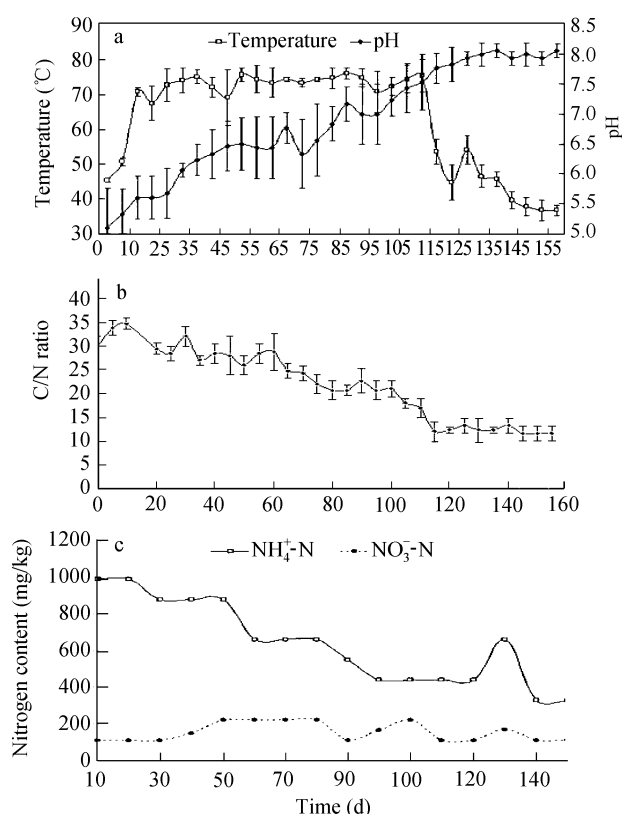


Fig. 1 Evolution of temperature and pH (a), trend of C/N ratio (b) and N content during the compost process of a mixture of vegetable residues (VR) and *P. oceanica* residues (PoR) at 70%: 30% (V/V).

2.2 Microbial dynamics

A large variety of mesophilic and thermophilic microorganisms were isolated from compost sampled at different periods during the composting process. These microorganisms can grow at temperatures range 10–70°C. Under aerobic conditions, temperature is a major factor determining microbial diversity and the intensity of metabolic activities (Amner *et al.*, 1988). During the mesophilic phase, there was a predominance of mesophilic bacteria with concentrations of 10^8 CFU/g dry matter (Fig. 2a). The natural microflora colonized the first substrate preferentially degrading the labile organic-matter. This idea was supported by the result of dehydrogenase activity (DHA) that was in the order of 4.8 mg TPF/g dry matter for MSW; this high value can be the result of a high oxidation rate of the organic matter. This intense metabolic activity increased the release of CO_2 and temperature (Hellmann *et al.*, 1997). During the thermophilic phase, the number of mesophilic bacteria decreased to 5×10^3 CFU/g dry matter. The mesophilic bacteria declined as thermophilic organisms increased to 10^7 CFU/g dry matter during the thermophilic phase. Mustin (1987) indicated that the microorganisms of compost, at any moment, create the conditions of their own destruction, which are optimal for the following microbial populations engaged in composting. During the cooling period, the number of thermophilic bacteria decreased appreciably to 10^5 CFU/g dry matter.

The pattern of CO_2 release during the composting cycle (Fig. 2b) did not show the same tendency. The process can be divided into two phases. The first one appeared during

the thermophilic period with a high release of CO_2 and the second appeared during the mesophilic period with low release. For example, VR released approximately 5000 mg CO_2 -C kg/dry matter after 70 d of composting, this value decreased at the end of biooxidative phase to 2000 mg C-CO_2 /kg dry matter. A stabilisation of the CO_2 release was observed during the maturity phase (after biooxidative phase). These results suggested that CO_2 release could be a determinant factor indicating compost maturity. Generally, the trends in respiration rates of the compost at different ages correspond to changes of the chemical and physical parameters observed during composting (Saviozzi *et al.* 2004). The results suggested that CO_2 could be an indicative parameter of compost maturity. For utilization in container media, a higher stability level (500 mg CO_2 -C /kg dry matter) may be required. Presently, composts are typically used on the basis of past experiences with specific products from given suppliers instead of their use on the basis of standard physical, chemical, and/or biological properties of the products that define quality.

Compost enzyme activities were considered to be sensitive to different experimental conditions and feed stock sources. Therefore, enzyme activities could be considered as effective indicators for abiotic stress (i.e., heavy metals) or management practices. It is also obvious that the relationship between an individual biochemical property and the total microbial activity is not always obvious, especially in the case of complex systems like compost, where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse. Dehydrogenase activity (DHA) has been used to

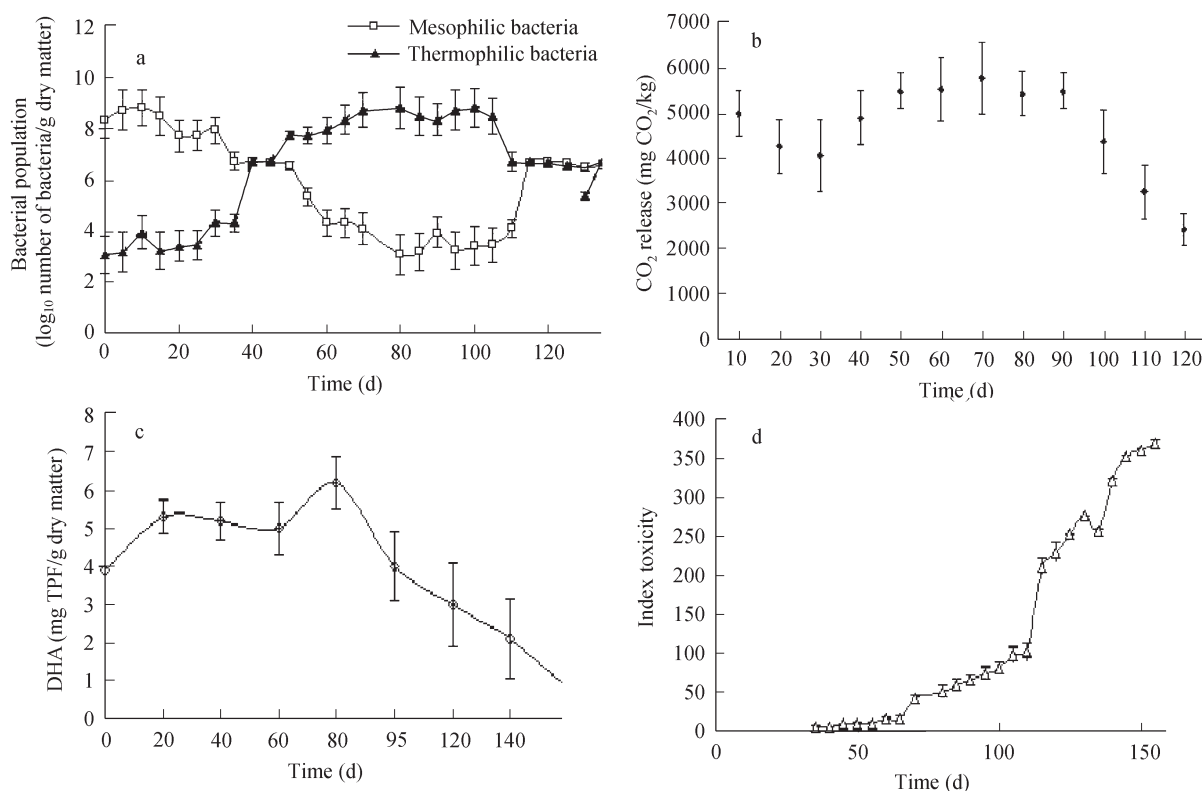


Fig. 2 Microbial and metabolic characteristics during the compost process of a mixture of VR and PoR at 70%:30% (V/V). (a) mesophilic and thermophilic bacterial populations; (b) evolution of CO_2 release; (c) dehydrogenase activity (DHA); (d) germination index (GI) evolution.

measure overall heterotrophic microbiological activity in soils (Tiquia *et al.*, 1992, Serra-Wittling *et al.*, 1995), and thus DHA measurement during composting can indicate compost stability. Tiquia (2005) proposed the determination of DHA during composting as a very reliable indicator of maturity and proposed a value below 35 µg TPF/g dry matter for highly mature composts. The mesophilic phase was characterized by a relatively weak DHA (Fig. 2c). At the beginning of the composting process, the rates of DHA in the studied piles were of the order of 4.5 mg TPF/g dry matter. During the thermophilic phase, a light increase of the DHA activity was observed and reached 6.5 mg TPF/g dry matter in the studied pile. During the cooling period, there is a net decrease of the DHA activity and at day 150 the values were stable (Fig. 2c), indicating a high maturity degree (Tiquia, 2005). The result indicated that the DHA activity was predictive of the compost maturity. The DHA activity varied almost positively with the amount of SOM. Indeed, the lower pH compost values associated to a higher C/N ratio, lead to a slow decomposition rate and consequently lead to a less DHA activity than that during the thermophilic phase.

The increase of the microbial activity of the compost can be the result of the high moisture content. Generally, there was a greater enzyme activity early in the composting period when compared to later. In the amended soil, the compost did not increase enzyme activity in an additive way. Dehydrogenase, a strictly endocellular enzyme, was the only one for which the activity in the amended soil increased significantly in proportion to the addition of compost. During the incubation, C mineralization and DHA activity were significantly correlated, indicating that DHA was a reliable indicator of global microbial activity (Serra-Wittling *et al.*, 1995). As mentioned by Skujins (1976), the appearance of DHA showed the average activity of the dynamic microbial population and depends on the total metabolic activity of microorganisms. Frankenberger and Dick (1983) reported that DHA often is correlated with microbial respiration when exogenous C sources are added to soils. In addition, some substances may seriously affect the DHA in composts, mainly volatile compounds frequently found in solid wastes such as CHCl_3 , benzene, and toluene. These chemical products strongly inhibit the activity of dehydrogenase. Ladd (1978) reported that some

alternate electron acceptors seemed to stimulate (Fe_2O_3 , MnO_2 , SO_4^{2-} , PO_4^{3-}) or to inhibit (NO_3^- , NO_2^-) the appearance of DHA in soil.

2.3 Pathogenic bacteria

One possible way to compost material is to eliminate pathogenic microorganisms. The optimal compost pasteurization values are often tested by inoculating with *Salmonella* or *E. coli* before compostion and determining when the pathogen is killed off (Soares *et al.*, 1995). The moisture of the final compost must be lower than 20% and C/N ratio must be lower than 15. Composting should suppress all pathogenic bacteria. In our study, *Salmonella* was absent at the end of the composting. These bacteria were destroyed when temperature reached 55°C (Martens, 2005), *Salmonella* came essentially from the food and meats. *Salmonella* species were regarded as a problem of the hygienic quality of the MSW compost.

2.4 Agronomic quality of composts

The salinity of the mature compost was low with EC values around 6.25 dS/m. Excessive salinity in compost can cause phytotoxicity and negatively affect the growth of salt intolerant plants. For composts having relatively high salt contents, repeated applications and/or elevated application rates can exacerbate soil salinity, leading to structural breakdown and/or plant growth inhibition (Smith *et al.*, 2001). We also noted that the compost was rich in N (13.0 g/kg), P (4.74 g/kg), K (7.48 g/kg) and MgO (15.80 g/kg), whereas MSW contained only 3.3 g/kg total P, this nutrient content is higher than the level of manner (6.2 g/kg) (Morel *et al.*, 2005). Immature compost, with a high C/N ratio can immobilize N (Garcia-Gomez *et al.*, 2003). Bacteriological analysis of the stable compost indicated a lack of pathogenic bacteria such as *Salmonellae* which appeared only at the beginning of composting cycle (data not shown). These bacteria disappeared when thermophilic phase started.

GI is a sensitive parameter to evaluate phytotoxicity and compost maturity. A GI value of 50% has been used as a marker of phytotoxin-free compost, while a value greater than 80% is thought to indicate mature compost (Campitelli and Ceppi, 2007). The mature compost in this study did not present phytotoxicity since the GI was higher

Table 1 Pearson's correlation coefficients between the nitrogen content (NH_4^+ -N and NO_3^- -N), CO_2 , DHA, composting time, and GI

	Time	NH_4^+ -N	NO_3^- -N	CO_2	DHA	C/N ratio	Temp.	Mb	Tb	GI
Time	1.000									
NH_4^+ -N	-0.911**	1.000								
NO_3^- -N	0.050	-0.096	1.000							
CO_2	-0.833**	0.651*	0.300	1.000						
DHA	-0.787**	0.758**	0.243	0.829**	1.000					
C/N ratio	-0.964**	0.814**	0.022	0.864**	0.715**	1.000				
Temp.	-0.737**	0.545	0.413	0.978**	0.798**	0.798**	1.000			
Mb	-0.383	0.551	-0.678*	-0.167	0.105	0.256	-0.289	1.000		
Tb	0.411	-0.576*	0.699**	0.105	-0.089	-0.285	0.241	-0.905**	1.000	
GI	0.944**	-0.832**	-0.164	-0.934**	-0.869**	-0.921**	-0.862**	-0.135	0.174	1.000

* Correlation is significant at the 0.05 level; ** correlation is significant at the 0.01 level. Correlations were based on average data of the 3 piles during composting.

Mb: Mesophilic bacteria; Tb: thermophilic bacteria.

than 50%. The mature compost had a value of GI higher than 80% (Fig. 2d). The start of maturity was indicated when the index attain 50%. This value was reached in the pile after 120 d. The index value of 80% (highly mature compost) was reached after 140 d.

Correlation tests were conducted among the measured chemical and biological properties of compost sampled during the composting process (Table 1). GI during compost cycle was best correlated to most of the parameters tested ($R \geq 0.89$) with the highest correlations found for C/N, DHA and CO_2 . The DHA activity correlated positively with CO_2 ($R_{\text{CO}_2} = 0.82$, $P = 0.01$) and NH_4^+-N ($R_{\text{NH}_4^+-\text{N}} = 0.75$, $P = 0.01$). The result showed DHA may be used as important criterion to define compost maturity of vegetable waste mixed with *Posidonia* residues.

3 Conclusions

Compost maturity is difficult to define by only one parameter since usually several parameters is needed crossbred. To be considered as a suitable criterion of compost maturity, a parameter must be a good indicator of composting and maturation processes and its value should change in a similar trend in all studied samples.

Various compost parameters (C/N, thermo stability, DHA, NH_4^+-N , NO_3^--N and CO_2) were used to evaluate compost development of a mixture of VR and PoR. Taking into account these parameters, the compost maturity was obtained from 120 d composting for VR and PoR compost. The period of 150 d indicate a high stabilisation of all parameters studied (C/N ratio < 15; NH_4^+-N concentration < 400 mg/kg; CO_2 -C concentration < 2000 mg CO_2 -C/kg; DHA activity < 1 mg TPF/g dry matter and GI > 80%). The mature compost was rich in N (13.0 g/kg), P (4.74 g/kg) and MgO (15.80 g/kg). Dead *P. oceanica* pose a problem in all Mediterranean countries, but mixing with VR, even at 30%, could be a useful tool to preserve the environment and increase the organic matter content in soil.

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