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Biodegradability of Seven Mediterranean Plants in a Composting Environment

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ABSTRACT

This study proposes to evaluate the biodegradability of branches and leaves from 7 Mediterranean plants: palm tree, olive tree, cypress, pine, bay tree, mimosa and plane tree. The chemical approach to biodegradability of each sample has been compared to its biochemical composition. During the first 5 days, biodegradation kinetics can be divided into 2 prevalent stages: the first one with a slow rate and the second with a fast rate. The difference between these 2 stages is more dependent on the plant's species than its different parts (leaves or branches). The study also confirms the role of lignin and the importance of granulometry in the biodegradation of green waste and the efficacy of the decomposing process in composting.

Keywords: biochemical composition, biodegradability, composting, green waste, lignin, valorization

INTRODUCTION

The biodegradability of green waste plays an important role in the composting process that converts organic matter into a useful product for ≤agricultural practices. The factors influencing the successful application of compost for agricultural use are its stability and maturity (Thuriès *et al.* 2002; Pansu *et al.* 2003; Domeizel *et al.* 2004; Sánchez-Monedero *et al.* 2004; Goyal *et al.* 2005; Tognetti *et al.* 2007; Kalamdhad *et al.* 2008). Since the biodegradability of organic matter is the principal process involved in the bio-stabilization of organic waste materials, compost stability often depends on the biodegradability of its components (Larré-Larrouy 2006; Smid *et al.* 2008; Fernandes *et al.* 2009).

Parameters influencing biodegradability are those involved in the activity of micro-organisms: moisture, oxygen, temperature, pH, granulometry as well as the quantity and quality of the degradable organic matter (Robin 1997; Tuomela *et al.* 2000; Ahtiainen *et al.* 2003; Komilis *et al.* 2005; Cayuela *et al.* 2006). Biodegradable compounds are natural organic compounds as opposed to human-made plastics for which no natural enzyme exists (Pagga 1998; Sawada 1998; Domenek *et al.* 2004).

However, the kinetics of biodegradation is not the same for all natural compounds (Chantigny *et al.* 2002; Bahri *et al.* 2008; Francou *et al.* 2008; Lin *et al.* 2008; López Alvarez *et al.* 2009). For example, the scientific community agrees that lignin degradation occurs more slowly than the degradation of other compounds such as, hemicellulose and/or cellulose (Kononova 1966; Tuomela *et al.* 2000; de Guardia *et al.* 2002; Said-Pullicino *et al.* 2007). Granulometry of the organic material, or the size of the fragments which determines the amount of contact area between enzymes and organic matter, significantly influences its biodegradability (Lhadi *et al.* 2006). All the previously cited parameters may play a role, alone or synergistically, according to all possible combinations.

Biodegrability can be determined via various methods. In the following sections, only a few references will be cited, partially. All of these methods are indirect and based on the micro-organisms activity. Some of them are *in vivo* tests where conditions are monitored but not controlled (Cayuela *et al.* 2006; Said-Pullicino *et al.* 2007; Kalambhad *et al.* 2008). Others are *in vitro* tests during which all parameters are controlled but the test conditions may differ from natural conditions (Vargas-García *et al.* 2007). However, there are some normalized methods (Ahtiainen *et al*. 2003). First, the concept of degradation kinetics primarily consists in defining biodegradability. Indeed, all materials which are biodegradable do so at different rates (of time). Studies were conducted over periods of 28, 60 or occasionally 90 days (Kusel *et al.* 1999; Miles *et al.* 2001). Biodegradation can be studied by (i) measuring the micro-organisms CO_2 exhaust during the degradation process (Sturm 1973; Struijs *et al.* 1990; Musmeci *et al.* 1994; Boni *et al.* 1998; Srinivasan *et al.* 2000) or (ii) the amount of O_2 consumed by the micro-organisms (Ianotti *et al.* 1994; Lasaridi *et al.* 2006; Lhadi *et al.* 2006). Monitoring the amount of CO_2 produced during the biodegradation of organic matter is less efficient because a fraction of the CO_2 is used by autotrophic micro-organisms for their metabolism, which leads to an underestimation of the biodegradation process. On the other hand, when the measurement of oxygen consumption is chosen to study the biodegradability of organic matter, the nitrification process can also lead to an underestimation of its biodegradation. A denitrifying agent can reduce this misleading production of nitrate.

In order to (i) better understand green wastes degradation, (ii) evaluate the impact of green waste on the composting process, (iii) identify mixtures adapted to the composting process, and (iv) take into account industrial constraints, we propose to:

- identify individual behavior of branches and leaves for 7 plant species, that is to say 14 samples (species have been chosen because they are common species used in compost process in the South of France);
- identify which compounds intrinsically influence the biodegradability of these 14 samples the most.

In this study, the samples' biodegradability was determined by measuring the mineralization rate of the amount of total organic carbon (TOC).

To attain these objectives, the biochemical composition

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and C/N ratio has been measured for each sample and compared with the TOC mineralization rate according to the work of other researchers (Robin 1997; Zmora-Nahum *et al.* 2005; Alburquerque *et al.* 2006; Hernández *et al.* 2006). The organic material biochemical composition corresponds to the quantification of different fractions: soluble fractions, hemicellulose, cellulose and lignin-cutin fraction (Vansoest *et al.* 1963; Robin 1997; Müller *et al.* 1998; Namour *et al.* 1998; Thuries *et al.* 2002; Pansu *et al.* 2003). The plant species we have selected are representative of the Mediterranean area: palm tree, olive tree, cypress, pine, bay tree, mimosa and plane tree (**Annex**). Many scientific studies, as previously noted, have addressed the biodegradation of green wastes in mixture, but only few studies have been carried out on the biodegradation of individual plant species, and especially on the difference between biodegradation of their branches and their leaves (Benitez *et al.* 2004; Don *et al.* 2005).

MATERIALS AND METHODS

Sampling

Random samples were taken in a green waste compost plant located in the South of France. Samples were separated first into plant species; and then into leaves and branches. Samples were stored after drying at 40°C (+/- 1°C) and crushed to particle size of 0.25 mm before analysis. All samples (1 kg per sample) from the compost plant were collected at once. Waste sampling was performed immediately upon their transport to the compost plant, before crushing and before the biodegradation process began. Thus, the physical alteration that makes the identification of studied species a rather complex process was avoided and the different components in the plants selected for sampling could be clearly identified. This way, samples representative of the different types of green waste could be collected.

Biodegradability determination

Biodegradability can be measured mainly using two kinds of methods: (i) automated methods based on the direct determination of carbon dioxide. (Boatman *et al.* 1986; Struijs *et al.* 1990; Buitron *et al.* 1993; Saudeco-Castaneda *et al.* 1994; Spérandio *et al.* 1997; Calmon *et al.* 2000; Komilis 2006; de Guardia *et al.* 2010), and (ii) methods monitoring the amount of O_2 consumed by the micro-organisms via a simple and fast measurement of the pressure drop induced by the oxygen consumption (Ianotti *et al.* 1994; Jackson *et al.* 1998; Lasaridi *et al.* 1998; Thibault *et al.* 2000; Adani *et al.* 2001; Miles *et al.* 2001). Techniques based on the direct monitoring of carbon dioxide emission, are not consistently reliable. For instance, micro-organisms such as autotrophic micro-organisms use a fraction of the carbon dioxide (CO_2) produced during the biodegradation of organic matter causing an underestimation of the amount produced and, subsequently, an error in the biodegradability estimation. As for methods based on monitoring oxygen consumption, the nitrification process also causes an error in the estimation of the biodegradability of the analyzed samples. Nevertheless, the use of an anti-nitrifying agent, such as alkyl thiourea (ATU) or 2-chloro-6-(trichloromethyl)-pyridine (TCMP), which can block the bacteria responsible for nitrification without damaging the micro-organisms responsible for the carbon biodegradation may be used to negate that effect. Thus, the amount of consumed O_2 corresponds only to the biodegradation of organic carbon.

While the currently employed techniques can eliminate or minimize the overestimation of oxygen consumption due to nitrification, the underestimation of CO_2 production cannot be quantified. Consequently, biodegradability determinations based on consumed O_2 are considered more reliable and more accurate than those based on CO_2 (Lasaridi *et al.* 1998; Jackson *et al.* 1998; Thibault *et al.* 2000; Adani *et al.* 2001).

In this study, the biodegradability of our samples was determined by measuring the mineralization rate of the amount of total organic carbon. This measurement is based on the determination of O_2 consumed by bacteria during the degradation of organic carbon.

We used an Oxitop®OC110 a very commonly used instrument in biodegradability studies (Reuschenbach *et al.* 2003; Komilis *et al.* 2009; Nolan *et al.* 2011). With it, a reliable respirometric test for assessing the biodegradability of chemical compounds can be performed. de Guardia *et al.* (2010) showed that the respirometric quotient of organic wastes is between 0.9 and 1.2.

The humidity was fixed at 50% and experiments occurred over a period of 28 days in a thermostated room at 20°C (Küsel *et al.* 1999). To be as close as possible to the microbiological community responsible for green waste degradation, the samples were seeded with a 10 day old green waste compost having a C/N ratio = 20, and rich in microbial activity. The compost sample used to study the biooxydation phase was collected in the same composting plant as the green waste samples analyzed. Each test consisted of mixing a quantity of matter (mass) extracted from this compost with a quantity of the studied sample so that the amount of total organic carbon contained in the studied sample exceeds 30% of the amount of total organic carbon contained in the compost used for seeding.

Oxygen consumption was calculated from the recorded pressure difference, using the principle of the ideal gas law, and logged separately for each respirometer. The pressure drop is a result of micro-organisms consuming oxygen to degrade organic material. The emission of CO_2 resulting from the biodegradation process is trapped by soda pellets inside the Oxitop®OC110. Drimal *et al.* (2007) in their study of polymers and de Guardia *et al.* (2010) in waste studies proved that the CO_2 emission and O_2 production were likewise comparable. In agreement with previous results, the amount of CO_2 emitted by a given sample can be calculated from the amount of O_2 consumed by the sample according to the formula below:

$$\text{mg of } CO_2 \text{ emitted} = \text{mg of } O_2 \text{ consumed} * 44/32$$

In this study, sample nitrification was tested by measuring the pressure drop inside the Oxitop®OC110 without soda pellets. The depression observed in the instrument is due to: (i) the biodegradation of organic carbon, which releases CO_2 trapped by the soda pellets (causing the depression) and/or (ii) the nitrification phenomenon. The absence of soda pellets prevents any pressure drop inside the Oxitop®OC110 subsequent to the degradation of the total organic carbon, because the volume of O_2 consumed by the micro-organisms is replaced by the CO_2 which is not trapped by the soda pellets. This is not the case in the nitrification process where oxygen is consumed and nitrate does not produce gaseous emission. Therefore, despite the absence of soda pellets inside the Oxitop®OC110, a nitrification process, that has developed sufficiently, creates a pressure drop inside the Oxitop®OC110 due to the high consumption of oxygen utilized to produce nitrate. As no depression was observed in this study: nitrification was deemed negligible. This observation is consistent with our measurements of nitric nitrogen performed on green wastes during composting (data not shown). Actually, green waste nitrification increases significantly after the 150th day of composting. Therefore, the use of a denitrifying agent was deemed unnecessary and the amount of oxygen consumed corresponds solely to the biodegradation of the amount of total organic carbon.

The CO_2 which should theoretically be released by the sample in the case of a complete oxidation of the carbon containing material, noted mg $ThCO_2$, is calculated using the following relationship (Pagga 1998; Calmon *et al.* 2000):

$$\text{mg } ThCO_2 = \text{mg C} * 44/12$$

where mg C is the quantity of total organic carbon present in the sample.

The mineralization rate (Rm) of total organic carbon can be calculated for the sample using the following formula (Calmon *et al.* 2000):

$$Rm = (\text{mg } CO_2)*100 / \text{mg}CO_2 \text{ theoretical}$$

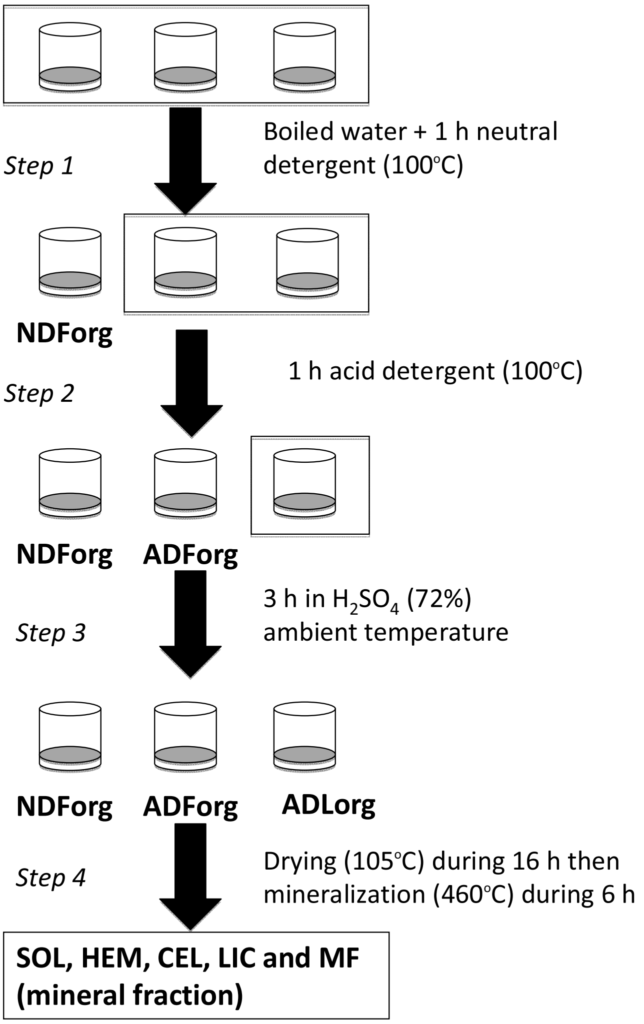

Fig. 1 Biochemical fractionation of organic matter according to the method of Van Soest and Wine (1963) (based on Robin 1997). SOL = 100–NDForg; HEM = NDForg–ADForg; CEL = ADForg– ADLorg; LIC = ADLorg.

Table 1 Degradation kinetics of studied green wastes.

Plant species	Rapid degradation kinetic between 0 and 5 days	Low degradation kinetic between 0 and 5 days
Bay tree	Leaves	Branches
Mimosa	Branches and leaves	
Olive tree	Branches and leaves	
Palm tree	Branches and leaves	
Cypress		Branches and leaves
Pine		Branches and needles
Plane tree		Branches and leaves

Table 2 Classification, according to values discrimination of 14 studied samples compared to: (i) kinetic slope in the first 5 days, (ii) soluble fraction (**SOL**), hemicellulose (**HEM**) and cellulose (**CEL**) and (iii) lignin (**LIC**).

Green wastes	Kinetic slope (classes)	SOL+HEM+CEL (classes)	LIC (classes)
Branches			
Bay tree	1	1	2
Cypress	1	1	2
Mimosa	2	2	1
Olive tree	2	2	1
Palm tree	2	2	1
Pine	1	1	2
Plane tree	1	1	2
Leaves			
Bay tree	2	2	1
Cypress	1	1	2
Mimosa	2	1	2
Olive tree	2	2	1
Palm tree	2	2	1
Pine	1	2	1
Plane tree	1	1	2

(“1” reference represents the lower value and “2” the higher value).

Analytical measurements

Total organic carbon has been measured by TOC-meter (Shimadzu) and nitrogen by the Total Kjeldhal Nitrogen method (ammonium measurement with an auto distillation analyzer after acid (H_2SO_4) digestion (ISO-11261)).

Biochemical fractionation

Biochemical fractionation of the organic matter (Van Soest *et al.* 1963; Robin 1997; Thuries *et al.* 2002; Pansu *et al.* 2003) corresponds to a sequential fiber analysis (based on the successive solubilization of organic compounds) that has allowed us to obtain 4 biochemical families, sorted from the least to the most soluble: soluble substances (SOL), hemicellulose-like substances (HEM), cellulose-like substances (CEL) and lignin/cutin-like substances (LIC) (**Fig. 1**).

RESULTS AND DISCUSSION

Degradation kinetics

Among the 14 samples we have studied, 2 types of behavior can be distinguished based on the kinetics of their biodegradation during the first 5 days rather than their Rm value after 28 days of incubation (**Fig. 2**). Seven samples had a slow degradation kinetics while the 7 others exhibited a rapid degradation kinetics. Discrimination is not observed between branches and leaves (**Table 1**) but rather between plant species. Our observations add up to very few published studies describing such an evolution of the mineralization kinetics. However, Komilis *et al.* (2009) showed that oxygen uptake was high during the first day of incubation of 6 composts analyzed for a four-day period and decreased in the second part of the experiment. Komilis (2006) studied the biodegradation of grass, leaves, branches, papers and wastes. He showed that the low level of CO_2 was due to the absence of a readily hydrolysable carbon fraction. When it was present, the hydrolysable fraction produced a high amount of CO_2. Unlike the results obtained with the present study, he showed that branches which contain a low hydrolysable carbon fraction and produce a low quantity of CO_2 are less biodegradable than leaves. Thus, the more abundant the hydrolysable fraction is, the higher the biodegrability is. In contrast, our results show that biodegradability difference is mainly attributable to plant species (**Table 2**) more than organ (branches versus leaves).

A statistical analysis based on values discrimination has been made in order to evaluate the impact of the biochemical composition of each sample on its degradation kinetics (**Table 2**). Comparison has been made of: (i) kinetics during the first 5 days, (ii) sum of HEM+SOL+CEL and (iii) LIC. Samples with slow kinetics (slope class 1) appear to have a rich LIC content (class 2). On the contrary, when the kinetics is fast (slope class 2), the samples contain a high proportion of HEM+CEL+SOL. These observations can be explained by the nature of the samples that are easily biodegradable and less resistant to micro-organism attacks in soluble fractions, and in cellulose-like substances and hemicellulose-like substances (Said-Pullicino *et al.* 2007; Vargas-García *et al.* 2007) and by natural resistance of lignin/cutin-like substances to the biodegradation (Tuomela *et al.* 2000; Komilis *et al.* 2003; Dignac *et al.* 2005). Therefore, the presence in a given material of a significant amount of lignin/cutin-like substances slows down the activity of microorganisms at the beginning of the experiment, but does not inhibit completely their capacity for mineralization. According to our knowledge and the bibliographical survey

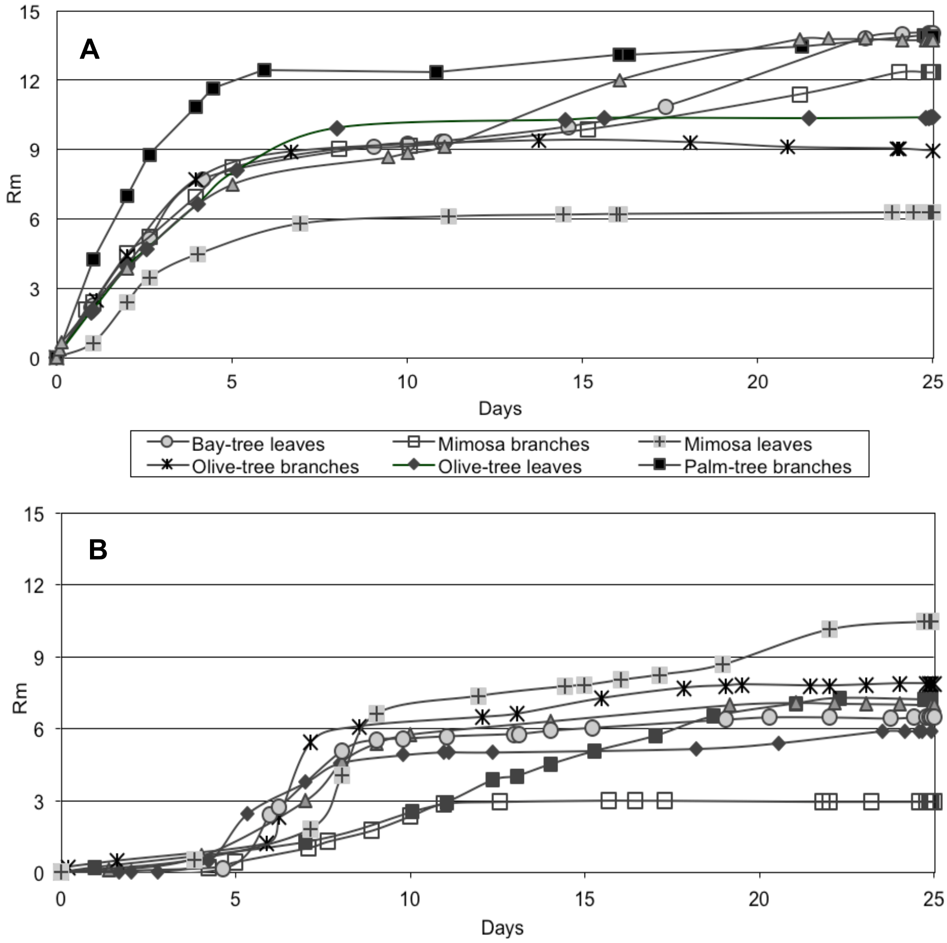

Fig. 2 Time evolution of mineralization rate (**Rm**) of 7 samples with rapid (**A**) and slow (**B**) degradation kinetics between initial time and 5 days mineralization.

carried out for this study, this lag time during the first 5 days of incubation has not previously been reported.

In comparison to the results of Don *et al.* (2005), the mineralization rates (Rm) of total organic carbon calculated after 28 days of incubation show that all the 14 samples are poorly biodegradable. We observe that leaves are not more rapidly biodegradable than branches and they present a comparable biodegradability behavior with respect to branches except concerning the bay-tree for which biodegradation occurs faster for leaves than branches. These observations suggest a negligible impact of the nature of the samples studied on their rate of mineralization. This result is in contradiction with those of de Guardia *et al.* (2002) who observed that the leaves, more nitrogen-rich than branches, are more biodegradable.

A particular attention is brought to the palm tree for which the laboratory study contradicts the observations made in the composting plant (results not shown): it showed a high biodegradability mineralization rate during laboratory tests while in an open air windrow, fragments of palm trees remained intact and were not biodegraded faster than other species. The differences observed between the 2 trials (composting plant and laboratory) can be explained by the optimum conditions selected in the laboratory. Ahtiainen *et al.* (2003) have shown that batch studies induce a different behavior of micro-organisms when samples are combined with carbon. The lack of carbon in substrate and thus the lack of an energy source in the natural conditions elicit the development of a different population of decomposers. The mesh of grinding in the composting plant, which is larger than the one chosen for the batch study, may also explain the difference observed, thus confirming the role of the particle size in the kinetics of degradation, a parameter that may explain different behaviors.

The degradation kinetics of pine needles exhibits an atypical response in our statistical study. The sum of SOL + CEL + HEM fractions corresponds quantitatively to the most important class (class 2), despite the slow degradation kinetics during the 5 first days (class 1). This result may be explained by the presence of aromatic compounds in needles as Don *et al.* (2005) also demonstrated. The study conducted by Don *et al.* (2005) on dissolved organic matter present in litter collected under maple, beech, ash, pine and spruce trees has shown that biodegradability is different depending on the species. In particular, it is increasingly important in the order: maple, beech, ash, spruce and pine. The quality and quantity of dissolved organic matter is also different according to the litter type. Regarding the extracts of pine needles, the authors explain the presence of dissolved organic carbon in quantities smaller than in the extracts of leaves is mainly due to the thick protective epidermic and hypodermic layer on needles._Although extracted in the fractions HEM+SOL+CEL, these organic compounds are less soluble in aqueous medium and therefore are less bioavailable for micro-organisms. Their biodegradation will be slowed down even more. Sequential analysis of fibers is based on the extraction procedure. It is difficult to identify with precision what kind of compounds have been extracted (Thuries *et al.* 2002). So, even if the protocol extraction is the same, extracted compounds depend on the initial sample composition. Concerning pine needles, it can be supposed that their biomaterials can easily be biodegraded into chemical compounds, particularly due to micro-organisms that degrade pine needles easily in natural conditions.

Comparing the mineralization rates and other parameters

The comparison of the mineralization rates (Rm) of the green wastes studied with their C to N ratio and biochemi-

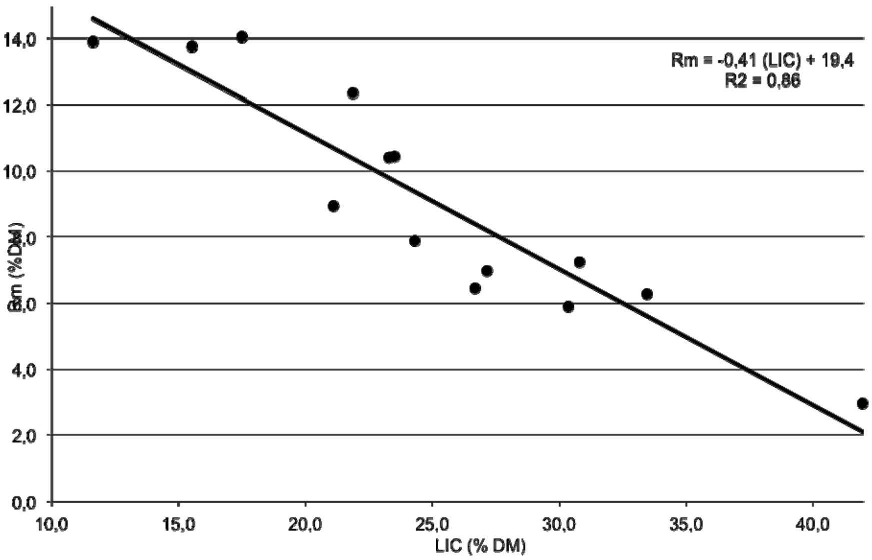

Fig. 3 Relationship between the mineralization rate (Rm) of the 14 samples of green waste and their lignin (LIC) content expressed as percents of dry matter (DM).

cal compositions, as well as statistical studies such as ANOVA, ANCOVA and multiple linear regression method were used to identify the concentrations of lignin/cutin-like substances as the main parameter influencing Rm. The C to N ratio and the quantities of soluble fraction, hemicellulose-like and cellulose-like substances, have been identified as the factors impacting Rm the least (results not shown, Khalil 2005). The work carried out has shown that the high levels of lignin/cutin-like substances present in the green waste studied (> 11.6% of the dry matter) explain the low rates of mineralization observed (<15% of total organic carbon). This result is in agreement with other studies (Argyropoulos *et al.* 1997; Robin 1997; Sánchez-Monedero *et al.* 2001; Komilis *et al.* 2003). The differences in lignin contents from one sample to another largely explain the variations observed in the mineralization rates. The linear regression presented in **Fig. 3** shows that Rm for the 14 studied samples is negatively correlated with their lignin content. This result confirms those of Robin (1997) who showed that the mineralization rate of a green waste with a content of lignin/cutin-like substances higher than 7% DM, can be estimated from its initial content of lignin.

CONCLUSION

Our study of green waste's biodegradability has confirmed the role of lignin on biodegradability, especially as a moderator of the biodegradation process. In this context, a better understanding of the green wastes and, in particular, better knowledge of their biodegradability can provide additional information to assist waste management. One underlying aim is to provide knowledge of blends of plant species and crushing sizes to optimize the composting practices. After composting and along the soil valorization process, it has been assumed that organic matter stabilization results not only from a decrease of the readily biodegradable fraction such as cellulose and hemicellulose but also from the relative increase of resistant compounds from the initial wastes (lignin for example) and from the microbial activity and humification.

Biodegradability has been studied here in the context of optimal conditions of degradation. Of course, this choice does not reflect the reality in the composting-industrial-process. The importance of crushing in *in vitro* study irreparably sends the reader back to the role of the waste particles' size in biodegradation, as illustrated in a study by Lhadi *et al.* (2006) showing the role of waste granulometry on the product quality with an increase of aromatic structures in presence of small fragments. All experimental conditions have remained the same throughout the study. Therefore, the biodegradability results obtained in this context may be relevantly used on a composting platform.

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Annex Common and Latin names of plants studied.

Common name	Latin name
Bay tree	*Nerium oleander*
Cypress	*Cupressus sempervirens*
Mimosa	*Acacia dealbata*
Olive tree	*Olea europea*
Palm tree	*Phoenix canariensis*
Pine	*Pinus sylvestris*
Plane tree	*Platanus X hispanicus*