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Assessment of organic matter biodegradation and physico-chemical parameters variation during co-composting of lignocellulosic wastes with *Trametes trogii* inoculation

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ABSTRACT

Lignin complexity molecule makes its biodegradation difficult during lignocellulosic wastes composting. So, the improvement of its biodegradation has usually been considered as an objective. This study aimed to determine the impact of *Trametes trogii* inoculation on organic matter and particularly on lignin and cellulose during green wastes co-composting with olive mill waste water sludge and coffee grounds. Three types of heaps (H1, H2 and H3) were investigated during 180 d. H3 and H2 were inoculated at the beginning of the process (t_0) and 120 d later (t_{120}), respectively while H1 was the control. Results showed the absence of pH stabilization in H3 during the first month. Also, in this period we observed a faster degradation of some easily available organic matter in H3 than in the other heaps. After 120 d, a better cellulose decomposition (25.28%) was noticed in H3 than in H1 and H2 (16%). Inoculation during the second fermentation phase induced supplementary lignin degradation in H2 with a percentage of 35% against 23 and 26% for H1 and H3, respectively. For all the runs, a Fourier Transform Infrared analysis showed aliphatic groups' decrease, OH groups' increase and lignin structural modification.

Keywords: Composting, Lignin degradation, Lignocellulosic wastes, Organic matter, *T. trogii*

1. Introduction

In Tunisia, the generation of urban solid wastes (SW) is rapidly increasing due to the growing population, the industrial development and the improvement of living standards. According to the ANGED [1], the national production of SW was about 7 million tons in 2012. A significant part (33,000 tons) of SW consisted in green wastes (GW) that were mostly produced from landscape maintenance (domestic gardens, park, etc.) and vegetables and fruits wholesale markets. In Tunisia, GW disposal is characterized by the lack of effective strategies for its valorization. In fact, traditionally, disposal of these lignocellulosic wastes was subjected to incineration, deposition in landfills and burying. Such strategies reduce the land use efficiency and cause environmental problems such as water contamination and odor pollution [2]. Following the approach of traditional GW disposal, concerns were raised both with environmental protection and economic development; therefore, GW disposal problem can be solved by composting. By involving natural biodegradation of organic materials in various stages, composting is widely practiced because it is rather economical. Thus, as reported by Rawoteea et al. [3], the composting of lignocellulosic wastes is also a practical way for disposing waste sustainably and producing a safe and stable product used as a soil amendment, and an organic fertilizer, or as a substitute for peat in soilless culture. This is especially true in many developing agricultural economies characterized by an arid climate and

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a chronic lack of organic fertilizers. However, the main problems in traditional composting of GW are the long duration of the processing time and the poor compost quality because of the high amounts of lignin and cellulose characterizing the substrate [4]. Hence, reducing the time required for green waste composting and improving the quality of the compost have become important goals in the use of composting for GW valorization. To this end, several authors have opted for various solutions like the addition of readily decomposable materials, such as animal manure and grease trap waste in order to initiate an intense microbial activity [5, 6], biosolids to improve on the microbial activity and GW biodegradation [7] or suitable lignolytic and cellulolytic microorganisms inoculation as a technique to accelerate the composting process and improve on the quality of the end product of the process known as compost [8]. Indeed, many reports have tackled the beneficial impact of fungal inoculation during GW composting and focused acceleration of compost maturity and to improve on its quality. In this respect, Haddadin et al. [9] indicated that inoculation with the lignocellulolytic fungi *Trichoderma harzianum* and *Phanerochaete chrysosporium* greatly reduced the time required for the compost to mature. Likewise, Wang et al. [10] reported that the inoculation with the lignocellulolytic fungus *Penicillium expansum* improved both the composting efficiency and the compost quality. Moreover, Awasthi et al. [11] observed that inoculation with the lignocellulolytic fungi *Trichoderma viride*, *Aspergillus niger*, and *Aspergillus flavus* enhanced the composting process. Additionally, the microbial inoculums which included a mixture of *Trichoderma* and White-rot fungi accelerated the decomposition rate [12]. Many studies confirmed that lignocellulose degradation by white-rot fungi is faster than that of any other organisms and that these microorganisms are responsible for most of the lignin decomposition in nature. They can cause selective or nonselective delignification through two major families of enzymes: peroxidases and laccases. There are thousands of species of white rot fungi, most of them Basidiomycotina, and a few Ascomycotina. *Trametes trogii* (*T. trogii)* is one of the basidomycete fungi capable of producing non-specific laccase. This enzyme is characterized by a high level of degradation of phenolic and non-phenolic compounds including lignin and many other various pollutants [13]. Due to these decomposition and detoxification properties, this strain has been widely applied in bioremediation including the decolorization of synthetic dyes [14], detoxification of landfill leachate [15] and decontamination of industrial effluents and soil polluted by herbicides and pesticides [16]. Nevertheless, the impact of *T. trogii* addition during composting has not been previously evaluated. Therefore, the current research dealt with the valorization of GW by its co-composting with OMWWs and CG in order to optimize the initial C/N ratio. These two wastes are both characterized by high lignin and phenolic compound content and they have not been efficiently valorized in Tunisia, yet. The effect of *T. trogii* inoculation at initial time and after 120 d of co-composting was also studied. The two major reasons that led choice of this inoculation time (t_{120}) included the thermosensitive character of the strain [13] which requires a low temperature (≤ 40℃) and the study of inoculation impact following natural beginning of lignin degradation. Such an impact included the effect of inoculation time on composting process (Temperature (T) and pH), total organic matter (OM) biodegradation and each fraction separately particularly lignin, cellulose, fats, soluble carbohydrates (WSCa) and soluble polyphenols (WSPH).

2. Materials and Methods

2.1. Preparation of Raw Materials

In order to facilitate the grinding of GW to 1-3 cm by the grinding mill BRO1VER, BV 400-5 and the crushing of OMWWs and therefore to increase the contact between substrate area and microorganisms, both wastes were air dried. In this study, the GW was mainly collected from dead leaves and tree branches gathered at the Ecole Nationale d'Ingénieurs de Sfax (ENIS) during greening maintenance. The solid blocks of OMWWs were brought from Sfax storage basins and were mechanically crushed and sieved to get 0.5 mm particles. The CG was collected from different cafeterias in Sfax and dried in the open air. Prior to drying, it was subjected to a refining step whereby impurities and contaminants such as cigarette butts, paper and plastic tips were removed.

The physico-chemical characteristics of the raw materials and the initial mixture were presented in Table 1 which showed that all wastes were characterized by acid pH and low electrical conductivity (EC) value. Despite their richness in total organic carbon (TOC) and some fertilizer elements, they contained a high level of lignin which gave them a recalcitrant characteristic.

2.2. Inoculum and Inoculation of H2 and H3

The *T. trogii* (CLBE55) isolate used in this study was obtained from the ENIS microbiology laboratory, Tunisia. It was stored at 4°C on malt extract agar (MEA) pH 5.5 containing 19 gL^{-1} malt extract and 15 gL^{-1} agar. From a culture grown on MEA in Petri dishes at 30℃ for 3 d, a pre-culture in M7 medium was prepared for 10 d at 30℃. This medium consisted of 10 gL^{-1} glucose, 5 gL^{-1} soy peptone, 1 gL^{-1} yeast extract, 2 gL^{-1} ammonium tartrate, 1 gL^{-1} KH_2PO_4, 0.5 gL^{-1} $MgSO_4.7H_2O$, 0.5 gL^{-1} KCl, 1 mLL^{-1} trace element solution and pH adjusted to 5.5. The trace elements solution contained 0.1 gL^{-1} $B_4O_7Na_2.10H_2O$, 0.01 gL^{-1} $CuSO_4.5H_2O$, 0.05 gL^{-1} $FeSO_4.7H_2O$, 0.01 gL^{-1} $MnSO_4.4H_2O$, 0.07 gL^{-1} $ZnSO_4.7H_2O$, 0.01 gL^{-1} $MoNH_4.4H_2O$. A volume (2%) was distributed on 1 L erlenmeyer flask containing wheat bran and olive cake (70%; 30%). In order to have semi-solid fermentation conditions, this mixture was moistened with Synthetic liquid medium (M7) with a ratio of (1; 2.5) (w; v) and maintained at 30℃ for 12 d.

2.3. Composting Process

Three types of heaps (H1, H2 and H3) were investigated in this study. Two heaps having these dimensions (H: 0.60 m, W: 1 m, L: 1.2 m) and weighing each 70 kg were constituted for each type. They were prepared with the same initial composition of wastes, put under the same conditions during composting and led to the production of C1, C2 and C3, respectively. To optimize their humidity (45-60%) and C/N ratio (30), the GW was mixed with OMWWs and CG at 47.38 and 15%, respectively. The composting process was held for 6 mon. In order to maintain aerobic

Table 1. Physico-Chemical Characteristics of Raw Materials and Initial Mixtures with Standard Deviation Dry Weight (DW) Basis

Parameters[a]	GW[b]	CG[b]	OMWWs[b]	Initial mixture[c]
pH	5.93	6.26	6.19	6.42
EC (mS/cm)	2.83 ± 0.51	0.59 ± 0.02	2.74 ± 0.053	1.84 ± 0.11
TOC (%)	37.66 ± 1.3	40.26 ± 1.3	29.22 ± 0.65	47.29 ± 0.38
Total Nitrogen (Nt)	0.882 ± 0.014	1.995 ± 0.028	1.595 ± 0.106	1.68 ± 0.08
C/N	42.68 ± 0.80	20.19 ± 0.93	18.38 ± 0.82	29.34 ± 0.89
Fats (%)	8.76 ± 0.76	7.43 ± 1.05	23.31 ± 2.21	20.24 ± 1.91
Lignin	31.13 ± 1.25	20.45 ± 0.96	nd	38.90 ± 0.48
Cellulose	14.06 ± 1.63	9 ± 1.05	nd	12.29 ± 0.96
WSPH	0.64 ± 0.21	0.45 ± 0.08	0.88 ± 0.12	0.59 ± 0.03
WSC	2.85 ± 0.05	1.02 ± 0.04	2.16 ± 0.08	6.49 ± 1.16
Na	0.04 ± 0.005	0.07 ± 0.009	0.15 ± 0.004	1.15 ± 0.06
Ca	0.64 ± 0.11	0.08 ± 0.001	0.34 ± 0.017	0.70 ± 0.03
K	0.67 ± 0.03	0.15 ± 0.003	0.81 ± 0.014	0.90 ± 0.07
Mg	0.18 ± 0.02	0.07 ± 0.003	0.09 ± 0.002	0.16 ± 0.02
P	0.08 ± 0.001	0.04 ± 0.002	0.05 ± 0.002	0.065 ± 0.01
Zn (ppm)	64.07 ± 10.91	4.93 ± 0.33	23.57 ± 1.24	64.17 ± 14.5
Cd	< 0.25	< 0.25	< 0.25	< 0.25

nd: not determined

[a] Electrical conductivity (EC), total organic carbon (TOC), total nitrogen (Nt), ratio of TOC and Nt, fats content (Fats), lignin content (lignin), cellulose content (cellulose), water soluble polyphenols content (WSPH), water soluble carbohydrates content (WSCa)

[b] Raw materials for composting GW, CG, OMWWs

[c] Average composition of heaps initial mixtures

conditions, the heaps were turned manually twice a week during the active phase and once a week during the maturation stage. Samples were collected at t_0 and monthly according to the method described in Gillet R [17]. Temperatures were daily measured at different points of the pile and its profiles corresponded to the weekly average. H1 was the control heap whereas H3 and H2 were inoculated with the white rot fungus *T. trogii* (10 gkg^{-1} waste) [18] at t_0 and at the maturation phase (t_{120}), respectively.

2.4. Analytical Methods

In this study, the equipments and the methods used to characterize the raw materials were the same as those used to characterize the compost samples. pH and EC were analyzed in a 1:10 (w/v) water soluble-extract using pH-meter type NeoMet, pH-220L and a conductivity meter type Cond 7110 inolab, respectively whereas the moisture content was calculated by a simple deduction of water loss. The chemical composition of the sub-samples including OM was determined by the weight loss at 550°C for 2 h of a dried sample while TOC was determined by the bichromate oxidation method and total Nitrogen (Nt) was calculated through Kjeldahl approach. The fat content was calculated by a four-hour extraction of 2.5 g of dried sample in a Soxhlet apparatus with 200 mL hexane. WSPH were determined from an aqueous extract in a 1:20 (w/v) according to the Folin-Ciocalteau method. For this, 2.5 mL of the aqueous extract, 1.25 mL of Folin-Ciocalteau reagent and 2.5 mL of 20% Na_2CO_3 were incubated in a 100 mL flask for one hour and put in darkness after adjusting its volume to 100 mL with distilled water. Absorbance was determined at 725 nm. WSCa were analyzed in 1:10 (w/v) water extract by the anthrone method [19]. Macro and micro elements analyses were carried out by atomic absorption (HITACHI, Z-6100 model) after hydrochloric and nitric acid digestion. Phosphorus was determined colorimetrically at 430 nm with a molybdo-vanadate phosphoric acid.

2.5. Fourier Transform Infrared Analysis

The Fourier transform infrared (FTIR) spectrum of each composting step was recorded between 4,000 and 600 cm^{-1} wave-numbers using a Cary 630 FTIR. In order to limit moisture interference, the compost samples were dried at 105°C for 24 h.

2.6. Lignin and Cellulose Content

The compost lignin contents were quantified by the Klason lignin method after being extracted with sulfuric acid (72%). The method was described in standard method TAPPI T222. Raw cellulose or Weende cellulose was determined based on a double treatment (acid and alkaline) according to the AFNOR [20] procedure using Fibertec System 1010 Heat Extractor.

2.7. Statistical Analysis

Basic statistical analyses of data were achieved using Excel and SPSS 20.0 programs for Windows. The Analysis of variance

(ANOVA) and the least significant differences (lsd) were calculated for the composting samples in order to determine changes in the parameters with time ($p < 0.05$). The averages were calculated from the values obtained from each repetition of each heap type and described as mean±SD.

3. Results and Discussion

3.1. Influence of Inoculation on the Temperature Profile of the Process

Temperature is considered as one of the most important parameters of the composting process. Its profiles at different composting steps for the three runs were given in Fig. 1.

Temperatures rose rapidly in all the heaps. They reached approximately 50℃ one week after the composting onset and kept this value in all the runs for a minimum of 10 d. This increase in temperature was caused by the generation of heat resulting from the aerobic microbial activities on readily available and high decomposable organic materials content in composting mixture [21].

According to Bernal et al. [22] such conditions (Temperature/Time) were enough for a proper disinfection of the waste materials (destruction of pathogens and weeds). A temperature peak (60℃) was observed for the initially inoculated run against 55℃ and 52℃ for H2 and H1, respectively. Still, no differences in the temperature profiles were noticed between the three heaps except for a temperature range slightly higher in H3 run until during the first three months of composting, which could be explained by an important biological activity originating from *T. trogii* inoculation or this was due to the higher content of carbohydrates of this treatment [23]. In fact, the intake of carbohydrates stimulated more the biological activity in the heap which generated more heat [24]. At the beginning of the composting process, the treatment seemed to increase the duration of the thermophilic stage. After the inoculation with *T. trogii*, (16 weeks of composting), temperature of run 2 increased, stabilized through two weeks and then gradually declined, while the temperature of runs 1 and 3 decreased. Probably, the heat was generated by the biological activity in the compost following inoculation. Gong et al. [8] reported similar results related to agricultural wastes inoculation. This increase, which might reflect lignin degradation, did not lead to a high temperature since it was in the maturation phase. The bio-oxidative phase of composting was considered to be finished when the heaps temperature stabilized close to that of the ambient (30℃). This occurred 23 weeks after the beginning of all the runs which was in accordance with the results of Khalil et al. [25] indicating that composting typically required 90-270 d to generate a stable product.

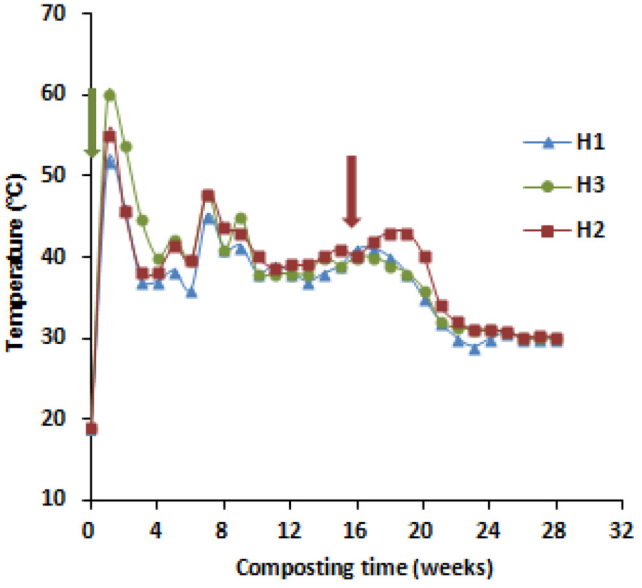

Fig. 1. Temperature development during composting time: Control heap (H1), initially inoculated heap (H3), and heap inoculated in maturation phase (H2).

3.2. Influence of Inoculation on the pH Profile of the Process

The composting process was carried out in a pH zone between 6.5 and 9 (Fig. 2). In the same heap, there were two successive phases: an alkalization (0-60 d) and a stabilization (60-210 d). During the first phase, the pH increased from the initial values of 6.5-7 to 9. Alkalization of the medium was essentially due to the disappearance of organic acids including the fatty and phenolic compounds whose degradation (Fig. 4 and Fig. 5(b)) took place in the same period (the first two months of the process). In fact, unlike H1 and H2, we noted the absence of a pH stabilization in the case of H3 between the 15th and 30th day. It seemed that inoculation at t_0 had the effect of strengthening the degradation of phenolic compounds and therefore, acted on the medium pH. Although other works have reported a pH acidification at the beginning of the process, our results were in agreement with those of El Fels et al. [26] which showed an alkalization during the first month of the process corresponding to the usual end value for the compost.

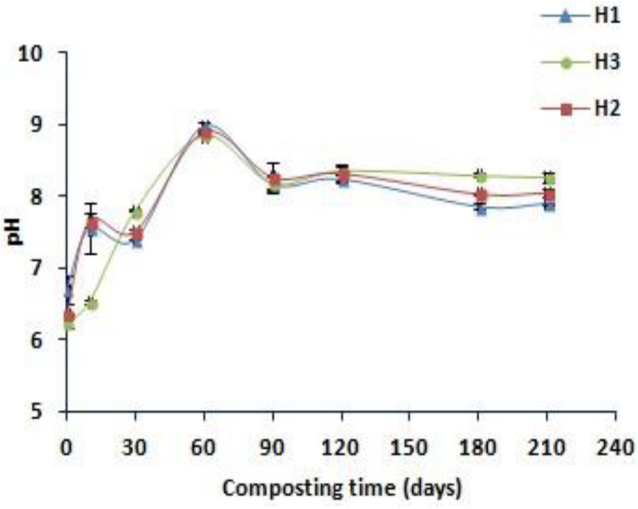

Fig. 2. pH variation: (H1) control heap, (H3) initially inoculated heap (H3) and (H2) heap inoculated in maturation phase (H2).

3.3. OM Degradation

According to Fig. 3, OM content (DW basis) decreased continuously in all the runs from approximately 85% on day zero to the following final rates: 65% (H1), 62% (H2) and 66% (H3). As reported by Shilev et al. [27], OM values having less than 60% of compost made from green waste was a usual indicator of a mature product. With the exception of H2 (62%), the final OM contents were slightly higher than 60% for the other two heaps. For each run, OM decreased following a linear function of zero order according to the Eq. (1), (2) and (3) below:

$$\text{H1: } \textit{Organic matter} = -0.8109\,t + 84.199 \quad R^2 = 0.9574 \quad (1)$$

$$\text{H2: } \textit{Organic matter} = -1.0238\,t + 87.768 \quad R^2 = 0.9748 \quad (2)$$

$$\text{H3: } \textit{Organic matter} = -0.8136\,t + 85.271 \quad R^2 = 0.8966 \quad (3)$$

Where, t = time composting on d and R^2 = determination coefficient

Increases in the decomposition rates over time were found in all the three heaps and a higher final rate was observed in run 2. From Fig. 3, we could also notice that after only one month, the biodegradation speed in the case of H3 was the most important with a rate of 13% compared to 6% for H1 and H2.

After three months, the decrease rate was lower for all runs. This might be the result of recalcitrant OM decomposition, such as lignin and cellulose [28]. After 120 d, the decomposition percentages were 17, 18 and 21% for H1, H2 and H3, respectively. Compared to H3, H1 and H2 showed a considerably lower OM degradation during this period. During this period, the difference in the OM decomposition was significant ($p < 0.05$) between H3 and H1and between H3 and H2. However no significant difference ($p > 0.05$) was noted between H2 and H1.

This indicated that *T. trogii* inoculation at the beginning of the process enhanced OM degradation and consequently accelerated the composting process. These results were similar to those of Lopez et al. [29] who reported that decomposition was more efficient in composts inoculated with lignocellulotic fungi than in untreated ones. In this study, we could say that the most easily available organic matter was affected by inoculation rather than other recalcitrant polymers. During maturation, normally characterized by molecules rearrangement and polymerization, we noticed a slight OM decrease in the case of H2 (14%) due to lignin degradation. Hence, after 28 weeks of composting, total organic matter losses were 24%, 30% and 28% in the runs 1, 2 and 3, respectively. This might suggest that *T.trogii* addition after the thermophilic phase promoted the microbial degradation of relatively stable substances that remained after the primary fermentation.

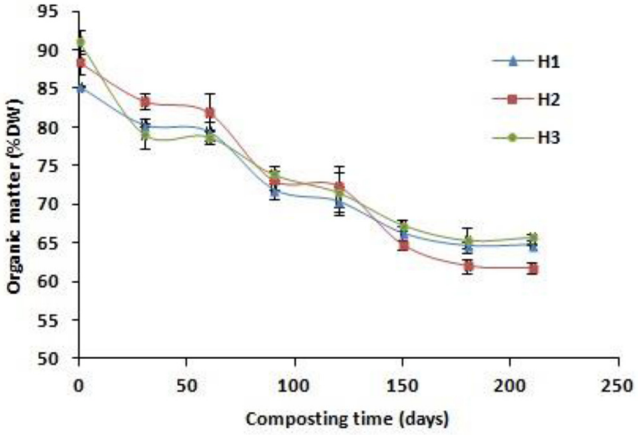

Fig. 3. Organic matter profile during composting time: (H1) control heap, (H3) initially inoculated heap, (H2) heap inoculated in maturation phase.

3.4. Changes in Total Fats

Total lipids evolution during composting is an important parameter in the quality of composts. In fact, the research work published by Annabi [30] showed that the presence of lipids in composts gave a certain hydrophobicity. Fig. 4 described the evolution of total lipids during the GW co-composting with solid blocks of OMWWs and spent CG in the three runs. Fig. 4 showed that total lipids have been significantly reduced. This decrease ranged between 85 and 90% within one month of composting, and reached more than 95% after two months and almost 100% at the end of co-composting. These results might lead us to conclude that lipids could be considered as one of the priority targets of OM for microorganisms through the first fermentation stage and that the inoculation in the third run at t_0 had no effect on this fraction decomposition inside the composting process. The total lipids reduction is attributed to metabolism via the intense activity of the microorganisms which is confirmed by many authors during waste composting [31].

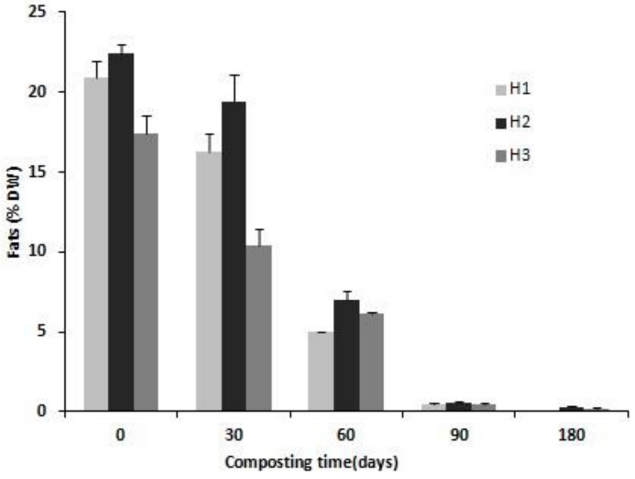

Fig. 4. Lipids degradation during heaps composting (H1), (H2) and (H3).

3.5. Lignin and Cellulose Behaviour

Table 2 summarized the rates of lignin and cellulose decomposition during composting, and showed a relatively high initial proportion of lignin in the three runs (around 40%) and a low cellulose content (approximately 11 to 13%). These results were in agree-

Table 2. Lignin and Cellulose Content during Composting (DW Basis)

Time[c] (d)	H1[e]		H2[e]		H3[e]	
	Lignin[a] (%)	Cellulose[b] (%)	Lignin[a] (%)	Cellulose[b] (%)	Lignin[a] (%)	Cellulose[b] (%)
0	38.07 ± 1.88	13 ± 0.22	39.51 ± 1.05	10.9 ± 0.4	38.85 ± 1.55	13.05 ± 0.22
90	41.69 ± 1.61	nd	43.1 ± 0.79	nd	44.79 ± 1.59	nd
120 degradation rate[d] (%) (0-120 d)	35.40 ± 1.58 7	10.88 ± 0.27 16.27	36.22 ± 0.25 8.31	9.13 ± 0.37 16.12	35.86 ± 1.57 7.68	9.75 ± 0.34 25.28
180 degradation rate[d] (%) (120-180 d)	29.2 ± 0.88 17.51	8.92 ± 0.12 18.07	25.54 ± 0.87 29.48	7.53 ± 0.41 17.52	28.92 ± 1.12 19.36	8.07 ± 0.59 17.2

nd: not determined
[a] Lignin content based on DW
[b] Cellulose content based on DW
[c] Composting time on days (0, 90, 120 and 180)
[d] Cellulose or Lignin degradation during (0-120 d) and (120-180 d)
[e] Control Heap (H1), heap inoculated in maturation phase (H2), initially inoculated heap (H3)

ment with other values related to the GW studies reported by Tuomela et al. [32] and Metzger et al. [33]. After 120 d of composting, cellulose degradation rates were 18, 15, and 25% for runs 1, 2 and 3, respectively. The greater cellulose degradation in H3 could be explained by the presence of cellulase and/or other enzymes capable of degrading cellulose in the culture medium and added to H3 when inoculating at t_0. This led to better global cellulose degradation in H3 (40%) compared to similar rates in H1 and H2 (33%). The obtained results from our experiments proved a little final degradation of cellulose allowing us to deduce that approximately 60-70% of this substrate remained undegraded for the three composts. The low degradation rate for cellulose could be attributed to an inhibition of cellulose degradation by the high content of lignin in the waste matrix [34]. Furthermore, Table 2 showed that the lignin amount was not only maintained but it even increased after three months of composting, which was a frequently faced phenomenon according to Eklind and Kirchmann [35]. Since lignin cannot be synthesized by microorganisms [36] and due to its extreme resistance to degradation, it is therefore more refractory to microbial decomposition than other biochemical constituents. Consequently, its concentration increased in the OM. Hence, the lignin degradation began slowly (8%) after three months of composting and most lignin degraded during the last two months of maturation phase with a highest rate in H2 (17%, 29% and 19% for H1, H2 and H3, respectively). This confirmed that under natural conditions, the degradation of lignocellulose, especially lignin, was slow. Therefore, the inoculation and the establishment of favorable conditions for the development of white rot fungi can ensure a better transformation. Previous studies have reported different and sometimes contradictory rates of lignin and cellulose degradation. The finding of the current work could be comparable to those reported by El Fels et al. [37] who noted that the maturation phase seemed to be more favorable to lignin degradation since most of the degradation (> 17%) took place during maturation while 8% took place during the thermophilic phase. In addition to the original lignin content and material thickness, these authors attributed this difference between the two co-composting phases to the conditions of the medium particularly the presence of nitrogen, actinomycetes and decomposing fungi.

3.6. WSPH and WSCa Changes

Carbohydrates balance during green waste co-composting is mainly due to the natural biological consumption phenomenon and the biodegradation of some parietal compounds such as cellulose. The soluble carbohydrate profile in Fig. 5(a) showed a general decrease in H1, H2 and H3.The high initial value in case of H3 (8% DW) could be due to the addition of the fungus inoculum containing carbohydrates. In fact the culture medium contained wheat bran which is characterized by high level of total carbohydrates (57%) with 8% of free sugar [23, 38-39]. The total decrease rate was about 70% for H3, 60% for H1 and 40% for H2. The most important reduction occurred during the first two months of composting, i.e. during the active fermentation. Thus sugesting that in this period, the mineralization process, microbial immobilization and non water-soluble formation from complex carbohydrates (cellulose, hemicellulose) were predominate [40]. Fig. 5(a), displayed that the decrease was highest in H3 (66%) compared to approximately 40% in H1 and H2. For the rest of the process (60-90 d), the WSCa increased by 45% in the three runs reflecting a compensation effect that occurred between the cited biological phenomena and the soluble carbohydrates formation from the cellulose and hemicellulose biodegradtion. A second slight rise in carbohydrates levels was also observed between 120 d and 150 d, reflecting a subsequent biodegradation of these constituents. Therefore, it could be concluded that the inoculation of H3 at t_0 accelerated the soluble carbohydrates consumption, but the inoculation of H2 during ripening had no effect on this kind of substrate.

Regarding the soluble phenolic compounds progress, Fig. 5(b) showed an initial rate of about 0.55-0.63% for all the runs. In the composts, the most important phenolic compounds elimination was during the first composting month. It varied from

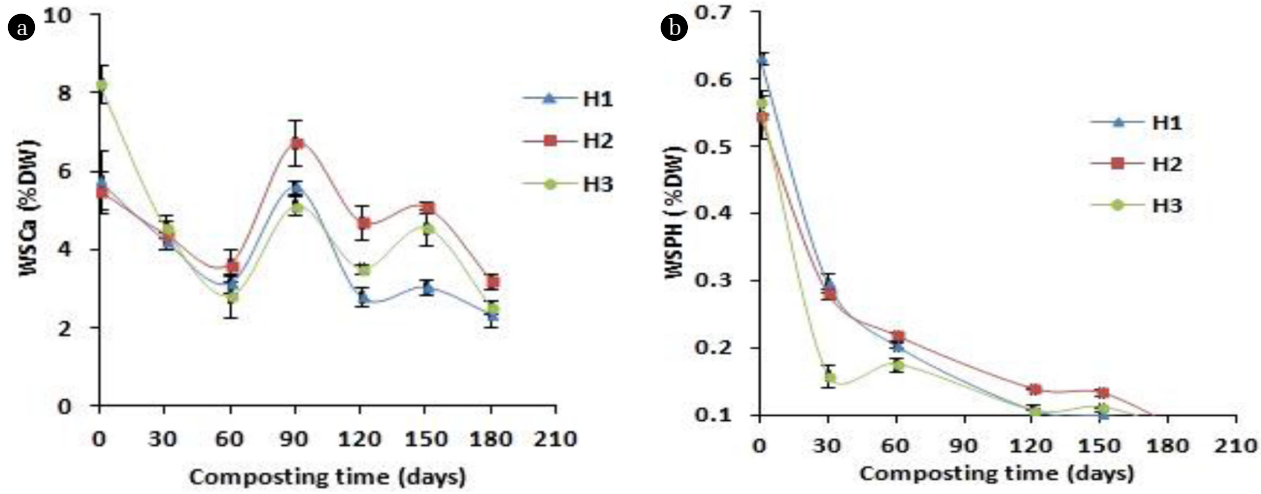

Fig. 5. Water soluble carbohydrates (WSCa) and water soluble polyphenols (WSPH) profiles during (H1), (H2) and (H3) composting: (a) WSCa profile, (b) WSPH profile.

48%, 53% and 70% in H2, H1 and H3, respectively during the first month of composting. This might enable us to conclude that inoculation in run 3 seemed to have an effect on WSPH biodegradation rate during the thermophilic phase. It should be pointed out that the relatively high rates of phenol elimination observed in H1 compared to H2 (54 and 48%) could be attributed to a slight difference in indigenous flora activities or to a sampling problem. Similar results were reported by Filippi et al. [41] with a significant reduction in the olive waste water SWPH content in the first composting stage. According to Zenjari et al. [42] this decrease in WSPH could be attributed to their oxidation with secondary metabolites to form humic substances. After 120 d, all the heaps showed a significant reduction in phenol exceeding 85%. In spite of the inoculations carried out in runs 2 and 3, WSPH compounds in the three obtained composts were mostly eliminated, corresponding to an overall reduction of 85% in the three heaps. During maturation, Fig. 5(b) showed no increase in WSPH rates despite the lignin degradation, probably because these substances were quickly consumed during humification. Indeed, according to Alkoaik and Ghaly [43] simple molecules resulting from complex compound degradations were re-synthesized again to form other complex compounds. After inoculation, there was a stable trend of the WSPH rate in run 2, showing an equilibrium between the two phenomena: reducing the levels of WSPH related to their use and/or polymerization, on the one hand, and the production of these compounds from the degradation of complex molecules such as lignin, on the other hand [44].

3.7. FTIR Spectra

Despite the difference in the relative intensity of some bands (Fig. 6), the FTIR spectra of the three obtained composts C1, C2 and C3 at different stages (t_0, t_{30}, t_{150} and t_{180}) recorded the same absorbance indicating that they went through the same steps during the composting cycle. According to Francou [45] the assignment of the main record bands was: A broad band between 3,000-3,600 cm^{-1} corresponding OH groups of alcohols and phenols, N-H groups of amids, amins and C-H of aromatics. Two peaks centered at 2,920 and 2,850 cm^{-1} were produced by C-H of aliphatics mainly lipids. A gathering of small peaks was recorded between 1,500-1,750 cm^{-1} corresponding to: C = C of lignin aromatics (1,500-1,525 cm^{-1}); N-H of amines, C = C of aromatics, C = O of quinones groups (1,600-1,650) and C = O produced by carboxylic acids (1,720-1,750 cm^{-1}). Another gathering peak was observed in the region of 1,000-1,460 cm^{-1} which corresponds to several chemical groups like carbonates, C-O of phenols and carboxylic acids and also some aromatics compounds generally characterizing C-H and C-C of aliphatic compounds, CH2 and CH3. The peak 1,020-1,100 cm^{-1} conformed the absorbance of C-C of aliphatics and C-O of polysaccharides but silicates can also be absorbed in this zone. For the peak that appeared at 874 cm^{-1}, it corresponded to carbonates. The main changes that have been recorded during composting were:

- An increase of the large band (3,000-3,600 cm^{-1}) for C1, C2 and C3 during the first three months of composting, which demonstrated the decomposition of low refractory organic matter [46]. This biodegradation was mainly based on oxidation reactions that release OH in the medium and that were deeply affected by the heap aeration. This band stabilized in the case of C1 but increased slightly in the cases of C2 and C3 by the end of the composting process despite the decrease of aeration frequency.
- A significant intensity decrease at 2,920 and 2,850 cm^{-1} during the first three months corresponding to the biodegradation of aliphatic compounds mainly lipids. These results were confirmed by the quantitative lipids dosage which showed that almost the totality of C1, C2 and C3 lipids were degraded only after three months of composting.
- A decrease of the 1,500-1,525 cm^{-1} band typical of lignin was noted for all the runs after three months of composting. This might lead us to suggest that a part of this substrate was modified or degraded during composting. Nevertheless, according to the quantitative analysis there was a slight degradation rate during the first composting phase and consequently this decrease

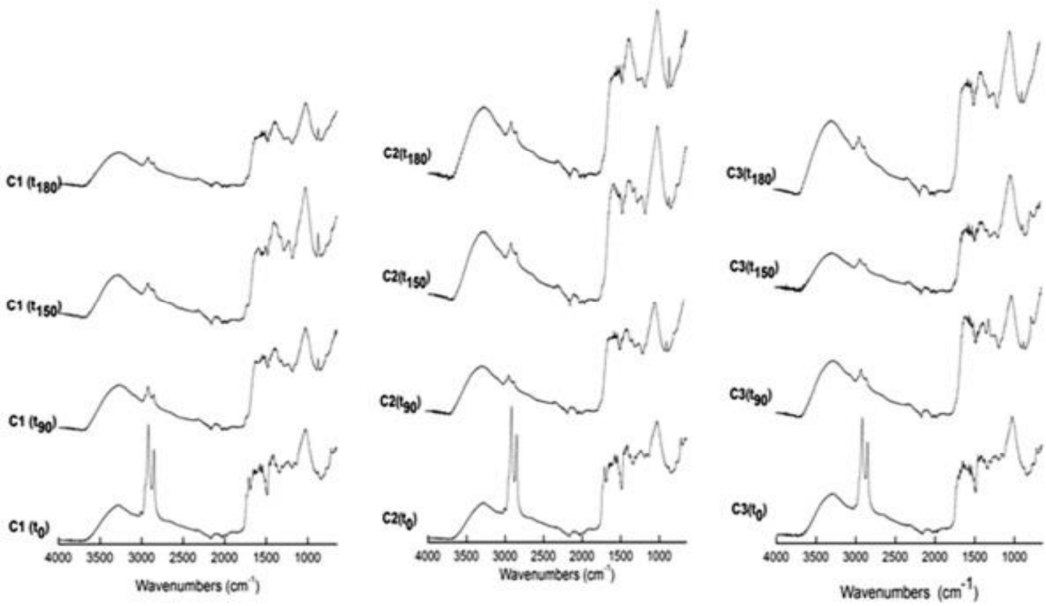

Fig. 6. FTIR spectra of composts C1, C2 and C3 from H1, H2 and H3, respectively at different stages of composting. Numbers (t_0, t_{30}, t_{150} and t_{180}) refer to composting time: 0, 30, 150 and 180 d.

was mainly due to the modification of its structure leading to an absorption capacity variation. According to Tuomela et al. [32], many bacterial strains can solubilize and modify the lignin structure without mineralization. Between 1,600 and 1,650 cm^{-1}, we noted the disappearance of some peaks which could be explained by the participation of organic compounds like quinones, aromatics and amines in the humification [47]. The intensity increase near 1,720 cm^{-1}, that was clear with C2 at t_{150}, corresponding the carboxylic acids might be the result of lignin degradation after fungal inoculation [48].

– The disappearance of some small peaks in the region of 1,100-1,400 cm^{-1} that characterized the initial state but that became more and more invisible during C1, C2 and C3 evolution. This could be related to the degradation of aromatic compounds, mainly the aliphatic ones vibrating in this zone [45]. The modification affecting 1,430-1,460 cm^{-1} that was more observable with C2 could be due to the aromatic degradation and methylation reaction including in the lignin biodegradation mechanism. In fact, as reported by Filley et al. [49] when lignin was degraded by wood decomposing fungi such as Basidomycetes, the main involved reactions were oxidation, methylation/demetoxylation and cleavage of an aromatic nucleus.

– For all composts, the 1,025 cm^{-1} peak which corresponded to polysaccharides increased at the end of the process which could be explained by cellulose degradation or contaminated composts by silicates vibrated also at 1,025 cm^{-1}.

4. Conclusions

The results of this work showed that the inoculation with the white rot fungus *T. trogii* had different impacts when achieved during different composting stages. The fungal addition at the beginning of the process didn't improve lignin degradation but increased the temperature and cellulose decomposition during the thermophilic phase and accelerated the biodegradation of easily available organic matter (soluble carbohydrates and soluble polyphenols). By contrast, during the maturation, *T. trogii* improved the lignin degradation which led to a higher degree of total OM decomposition in H2 than the two other heaps but had no impact on cellulose rate reduction. Unlike cellulose, lignin degradation occurred mainly at the maturation phase for all the runs but it showed a greater resistance during the active fermentative phase. During this phase, it was subjected to a partial biotransformation which modified its infrared spectra absorption. In this work, composting physico chemical study was conducted in order to understand the global effect of *T. trogii* inoculation on organic matter particularly on lignin degradation. Although this study did not really introduce new concepts about the composting process but it confirmed that lignin degradability is still a complex and unpredictable phenomenon. In depth study of microbial communities and enzymatic pathways implicated in composting are needed to better understand the biodegradation process. As a potential application, the resulting composts will be used as organic fertilizers in order to improve sandy soil properties.

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