"GREEN" EMPLOYMENT IN THE MANAGEMENT OF BIOWASTES

PROJECT ACRONYM: Green_Crew

https://www.serres.gr/greencrew/el/green-crew/

WP 4: Recycling of organic waste

Deliverable 4.2.3
Physicochemical characterization of waste lignocellulosic biomass and produced compost

October 2020

Lead Beneficiary	Municipality of Serres
Project Partners	Municipality of Serres
	Aristotle University of Thessaloniki – Special Account for Research Funds
	Municipality of Nestos
	Municipality of Blagoevgrad

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SUMMARY

The current Deliverable (D 4.2.3) reports the experimental protocols for the physicochemical characterization and chemical composition analysis of raw materials (waste lignocellulosic biomass) used a feedstock and compost end-products produced as part of the composting technology demonstrated in the “Green –Crew” project which is implemented within the context of the Interred V-A Cooperation Programme ‘’Greece – Bulgaria 2014-2020’’. Furthermore, a short overview of the composting process is also provided. It is part of WP4 “Recycling of organic waste” which focuses on technical research for the development, establishment, and operation of a pilot compost production unit.

The conducted experimental study has revealed the following main guidelines:

- Characterization of biomass wastes is useful for the design of efficient composting processes by selecting the appropriate feedstocks (mixtures) and conditions.
- Analysis of biomass and compost samples showed that the carbohydrates are digested faster than lignin – its content remains relatively high in the compost product – the antioxidant and antimicrobial properties of lignin may be also utilized.
- Composting of relatively high C/N lignocellulosic waste may offer high quality compost with increased N relative content.
- The content and type of inorganics present in biomass may also increase the nutritional properties of compost.

In general, composting represents an economically and environmentally sustainable approach for the on-site management and valorization of biomass related wastes and residues, which can however be significantly improved when knowledge and experience from fundamental biological and chemical sciences studies are utilized. Training of young people to implement knowledge-based protocols and methods is of paramount importance and promotes “green employment and entrepreneurship”.

1. Introduction

Biomass waste valorization is a key aspect in Circular Economy. Biomass waste or bio-waste such as agricultural wastes and residues, municipal green wastes, sludge, wastewater and food wastes, are mostly seen as low-valued materials that are mainly disposed toward landfills or incineration for heat and energy production. Traditional waste management strategies lead to serious environmental problems such as space occupancy, declining air quality, water and soil contamination. Approximately, 4.9 tons of wastes correspond to each EU inhabitant; including ~500 kg of municipal waste. The EU Framework on Circular Economy[1], the EU directive 1999/31/EC[2] and 2003/33/EC[3] require member states to separately collect and reduce the organic fraction allocated in landfills, favoring the development and application of biotechnologies in transforming organic wastes to agricultural products. Therefore composting, the microbial process where organic wastes are converted to a stable soil conditioner is an effective alternative strategy to reduce the volume of organic waste and produce valuable end-products that improve soil quality. WP4 “Recycling of organic waste” focuses on technical research for the development, establishment, and operation of a pilot compost production unit. This deliverable (D 4.2.3) reports the experimental protocols for the physicochemical characterization and chemical composition analysis of raw materials (waste lignocellulosic biomass) used a feedstock and compost end-products produced as part of the composting technology demonstrated in the *Green_Crew Interreg Greece – Bulgaria Project*. Furthermore, a short overview of the composting process is also provided.

1.1 Potential for biomass valorization towards compost

Composting of bio-wastes is generally accepted as an inexpensive, simple and environmentally sound process for waste processing and reuse. Several composting techniques have been developed and evaluated for municipal- and agricultural waste, food waste, sludge and wastewaters.
The benefits of composting are summarized below:

- Reduction of the municipal and agricultural trimming load otherwise directed towards landfill and incineration
- Production of valuable soil conditioners and amendments
    - Composts are used increasingly for their nutrient value, ability to build up soil organic matter, control soil erosion and also for their ability to suppress plant diseases.
- Contribution to circular economy

[1] http://ec.europa.eu/environment/circular-economy/index_en.htm
[2] https://eur-lex.europa.eu/eli/dir/1999/31/oj
[3] http://data.europa.eu/eli/dec/2003/33(1)/oj

- Job growth through integrated “green” bio-waste management and social enterprises
    - Composting is an economically valuable management tool for local government, nurseries and other sectors of horticulture that generate organic waste.
- Environmental awareness

Composting procedures greatly vary and tend to be highly empirical due to variation of the chemical composition of the raw materials and the environmental factors (O_2 diffusion, pH, C/N ratio, temperature and moisture content). Composting effectiveness and end-product optimization, therefore, requires not only understanding the composting process but evaluating the characteristics of the feedstock and end-product as well in all steps of composting.

1.2 Biological, chemical and physical processes of composting

Composting is a natural process that converts organic residues to stable organic matter while at the same time releasing nutrients[4]. Composting is a combination of biological, chemical and physical processes with micro-organisms having a key role. Industrial composting is utilizing the aerobic process by majority and to a lesser degree the anaerobic process. Aerobic process requires O_2 for microorganisms to function (Reaction 1), whereas anaerobic process utilizes alternative to O_2 pathways (anoxic or low O_2 environments; Reaction 2). Anaerobic composting processes are also known as digestion or fermentation.

$$\boldsymbol{Organic\ Waste} + \boldsymbol{O_2} \xrightarrow{\boldsymbol{microorganisms}} \boldsymbol{CO_2} + \boldsymbol{NH_3} + \boldsymbol{Compost\ product} + \boldsymbol{Energy}\ (\boldsymbol{heat})$$

Reaction 1. Schematic of the aerobic composting process, aerobic microbes consume organic waste and O_2 to produce a stable compost end-product. Through this biological process CO_2 and NH_3 are emitted and heat is released. NH_3 contributes to odor and air particle pollution.

$$\boldsymbol{Organic\ Waste} \xrightarrow{\boldsymbol{anaerobic\ microorganisms}} \boldsymbol{CO_2} + \boldsymbol{NH_3} + \boldsymbol{N_2O} + \boldsymbol{H_2S} + \boldsymbol{CH_4} + \boldsymbol{Compost\ product} + \boldsymbol{Energy}\ (\boldsymbol{heat})$$

Reaction 2. Schematic of the anaerobic composting process, under low or no O_2 environment anaerobic microbes consume organic waste to produce a stable compost end-product. Through this biological process besides CO_2 and NH_3 other by-products are emitted (N_2O, CH_4 and H_2S) and heat is released. N_2O and CH_4 are considered potent greenhouse gasses, and H_2S a strong corrosive and strong odor contributor. However, CH_4, that can be recovered from the composting biogas can be utilized for all the known applications of natural gas, i.e. heating, power production, etc.

[4] Goyal *et al.*, (2005) Biores. Technol. 96(14): 1584-1591

Typically raw materials (municipal- and agro-bio-wastes) are collected, mixed and size-reduced, gathered into a pile or a composting drum and then microbial degradation begins, until the maturation of a stable composting end-product (Figure 1).

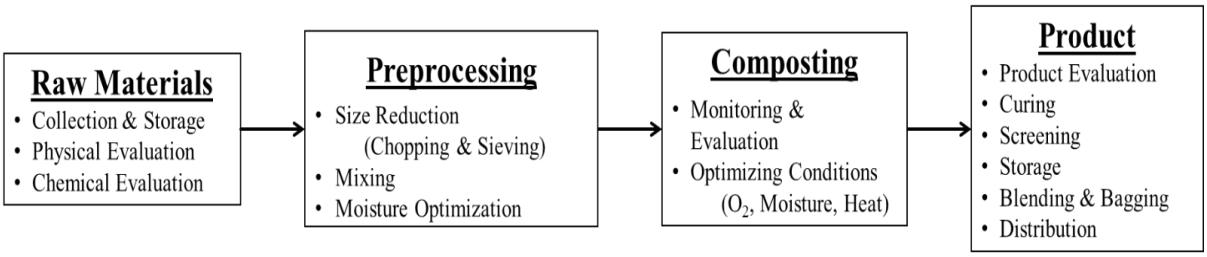

Figure 1. Chart flow diagram of common operations in a typical composting system

Temperature is the primary factor affecting microbial activity in composting. Therefore, composting process is typically divided in four stages: early, mid-exponential, thermophilic and curing, and maturing stage with characteristic heat profiles ranging from ambient – mesophic to thermophilic (15 – 75 ^{o}C).

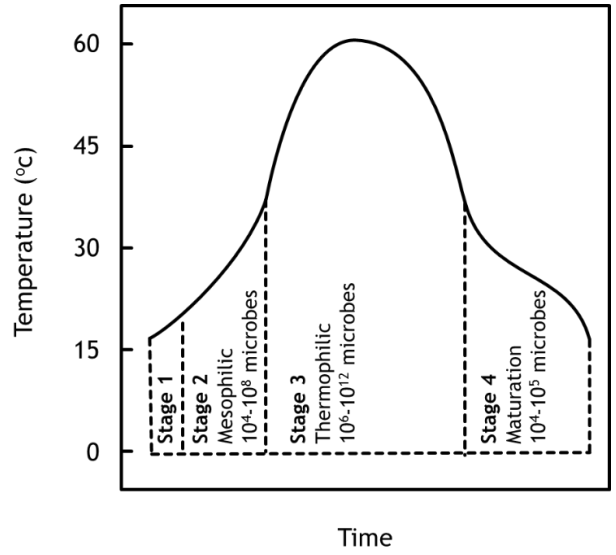

Figure 2. Schematic patterns of temperature and microbial growth in compost piles during aerobic composting.

Different microbes such as bacterial and fungi dominate each stage, however the end-product has low microbial population and in most cases, it is pathogen free. As the composting temperature rises from stage 2 to stage 3, pathogens are usually destroyed as the temperature reaches their thermal death points (50 – 60 ^{o}C). Temperature and microbial activity are excellent indicators to monitor the various stages of composting. Temperature is recorded with the use of long temperature probes either manually or automatically with the use of a data logger.

Moisture is also a critical criterion for optimum composting. It appears that moisture content of 50 – 60% is desirable. Water is essential for microbial growth, not only for microbes themselves, but also as a nutrient eluent prior to microbial uptake.

During composting heat may vaporize notable amounts of water. For example, the decomposition of 1 g of organic waste releases 25 kJ (heat) energy, which is enough to vaporize 10 g water. Consequently, moisture content should be monitored and maintained by water addition. The amount of water in a compost sample is usually determined gravimetrically after drying.

Microbial activity during composting chemically alters the composition of the organic waste. The chemical and elemental composition of raw material and end-products depends on the types of the compost feed materials. For that reason and simplicity, C and N are commonly used to monitor the composting process. The primary energy source is provided by C mineralization (microbial assimilation and respiration – CO_2), and N is critical for microbial assimilation and growth. Although various organic waste may have a wide range of C/N ratios (15 – 500), as shown in Figure 3, a much narrower C/N ratios (25 – 35) are required for effective composting. Low C/N ratios favor NH_3 emissions and high C/N ratios slow the decomposition process (Figure 4). Although mixing of various feedstocks to obtain a balanced and desirable C/N ratio of the mixture can be applied, this approach may not be appropriate for all organic material due to differences in their chemical composition and bio-degradability. Differences in bio-degradability can, in part, be explained in terms of cellulose, hemicellulose and lignin content of the organic waste (Figure). The total amounts of elemental C and N are determined by elemental analysis, whereas the total organic (volatiles) and inorganic fractions (ash) are determined by thermal/calcination protocols. The chemical and structural composition of the organic fraction biomass (lignocellulose) is determined by a two–stage acid hydrolysis protocol. All these protocols are described below in detail and have been applid for the analysis of representative waste biomass samples.

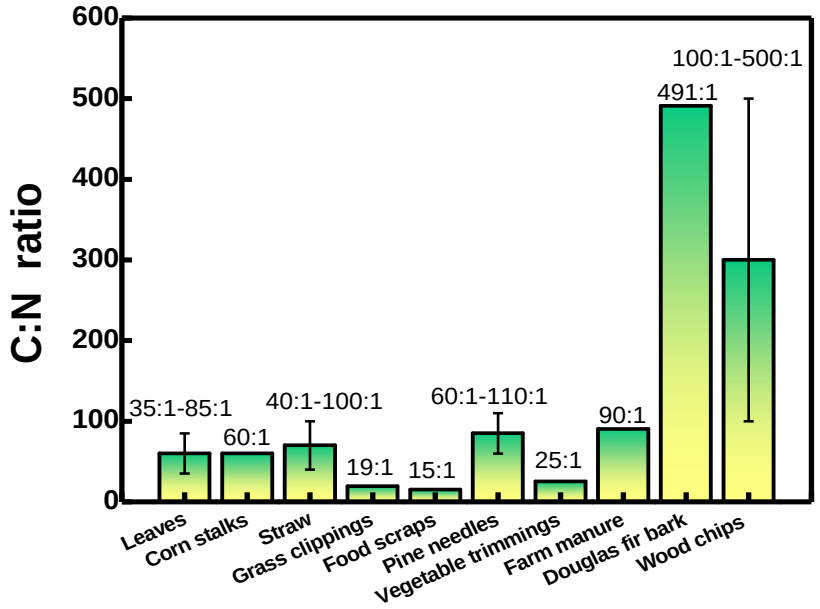

Figure 3. Differentiation of C/N ratio for a variety of biomass wastes. Based on data from ref.[5,6]

[5] http://compost.css.cornell.edu/chemistry.html
[6] Understanding the Composting Process, University of Arkansas, United States Department of Agriculture, and County Governments Cooperating

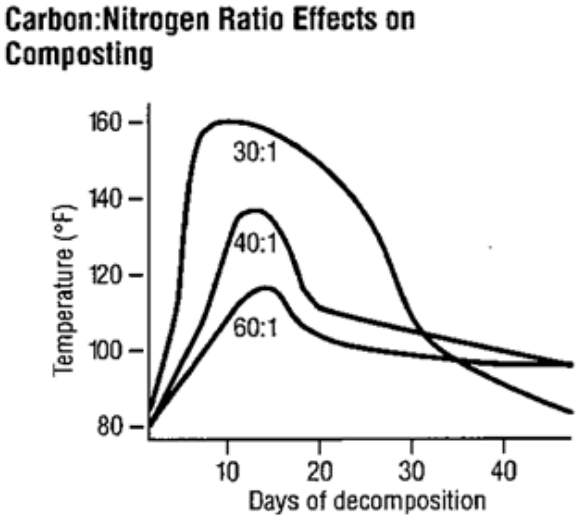

Figure 4. Effect of C/N ratio on compost process time[7].

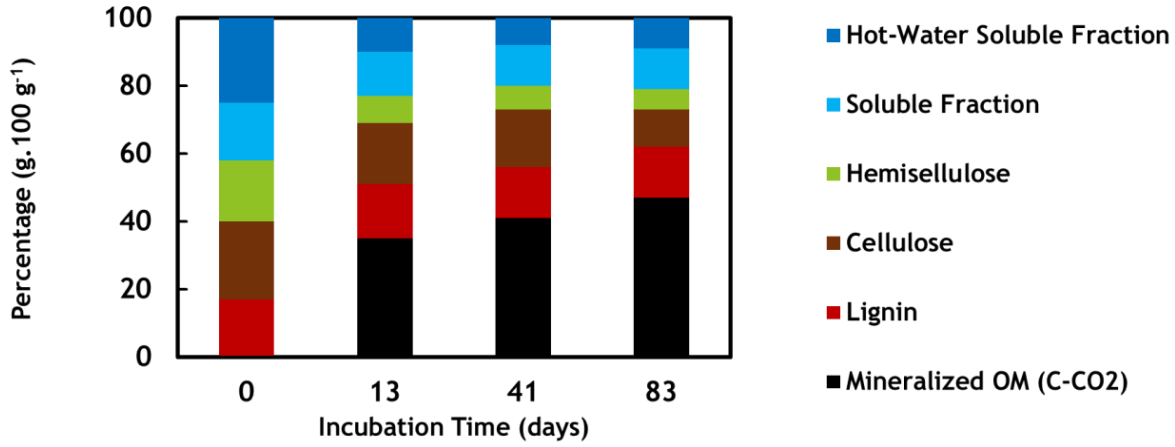

Figure 5. Bio-waste compositional changes during composting[8]

Another important parameter in the composting process is the particle size of the raw biomass feedstocks. Generally, smaller biomass particles allow the microorganism to digest more material and in shorter process time. As a consequence, chopping, shredding and chipping can accelerate the composting process, as can be observed in Figure 6. Particle size ranging between 1-8 cm is recommended.

[7] http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.htm
[8] **Lashermes *et al.*, 32 (2012): 271–277**

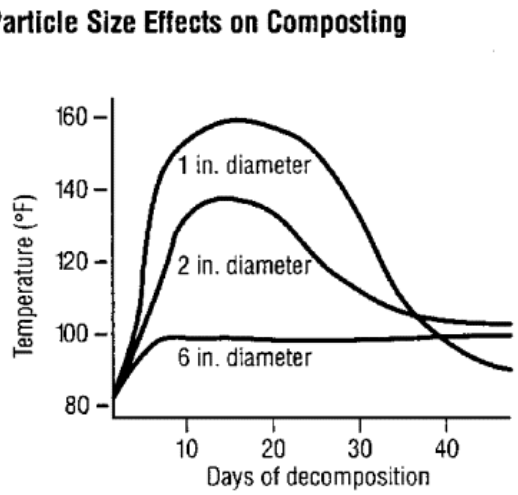

Figure 6. Effect of particle size on composting[9].

1.3 Importance of compost monitoring

It is critical and essential as the role of composting is developing in various economic sectors such as municipal and agricultural waste management to improve the capacity to reliably produce compost of consistent quality and the volumes required by each industry and end user. This can be done through consistent monitoring and evaluation guidelines of the raw material feedstock and end-product[10]. Although there is no single indicator to monitor the composting process, the integration of several physical, chemical and biological measurements could support composting optimization toward high quality products.

2. Materials (feedstock biomass wastes and related composts)

The lignocellulosic biomass wastes and related composts studied in this project can be divided into three groups: A) representative agricultural wastes, such as olive tree, vineyard and bitter orange pruning, almond shells, apricot kernels, and wheat stems, B) olive tree pruning (branches and leaves) and the respective compost samples at different stages (time) of composting, and C) mixtures of biomass wastes and related composts originated from the pilot compost facilities in the municipalities of Serres and Nestos.

Group A: Residues from representative agricultural crops, typically found in Northern Greece: olive tree prunings and leafs (Olea europaea L. cv. Chondroelia Chalikidikis), vineyard prunings (Vitis vinifera cv. Xinomavro), Bitter orange tree prunings and leafs (Citrus × aurantium cv. Seville), almond shells (Prunus amygdalus cv. Texas), apricot kernels (Prunus armeniaca) and wheat stems (Triticum aestivum). The samples are shown in Figure 7 and Table 1.

[9] http://whatcom.wsu.edu/ag/compost/fundamentals/needs_particle_size.htm
[10] Bernal et al., 1998 Maturity and stability parameters of composts prepared with a wide range of organic wastes. BIORESOURCE TECHNOLOGY, 63:1, 91-99

Olive tree prunings
Olea europaea L. cv. Chondroelia Chalikidikis

Vineyard prunings
Vitis vinifera cv. Xinomavro

Bitter orange tree prunings
Citrus × aurantium cv. Seville

Almond shells
Prunus amygdalus cv. Texas

Apricot kernels
Prunus armeniaca

Wheat stems
Triticum aestivum

Figure 7. Biomass residues from agricultural crops

Table 1. Representative agricultural wastes selected for chemical analysis and characterization

Common name	Scientific name	Agricultural green waste
Olive tree	Olea europaea L. cv. Chondroelia	Prunings (leaves & branches)
Populus tree	Populus deltoides	Prunings (braches)
Vineyard	Vitis vinifera cv. Xinomavro	Prunings (branches)
Almond shells	Prunus amygdalus cv. Texas	Shells
Bitter-orange tree	Citrus × aurantium cv. Seville	Prunings (leaves & branches)
Apricot kernels	Prunus armeniaca	Kernels
Wheat	Triticum aestivum	Straws

Group B: Compost samples from olive tree prunings (branches and leaves) at different composting stages: 3, 6, 9 and 12 months (Figure 8 and Table 2). Over 750 million olive trees (*Olea europaea*) are cultivated worldwide, 95% of which are in the Mediterranean region. Greece devotes 60% of its cultivated land to olive tree growing, approx. 1 million ha producing 350000 tn olive oil and holding 16% of the global olive oil production. Olives are grown for oil in Greece, with Peloponnese being the source of 65% of Greek production, as well as in Crete, the Aegean Islands and Ionian Islands. Since mid-19th century, olive tree cultivation in Northern Greece was restricted in the Chalkidiki region. In the latter years, olive tree cultivation has expanded across northern Greece. Currently, in the region of Serres, 4.7 thousand ha are cultivated for olive oil production. The most common cultivars are Chondroelia, Leukoelia and Petroelia. Olive tree cultivation requires soil preparation, fertilization, harvesting and pruning activities. Typically, 1 – 3 ton.ha^{-1} pruning residues are produced that consist of branches and leaves (Figure 9). Approx. 50 kg of fresh olive tree pruning were size-processed through a commercial type

shredder (~1-4 cm). Olive tree chippings were mixed with a small portion of compost inoculum (1 Kg) and were incubated for 12 months at ambient Mediterranean temperatures (September 2017 until September 2018). Every two weeks the compost pile was turned over. Samples were taken at 3, 6, 9 and 12 months.

Figure 8. Olive tree pruning (branches and leaves) and compost samples at different stages of composting

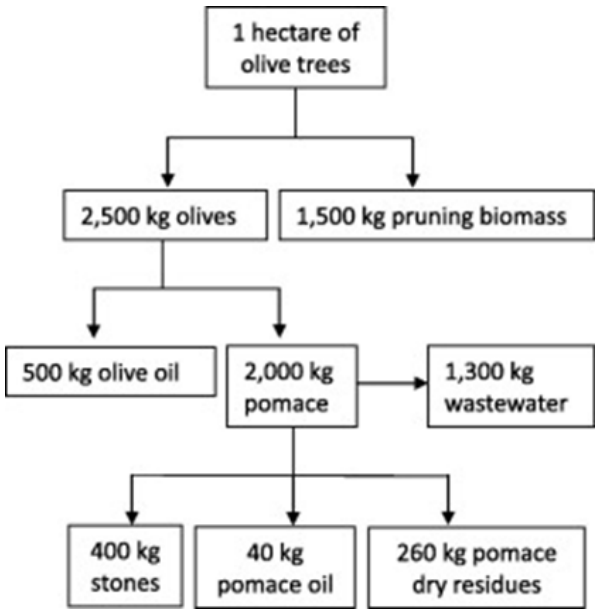

Figure 9. Approximate mass distribution of biomass residues evolved during cultivation and processing of olive trees and production of olives/olive oil.

Table 2. Compost samples (at different stage of composting) from olive tree pruning

Common name	Scientific name	Product
Olive tree	Olea europaea L. cv. Chondroelia	Compost 3 months

-//-	-//-	Compost 6 months
-//-	-//-	Compost 9 months
-//-	-//-	Compost 12 months

Group C: Biomass and compost samples from Nestos and Serres region. Samples from three different composting stages (initial biomass samples, early stage-2 months and final stage-9 months old compost - Figure 10) were collected from the municipality of Nestos and the composting facility that was developed within the frame of the "GreenCrew" project. The initial biomass wastes were a mixture of olive, plane and cypress tree pruning. One compost sample derived from plane and pine trees, lagerstroemia plant, vegetable wastes and wheat straws was collected from the small scale composting facility operated at the municipality of Serres (Figure 11).

Figure 10. Compost samples from the facility in the municipality of Nestos

Early stage compost samples *(2 months old, March 2020)*

Figure 11. Compost samples from the facility in the municipality of Serres.

3. Analytical protocols for the characterization of waste biomass feedstock and compost products

The analytical protocol followed for the characterization of raw biomass feedstocks and compost samples is shown in Figure 12.

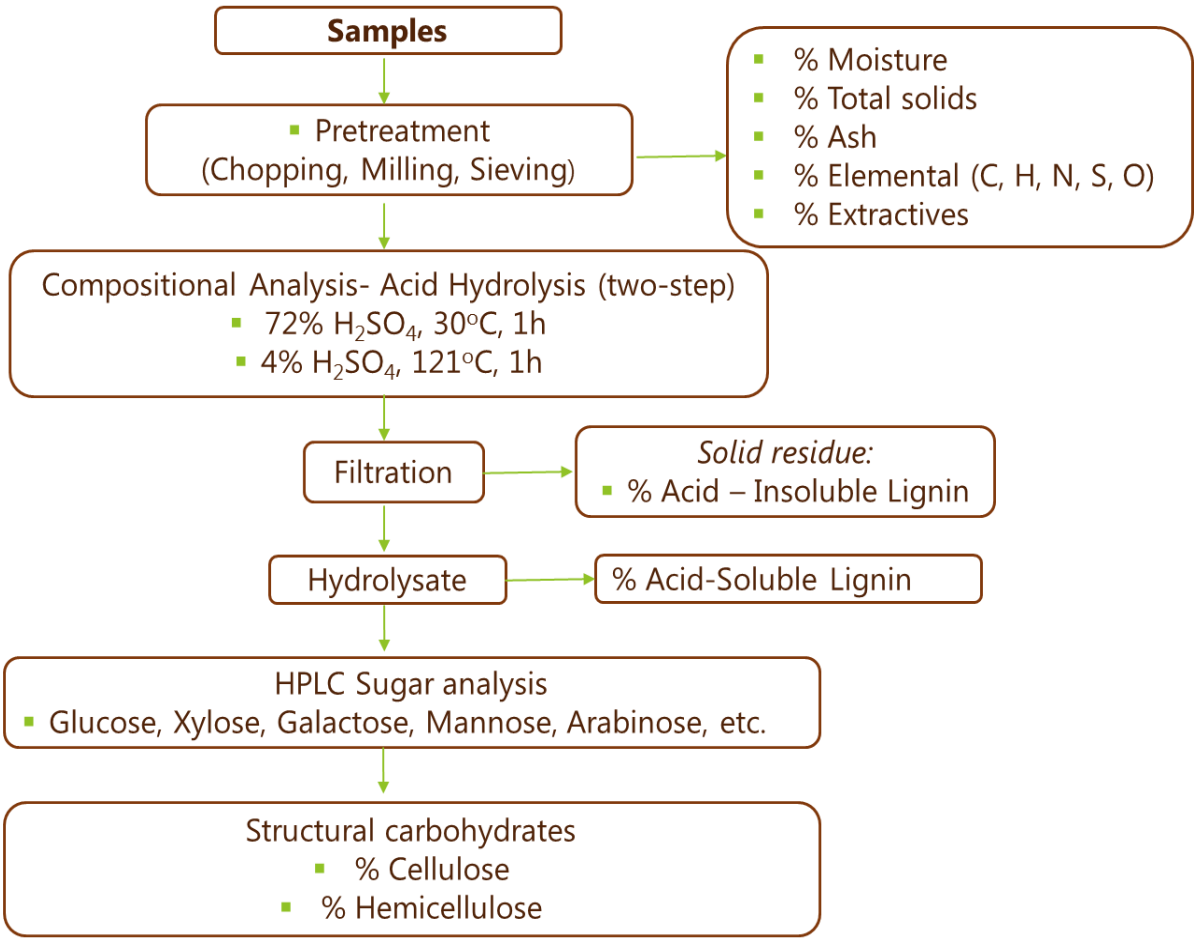

Figure 12. Schematic flow chart of typical physio-chemical characterization pipeline of raw materials and compost end-products.

3.1. Drying of raw biomass materials and compost samples prior to chemical analyses[11]

Drying is required to reduce sample moisture <10 % prior to any analysis and characterization. Composting facilities typically apply air – drying, whereas further drying in analytical laboratories require oven drying.

Air – drying procedure

Air – drying requires relatively high ambient temperatures and relatively dry air (~24 ºC, <50 % relative humidity). Biomass samples are spread evenly on a clean surface and turned over on a daily basis to ensure drying evenness and inhibit microbial growth. Air – drying may last up to 2 months or longer depending on the air properties. This procedure should produce samples with moisture contents below 10%. Dried samples are stored in air – tight containers for further analysis.

Oven – drying procedure

Oven drying requires a constant flow of dry air (45 ºC, 0-5 % RH) that is typical achieved with a laboratory oven. Alternatively, heating of the samples under static conditions and good aeration of the oven can also be applied effectively. Samples are spread evenly on ceramic or metal dishes and oven-dried until the change in the sample weight is less than 1% in 24 h. This process typically requires 48 h for drying small samples (1-2 kg). Dried samples are stored in air – tight containers for further analysis.

Important note: Drying the samples at relatively high temperatures (i.e. higher than ca. 100ºC) is not suitable for biomass analysis, because structural and chemical changes of the lignocellulosic components may occur upon heating.

3.2. Size-reduction

Size-reduction of the raw materials and compost products increases the particle surface area that is easily accessible for degradation, digestion or hydrolysis. This process is also essential in routine analysis to determine the chemical composition of the raw organics wastes and compost end-products. Size reduction is done initially towards a size range of ca. 1-5 cm, followed by further cutting towards particles of ≤ 2 mm (or even below 500 μm for specific analytical protocols, i.e. elemental analysis, etc.). The chips produced at the first stage are also appropriate for composting while the finer chips of smaller size are appropriate for physicochemical analysis as well as other (bio)chemical valorization processes, such as enzymatic hydrolysis, pyrolysis, carbonization, etc. Sieves of varying apertures are used to determine the size fractions of the chips.

[11] https://www.nrel.gov/docs/gen/fy08/42620.pdf

Size reduction for analytical protocol

Equipment:

1. Shredder (1st stage, rotary blades 2-10 mm; 2500 W)
2. Knife milling (2nd stage, ≤ 2 mm)
3. Grinder (for elemental analysis – fine powder ≤ 500 μm; IKA Tube mill, 20000 rpm)
4. Sieves with various mesh sizes i.e. Ø500 – 2000 μm
   Analytical balance (≤0.1 g resolution)

Procedure:

1. Collect approximately 5 Kg of raw biomass material or compost end-product and record their attributes (collection place and date, material and purity)
2. Shred material to ~2 – 10 mm size using a rotary shredder
3. Air – dry samples until constant moisture ≤ 10 % dw
4. For acid hydrolysis protocols (below) use knife-mill treated samples (Ø2000 μm)
5. For elemental analysis, grind ~ 10 g sample to fine powder using IKA Tube Mill (20000 rpm, 3 mins; 1 min hand sieving Ø 500 μm; x 3 times)
6. Store processed samples in air – tight containers

3.3. pH

pH is the in a solution and it is determined by immersing a pH probe into the solution. pH is either measured in the extracted compost liquid or in specified dilution volume (1:1 – 1:5 compost to liquid) with dH_2O or 0.001 M $CaCl_2$. The pH value of the compost end-product is very important as acidic pH inhibits seed germination and crop growth. The pH of finished composts products ranges from 6 – 7 and depends on the biomass feedstock. Compost feedstocks such as wood may be quite acidic, while others treated with lime may be a significant source of alkalinity. The pH determination of samples was carried out in extracted compost liquid.

Equipment:

1. pH probe with controller
2. Filter papers

Procedure:

1. Weight about 1g of compost sample
2. Add 10 ml d. H_2O and leave the suspension under stirring for 1h.
3. Afterwards, decant the liquid and measure the pH with the pH meter.

3.4. Moisture content determination[12]

Moisture is a critical factor not only describing the physical properties of the feedstock and compost end-product but also important regulator of microbial degradation, as dry conditions inhibit microbial growth whereas too wet conditions may favor anaerobic hotspots and poor air permeability slowing the composting process. Moisture content is easily and accurately determined by drying and weighing a sample.

Moisture content determination protocol

Equipment:

1. Drying oven (≥105 ^{o}C) with programmable temperature settings
2. Porcelain crucibles Ø50 mm or Ø117 mm
3. Analytical balance (≤0.1 g resolution)

Procedure:

1. Weigh approx. 10 g (± 0.1 g) of biomass sample (after equilibration for 1 month at ambient conditions) in dry porcelain crucibles.
2. Dry samples in an oven at 105 ± 5 oC for ≥ 12 h and until constant weight (preferably overnight 24 h).
3. Calculated moisture content (%) based on the weight difference before and after drying, using the following equation.
4. Dried samples can be stored for further analysis, i.e. ash, volatile solids and chemical composition.

$$Moisture\ (\%) = 100 * \frac{W_{(sample+cup)} - W_{105(sample+cup)}}{W_{(sample+cup)}}$$

3.5. Ash content and volatile solids [13]

Ash content (AC) is the inorganic content of the sample per dry weight (% dw), whereas volatile solids (VS) refers to organic fraction (indication of organic matter in the sample) of the sample lost during ignition (calcination). The combustion/calcination method relies on high temperature oxidation (≥550^{o}C) and direct weight measurements of the volatile organic fraction lost. Ash content and volatile solids was determined according to NREL TP-510-42622 standard operating protocol (SOP) which is similar to ASTM E1755-01.

Ash content and volatile solids determination protocol

Equipment:

1. Muffle furnace (≥575 ^{o}C) with programmable temperature settings

[12] https://www.nrel.gov/docs/gen/fy08/42621.pdf
[13] https://www.nrel.gov/docs/gen/fy08/42622.pdf

2. Porcelain crucibles
3. Analytical balance (≤0.1 mg resolution)

Procedure:

1. Weigh approx. 3 g of oven-dry biomass or compost in dry porcelain crucibles.
2. Combust samples in a muffle furnace following the program below (
3. $Ash\ (\%\ dw) = 100 * \frac{W_{575\ (cup+ash)} - W_{cup}}{dw_{sample}}$

$$Volatile\ Solids\ (\%\ dw) = 100 - Ash\ (\%\ dw)$$

4. Table 3).
5. Calculated ash and volatile solids content (%) as the weight difference before and after the loss-on-ignition per dry basis using the following equations.
6. Ash samples can be stored for further analysis, i.e. trace metal determinations by ICP-OES/AES.

$$Ash\ (\%\ dw) = 100 * \frac{W_{575\ (cup+ash)} - W_{cup}}{dw_{sample}}$$
$$Volatile\ Solids\ (\%\ dw) = 100 - Ash\ (\%\ dw)$$

Table 3. Combustion temperature program for ash and volatiles determination

Temperature (Set-point; ^oC)	**Hold Time** (mins)	**Ramp Rate** (^oC/min)
105	12	---
250	30	10
575	180	20
105	Hold	---

The semi-quantitative analysis of the chemical composition of ash (expressed as % element of the metals) was performed by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS), using a JEOL 6300 microscope equipped with an energy dispersive spectroscopy (EDS) system for X-ray microanalysis (OXFORD ISIS 2000).

3.6. Elemental analysis

Total C and N are related to feedstock and end-product quality. Total C and N is the sum of inorganic and organic forms of C and N in the sample, respectively. The combustion method is a simple, fast precise and direct method of measuring C and N percentage. The sample is rapidly oxidized at high temperatures (e.g. 950-1000^{o}C) and the produced gases are reduced, allowing the determination of C as CO_2 and of N as N_2 (derived from NOx reduction) by a thermal conductivity detector.

Elemental Carbon (C) and Nitrogen (N) Determination Protocol

Equipment:

1. Total Elemental Analyzer (EA)
2. Analytical balance with ≤0.01 mg resolution

Procedure:

1. Weigh approximately 1 – 5 mg of sample
2. Carefully enclose the sample in a tin capsule
3. Select the appropriate method (defined by the manufacturer) and standards for total elements analysis
4. C and N content is typically reported as % per dry basis.

3.7. Determination of extractives[14]

Extractives of lignocellulosic biomass could be determined via the two-step extraction procedure using water as ethanol as solvents. The water extractives are initially isolated using a Soxhlet apparatus and the procedure is repeated with ethanol as solvent.

Equipment:

1. Analytical balance (with ≤0.1 mg resolution)
2. Oven (heating at 105 ^{o}C)
3. Vacuum oven (heating at 40 °C)
4. Soxhlet apparatus
5. Heating mantles
6.Condensers with appropriate fitting for Soxhlet tubes
7.Single thickness cotton cellulose thimbles, 94 mm external length by 33 mm internal diameter
8. Rotary evaporator with trap and water bath set to 40 °C

Procedure:

1. Dry flasks and all glassware at 105 °C in a drying oven for a minimum of 12 hours. Remove the glassware and allow it to come to room temperature in a desiccator; record the dry weight of the flasks; then add boiling stones to the flasks.
3. Add 2-10 g of sample to a tared extraction thimble. Record the weight to the nearest 0.1 mg.
4. Assemble the Soxhlet apparatus.
5. Add 190 mL of HPLC grade water to the tared receiving flask. Place the receiving flask on the Soxhlet apparatus. Adjust the heating mantle temperature to provide a minimum of 4-5 siphon cycles per hour.
6. Reflux for 6-24 hours.

[14] https://www.nrel.gov/docs/gen/fy08/42619.pdf

7. When reflux time is complete, turn off the heating mantle and allow the glassware to cool to room temperature.
8. Leave the thimble in the Soxhlet extractor, removing as much residual water from the Soxhlet tube as possible.
9. Add 190 mL ethyl alcohol to the tared ethanol receiving flask. Place the receiving flask on the Soxhlet apparatus. Adjust the heating mantle temperature to provide a minimum of 6-10 siphon cycles per hour.
10. Reflux for 16-24 hours.
11. When reflux time is complete, turn off the heating mantle and allow the glassware to cool to room temperature.
12. Remove the thimble and transfer the extracted solids, as quantitatively as possible, onto cellulose filter paper in a Buchner funnel. Wash the solids with approximately 100 mL of fresh ethanol. Allow the solids to dry using vacuum filtration and subsequently ambient air.

3.8. Acid Hydrolysis (two – stage) for structural sugar analysis of biomass samples[15]

The lignocellulosic biomass samples, after the water/ethanol extraction step described in 3.7, comprises of the three main structural components, i.e. cellulose, hemicellulose and lignin. The determination of their content is based on a two-step acid hydrolysis procedure according to the NREL TP-510-42618 protocol. During hydrolysis the polymeric carbohydrates are hydrolyzed into the monomeric sugars, which are determined by high pressure liquid chromatography (HPLC). At the same time, a small portion of lignin dissolves, providing the acid soluble lignin (ASL) and the acid insoluble material, which in addition to the acid insoluble lignin (AIL) may also include ash, which must be accounted for the compositional balance. The ASL is measured by UV spectroscopy.

Important note: In order to determine the content of the three main structural components/biopolymers, i.e. cellulose, hemicellulose and lignin, the extractives (and inorganics/ash if present at high content) should be first removed. It also recommended to use dry biomass samples during the acid hydrolysis protocol in order not to affect the acid concentration.
In case that the acid hydrolysis protocol is applied directly to the parent biomass, with removal of the extractives, then the determined sugars represent the content of the total sugars and not of the “structural” sugars, i.e. those present in cellulose and hemicellulose.

Acid Hydrolysis (two – stage) Protocol

Equipment:

[15] https://www.nrel.gov/docs/gen/fy13/42618.pdf

1. Pressure reaction (glass) vessels
2. Autoclave (pressure cooker)
3. Water bath with temperature controlled at 30 ^{o}C
4. Vacuum pump and Buchner filter
5. Filter papers (1-2 μm)

Procedure:

1. Weigh approximately 350 mg of sample into pressure vessel
2. Digest sample with concentrated acid (72% sulphuric acid H_2SO_4), mix well with a glass rod or votrex
3. Incubate samples at 30 ^{o}C for 1 h in a water-bath
4. Dilute acid to 4% by adding 84 ml dH_2O
5. Autoclave samples at 120 ^{o}C for 1 h
6. Let samples to cool down or use compressed air to aid faster cooling
7. Separate liquids from solids using pre-weighted (dry) filters in a Buchner vacuum system
8. Collect liquid samples (hydrolysates), neutralize a portion with $CaCO_3$*
9. Store (-20 ^{o}C) liquid samples for further analysis.
10. The recovered solids are further treated as described in 3.9.

***Important note:** Proceed with $CaCO_3$ neutralization and HPLC analysis as soon as possible after acid hydrolysis. Hydrolysates are not stable and sample (sugars) degradation is possible.

3.9. Acid Insoluble Lignin (AIL) in biomass[15]

Acid insoluble lignin (AIL), frequently referred as *Klason* lignin, is considered as high molecular weight lignin. This is the main portion of the biomass remaining after the acid hydrolysis, the (minor) rest being the inorganics/ash.

Acid Insoluble Lignin Determination Protocol

Equipment:

1. Drying oven (105 ^{o}C)
2. Analytical Balance (≤ 0.1 mg resolution)

Procedure:

1. Collect the acid insoluble solid fraction from step **7** of protocol **3.8** on a pre-weighted dry filter
2. Dry the filter and the containing solids overnight at 105 ^{o}C
3. Calculate the acid insoluble solids (AIS) % on dry biomass following the equation bellow.

$$AIS\ (\%\ dw) = 100 * \frac{W_{105(Filter+solids)} - W_{Filter}}{W_{dw}\ (biomass\ sample)}$$

4. Conduct the protocol 3.5. for ash content determination, on the recovered dry AIS, in order to determine which portion (usually very low) is attributed to inorganics/ash and which to volatile acid insoluble lignin (AIL).
5. Calculate the AIL (%) content on dry biomass basis, using the measured AIL weight in the equation above, instead of AIS.

3.10. Acid Soluble Lignin (ASL) in biomass

Acid soluble lignin (ASL) is low molecular weight lignin that is solubilized during the acid hydrolysis step (see 3.8).

Acid Soluble Lignin Determination Protocol

Equipment:

1. UV Spectrophotometer and HPLC with Diode Array UV-Vis Detector

Procedure:

1. Collect the soluble fraction from step 7 protocol 3.8
2. Select the appropriate standards and method for the determination of phenolics/ASL
3. Run a background dH_2O or 4 % H_2SO_4 blank on the UV Spec
4. Aliquot 2 ml of hydrolysis liquid to a UV cuvette (Quartz crystal)
5. Determine the absorption at 205 nm; if absorption > 0.8 dilute sample with dH_2O or 4 % H_2SO_4
6. Calculate the Acid Soluble Lignin (ASL %) percentage on dry biomass basis following the equation bellow.

$$ASL\ (\%\ dw) = \frac{ABS_{205} * Volume(ml) * Dillution}{\varepsilon\ (HW\ or\ SW) * W_{dw}(mg) * Wavelength\ (cm)}$$

With emission factor (ε) for hardwood biomass ε_{HW} = *25 or softwood biomass* ε_{SW}=*12* $L.g^{-1}.cm^{-1}$

3.11. Structural Sugars [15]

The determination of structural sugars, i.e the contents of cellulose and hemicellulose, was performed by analysis of the acid hydrolysates of 3.8 with HPLC.

Structural Sugars Determination Protocol

Equipment:

1. HPLC (Prominance; Shimadzu) with Refractive Index Detector (RID; Shimadzu)

Procedure:

1. Select the appropriate standards and method for the determination of sugars (column SP0810) and organic acids, mainly acetic acid (column SH1011)
2. Collect the soluble fraction (hydrolysate) from step 7 protocol 3.8
3. Transfer approximately 20 ml in a 50 ml Erlenmeyer flask, and neutralize sample to pH 5 – 6 with calcium carbonate, allow the solids to settle and collect the supernatant.
4. Pass the supernatant through a 0.2 μm filter prior to HPLC analysis
5. Select the appropriate method and calibration line for each compound
6. Determine the concentration of each monomeric sugar and organic acid (if present) in mg/ml.
7. The cellulose content is calculated (based on the equation below) from glucose concentration using a correction factor of 0.9.
8. The hemicellulose content is calculated (based on the equation below) from the concentration of arabinose, galactose, xylose, and mannose, using a correction factor of 0.88 for the C-5 sugars and 0.90 for the C-6 sugars.
9. The determined concentration of acetic acid represents the acetyl units existing in the lignocellulose structure and are being considered in closing the analysis mass balance.

Note: cellobiose concentration > 3 $mg.ml^{-1}$ indicates incomplete hydrolysis

$$Carbohydrate\ (cellulose)(\%\ dw) = 100 * \frac{[glucose\ conc.(mg.ml^{-1})] * 0.9\ (Correction\ factor) * Hydrolysate\ vol.(ml)}{SRSglucose * W_{dw}(mg) * 1000}$$

Where:
SRS = Sugar Recovery solution = concentration of sugar, i.e. glucose, determined by HPLC (mg/ml) in a solution with known concentration of the sugar that has been subjected to the acid hydrolysis protocol, as described for the biomass samples, divided by the known sugar concentration

W_{dw} = oven dry weight = biomass sample weight excluding the determined moisture

3.12. Bulk density of compost

The bulk density of compost was measured according to the following procedure: 2.5 g of solids (particle size ≤ 1 mm) were added in a volumetric cylinder, slightly compacted with a rod to avoid large void spaces and the volume was measured. The bulk density was calculated by dividing the weight of solid by the volume of the solid in the cylinder.

3.13. Porosity characteristics of compost

The porosity of the compost was determined via nitrogen adsorption-desorption porosimetry at -196 ^{o}C using an Autosorb 1MP (Quantachrome) porosimeter. More specifically, the BET surface area and the total pore volume were determined by N_2 porosimetry, with these two parameters being indicative of the degradation degree of the initial rigid lignocellulose structure and the available surface for microbes to act. It also provides an indication of the sorption capabilities of the produced compost. Prior to the measurements, all samples were outgassed at 80 ^{o}C for 19 h.

4. Results

Representative results of the physico-chemical characterization and analysis of the parent lignocellulosic biomass wastes and related composts are reported and discussed in this section. The results have been organized in accordance to the three groups of materials described in section 2.

4.1. Residues from representative agricultural crops

The moisture, ash and volatile solids contents of representative agricultural residues from Northern Greece are shown in Table 4. All the biomass samples studied exhibited moisture content less than 10% and could be further used in the acid hydrolysis protocol for the determination of their chemical/structural composition. The ash content ranged between 0.4 and 9.3%. The less ash content is observed for apricot kernels while the higher amount was measured for the bitter orange tree prunings (branches and leaves). The semi-quantitative chemical composition analysis of ash by SEM-EDS, showed the presence of Na, Mg, Al, Si, P, S, Cl, K and Ca. The elemental distribution in ash is shown in Figure 13. The most abundant elements in ash samples are potassium (K) and calcium (Ca) while the less abundant is chlorine (Cl), with the exception of wheat straws which exhibited higher amounts of Cl and sulfur (S) as well as relatively high content of Si and Mg. In order to determine the ratio C/N, essential for the compost process, elemental analysis was performed on the agricultural wastes. Carbon content was found to be in the range of 45-54 wt.%, hydrogen content in the range of 5.6-8.0 % and nitrogen between 0.3-1.5%. The C/N ratio ranges between 32-159. Taking into account that the recommended C/N ratio is ~30, poplar prunings (branches) and bitter orange prunings (branches & leaves) appear to be more suitable for efficient composting.

Table 4. Moisture, ash, volatile solids and elemental analysis on dry basis of selected agricultural wastes.

Biomass feedstock	% Moisture	% Ash	% Volatile solids	%N	%C	%H	%S	%O	C/N
Olive tree prunings-branches & leaves	7.8	3.5	96.5	0.9	49.1	6.6	0.1	43.3	52
Vineyard pruning/branches	9.7	3.2	96.8	0.5	44.9	7.9	-	46.7	88
Poplar prunings/branches	6.9	6.8	93.2	1.5	48.2	6.2	0.1	44.1	32
Bitter orange tree prunings-branches & leaves	8.2	9.3	90.7	1.3	46.2	5.6	0.1	46.8	36

Wheat straws	9.7	4.6	95.4	0.5	43.1	7.4	-	49.0	91
Almond shells	9.8	1.6	98.4	1.4	54.1	6.6	0.1	43.3	52
Apricot kernels	5.4	0.4	99.6	0.3	49.7	8.0	-	42.0	159

Note: %O is calculated as the mass difference among the sample and its detected elements.

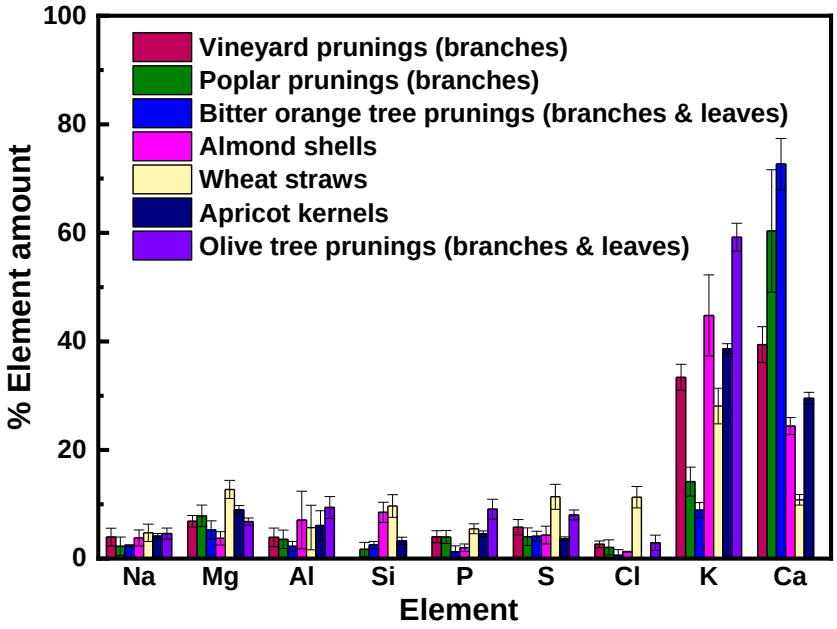

Figure 13. Element distribution in ash samples of the agricultural residues.

The composition (sugar monomers, cellulose, hemicellulose, lignin and extractives) of selected agricultural wastes was determined via the initial extraction protocol (section 3.7) and the acid hydrolysis protocol (section 3.8). Total extractives were determined as the sum of water and ethanol extractives, total lignin as the sum of acid insoluble (AIL) and acid soluble lignin (ASL) and total sugars as the sum of glucan (Glu), xylan (Xyl), galactan (Gal), arabinan (Ara) and manan (Man) compounds. Two typical chromatographs of a hydrolysate and the standard curves are shown in Figure 14 and 15, respectively.

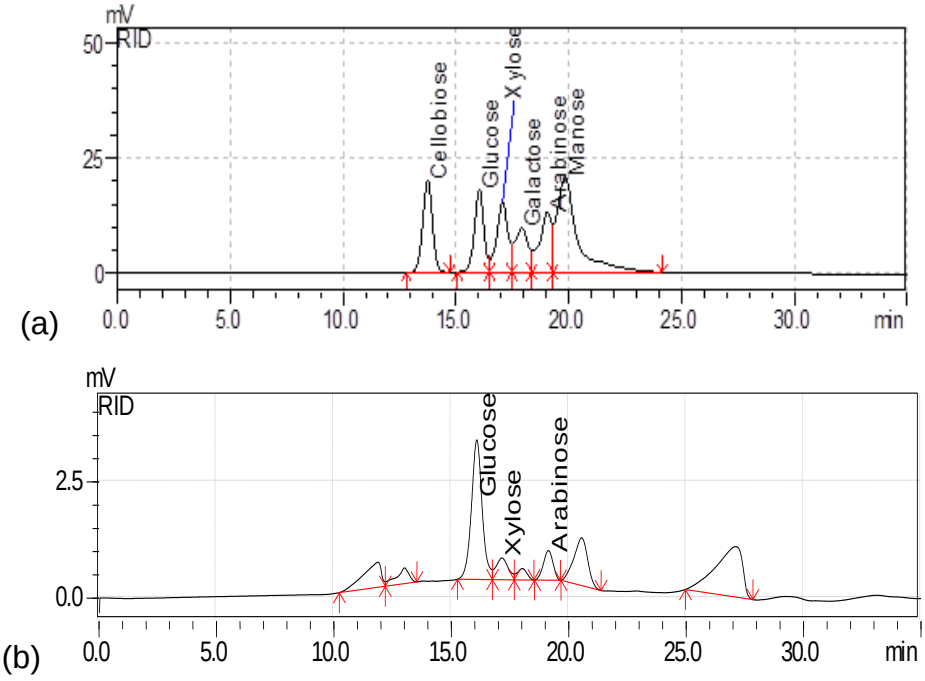

Figure 14. Typical HPLC chromatographs of (a) sugars standards solution (2.5 mg.ml^{-1}) and (b) hydrolysate of an olive tree pruning compost sample.

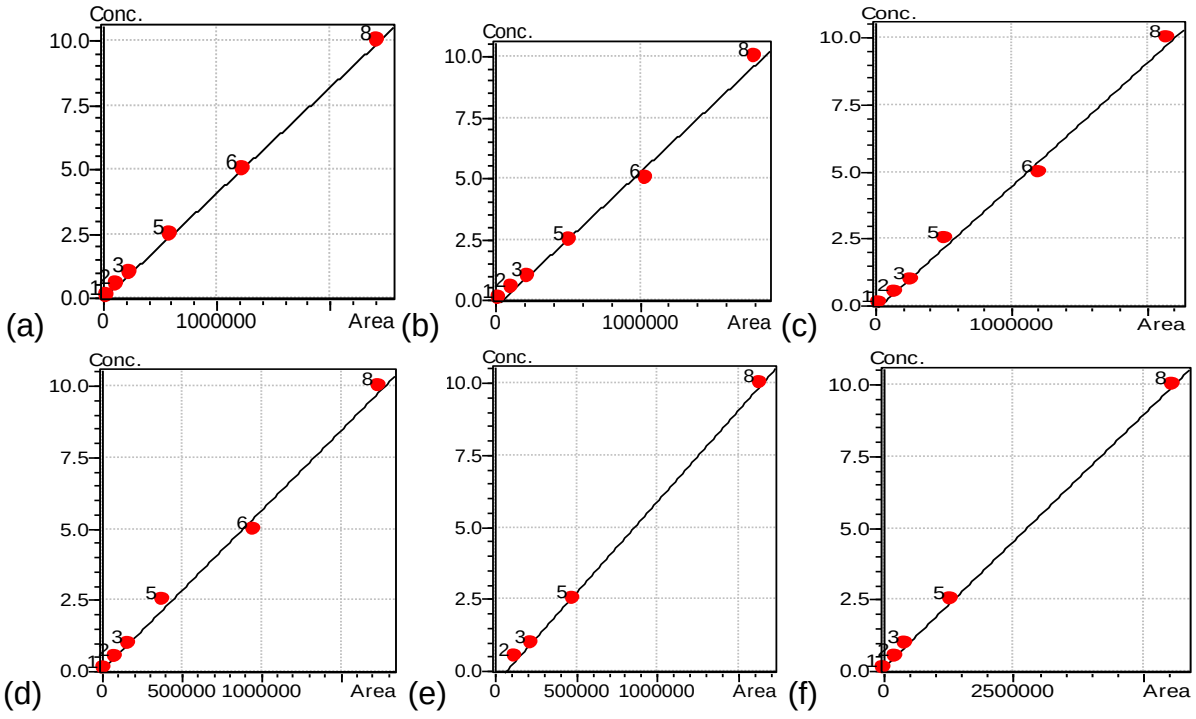

Figure 15. Standard curves of (a) cellobiose - retention time 13.66 min – r^2=0.99, (b) glucose - retention time 16.05 min – r^2=0.99, (c) xylose - retention time 17.05 min – r^2=0.99, (d) galactose - retention time 18.16 min – r^2=0.99, (e) arabinose - retention time 19.09 min – r^2=0.99 and (f) mannose - retention time 19.95 min – r^2=0.99.

With regard to the extractives of agricultural wastes, the higher amount is exhibited by olive tree prunings (branches and leaves) (21.6 %wt). Generally, the lignocellulosic biomass extractives are composed of soluble sugars (glucose, etc.), pectins, fatty acids, esters of fatty acids, etc.

With regard to the total sugars and lignin contents (Table 5, wt.% dry basis), the wheat straws, almond shells and poplar prunings (branches) exhibited the higher sugar content while olive tree prunings (branches and leaves) showed the higher lignin content. The high sugar content and the less lignin content will accelerate the composting process, facilitating the microorganism growth and activity. In addition to the analysis of the parent biomass, the analysis of the extractives-free olive tree prunings was also performed and the obtained results are also shown in Table 5 (second row). It is clear that when applying the acid hydrolysis protocol to an extractives-free biomass (at least for the olive tree prunings), the mass balance closes at much higher values, closer to 100%, as this protocol is better suited for “pure” lignocellulosic materials with no pectins, fatty acids, etc.

Table 5. Composition of selected agricultural wastes (wt.%, dry base).

Biomass feedstock	% Extractives	AIL	ASL	Total Lignin	Glu	Xyl	Gal	Ara	Man	Total Sugars	Mass(**) balance
Olive tree prunings/ branches & leaves	21.6	39.3	4.9	44.2	13.1	4.3	2.8	-	3.3	23.5	71.4
-//- (*)	-	40.5	3.3	43.8	38.4	3.7	2.5	-	2.2	46.8	91.0
Vineyard prunings/ branches	17.8	19.1	2.0	21.1	32.6	8.1	2.7	2.1	-	45.5	70.2
Poplar prunings/ branches	18.9	18.0	1.0	19.0	40.5	14.1	0.9	1.5	3.8	60.8	80.1
Bitter orange tree/branches & leaves	n.d	24.8	0.1	24.9	39.5	14.5	-	-	-	54.1	-
Wheat straws	19.3	6.2	3.2	9.4	30.8	18.6	2.1	9.8	0.2	61.6	75.7
Almond shells	9.5	30.3	2.4	32.7	28.2	22.7	6.2	1.5	2.9	61.5	95.5
Apricot kernels	6.2	33.8	2.7	36.5	19.2	15.8	10.9	2.1	1.4	49.4	85.8

(*) Extractives-free olive tree prunings

(**) The ash content (from Table 4) have been included in the mass balance estimation whereas the content of acetyl units (determined by HPLC via measuring the acetic acid in the hydrolysates), typically being between ca. 1-5 wt.% on dry biomass, should be added on top of the mass balance values shown in Table 5. The content of extractives is not included in the mass balance.

4.2. Compost samples from olive tree prunings (branches and leaves) at different composting stages

The physical properties of olive tree prunings (branches and leaves) derived compost samples examined were moisture, bulk density and porosity. Moisture content of all compost samples (i.e. produced after a composting period of 3, 6, 9 and 12 months) which have been previously equilibrated for 1 month at ambient conditions, was relatively lower than that of the initial biomass feedstock, ranging from 4.7-7.2 wt.%. Bulk density values were similar for all samples, i.e. ~ 0.40-0.41, as can be also seen in Table 6, thus indicating that the bulk density does not change significantly over the 12-months period of composting compared to the density of the parent biomass, at least for this type of feedstock (olive tree branches and leaves). The surface area and total pore volume, determined by N_2 porosimetry (Table 6), of the compost samples were higher compared to those of the parent biomass, but with no systematic increase with composting time.

Table 6. Physical properties of olive tree prunings-leafs compost samples at different compost stages.

Biomass feedstock	% Moisture	Bulk density (g/ml)	S_{BET} (m_2/g)	V_p (cm^3/g)
Olive tree prunings (branches & leaves)	7.8	0.409	0.9	0.004
Compost 3 months old	4.7	0.406	2.3	0.006
Compost 6 months old	6.0	0.401	1.3	0.009
Compost 9 months old	7.0	0.412	1.1	0.009
Compost 12 months old	7.2	0.402	1.5	0.010

The chemical properties and characteristics of the compost samples produced from olive tree prunings (branches and leaves), that were studied were the pH, ash and volatile content as well as the elemental (N, C, H, S, O) composition (Table 7). The pH increases substantially at the initial stages of composting (e.g. first 6 months) from 5.2 (parent biomass) to 7.3, and then remains almost stable, with values of 7.4-7.5 at 12 months composting. Similar results are also presented in the literature and the optimum pH values of compost were found to be 5.5-8.0[16]. Ash content is also increased due to the elimination of organic matter and enrichment of samples in inorganic compounds. From the elemental analysis, carbon was shown to be decreased as a consequence of composting while nitrogen was increased. C/N ratio was also decreased. Summarizing the chemical properties of compost, in Figure 16, the pH showed a significant increase with the time of composting process while carbon content and C/N ratio decreased.

[16] Jain et al. Sustainable Environment Research (2019) 29:9

Table 7. Chemical properties of the compost samples produced from olive tree prunings (branches and leaves) at different stages of composting

Biomass feedstock	pH	% Ash	% Volatile solids	%N	%C	%H	%S	%O	C/N
Olive tree prunings/branches and leaves	5.2	3.5	96.5	0.9	49.1	6.6	0.1	43.3	52
Compost 3 months old	6.2	4.3	95.7	1.3	50.4	8.5	-	39.8	39
Compost 6 months old	7.3	10.6	89.4	1.3	46.3	7.6	-	44.7	35
Compost 9 months old	7.5	8.7	91.3	1.6	44.9	7.8	-	45.7	27
Compost 12 months old	7.4	8.9	91.1	1.5	40.1	6.9	-	51.6	27

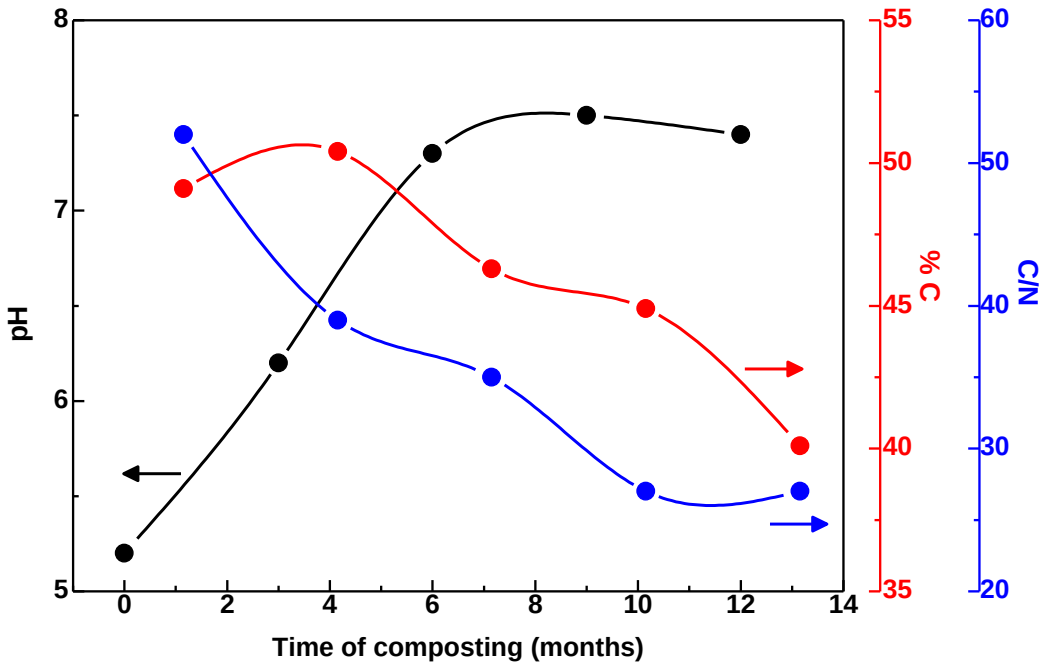

Figure 16. Variation of chemical properties with composting time (of olive tree prunings – banches and leaves)

The process of biomass degradation, and especially of cellulose, was also monitored by X-ray powder diffraction. In the patterns of Figure 17, it can be observed that the intensity of the peaks at 2theta ≈ 16 and 22^{o} corresponding to crystalline cellulose, is significantly decreased, at the latter stages of composting.

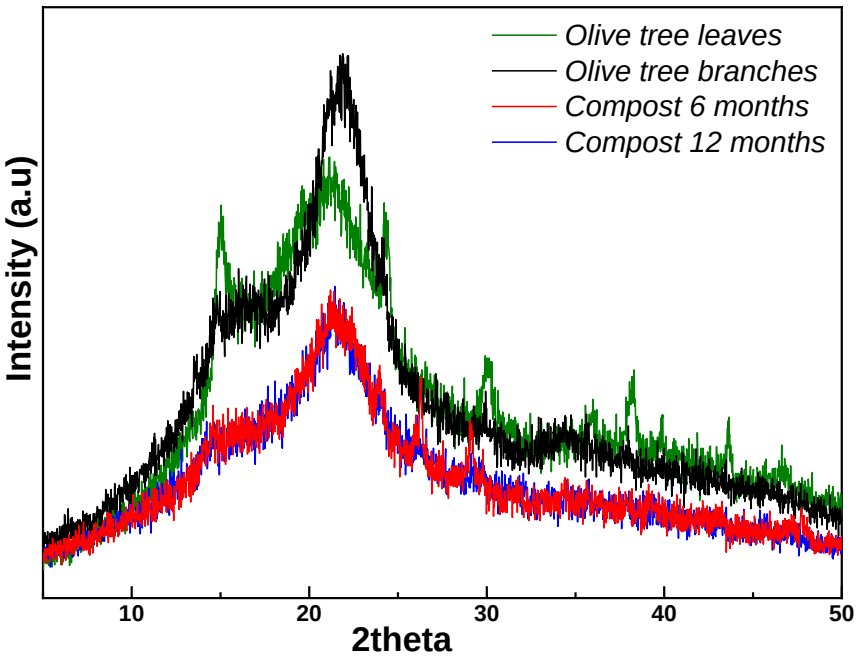

Figure 17. XRD patterns of olive tree prunings compost samples.

The composition analysis results of the parent biomass and of the related compost sample produced after 12 months composting time, are shown in Table 8. The analysis data was reported for both the initial biomass and compost samples, as well as after removal of the extractives. In the initial biomass feedstock, total sugars are about 23.5% and total lignin 44.2%. The composting process resulted in decrease of total sugars due to their consumption by the microorganisms while lignin content was increased, as lignin is not easily digested by the bacteria and fungi involved in the composting process. The extractives were also decreased indicating that sugars and other components in the extractives can be consumed by the microorganisms.

In the extractive-free biomass sample, total lignin was similar to that of the initial biomass sample. However, the ASL is higher for the initial sample, indicating that some soluble phenolics are present in the extractives. The content of sugars (mainly of glucose) is relatively low in the analysis of the initial biomass sample, as well as of the initial compost sample, and this needs further systematic investigation. When compared the analysis data of the two extractives-free samples, it is shown that structural sugars are also decreased (as the total sugars) and lignin is increased.

Table 8. Composition of olive tree prunings (brances & leaves) and the derive compost after 12 months (wt.%, dry base).

Biomass feedstock	% Extractives	AIL	ASL	Total Lignin	Glu	Xyl	Gal	Ara	Man	Total Sugars	Mass(**) balance
Olive tree prunings/branches & leaves	21.6	39.3	4.9	44.2	13.1	4.3	2.8	-	3.3	23.5	71.4
-//- (*)	-	40.5	3.3	43.8	38.4	3.7	2.5	-	2.2	46.8	91.0
Compost 12 months old	13.9	44.7	5.2	49.9	8.9	4.3	3.0	-	3.2	19.4	78.2
-//- (*)	-	41.3	3.8	45.1	33.8	4.3	3.3	-	2.7	44.1	98.7

(*) Extractives-free samples of olive tree prunings and of 12-months old compost

(**) The ash content (from Table 4) have been included in the mass balance estimation whereas the content of acetyl units (determined by HPLC via measuring the acetic acid in the hydrolysates), typically being between ca. 1-5 wt.% on dry biomass, should be added on top of the mass balance values shown in Table 8. The content of extractives is not included in the mass balance.

4.3. Biomass and compost samples from Nestos and Serres region

The physical properties of the biomass and compost samples from Nestos and Serres regions are shown in Table 9. Moisture content of all samples (after 1 month equilibration at ambient conditions) ranged from 6.2 to 9.4 %wt. It should be noted that the values presented in Table 9 are the average values of different samples of each compost received from the facilities in the municipalities of Serres and Nestos. With regard to the porous properties of the composts, the specific surface area (S_{BET}) and the pore volume (V_p) were increased compared to the parent biomass, similarly to the compost samples of the olive tree prunings presented in the previous section. The extent of changes/increase depend on time of composting and/or nature of the parent biomass feedstock used (different in the two facilities, see section 2).

Table 9. Physical properties of compost samples from Nestos and Serres regions.

Biomass feedstock	% Moisture	Bulk density (g/ml)	S_{BET} (m_2/g)	V_p (cm^3/g)
Nestos initial biomass samples	6.2	0.455	0.8	0.005
Nestos compost (2 months old)	7.0	0.419	1.1	0.009
Nestos compost (9 months old)	9.3	0.361	1.4	0.010
Serres compost (2 months old)	9.4	0.371	1.9	0.015

Furthermore, the chemical properties of compost samples are presented in Table 10. The pH values of Nestos samples showed a gradual increase from 6.3 (initial waste biomass feedstocks) to 7.4 (after 9 months of composting) while the compost from Serres exhibited a higher pH value equal of 8.1. This behavior has been also identified in

previous works for similar waste composting processes. Indicatively, compost samples obtained from home composting of grass, leaves and kitchen wastes showed pH close to neutral towards the final stages of composting process[17] while composts produced from straw and maize stover exhibited alkaline pH equal to 8.65 and 8.05, respectively[18]. Ash content of initial biomass and early stage compost from Nestos was high and equal to 32.2 and 34.4% wt, respectively. However, the ash content of the early stage compost from Nestos showed a wide differentiation in the range of 2.8 to 60.4 wt.% between the four samples tested (from four different sampling points). This observation can explain the substantially lower ash content of the 9-months compost from Nestos. A narrower range (11.7-16.5 wt.%) of ash content was observed for the compost from Serres. From the elemental analysis, carbon was slightly decreased from 46.7 to 43.4% while nitrogen increased from 0.7 to 1.2%, upon 9 months composting in Nestos facility. C/N ratio was also decreased from 63 to 35. These results show the same trends with the composting process of the olive tree prunings (see section 4.1).

Table 10. Chemical properties of compost samples from Nestos and Serres regions.

Biomass feedstock	pH	% Ash	% Volatile solids	%N	%C	%H	%S	%O	C/N
Nestos initial samples	6.3	32.2	67.8	0.7	46.7	6.1	0.1	46.4	63
Nestos compost (2 months)	7.2	34.4	54.8	0.8	46.4	6.2	-	46.7	61
Nestos compost (9 months)	7.4	6.1	93.9	1.2	43.4	5.4	-	49.9	35
Serres compost	8.1	11.7	88.3	1.5	37.4	4.6	0.2	56.4	25

With regard to the composition of both the initial biomass and compost samples at different composting time, the results are shown in Table 11. In the initial biomass feedstock from Nestos region, total sugars are 32.9% and total lignin 33.5%. The composting process resulted in decrease of total sugars to 25.5% after 2 months and 12.1% after 9 months due to their degradation and consumption by the microorganisms. The lignin content increased up to 51.4% due to slower degradation from microorganisms. The compost from Serres region exhibited lignin content in the range of 44.6-52.5% and total sugars ranging from 9.2 to 17.3%. The differentiations due to the inhomogeneity of the initial lignocellulosic biomass feedstock and/or the variation in the effectiveness of composting, can be revealed from the analysis data presented in Table 11 for three different samples from the Serres facility. The lignin content of the compost ranges between 44 and 52 wt.% and the total sugars between 9 and 17 wt.%.

Table 11. Composition of biomass wastes and compost samples from Nestos and Serres regions (wt.%, dry base)

[17] Manohara B and Belagali S L, Curr Trends Biomedical Eng & Biosci., 6, 3, (2017)
[18] Barus J., Journal of degraded and mining lands management, 3, 4 (2016)

Biomass feedstock	% Extractives	AIL	ASL	Total Lignin	Glu	Xyl	Gal	Ara	Man	Total Sugars	Mass (*) balance
Nestos initial samples	13.8	31.4	2.1	33.5	16.9	9.3	2.1	-	4.6	32.9	88.2
Nestos compost (2 months)	6.6	30.7	1.8	32.5	17.7	2.9	1.9	-	2.9	25.5	94.4
Nestos compost (9 months)	7.0	47.7	3.7	51.4	2.6	5.3	2.1	1.9	0.2	12.1	71.0
Serres compost-a	8.3	50.7	1.8	52.5	9.2	5.2	1.8	0.9	0.2	17.3	81.7
Serres compost-b	n.d.	42.4	2.2	44.6	5.5	4.5	2.0	1.5	-	13.5	70.0
Serres compost-c	n.d.	50.4	2.1	52.5	-	4.3	1.9	0.9	2.1	9.2	68.0

(*) The ash content (from Table 4) have been included in the mass balance estimation whereas the content of acetyl units (determined by HPLC via measuring the acetic acid in the hydrolysates), should be added on top of the mass balance values shown in Table 11. The content of extractives is not included in the mass balance.

5. Conclusions

The main outcome and conclusions of the work conducted in this deliverable that can be utilized as helpful recommendations for those interested in applying the composting process for the management and valorization of lignocellulosic bio-wastes, are summarized below:

- Analysis of the parent waste biomass and the compost samples showed that the carbohydrates, i.e. sugars in extractives or in the structural components cellulose and hemicellulose, are digested and consumed by the composting microorganisms faster than lignin.
- Lignin is eventually transformed to the known “humus”, a relatively condensed polyaromatic structure with several chemical functionalities, which is the final, stable form of compost, according to the relevant literature data.
- Lignin may also offer additional properties to the soil/plant due to its antioxidant and antimicrobial properties.
- Regardless of the nature of the lignocellulosic biomass feedstock, the C content is decreasing upon composting while the N content is increasing, leading to decreased C/N values in the composts.
- The increased content of N is beneficial for the soil conditioning and pesticide properties of compost.
- The different nature/type of waste biomass feedstock provides different amounts of inorganics (i.e. Ca, K, Si, Mg. etc) which can be utilized as nutrients in the soil.
- The porosity of compost is increased compared to that of the parent waste biomass feedstock, providing more space/volume for adsorbing humidity and various nutrients in order to release them in a controlled manner. Furthermore, the progressive “opening” of the lignocellulosic structure facilitates the action of microorganisms in the process of biomass degradation. It should be pointed out

though, that the surface area and pore volume of compost are 100-1000 times less that those of activated carbons and (bio)chars. Still, the small changes induced in comparison to the initial biomass are considered as beneficial for compost production and use.

Thus, appropriate physicochemical characterization and analysis of the parent waste biomass feedstocks is useful for the design of efficient composting processes by selecting the appropriate feedstocks (mixtures) and conditions.

In addition to the physicochemical properties of the parent waste biomass and the produced compost, the utilization of the latter as soil conditioner and natural pesticide requires dedicated and specific tests, such as seed germination tests to assess any phytotoxic effects of the compost end products prior to field application. For example, compost made out of olive tree chipping may contain large amounts of plant inhibitors, such as phenols. Chipping and composting of olive tree residual biomass is promoted by local agricultural association and authorities. A large number of Greek farmers have switched from burning pruning waste to chipping and composting. However, farmers are concerned that the accumulated olive tree compost may generate risks of pest contamination. For more information concerning farmers approach towards compost product please refer to OliveClima Project[19].

As a take-home message, composting represents an economically and environmentally sustainable approach for the on-site management and valorization of biomass related wastes and residues, which can however be significantly improved when knowledge and experience from fundamental biological and chemical sciences studies are utilized. Training of young people to implement knowledge-based protocols and methods is of paramount importance and promotes “green employment and entrepreneurship”.

[19] LIFE11 ENV/GR/942 oLIVE-CLIMA http://www.oliveclima.eu/