

# Chemical characterization of tropical ramial and trunk woods and their lignins in view of applications in soil amendments

Rodrigue Daassi<sup>a,b,c</sup>, Pierre Betu Kasangana<sup>a</sup>, Damase P. Khasa<sup>b</sup>, Tatjana Stevanovic<sup>a,c,\*</sup>

<sup>a</sup> Renewable Materials Research Center (CRMR), Université Laval, Quebec, QC, G1V 0A6, Canada

<sup>b</sup> Centre for Forest Research and Institute of Integrative and Systems Biology, Université Laval, Quebec, QC, G1V 0A6, Canada

<sup>c</sup> Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec, QC, G1V 0A6, Canada

## ARTICLE INFO

### Keywords:

*Gmelina arborea*

*Sarcocephalus latifolius*

Ramial chipped wood

Chemical composition

Lignins

S/G ratio

Soil

## ABSTRACT

We have determined the chemical properties of *Gmelina arborea* and *Sarcocephalus latifolius* trunk wood (TW) and ramial chipped wood (RCW), in order to examine the applicability of RCWs in soil amendments. The results of the study of *S. latifolius* represent the first report on this species. Characterizations of TW and RCW samples included determination of extractives, Klason and acid soluble lignin, major monosaccharides, ash, and mineral constituents. Higher contents of total lignins and monosaccharides were found in RCW than in TW for *S. latifolius*, while opposite results were obtained for *G. arborea*. The organosolv lignins isolated from the studied samples were analyzed by HPLC, FTIR, GPC, <sup>31</sup>P NMR and HSQC methods. The studied lignins belong to guaiacyl-syringyl (G-S) type, with a predominance of G units in *S. latifolius* and S units in *G. arborea* lignins. Based on obtained results, RCW samples from *S. latifolius* could be anticipated performing well in soil amendment trials, as indicated by their lower C/N ratios and higher mineral and lignin contents.

## 1. Introduction

Nowadays, due to the high soil degradation level all over the world (Baude et al., 2019), ecological and sustainable farming practices such as the use of cover crops, green manures or ramial chipped wood (RCW) are promoted to restore soil fertility (Félix et al., 2018; Manlay et al., 2007). RCW application is a sustainable technique which consists of application of lignocellulosic materials in soil to improve its fertility, microbial diversity and water economy, while contributing to stable soil carbon (Barthès et al., 2010; Félix et al., 2018). RCW refers to twigs and undried branches (without foliage), with diameter lower than 7 cm, subsequently fragmented, by a technique developed at Laval University in the 1980's (Lemieux, 1986). The chemical composition of these materials consists of cellulose, hemicelluloses and lignin, as structural components, along with simple sugars, amino acids, proteins, vitamins and essential nutrients, accompanied by extractable matter composed of polyphenols, terpenoids etc. (Senga et al., 2016; Stevanovic and Perrin, 2009; Suansa and Al-Mefarrej, 2020). Recent studies have demonstrated that ramial chipped woods (RCWs) represent a key material for restauration of a degraded soil (Barthès et al., 2010; Félix et al., 2018; Kindiela et al., 2019; Soumare et al., 2002). However, their chemical and nutrient composition varies considerably between the species and is different from that of the trunk wood (Tissaux, 2001).

RCWs represent a major source of energy and contribute to stable soil carbon through lignin degradation in soil. RCW application (incorporation or mulch) has been thoroughly evaluated mainly in Canada and temperate regions of Europe and recently in a few African regions where several studies highlighted its beneficial effect on physico-chemical and biological properties of soil (Félix et al., 2018; Soumare et al., 2002) and on crop production (Barthès et al., 2010). Despite their interest, none of those studies has addressed the investigation of RCW lignin from different tropical trees in order to understand the mechanisms by which the RCWs amendments may provide benefits to soils and crops in sub-Saharan Africa. The application of the RCWs from the two selected tropical hardwoods to the soils in Benin, is anticipated to improve the soil fertility through the stimulation of the soil biodiversity, the enhancement of the soil water economy through the introduction of the hydrophilic lignocellulosic material, while contributing to the stable carbon in soil on the long run. The technique of the RCWs does not comprise any use of synthetic fertilizers and thus it represents a contribution to organic farming and sustainable agriculture.

Lignins, the second most abundant biopolymers on Earth, represent the most recalcitrant constituents of lignocellulosic materials, contributing importantly to soil organic matter, thus influencing its pool-size and turnover (Shamim et al., 2014; Thevenot et al., 2010).

\* Corresponding author at: Renewable Materials Research Center (CRMR), Université Laval, Quebec, QC, G1V 0A6, Canada.

E-mail address: [tatjana.stevanovic@sbf.ulaval.ca](mailto:tatjana.stevanovic@sbf.ulaval.ca) (T. Stevanovic).

Annually, lignin biopolymers account for nearly 30 % of the carbon sequestered in plant materials (Austin and Ballaré, 2010). Woody plants contribute also, in important way, to soil organic carbon sequestration, by natural bioconversion from forest litter. Lignins are postulated to play important roles in the cycling and stabilization of soil organic carbon and to contribute to humus formation (Xiao et al., 2007), due to their high carbon content and the abundance of aromatic structures (Stevenson, 1994). It has been demonstrated that the addition of the Kraft lignin from rice straw, improved the soil aggregation and its chemical properties suggesting that this lignin-rich materials could be beneficial for soil amendments (Xiao et al., 2007). As it has been established in several studies, lignins are of huge importance for humus formation in soils receiving lignocellulosic residues, which is of importance for sustainable soil fertility (Kleber and Lehmann, 2019; Shamim et al., 2014; Thevenot et al., 2010). Lignin is the main carbon sink in the soil. Thus, by using ramial chipped woods as soil amendments, their lignins will contribute to soil carbon storage while the process of biodegradation of the lignocellulosic materials will favor the release of nutrients, especially nitrogen, necessary for plant nutrition, by white-rot fungi in particular. Basically, it is through the biodegradation of the lignocellulosic biomass that the macromolecules, among which lignins, are partly or completely depolymerized and mineralized, in case of lignins, mainly under the influence of white rot fungi through extracellular enzymatic system (peroxidases and laccase) (Datta et al., 2017; Knežević et al., 2013).

Many studies performed on chemical properties of lignins isolated from biomass have been using different processes under various conditions. Advantages of organosolv processes in comparison with other methods (Kraft and acidic sulphite) reside mainly in the uses of an organic solvent as delignifying agent to separate biopolymers from the lignocellulosic biomass. The organosolv process using ethanol-water solvent has been explored since 1990's and the  $\text{FeCl}_3$  as catalyst has also been used but that mainly in order to get access to the cellulose for further hydrolysis and conversion into cellulosic ethanol (Kim et al., 2010; Zhang et al., 2018). However, we recently optimized the organosolv process in the aim of obtaining the organosolv lignin with high purity and excellent yield, therefore convenient for structural studies. The originality of our organosolv process resides in the pre-extraction step using the same solvent system (ethanol-water) before pulping (Koumba-Yoya and Stevanovic, 2016). This pre-extraction consists of removing the extractive components from biomass prior to the catalytic process, in order to preserve the efficacy of the catalyst notably by avoiding its interacting with phenolic extractives in particular. This explains our choice to apply this process providing access to a high purity lignin from the starting lignocellulosic material, which is therefore adequate for structural studies.

This study is a preliminary investigation focusing on chemical characterization of the ramial chipped wood (RCW), in comparison with the trunk wood (TW) of *Gmelina arborea* and *Sarcocephalus latifolius* chosen for the field studies in Benin. We are concentrating in this study on the properties of lignins from the studied samples as lignins are associated with recalcitrant organic matter in soils. We have chosen the organosolv process, developed in our laboratory, for lignin isolation, since our previous studies have demonstrated that this process allowed for an access to pure lignins, mainly exempt of residual carbohydrates and ashes (Kasangana et al., 2020; Koumba-Yoya and Stevanovic, 2016). These lignins are chosen as good samples for study of lignin structural changes after incorporation in soils, which makes part of ultimate goals of our research on the impact of RCW application in soil amendment trials.

In order to anticipate the potential performance of the RCWs in application as soil amendments, the C/N ratios, the total lignin and mineral contents along with the lignin/N ratios are determined in this study, in comparison with the corresponding TW from two studied tropical hardwoods, *Gmelina arborea* Roxb. ex Sm., and *Sarcocephalus latifolius* (Sm.) E. A.

## 2. Materials and methods

### 2.1. RCW and TW sample preparation

Ramial chipped wood (RCW) and trunk wood (TW) of *G. arborea* and *S. latifolius* were collected in May 2018 from a 5-year plantation at the Mèdji Agricultural High School in Sékou (06°41'64" N; 02°34'82" E) and a 10-year plantation located in the area around the Kika Agricultural Technical High School (09°17'09 "N; 2°45'06" E), respectively, in the Republic of Benin. Both species are chosen for this research because of their availability in the study area. RCW of each species was obtained by chipping branches of less than 7 cm diameter, without foliage and using a locally manufactured shredder. The air-dried chips of each sample were ground using a hammer mill and sieved through a shaking screen, and the fraction of sawdust between 40 and 60 mesh was collected prior to chemical analyses.

### 2.2. RCW and TW chemical composition determination

The RCW and TW sawdust were first Soxhlet extracted for 6 h with anhydrous toluene-ethanol system (0.427:1.000, v/v), and after air drying, were then extracted with hot water in order to determine the total extractives content in the two species. The Klason and acid soluble lignin content in sawdust of both species was determined according to standard methods (ASTM D1106-96, 2013). Carbohydrate analysis was performed by high performance liquid chromatography- refractive index detector (HPLC-RID), using an Agilent Technologies 1200 series equipped with a Rezex RHM-Monosaccharide H+ (8%) (300 × 7.8 mm) column according to National Renewable Energy Laboratory (NREL) procedure (Sluiter et al., 2008). The calibration curves were performed using the monosaccharide standards (Sigma-Aldrich). Moisture and ash contents were computed using the ASTM standard method described in Senga et al. (Senga et al., 2016). In the various samples, mineral contents (P, K, Ca, Mg, Na, Mn, Fe, Zn, Al, Si) were analysed by inductively coupled plasma (ICP) atomic emission spectrometry on optimum machine DV 4300 (Perkin Elmer) with a Scott type of nebulizer. Elemental analyses were carried out using a PerkinElmer® 2400 Series II to analyse C, H and N. Finally, pH measurement of studied samples was performed as follows: 20 g of sawdust from each sample were covered with 200 mL of distilled water; after soaking overnight at room temperature, the pH value was measured using a pH meter (Fisher Scientific accumet AB15 Basic) (Sitholé, 2005). Each result corresponds to the mean of three replicates.

### 2.3. Organosolv lignin isolation

Lignin isolation was performed following the catalytic organosolv process developed in our laboratory (Koumba-Yoya and Stevanovic, 2016).

As illustrated in Fig. 1, 150 g of sample were pre-extracted with 150 mL of ethanol-water mixture (1:1, v/v) in a Soxhlet extractor at 80 °C for 6 h. After air drying, 50 g of extractive free RCW or TW were treated with 0.5 L of ethanol-water system (1:1, v/v) in Parr reactor series 4842 (2L), using 6 mmol of ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) as a catalyst at 180 °C for 90 min. After cooling, organosolv pulp was then separated from residual liquor by vacuum filtration, and subsequently washed three time with 200 mL of hot ethanol-water mixture (1:1, v/v). The residual liquor was then first evaporated to remove ethanol and then organosolv lignin finally precipitated by acidification with 2 M HCl to pH = 1.5. The organosolv lignins of studied samples were recovered in form of powder (dark brown for RCW; lighter brown for TW), after final filtration and drying in an oven at 40 °C overnight. The lignin recovery was computed using the following Eq. (1).

$$\text{Lignin recovery} = \frac{\text{Isolated Lignin mass}}{\text{Biomass weight} \times \text{initial Klason lignin}} \times 100 \% \quad (1)$$

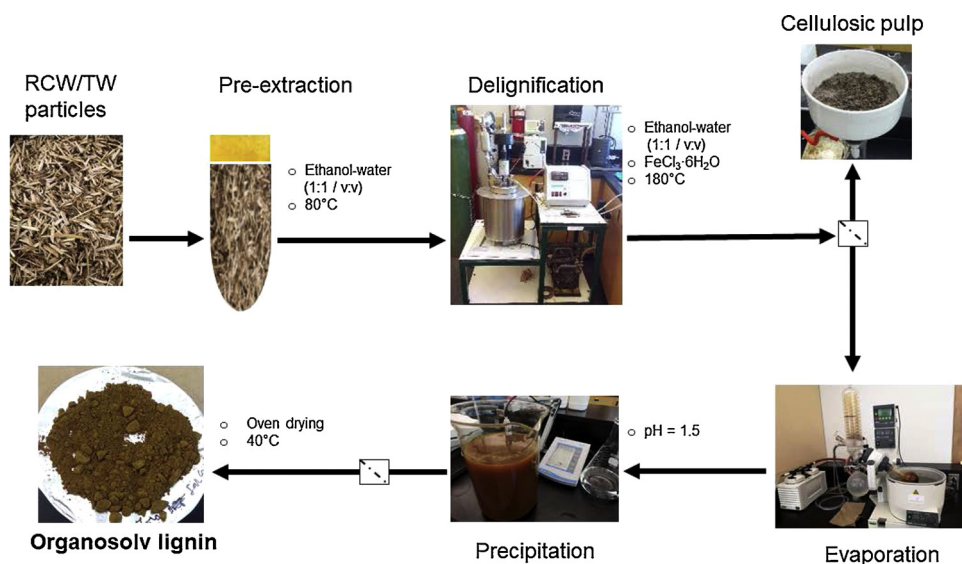


Fig. 1. Schematic representation of catalytic organosolv process.

#### 2.4. Analytical methods

The molecular weight analysis of studied lignins were performed by gel permeation chromatography (GPC) on an Agilent 1200 series equipped with column PL gel 5  $\mu$ m Mixed-D 300  $\times$  7.5 nm and using tetrahydrofuran (THF) as eluent (0.5 mL/min) and polystyrene as standard (580–28770 Da, Agilent). Prior to injection, 20 mg of sample were dissolved in 2 mL of THF and filtered through 0.45  $\mu$ m porosity filter (Tolbert et al., 2014).

For Fourier transform infrared (FTIR) spectroscopy analysis, spectra were recovered from 4000–650  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  and 64 scans per sample with PerkinElmer Spectrum 400. Condensation indices were calculated using Eq. (2) (Faix, 1992)

$$\text{Condensation Indices} = \frac{\sum \text{all minima between } 1500 \text{ and } 1050 \text{ cm}^{-1}}{\sum \text{all maxima between } 1600 \text{ and } 1030 \text{ cm}^{-1}} \quad (2)$$

The Nuclear Magnetic Resonance ( $^{31}\text{P}$  NMR) analyses were carried out by spectra acquisition on a Varian NMR spectrometer at 500 MHz after derivatization of organosolv lignins with 2-chloro-4,4,5,5-tetra-methyl-1,2,3-dioxaphospholane (Cl-TMDP) (Meng et al., 2019). The parameters selected for the analyses were: relaxation time of 15 s, pulse width of 45° and line broadening of 4 Hz. The 2D ( $^{13}\text{C}$ – $^1\text{H}$ ) hetero-nuclear single-quantum coherence (HSQC) experiment was also used to characterize organosolv lignins studied. Concisely, 80 mg of lignin were

dissolved in 0.7 mL of dimethylsulfoxide- $\text{d}_6$  (DMSO- $\text{d}_6$ ) and spectra were recorded using a Varian NMR spectrometer at 500 MHz. A semi-quantitative analysis of the HSQC spectra was performed using Mes-tReNova 14.1.2 processing software. The cross-signals related to the structural units and linkages in the HSQC spectra were assigned by comparison with the available literature data (Jiang et al., 2018; You et al., 2013; Yue et al., 2018; Zhang et al., 2017; Zhao et al., 2019). The S/G ratios were calculated from the HSQC spectra using the G  $\text{C}_2$ – $\text{H}_2$  signals ( $\text{G}_2$ ) as internal standard. Results are reported as percentage of interunit linkages or lignin subunits from 100 aromatic units (C9 units) (Zhao et al., 2019).

#### 2.5. Statistical analysis

Differences in chemical properties between RCW and TW and between different OH group types of the studied lignins were assessed using one-way analysis of variance (ANOVA), followed by least significant difference multiple comparison tests. These analyses were performed using Statistical Analysis System (SAS) version 9.4 and  $p$ -value < 0.05 was considered statistically significant.

Table 1

Chemical constituents of ramial chipped wood (RCW) and trunk wood (TW).

Entry	Chemical constituents	<i>G. arborea</i>		<i>S. latifolius</i>	
		RCW	TW	RCW	TW
1	Total Extractives	16.1 $\pm$ 0.4 a	8.9 $\pm$ 0.4 d	12.7 $\pm$ 0.4 b	11 $\pm$ 0.3 c
	EtOH-Tol Ext <sup>a</sup>	4.9 $\pm$ 0.3 b	6 $\pm$ 0.3 a	3 $\pm$ 0.3 c	5.8 $\pm$ 0.2 a
	Hot water Ext	11.2 $\pm$ 0.1 a	2.9 $\pm$ 0.1 d	9.7 $\pm$ 0.1 b	5.2 $\pm$ 0.1 c
2	Total lignin	24.6 $\pm$ 0.2 c	26.7 $\pm$ 0.3 b	35 $\pm$ 1.1 a	28.3 $\pm$ 0.1 b
	Klason lignin	21.6 $\pm$ 0.1 b	21.7 $\pm$ 0.2 b	33.3 $\pm$ 1 a	26.8 $\pm$ 0.1 b
	Acid soluble lignin	3 $\pm$ 0.1 b	5 $\pm$ 0.1 a	1.7 $\pm$ 0.1 c	1.5 $\pm$ 0 c
3	Total sugar <sup>b</sup>	50.7 $\pm$ 1.1 d	72.4 $\pm$ 0.1 a	66.9 $\pm$ 1.2 b	56.8 $\pm$ 0.2 c
	Glucose	33.6 $\pm$ 0.7 c	40.4 $\pm$ 0.2 a	23.7 $\pm$ 0.6 d	35.9 $\pm$ 0.1 b
	Xylose	14 $\pm$ 0.4 c	17.9 $\pm$ 0.1 a	14.1 $\pm$ 0.3 b	17.7 $\pm$ 0.1 a
	Arabinose	3.2 $\pm$ 0.1 c	2.1 $\pm$ 0.1 b	3.2 $\pm$ 0.2 a	2.4 $\pm$ 0.5 c
4	Ash	3.3 $\pm$ 0.1 b	0.7 $\pm$ 0.1 d	6.5 $\pm$ 0.1 a	1.1 $\pm$ 0 c

<sup>a</sup> Ethanol-toluene; <sup>b</sup> = Sum of major monosaccharides; All results of measurements are expressed as means  $\pm$  standard error of triplicates; mean with same letter are not significantly different by line; LSD test  $p$  < 5%.

### 3. Results and discussion

#### 3.1. Chemical characterization of ramial chipped wood and trunk wood

Significant variations of chemical indices were found between the RCW and the TW samples for studied wood species, as shown in Table 1.

The total extractives content was higher in RCW than in TW for both species, which could be explained by the composition of tree branches, generally rich in extractable primary metabolites (amino acids, sugars, proteins, hormones, etc.) (Stevanovic and Perrin, 2009). One the other hand, small branches constituting RCW contain also high proportions of bark tissue (Dossa et al., 2018), which are rich sources of extractives, especially polyphenols according to the literature (Francezon and Stevanovic, 2017). This observation confirms that nutrients are mostly stored in twigs (Tissaux, 2001) and that the high total extractive content is related to high proportions of bark in RCW samples. In addition, the extractive contents determined in tropical hardwoods are higher than typical extractive contents ( $5 \pm 3\%$ ) of temperate zone hardwoods. The results obtained in the present study corroborate well with the results published on extractive contents of different parts (fruit, branch, bark wood, leaf) of *G. arborea* from tropical region (Fuwaape, 1989). Hot water extractive content was higher than the ethanol toluene extract for RCW for both species, while the inverse result was obtained for TW. The latter result is similar to that reported by Doat (Doat, 1976), who found that the average benzene alcohol extract ( $6.1\%$ ) for *G. arborea* wood samples was higher than that obtained with hot water ( $1.5\%$ ).

The total lignin content (Klason plus acid soluble lignin) was determined to be significantly higher in *S. latifolius* samples than in *G. arborea* samples, while different total lignin contents were found between RCW and TW, as it has been reported in several studies on different wood species (Senga et al., 2016; Tissaux, 2001; Xiao et al., 2019). In tree, lignification degree differs not only depending on the species, but also on different plant parts (Boudet, 2000). The values on lignin contents presented in Table 1 for studied tropical species are higher than those ( $20 \pm 4\%$ ) reported for temperate zone hardwoods, but are in the range ( $20\text{--}35\%$ ) of values reported for other tropical hardwoods (Xiao et al., 2019).

As for the total carbohydrate content (glucose, xylose and arabinose), a significant difference was found between the samples. The higher carbohydrate content in TW than in RCW was determined for *G. arborea*, while opposite trend was found for *S. latifolius* samples. This result illustrates that carbohydrates distribution within the individual tissues (branch, wood, bark etc.) varies depending on species (tree or shrub) and depends on ontogenetic (maturation stage) and environmental impacts (climate, mineral nutrition etc.) as proposed by Magel and coauthors (Magel et al., 2000).

RCW samples were determined to have significantly higher ash content than corresponding TW and that trend was more important for *S. latifolius* than for *G. arborea*. These ash contents are close to those ( $1.2\text{--}7.9\%$ ) reported in previous studies on RCWs (Demeyer et al., 2001; Senga et al., 2016; Tissaux, 2001). The high ash content of RCW compared to TW indicates the mineral nutrient richness, which is a good property regarding the application of RCW in soil amendment treatments as it is favorable to soil fertility, supported by high inputs of phosphorous, potassium and calcium determined in RCW (Table 2 C).

#### 3.2. Elementary chemical composition, mineral content and pH of RCW and TW of *G. arborea* and *S. latifolius*

Results of the elemental analysis and, degradability (C/N ratio of RCW and TW) of *G. arborea* and *S. latifolius* are also shown in Table 2.

The N content was determined to be significantly higher in RCW than in TW for both species, the RCW of *S. latifolius* ( $0.6 \pm 0.0\%$ ) having higher content than *G. arborea* ( $0.5 \pm 0.1\%$ ). Similar results

are reported from other studies showing a higher concentration of nitrogen in branches than in trunk wood (Barthès et al., 2010; Kim, 2019; Senga et al., 2016). In addition, Louppe and coworkers, have found in *G. arborea* the following distribution of N content (%): 0.21, 0.4, 0.5 in TW, branches and bark respectively (Louppe et al., 1998), which corroborate well with our results. The high N content of RCW can be taken as a good indicator that, once applied to the soil, RCW would potentially improve nutritional availability of nitrogen, particularly at a pH values above 5.5. The C and H contents of both species were higher in TW than in RCW and were in accordance with those reported in the literature for temperate zone hardwoods ( $42\text{--}50\%$ ) (Senga et al., 2016; Xiao et al., 2019) and softwoods ( $46\text{--}57\%$ ) (Stevanovic and Perrin, 2009; Xiao et al., 2019). One should notice, however, that our results are closer to those reported for softwoods, while the S/G ratios determined for samples studied in this research do confirm that we are dealing with hardwood species, from tropical zone though. The C/N ratio for both species was higher in TW than in RCW and lower in RCW of *S. latifolius* than that of *G. arborea*. These values are close to those reported for tropical (Félix et al., 2018; Senga et al., 2016; Soumare et al., 2002) and temperate zone hardwoods (Pei et al., 2019; Tissaux, 2001). All these values are, however, well above the optimum C/N ratio required for fast decomposition and N mineralization in soil which is required to be beneath 30 (Shamim et al., 2014). We could suspect more nutrient immobilization when RCWs of *G. arborea* are applied to soil due to their higher C/N ratio value ( $94.9 \pm 5.2$ ) than when RCWs of *S. latifolius* ( $82.8 \pm 0.5$ ) are applied. This is also confirmed by the lignin/N ratio, which is significantly, higher for the TW than for the RCWs, as can be seen in Table 3. In addition to the C/N ratio, the lignin/N ratio is often used as a good predictor of lignocellulosic materials decomposition in soil, particularly for lignin-rich materials (Pei et al., 2019; Taylor et al., 1989). Lignins are resistant to enzymatic attacks and they provide physical protection to cellular constituents against degradation. This explains how they control the decomposition rate of lignocellulosic materials in soil (Shamim et al., 2014). The lignin/N ratios were determined to be lower for RCW than for TW for both studied species. One should keep in mind, however, that some of the nitrogen from RCW is chemically bonded in lignin, as it will be demonstrated further from the elementary analyses results of organosolv lignins isolated from the studied samples (Table 3).

For both species, all minerals analyzed (Table 2 C) were significantly more concentrated in RCW than in TW. The highest contents of all nutrients were determined in RCWs of *S. latifolius*, which is also consistent with their higher ash content (Table 1). The contents of primary (P, K), secondary (Ca, Mg, Na) and trace (Fe, Mn, Al, Zn, Si) mineral elements in the studied samples (RCW versus TW) for both species were close to those reported in the literature. It has been reported previously that the minerals are more concentrated in RCW than in TW (Augusto et al., 2000; Louppe et al., 1998; Senga et al., 2016). In addition, other studies showed that except for Ca, nutrient contents in different parts of tree (stem, branch, bark, leaf) decrease exponentially with increasing diameter (Hendrickson, 1987; Tissaux, 2001). According to Hendrickson, the process of storing nutrients in the tree, some of which are recycled to produce new shoots, could explain the significant decrease in nutrient content in wood and bark of larger diameter branches (Hendrickson, 1987), while explaining the higher nutrient content of RCW compared to TW. Similarly, the translocation of some minerals (e.g. phosphorus) from leaves to twigs is an important tree strategy for increasing the efficiency of use of limiting soil mineral resources (Johnson and Turner, 2019). The deficiency of most minerals in soil requires trees to set up a recycling mechanism to limit losses (Johnson and Turner, 2019) and therefore explains the interest of its high concentration in the branches. Ca being concentrated in the bark moves slowly or not at all from bark into another part of the tree (Fromm, 2010); which explains its high content in RCW compared to TW. K, Ca and Mg, which play decisive physiological roles in plant development and function (Fromm, 2010), were determined to be the



**Table 2**

CHN composition (A), C/N and Lignin/N ratio (B) and chemical constituents (C) of ramial chipped wood (RCW) and trunk wood (TW) of studied species.

	<i>G. arborea</i>		<i>S. latifolius</i>	
	RCW	TW	RCW	TW
(A) CHN contents (%)				
C	49.1 ± 0.1 b	51.0 ± 0.7 a	50.5 ± 1.1 a	51.6 ± 0.4 a
H	5.7 ± 0.1 a	5.9 ± 0.0 a	5.1 ± 0.2 b	5.7 ± 0.1 a
N	0.5 ± 0.1 b	0.1 ± 0.0 d	0.6 ± 0.0 a	0.4 ± 0.0 c
O	44.7 ± 0.8 a	43.1 ± 0.2 c	43.8 ± 0.3 b	42.3 ± 0.5 d
Lignin <sup>a</sup>	24.6 ± 0.2 c	26.7 ± 0.3 b	35.0 ± 1.1 a	28.3 ± 0.1 b
(B) Degradability indices (ratio)				
C/N	94.9 ± 5.2 c	541.9 ± 93.8 a	82.8 ± 0.5 d	123.0 ± 2.8 b
Lignin <sup>a</sup> /N	47.5 ± 2.3 d	285.9 ± 54.6 a	57.5 ± 2.3 c	67.4 ± 1.6 b
(C) Mineral contents (mg/kg) and pH				
P	537.2 ± 4.0 b	190.4 ± 4.0 c	1061.9 ± 18.9 a	517.7 ± 8.8 b
K	5149.1 ± 36.1 b	1545.9 ± 13.0 d	8852.6 ± 14.4 a	3275.9 ± 16.4 c
Ca	6059.9 ± 30.7 b	953.3 ± 13.4 c	7110.8 ± 61.9 a	125.6 ± 6.8 d
Mg	2150.9 ± 27.7 b	482.9 ± 5.8 c	3360.1 ± 36.3 a	89.3 ± 2.1 d
Na	205.5 ± 4.1 b	76.4 ± 3.5 d	214.9 ± 0.5 a	89.2 ± 0.5 c
Fe	94.5 ± 4.1 b	32.7 ± 2.4 c	1040.4 ± 33.1 a	11.8 ± 1.1 c
Mn	61.8 ± 0.7 a	1.5 ± 0.3 b	59.7 ± 1.2 a	ND
Zn	16.1 ± 1.2 a	6.5 ± 1.1 b	13.7 ± 0.4 a	ND
Al	107.9 ± 1.7 b	21.3 ± 1.7 c	271.5 ± 8.1 a	3.7 ± 0.0 d
Si	181.3 ± 5.9 b	14.7 ± 0.5 c	701.7 ± 3.7 a	14.7 ± 1.0 c
pH	5.0 ± 0.01 c	5.2 ± 0.01 b	6.5 ± 0.05 a	5.2 ± 0.02 b

<sup>a</sup> Based on total lignin from Table 1. See also the results for organosolv lignins further. ND: not determined. All content measurements are expressed as means ± standard error of triplicates; mean with same letter are not significantly different by element; LSD test  $p < 5\%$ .

most concentrated macronutrients, accounting for more than 80 % of the total inorganic content in RCW from both studied species (Table 2).

Finally, the pH values determined for the RCW of *S. latifolius* ( $6.5 \pm 0.05$  %) were higher than those determined for the trunk wood counterpart ( $5.2 \pm 0.02$  %), while a contrary trend was found for the RCW ( $5.0 \pm 0.01$  %) and the TW ( $5.2 \pm 0.01$  %) of *G. arborea* (Table 2 C). Lower acidity of lignocellulosic samples studied in our research was found than that reported in other studies on lignocellulosic materials (pH, 3.3–5.8) (Sitholé, 2005). In general, the more acidic the soil (pH = 5.5 and lower), the higher the exposure of plants to significant nutrient deficiencies. On the other hand, in most mineral soil fields, the availability of nutrients and the activity of microorganisms are favoured when the pH is between 5.5 and 7.

### 3.3. Yield, purity and elemental analysis of organosolv lignins

We have opted to apply the organosolv process developed in our laboratory, since the results of our previous studies confirmed that it provided access to high purity lignins (Kasangana et al., 2020; Koumba-Yoya and Stevanovic, 2017). The applied procedure consists of a pre-extraction step with the same solvent system (ethanol water) as the

main catalytic (with Lewis acid as catalyst) organosolv treatment, which is performed at 180 °C, as presented in Fig. 1. The results obtained on lignin isolation and characterization are presented in Table 3.

The yield of organosolv lignins from all samples was determined to be above 15 % (with lignin recovery superior to 60 %). The higher yields of lignin were achieved from TW than from the RCW of both species. This indicated that the lignin from trunk is more easily accessible for organosolv process than that from RCWs. Based on the initial Klason lignin content, the lignin recovery of *G. arborea* (RCW, TW) was better than that from *S. latifolius* (RCW, TW). One should note that we have applied the conditions used previously for pulping of the hardwood bark examined in a previous study, taking into consideration the fact that the RCW is a material very rich in bark (Koumba-Yoya and Stevanovic, 2017). The same pulping conditions were applied to wood and bark-rich RCW in this research. Therefore, it is not surprising that the highest recovery of lignin was achieved from TW of *G. arborea* with the least original lignin content. The purity of all studied lignin samples was determined to be higher than 92 % (assessed as sum of Klason plus acid soluble lignin of the isolated lignins), which confirms the findings of our previous studies (Kasangana et al., 2020; Koumba-Yoya and Stevanovic, 2017). These results confirm that we have made the proper

**Table 3**

Properties and elemental analysis of organosolv lignins from RCW and TW of studied species.

	<i>G. arborea</i>		<i>S. latifolius</i>	
	RCW	TW	RCW	TW
Lignin yield <sup>u</sup> (%)	15.9 ± 0.9 a	18.6 ± 1.1 a	16.3 ± 0.5 a	18.2 ± 0.8 a
Lignin recovery <sup>v</sup> (%)	77.4 ± 3.3 b	94.7 ± 0.8 a	60.4 ± 1.7 c	75.6 ± 0.4 b
KL (%)	95.2 ± 0.3 a	92.5 ± 0.0 c	93.2 ± 0.3 b	89.9 ± 0.0 d
ASL (%)	4 ± 0.2 c	7.4 ± 0.1 a	5.2 ± 0.2 b	2.6 ± 0.1 d
Glucose (%)	1.2 ± 0.1 a	0.8 ± 0.2 a	1.2 ± 0.1 a	0.8 ± 0.1 a
Ash (%)	0.3 ± 0.0 b	0.2 ± 0.0 c	0.6 ± 0.0 a	0.3 ± 0.0 b
C (%)	65.86 ± 0.06 d	66.86 ± 0.03 a	66.38 ± 0.02 b	66.12 ± 0.02 c
H (%)	5.85 ± 0.02 a	5.51 ± 0.01 d	5.76 ± 0.02 b	5.67 ± 0.01 c
N (%)	0.81 ± 0.01 a	0.28 ± 0 c	0.78 ± 0.01 ab	0.76 ± 0.01 b
O (%)	27.44 ± 0.27 a	27.31 ± 1.22 a	27.05 ± 1.65 a	27.33 ± 1.64 a

<sup>u</sup> Based-on oven dry mass.

<sup>v</sup> Calculated relative to the total lignin contents of RCW and TW, KL, Klason lignin, ASL, acid soluble lignin, ash and glucose (% of organosolv lignin), all measurements are expressed as means ± standard error of triplicates; mean with same letter are not significantly different by lignin sample; LSD test  $p < 5\%$ .

choice to apply organosolv process to get access to representative lignin samples from the studied materials. According to ash and residual glucose contents, the organosolv lignin from the trunk wood of *G. arborea* had higher purity than that from *S. latifolius*. The results obtained in this research confirm that the organosolv process based on ethanol-water mixture using  $\text{FeCl}_3$  as catalyst provides access to a high purity lignin (sugar content lower than 1.3 % and ash content lower than 0.6 %) which is a suitable lignin sample for structural studies.

CHN elemental analyses performed on organosolv lignins provided information about significant differences in C, H, O, and N contents. The TW organosolv lignin of *G. arborea* was determined to have the lowest N content compared to other lignin samples (Table 3). It is important to note the higher relative concentration of nitrogen in all lignin samples than in corresponding wood (RCW and TW) samples from which they have been isolated (Table 2). This could be attributed to the potential protein-lignin complexes formation during organosolv pulping process, especially for the RCW samples as described by Latif et al. (Latif et al., 2019). This high nitrogen content in studied lignins could also be related to a natural occurrence of N in lignins, as we are dealing with lignins originating from samples containing living cells and therefore rich in N. This hypothesis could be confirmed by higher nitrogen contents both in the starting RCW compared to the TW (Table 2) and by the higher N content in the RCW lignin compared to the TW lignin from both species. The high N content of the RCW lignin compared to the TW lignin from both species could be therefore taken as a favorable feature for the application of RCW as an organic soil amendment, rich in carbon (lignins are the carbon richest polymers from lignocellulosic biomass) but also containing nitrogen.

### 3.4. Polymer properties of lignin samples

The polymer properties of organosolv lignins were studied by GPC analyses and the obtained results, presented in Fig. 2 indicate that the lignin samples of *S. latifolius* have higher average molecular weight (Mw, range 2020–3014) than those from *G. arborea* (Mw, range 1743–1948).

The higher Mn and Mw values were determined for RCW lignin (645 g/mol, 1948 g/mol) from *G. arborea* than those for its TW counterparts (535 g/mol, 1743 g/mol) while the opposite trend was obtained for the *S. latifolius* samples. Different levels of lignification

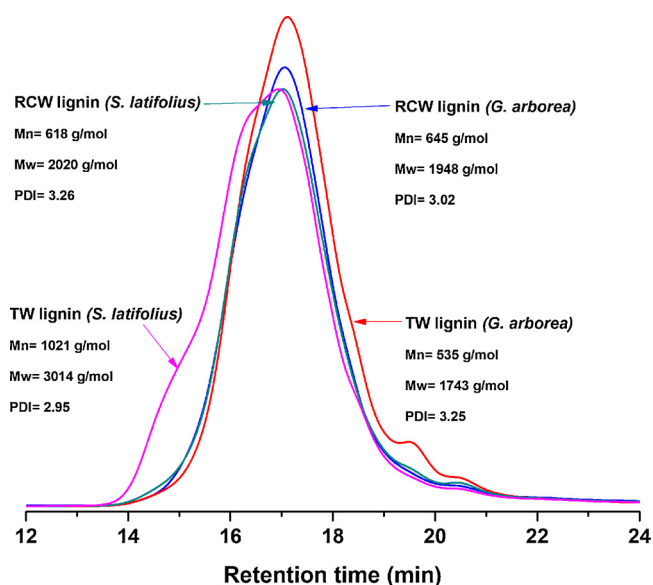


Fig. 2. Polymer properties of organosolv lignins from RCW and TW from *G. arborea* and *S. latifolius* as determined by GPC.

Mn: number average molecular mass, Mw: weight average molecular mass, PDI: polydispersity index (Mw/Mn).

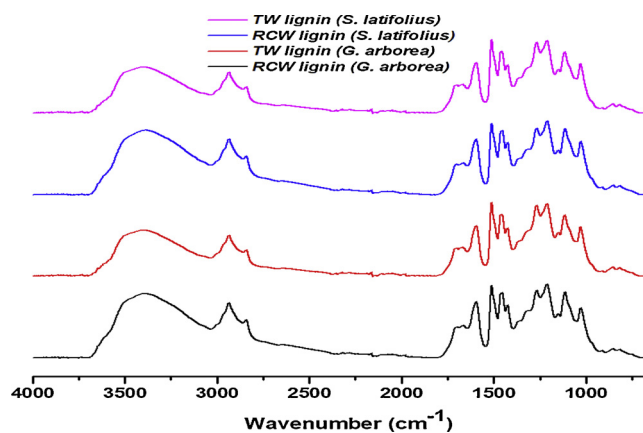


Fig. 3. FTIR spectra of RCW and TW lignin of *G. arborea* and *S. latifolius*.

between tree and shrub as well as between trunk and branches could explain these results. As for the polydispersity indices (PDI) lower value was determined for RCW organosolv lignin than for TW lignin from *G. arborea*, while opposite trend was determined for *S. latifolius* lignin samples. These results suggest that the lignin of RCW from *S. latifolius* might have larger particle size and are more heterogeneous than those from *G. arborea* (Tolbert et al., 2014). The higher values determined for Mw indicate that the initial materials (RCW, TW) of *S. latifolius* would have higher recalcitrance to degradation than the *G. arborea* samples, once in soil.

### 3.5. FTIR spectral analyses

Lignin structural properties are commonly analyzed by FTIR spectroscopy. The FTIR spectra of the *G. arborea* and *S. latifolius* organosolv lignins are presented in Fig. 3, while the assignments of the observed bands are made according to previous studies (Faix, 1992, 1991; Xiao et al., 2019) and are listed in Table S1 (Supporting data).

The FTIR spectra of the studied lignins (Fig. 3) exhibit typical hardwood lignin patterns. The intensities of the majority of the spectral bands in the FTIR spectra of the RCW lignins were higher than corresponding band in the spectra of the TW lignins, for both studied species. The lignins' spectra contained characteristic absorption bands of G-S type lignin as shown in Fig. 3. However, the aromatic CH in plane deformation band ( $1140\text{ cm}^{-1}$ ), (typical of guaiacyl condensed units) is present only in spectra of *S. latifolius* lignins and absent from the spectra of *G. arborea* lignin samples. This result indicates that guaiacyl unit could be more abundant in *S. latifolius* lignin than in *G. arborea* lignin. This absorption band is also more important in FTIR spectra of *S. latifolius* RCW lignin than that of its TW counterpart, for the same species. This result is further confirmed by the condensation indices calculated from the FTIR spectral data for the *S. latifolius* lignins (0.90 and 0.81 for the RCW and the TW lignins, respectively). Similarly, the differences between the spectra for the RCW and the TW lignins were observed for the bands corresponding to C=O stretching vibrations of unconjugated ketones, esters or carbonyl groups ( $1738\text{--}1709\text{ cm}^{-1}$ ) and OH stretching ( $3460\text{--}3412\text{ cm}^{-1}$ ) (Bauer et al., 2012). These groups are estimated to be present in higher concentration in the RCW lignin than in the TW lignins for both species. These findings indicate that the RCW lignins might contain more aliphatic hydroxyl and carbonyl groups originating from carbohydrates, the contents of which was determined to be higher in the RCW than in the TW lignins, thus independently confirming the results presented in Table 3.

### 3.6. $^{31}\text{P}$ NMR analyses of lignin samples

The data obtained by the quantitative  $^{31}\text{P}$  NMR spectral analyses of the studied lignins are presented in Table 4 and the main integration

**Table 4**  
Contents of various hydroxyl groups by quantitative  $^{31}\text{P}$  NMR of lignin.

Hydroxyl groups content (mmol/g)	<i>G. arborea</i> lignin		<i>S. latifolius</i> lignin	
	RCW	TW	RCW	TW
Aliphatic	1.18 $\pm$ 0.12 a	0.75 $\pm$ 0.19 a	1.39 $\pm$ 0.33 a	1.02 $\pm$ 0.37 a
S	2.61 $\pm$ 0.2 ab	3.46 $\pm$ 0.9 a	1.93 $\pm$ 0.42 b	1.55 $\pm$ 0.53 b
G	1.2 $\pm$ 0.12 a	1.45 $\pm$ 0.35 a	2.04 $\pm$ 0.42 a	2.03 $\pm$ 0.71 a
COOH	0.22 $\pm$ 0.03 a	0.18 $\pm$ 0.04 b	0.23 $\pm$ 0.06 a	0.11 $\pm$ 0.03 b
S/G (phenolic-OH)	2.16 $\pm$ 0.05 b	2.39 $\pm$ 0.03 a	0.96 $\pm$ 0.03 c	0.79 $\pm$ 0.02 d

S: syringyl unit; G: guaiacyl unit; COOH: carboxylic acid. All contents are expressed as means  $\pm$  standard error of triplicates, mean with same letter are not significantly different by lignin sample; LSD test  $p < 5\%$ .

areas for each type of hydroxyl group were assigned according to the data from the literature (Balakshin and Capanema, 2015; Crestini and Argyropoulos, 1997; Meng et al., 2019).

The results of the quantitative  $^{31}\text{P}$  NMR spectral analyses of the lignin samples are presented in Fig S. 2. As shown in Table 4, the aliphatic hydroxyls (150–145.5 ppm) and carboxylic hydroxyls (136–133.6 ppm) contents are higher in the RCW lignins than in the TW lignins for both species. The high contents of aliphatic hydroxyls in RCW lignins could be related to residual carbohydrates in these lignins, which is higher than in lignins isolated from the TW counterparts of both species (Table 3). The carbohydrates are rich in aliphatic hydroxyls which is a plausible explanation based on results of Table 3. Interestingly, however, this result could also be related to our previous comparative study of organosolv lignins from bark and wood of sugar maple, in which the important contents of aliphatic hydroxyl groups were found in sugar maple bark lignin in contrast with the results on the lignin from wood of the same species (Koumba-Yoya and Stevanovic, 2017). The present results could be perhaps correlated to that study, as the RCW are all materials relatively rich in bark, due to small diameters of branches (higher proportions of bark in branches than in trunk). The  $^{31}\text{P}$  NMR spectra revealed two distinct bands in the phenolic region (137.6–144 ppm). The broad peaks observed around 142.3 and 139.5 ppm are assigned to phenolic hydroxyls, present in syringyl and guaiacyl units respectively (Balakshin and Capanema, 2015; Meng et al., 2019; Wen et al., 2015). The peak corresponding to the p-hydroxyphenyl H unit (137.2–138.1 ppm) was, however, absent from the spectra analyzed in this study. Taken together, these results seem to confirm that all studied lignins belong to the G–S type as already indicated by the FTIR analysis. Both lignins isolated from *G. arborea*, the RCW lignin and TW lignin, seem to be dominated by syringyl hydroxyl groups (2.61  $\pm$  0.2 mmol/g; 3.46  $\pm$  0.9 mmol/g), while containing less guaiacyl hydroxyls (1.2  $\pm$  0.12 mmol/g; 1.45  $\pm$  0.35 mmol/g). The higher concentrations of syringyl hydroxyls in TW lignin as compared to RCW is also consistent with the lower concentration of lignin determined in the *G. arborea* samples (Table 1). On the contrary, guaiacyl hydroxyl units (2.03  $\pm$  0.71 mmol/g; 2.04  $\pm$  0.42 mmol/g) are much more important in lignins from *S. latifolius*, especially in that from RCW, than the syringyl hydroxyls (1.55  $\pm$  0.53 mmol/g; 1.93  $\pm$  0.42 mmol/g), as also shown by the calculation of the S/G ratio (phenolic–OH) for the same species. The contrasting results between the two species demonstrate the well-known variability of different types of phenolic hydroxyls in lignins depending on wood species and different tree parts (bark, trunk, branch) (Crestini and Argyropoulos, 1997). To the best of our knowledge, the results reported here on the chemical composition of *S. latifolius* samples and particularly on their lignins, are the first reports of this kind on this species.

In addition to the higher pH value of RCW from *S. latifolius* (pH = 6.5), the presence of more guaiacyl units in *S. latifolius* could have a positive effects on stable carbon in soil, following the treatment of soils with the RCW from *S. latifolius*. Indeed, guaiacyl units are well-known for their high reactivity by oxidative coupling reactions with

phenols and other molecules. Therefore, the abundance of G moieties in this tropical hardwood lignin could have favorable effect on stable carbon storage upon application of *S. latifolius* RCWs in soil amendment trials, through the introduction of this carbon-rich material and its biodegradation in soil.

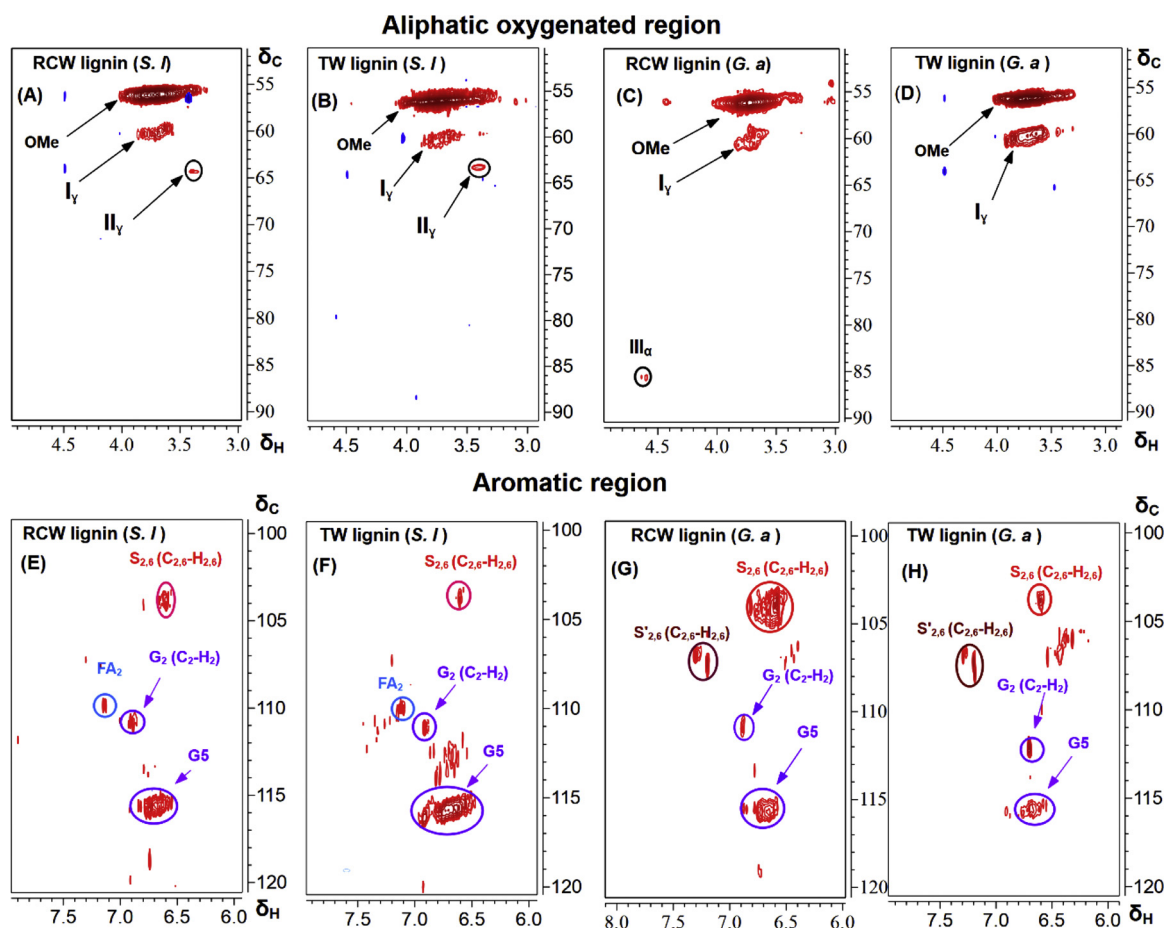
### 3.7. 2D HSQC NMR analyses of lignin samples

The 2D HSQC NMR was carried out on organosolv lignins from *S. latifolius* and *G. arborea* for elucidating lignin subunit composition and lignin inter-unit linkages, as well as for identifying the impurities related to the presence of carbohydrates in the lignins. Thus, carbohydrate anomeric signals which typically appear in the  $\delta_{\text{C}}/\delta_{\text{H}} = 4.0\text{--}6.0/99\text{--}105$  region are clearly absent from the HSQC spectra of all lignin samples studied here. This could be explained by the absence or even small amount of polysaccharides in the lignins as shown in Table 3. Nevertheless, this result confirms, once again, the effectiveness of our organosolv process in term of high purity of isolated organosolv lignin.

In addition, as shown in Figs. 4 and 5, the detailed analysis of aliphatic-oxygenated regions (side chains,  $\delta_{\text{C}}/\delta_{\text{H}} = 50\text{--}90/2.5\text{--}6.0$ ) of the lignins isolated from RCW and TW revealed the similarities between these two lignins for both *S. latifolius* and *G. arborea*. Beside methoxyl groups, the correlation peaks for  $\beta\text{--O--}4'$  substructures ( $\text{I}_{\text{V}}$ ) at around  $\delta_{\text{C}}/\delta_{\text{H}} = 60.03/3.74$  ppm were the most prominent in the HSQC spectra.

The  $\beta\text{--O--}4'$  linkage (Fig. 5) is well-known to be the most prominent inter-unit linkage in lignins of all origins and the one readily cleaved chemically in various pulping procedures (You et al., 2013; Yue et al., 2018; Zhang et al., 2017). Moreover, the  $\beta\text{--}5'$  linkages in phenylcoumaran substructures ( $\text{II}_{\text{V}}$ ) detected in  $\text{C}_{\text{V}}\text{--H}_{\text{V}}$  correlations at around  $\delta_{\text{C}}/\delta_{\text{H}} = 63.80/3.36$  ppm are only present in *S. latifolius* lignins (both in RCW and TW, lignins), which are reflecting condensed structures in guaiacyl residues. This feature has already been confirmed by higher condensation indices from the FTIR results and, more pronounced guaiacyl character of these lignins. In addition, ferulate ( $\text{FA}_2$ ) substructures were also found in *S. latifolius* lignin at around  $\delta_{\text{C}}/\delta_{\text{H}} = 109.6/7.15$  ppm. In contrast, low signals of  $\beta\text{--}\beta'$  linkages in resinol substructures ( $\text{III}_{\text{a}}$ ) were detected and confirmed by  $\text{C}_{\alpha}\text{--H}_{\alpha}$  correlations at  $\delta_{\text{C}}/\delta_{\text{H}} = 85.54/4.61$  ppm only in RCW lignins extracted from *G. arborea*.

In the aromatic region ( $\delta_{\text{C}}/\delta_{\text{H}} = 90\text{--}120/6.0\text{--}8.0$ ) of HSQC spectra of all studied lignins, the signals from S, G units were clearly distinguished, with no signals for H units, indicating that the lignins from two tropical species studied are essentially G–S type lignins. This is also in harmony with the results obtained by FTIR and quantitative  $^{31}\text{P}$  NMR analysis. In HSQC spectrum, S units exhibit a more prominent signal in *G. arborea* lignins, as indicated by the  $\text{C}_{2,6}\text{--H}_{2,6}$  correlation at around  $\delta_{\text{C}}/\delta_{\text{H}} = 104.01/6.60$  ppm than it is the case for *S. latifolius* lignins. More interestingly, the signals for  $\text{C}_{\alpha}\text{--oxidized}$  ( $\text{C}_{\alpha}\text{OOH}$ ) S units ( $\text{S}'$ ) were detected at  $\delta_{\text{C}}/\delta_{\text{H}} = 107.30/7.20$  ppm, especially in *G. arborea* lignins (Fig. 5). In contrast, the signals for G units were more important in *S. latifolius* lignins than in *G. arborea* lignins with the correlations for



**Fig. 4.** The 2D HSQC NMR spectra of RCW and TW lignin from *S. latifolius* and *G. arborea* (A-H). (A-D) Side chain ( $\delta_C/\delta_H$  50-90/2.5-5.8) and (E-H) aromatic ( $\delta_C/\delta_H$  90-120/5.5-8.0) regions in the 2D HSQC NMR spectra of RCW and TW lignin from *S. latifolius* (*S. l.*, left) and *G. arborea* (*G. a.*, right), respectively.

C<sub>2</sub>-H<sub>2</sub> at around  $\delta_C/\delta_H = 111.69/6.72$  ppm, and C<sub>5</sub>-H<sub>5</sub> at  $\delta_C/\delta_H = 115.68/6.67$  ppm, respectively. The values of S/G ratios of all studied lignins obtained from HSQC spectra are presented in Table 5.

The S/G ratios of 0.82 and 0.53 were determined for *S. latifolius* lignins (from RCW and TW, respectively) and 1.67 and 1.3 for *G. arborea* lignins (from RCW and TW, respectively). It is important to note that the S/G ratios obtained from the  $^{31}\text{P}$  NMR spectral analyses do not reflect the actual S/G ratios of lignins as is the case with the S/G ratios obtained from HSQC spectra. The interest in S/G values from  $^{31}\text{P}$  NMR spectra, which correspond to lignin units with free phenolic hydroxyls, resides in the fact that those units are recognized as important sites for lignin biodegradation. Altogether, the results from HSQC studies are in harmony with the results of the FTIR and  $^{31}\text{P}$  NMR analyses, which confirmed the dominance of G-units in *S. latifolius* lignins, while the *G. arborea* lignins are rather dominated by S units.

#### 4. Conclusions

Several properties of RCW, determined in this research, would be advantageous for soil amendment applications: higher mineral content, more favorable degradability indices in comparison with their TW counterparts and the contents and properties of their lignins. The RCW and the TW lignins isolated from the studied samples are determined to belong to the G-S type of lignins, with the dominance of the guaiacyl units in the *S. latifolius* lignins and the syringyl units in the *G. arborea* lignins. This is the first report on the chemical properties of the RCW and the TW from *S. latifolius* and its lignins. Interestingly, the lignins obtained from the studied tropical hardwoods were determined to contain nitrogen, those from the RCW in higher proportion than their

counterparts from the TW. Between studied lignocellulosic samples, the RCWs from the *S. latifolius* could be anticipated to perform well in soil amendment trials, based on their lower acidity, lower ratio C/N, and higher total mineral, and lignin content. The structural properties of the *S. latifolius* RCW lignin, such as the abundance of G-units in this otherwise S-G lignin, along with its nitrogen content, could prove favorable to retention of organic matter and carbon storage in soil, while improving soil stability and soil health.

#### CRediT authorship contribution statement

**Rodrigue Daassi:** Conceptualization, Methodology, Visualization, Software, Writing - original draft. **Pierre Betu Kasangana:** Methodology, Visualization, Software, Writing - review & editing. **Damase P. Khasa:** Supervision, Writing - review & editing, Funding acquisition. **Tatjana Stevanovic:** Conceptualization, Methodology, Validation, Supervision, Funding acquisition, Writing - review & editing.

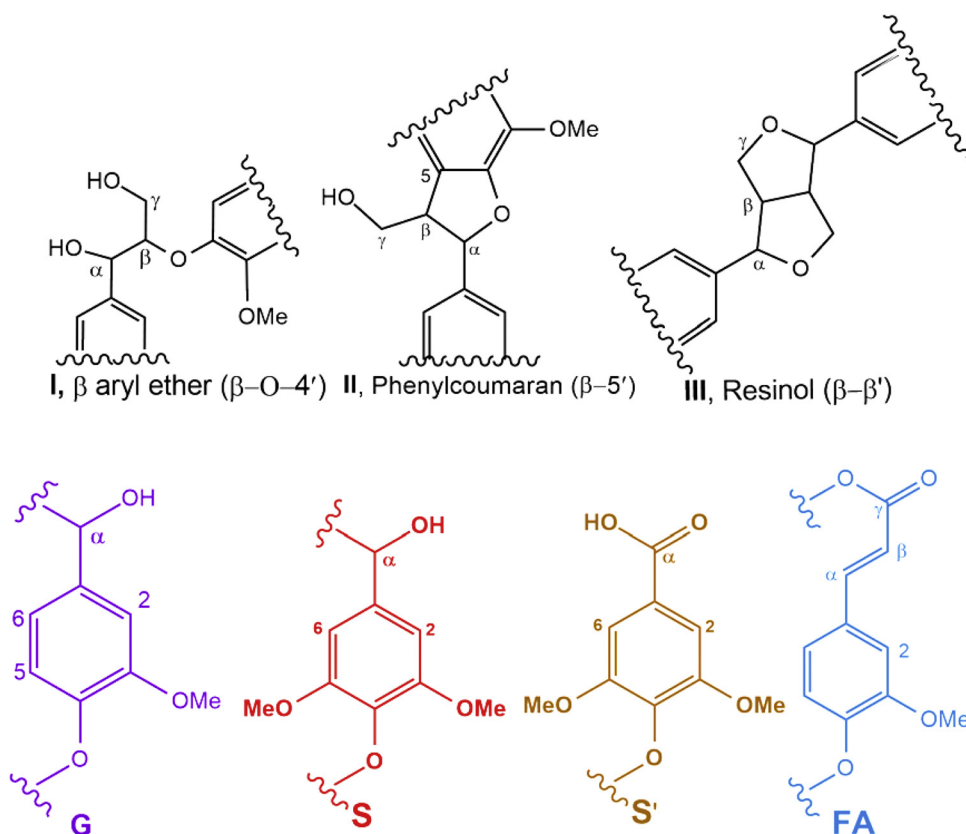
#### Declaration of Competing Interest

The authors report no declarations of interest.

#### Acknowledgments

This research is made possible thanks to the financial support from the Ministère de l'Environnement et de la Lutte contre les changements climatiques (MELCC) du Québec (Grant number: PCCI015), as part of the 2013-2020 Action Plan on Climate Change, funded by the Green





**Fig. 5.** Main substructures of organosolv lignins isolated from RCW and TW of *G. arborea* and *S. latifolius* identified by HSQC NMR. The recovered lignin fragments: I.  $\beta$  aryl ether structure formed by  $\beta$ -O-4' linkages; II. Phenylcoumaran structures formed by  $\beta$ -5' linkages; III. Resinol structures formed by  $\beta$ - $\beta'$ ; G guaiacyl units, S syringyl unit; S' oxidized syringyl units bearing a carboxyl group at C $\alpha$ ; FA ferulates.

**Table 5**

Lignin aromatic units quantification (%) from Integration of  $^{13}\text{C}$ - $^1\text{H}$  correlation signals in the HSQC Spectra of lignin isolated from RCW and TW of *G. arborea* and *S. latifolius*.

	<i>G. arborea</i> lignin		<i>S. latifolius</i> lignin	
	RCW	TW	RCW	TW
<i>Lignin aromatic units by HSQC (%)</i>				
G	37.52	42.56	54.65	65.35
S <sup>a</sup>	62.68 <sup>a</sup>	57.44 <sup>a</sup>	45.35	34.65
S/G	1.67	1.34	0.82	0.53

S: syringyl unit; G: guaiacyl unit; <sup>a</sup>: This amount of S was calculated by adding that of S' (oxidized syringyl at  $\delta_{\text{C}}/\delta_{\text{H}} = 107.30/7.20$  ppm) which is 16.42 % for RCW and 22.18 % for TW *G. arborea* lignins.

Fund. The technical support provided by Mr. Yves Bédard (Renewable Material Research Center, Université Laval) is highly acknowledged.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2020.112880>.

## References

- ASTM D1106-96, 2013. Standard test method for acid-insoluble lignin in wood. ASTM Int. 04 2013.
- Augusto, L., Ranger, J., Ponette, Q., Rapp, M., 2000. Relationships between forest tree species, stand production and stand nutrient amount. *Ann. For. Sci.* 57, 313–324.
- Austin, A.T., Ballaré, C.L., 2010. Dual role of lignin in plant litter decomposition in terrestrial ecosystems. *Proc. Natl. Acad. Sci. U. S. A.* 107, 4618–4622.
- Balakshin, M., Capanema, E., 2015. On the quantification of lignin hydroxyl groups with  $^{31}\text{P}$  and  $^{13}\text{C}$  NMR spectroscopy. *J. Wood Chem. Technol.* 35, 220–237.
- Barthès, B., Manlay, R., Porte, O., 2010. Effets de l'apport de bois raméal sur la plante et le sol: Une revue des résultats expérimentaux. *Cah. Agric.* 19, 280–287.
- Baude, M., Meyer, B.C., Schindewolf, M., 2019. Land use change in an agricultural

- landscape causing degradation of soil based ecosystem services. *Sci. Total Environ.* 659, 1526–1536.
- Bauer, S., Sorek, H., Mitchell, V.D., Ibáñez, A.B., Wemmer, D.E., 2012. Characterization of *Miscanthus giganteus* lignin isolated by ethanol organosolv process under reflux condition. *J. Agric. Food Chem.* 60, 8203–8212.
- Boudet, A.M., 2000. Lignins and lignification: selected issues. *Plant Physiol. Biochem.* 38, 81–96.
- Crestini, C., Argyropoulos, D.S., 1997. Structural analysis of wheat straw lignin by quantitative  $^{31}\text{P}$  and 2D NMR spectroscopy. The occurrence of ester bonds and  $\alpha$ -O-4 substructures. *J. Agric. Food Chem.* 45, 1212–1219.
- Datta, R., Kelkar, A., Baraniya, D., Molaei, A., Moulick, A., Meena, R.S., Formanek, P., 2017. Enzymatic degradation of lignin in soil: a review. *Sustain.* 9.
- Demeyer, A., Voundi Nkana, J.C., Verloo, M.G., 2001. Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. *Bioresour. Technol.* 77, 287–295.
- Doat, J., 1976. Caractéristiques papetières d'une essence tropicale de reboisement, le Gmelina arborea. *Rev. Bois Forêts des Trop.* 168, 47–63.
- Dossa, G.G.O., Schaefer, D., Zhang, J.L., Tao, J.P., Cao, K.F., Corlett, R.T., Cunningham, A.B., Xu, J.C., Cornelissen, J.H.C., Harrison, R.D., 2018. The cover uncovered: bark control over wood decomposition. *J. Ecol.* 106, 2147–2160.
- Faix, O., 1991. Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforschung* 45, 21–27.
- Faix, O., 1992. Fourier transform infrared spectroscopy. In: Lin, S.Y., Dence, C.W.E. (Eds.), *Methods in Lignin Chemistry*. Springer Verlag, Berlin, pp. 83–109.
- Félix, G.F., Clermont-Dauphin, C., Hien, E., Groot, J.C.J., Penche, A., Barthès, B.G., Manlay, R.J., Titttonell, P., Cournac, L., 2018. Ramial wood amendments (Piliostigma reticulatum) mitigate degradation of tropical soils but do not replenish nutrient exports. *L. Degrad. Dev.* 29, 2694–2706.
- Francezon, N., Stevanovic, T., 2017. Integrated process for the production of natural extracts from black spruce bark. *Ind. Crops Prod.* 108, 348–354.
- Fromm, J., 2010. Wood formation of trees in relation to potassium and calcium nutrition. *Tree Physiol.* 30, 1140–1147.
- Fuwape, J.A., 1989. Gross heat of combustion of Gmelina (Gmelina arborea (Roxb)) chemical components. *Biomass* 19, 281–287.
- Hendrickson, O., 1987. Winter branch nutrients in northern conifers and hardwoods. *For. Sci.* 33, 1068–1074.
- Jiang, B., Zhang, Y., Guo, T., Zhao, H., Jin, Y., 2018. Structural characterization of Lignin and Lignin-Carbohydrate Complex (LCC) from Ginkgo shells (Ginkgo biloba L.) by comprehensive NMR spectroscopy. *Polymers*. 10.
- Johnson, D.W., Turner, J., 2019. Nutrient cycling in forests: a historical look and newer developments. *For. Ecol. Manage.* 444, 344–373.
- Kasangana, P.B., Bhatta, S., Stevanovic, T., 2020. Effect of pre-extraction on composition of residual liquor obtained from catalytic organosolv pulping of sugar maple bark. *Sustain. Chem.* 1, 1–11.
- Kim, C., 2019. Carbon and nitrogen distribution of tree components in larix kaempferi

- carriere and *Quercus variabilis* blume stands in Gyeongnam province. J. Korean Soc. For. Sci. 108, 139–146.
- Kim, Y., Yu, A., Han, M., Choi, G.W., Chung, B., 2010. Ethanol solv pretreatment of barley straw with iron(III) chloride for enzymatic saccharification. J. Chem. Technol. Biotechnol. 85, 1494–1498.
- Kindiela, B.W.N., Bakouétla, G.F.M., Yebas, L.M.F., Bitá, A.M., 2019. Impact de bois raméal fragmenté de *Bridelia ferruginea* et *Acacia mangium* sur la croissance et la production du manioc sur sol ferrallitique au Congo. J. Anim. Plant Sci. 40, 6636–6647.
- Kleber, M., Lehmann, J., 2019. Humic substances extracted by alkali are invalid proxies for the dynamics and functions of organic matter in terrestrial and aquatic ecosystems. J. Environ. Qual. 48, 207. <https://doi.org/10.2134/jeq2019.01.0036>.
- Knežević, A., Milovanović, I., Stajić, M., Lončar, N., Brčeski, I., Vukojević, J., Čilerdžić, J., 2013. Lignin degradation by selected fungal species. Bioresour. Technol. 138, 117–123.
- Koumba-Yoya, G., Stevanovic, T., 2016. New biorefinery strategy for high purity lignin production. ChemistrySelect 1, 6562–6570.
- Koumba-Yoya, G., Stevanovic, T., 2017. Study of organosolv lignins as adhesives in wood panel production. Polymers. 9, 1–13.
- Latif, N.H.A., Rahim, A.A., Brosse, N., Hussin, M.H., 2019. The structural characterization and antioxidant properties of oil palm fronds lignin incorporated with p-hydroxyacetophenone. Int. J. Biol. Macromol. 130, 947–957.
- Lemieux, G., 1986. Le bois raméal et les mécanismes de fertilité du sol. Ministère de l'Énergie et des Ressources, Faculté de Foresterie, Université Laval, Québec.
- Loupe, D., Ouattara, N., Oliver, R., 1998. Maintien de la fertilité dans trois jachères arborées. Bilan minéral (Korhogo, Nord de la Côte d'Ivoire). agritrop.cirad.fr 18, 8.
- Magel, E., Einig, W., Hampp, R., 2000. Carbohydrates in trees. In: Gupta, A.K., Kaur, N.B.T.-D. (Eds.), C.S. Carbohydrate Reserves in Plants. Elsevier, pp. 317–336.
- Manlay, R.J., Feller, C., Swift, M.J., 2007. Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agric. Ecosyst. Environ. 119, 217–233.
- Meng, X., Crestini, C., Ben, H., Hao, N., Pu, Y., Ragauskas, A.J., Argyropoulos, D.S., 2019. Determination of hydroxyl groups in biorefinery resources via quantitative <sup>31</sup>P NMR spectroscopy. Nat. Protoc. 14, 2627–2647.
- Pei, G., Liu, J., Peng, B., Gao, D., Wang, C., Dai, W., Jiang, P., Bai, E., 2019. Nitrogen, lignin, C/N as important regulators of gross nitrogen release and immobilization during litter decomposition in a temperate forest ecosystem. For. Ecol. Manage. 440, 61–69.
- Senga, P.M., Prado, M., Khasa, D., Stevanovic, T.J., 2016. Contribution to chemical study of stem and branches of *Trema orientalis* L. (Blum) and *Leucaena leucocephala* (Lam.) De Wit. Nat. Prod. Chem. Res. 04, 4–9.
- Shamim, G., Sandra, Y.F., Joann, K.W., 2014. Lignin controls on soil ecosystem services: implications for biotechnological advances in biofuel crops. In: Fachuang, Lu (Ed.), Lignin. Nova Science Publishers, New York, pp. 375–416.
- Sitholé, B., 2005. New method of measuring the pH of wood chips. Pulp Pap. Canada 106, 42–45.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass. Lab. Anal. Proced. 1617, 1–16.
- Soumare, M.D., Mnkeni, P.N.S., Khouma, M., 2002. Effects of *Casuarina equisetifolia* composted litter and ramial-wood chips on tomato growth and soil properties in Niayes. Senegal. Biol. Agric. Hortic. 20, 111–123.
- Stevanovic, T., Perrin, D., 2009. Chimie du bois. Presses polytechniques et universitaires romandes.
- Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions. John Wiley & Sons.
- Suansa, N.I., Al-Mefarrej, H.A., 2020. Branch wood properties and potential utilization of this variable resource. BioResources 15, 479–491.
- Taylor, B.R., Parkinson, D., Parsons, W.F.J., 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70, 97–104.
- Thevenot, M., Dignac, M.F., Rumpel, C., 2010. Fate of lignins in soils: a review. Soil Biol. Biochem. 42, 1200–1211.
- Tissaux, J.-C., 2001. Caractérisation de bois raméaux fragmentés et indices de décomposition. Mémoire pour l'obtention du grade de maître es sciences (M.Sc.) Département des sciences du bois et de la forêt, Université Laval.
- Tolbert, A., Akinoshio, H., Khunsupat, R., 2014. Review: characterization and analysis of the molecular weight of lignin for biorefining studies. Biofuels, Bioprod. Biorefining 8, 836–856.
- Wen, J.L., Sun, S.L., Yuan, T.Q., Sun, R.C., 2015. Structural elucidation of whole lignin from *Eucalyptus* based on preswelling and enzymatic hydrolysis. Green Chem. 17, 1589–1596.
- Xiao, C., Bolton, R., Pan, W.L., 2007. Lignin from rice straw Kraft pulping: effects on soil aggregation and chemical properties. Bioresour. Technol. 98, 1482–1488.
- Xiao, M.-Z., Chen, W.-J., Hong, S., Pang, B., Cao, X.-F., Wang, Y.-Y., Yuan, T.-Q., Sun, R.-C., 2019. Structural characterization of lignin in heartwood, sapwood, and bark of *eucalyptus*. Int. J. Biol. Macromol. 138, 519–527.
- You, T.T., Mao, J.Z., Yuan, T.Q., Wen, J.L., Xu, F., 2013. Structural elucidation of the lignins from stems and foliage of *Arundo donax* Linn. J. Agric. Food Chem. 61, 5361–5370.
- Yue, P.P., Hu, Y.J., Fu, G.Q., Sun, C.X., Li, M.F., Peng, F., Sun, R.C., 2018. Structural differences between the lignin-carbohydrate complexes (LCCs) from 2- and 24-month-old bamboo (*Neosinocalamus affinis*). Int. J. Mol. Sci. 19, 1–14.
- Zhang, Y., Hou, Q., Xu, W., Qin, M., Fu, Y., Wang, Z., Willför, S., Xu, C., 2017. Revealing the structure of bamboo lignin obtained by formic acid delignification at different pressure levels. Ind. Crops Prod. 108, 864–871.
- Zhang, H., Zhang, S., Yuan, H., Lyu, G., Xie, J., 2018. FeCl<sub>3</sub>-catalyzed ethanol pretreatment of sugarcane bagasse boosts sugar yields with low enzyme loadings and short hydrolysis time. Bioresour. Technol. 249, 395–401.
- Zhao, C., Huang, J., Yang, L., Yue, F., Lu, F., 2019. Revealing structural differences between alkaline and Kraft lignins by HSQC NMR. Ind. Eng. Chem. Res. 58, 5707–5714.