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REPLICATION

Wood and bark lignin contents of trees from deciduous forests of eastern India

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Abstract
Lignin is a polyphenolic compound found in plant tissues, especially wood and bark. The lignin content determines the quality of wood biochar in agroecological uses, and is used in the production of synthetic resins and adhesives. Despite its importance in plant physiology and its agricultural and industrial utility, there exists a wide gap of knowledge of lignin contents of tropical hardwood trees of South Asia, except for a few species. We present here the first estimation of lignin content in wood and bark of 48 species from tropical deciduous forests of India. We show that some species are characterized by greater wood lignin (WL) compared with bark lignin (BL) content, contrary to the generalization held for hardwood trees, and suggest a plausible correspondence between the WL to BL ratio and timber quality.

Keywords: Deciduous Forest; India; Lignin; Wood;

1. Introduction

Lignin, a polyphenol compound, is found in plant cell wall, and is the second most abundant plant biopolymer after cellulose. It is found especially in the bark and wood fibers. Lignin is formed by variously organized phenyl-propane units, linked by alkyl-aryl ether and C–C bonds, and is derived chiefly from p-coumaryl, coniferyl, and sinapyl alcohols. The proportion of these aromatic units in the macromolecular structure depends on the taxonomic group of the plant and its anatomical part. The guaiacyl type is found predominantly in softwoods and guaiacyl-syringyl in hardwoods (Novaes et al., 2010; Santos et al., 2012). The lignin in wood contributes to its mechanical strength, enabling the trees to support more than 2,000 metric tons of aboveground biomass (Fry & White, 1938). Lignin is industrially utilized for making adhesives for composite wood products, such as particleboard and linoleum, and as an active ingredient of phenolic resins (Çetin & Özmen, 2002). Higher lignin content of wood is known to yield greater quantity and quality (calorific value) of biochar for improving farm soil quality (Cagnon et al., 2009; Novotny et al., 2015). For its importance in production of biochar (Deb et al., 2016; Spokas et al., 2012) and industrially valued products (Chung & Washburn, 2012), information of lignin content in different species would facilitate appropriate feedstock selection.

2. Objective

Tropical deciduous forests constitute the most widespread forest type in India (Champion & Seth, 1968), and is the habitat of at least 70 indigenous hardwood tree species. Wood lignin (WL) profiles of these trees are almost invisible in the available wood chemistry literature, except for only three species, namely, *Shorea robusta* Gaertn., *Tectona grandis* L.f., and *Dalbergia sissoo* Roxb. Ex DC from central India (Narayanamurti & Das, 1955). We intend to fill this gap in knowledge of lignin content of major trees of India's deciduous forests, covering 68% of the total forest cover in India (Reddy et al., 2015). We further intend to examine if the tropical Asian hardwood lignin contents conform the generalizations about WL and bark lignin (BL) contents, built primarily on tree lignin data from neotropical, temperate, and alpine zones of the world.

3. Materials and methods

3.1. Site selection

Since lignin content of hardwood trees (except *S. robusta, T. grandis, and D. sissoo*) from deciduous forests of India are yet unknown, we selected a dry deciduous forest of Bankura district in West Bengal and a moist mixed deciduous forest of Rayagada district in Odisha (Figure 1). The forest type and species composition of the sampling sites are given in Table 1.

3.2. Sample collection

A total of 48 species of native hardwood trees were sampled. Mature trees older than 10 years, with girth of >25 cm at 1.2 m stem height were selected for sampling. To avoid felling of any live tree, we did not collect samples of mature stem wood; instead, *ca.* 15 cm pieces of wood of *ca.* 5 cm diameter with intact bark were collected from matured branches.

3.3. Chemical analyses

Six samples of wood and bark of each species from each habitat were examined. The samples were prepared using C-TAB, following Van Soest (1963). Acid insoluble (Klason) lignin was estimated following Obst et al. (1988); 0.3 g of powdered sample was treated with 3 ml 72% sulfuric acid for 1 hr at 30°C. Subsequently, the solution was diluted to 4% for acid hydrolysis in an autoclave for 1 hr, followed by filtration using Whatman-1 filter paper to separate the soluble and insoluble fraction.

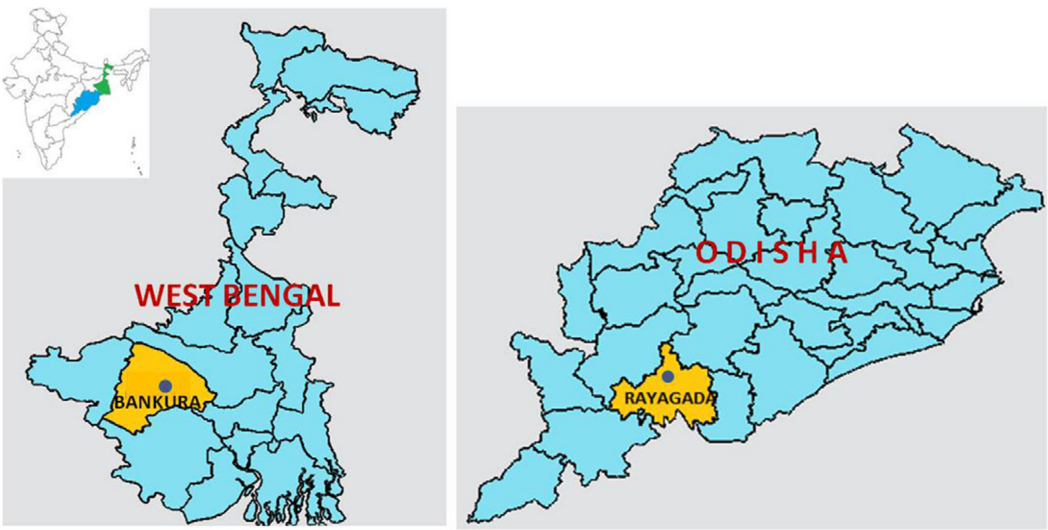

Figure 1. Map showing locations in two States of India. Dots indicate the locations in the districts (yellow) from which wood samples were collected.

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Table 1. Deciduous forest types in Odisha and West Bengal with dominant series

State	District	Forest type	Sampling point coordinates[a]	Dominant species (in order of relative abundance)[b]
Odisha	Rayagada	Tropical moist mixed deciduous	19°33′19.13”N 83°26′16.7″E 19°40′08.96”N 83°29′24.02″E	*Diospyros melanoxylon, Cleistanthus collinus, Anogeissus latifolia, Madhuca latifolia, Buchanania lanzan, Holarrhena antidysenterica, Shorea robusta, Terminalia tomentosa, Pterocarpus marsupium, Syzygium cumini, Bridelia retusa*
West Bengal	Bankura	Tropical dry deciduous	23°13′07.3″N 87°15′50.8″E 23°12′46.1″N 87°16′24.18″E	*S. robusta, D. melanoxylon, H. antidysenterica, B. lanzan, A. latifolia, Lagerstroemia parviflora, M. latifolia, Terminalia belerica, Albizzia procera, Cassia fistula*

[a]Point samples with 100 m radius.
[b]Based on each species density estimated in 5 × 1,000 m^2 transects.

To remove interference from different pigment compounds from the wood of *Diospyros* and *Terminalia* spp., which showed reddish to black filtrates after acid hydrolysis, we modified the extraction method by repeated washing of the filtrate with 1 L hot water (approx. 50°C), followed by 10–15 ml acetone and 1 L water, successively, before drying the filter paper and the precipitate, weighing and preparing the substance for gravimetric analysis.

The acid soluble lignin content (*S*) was estimated using UV–vis spectrophotometer (JASCO V-730 Bio, Tokyo, Japan) at 215 and 280 nm, and calculated from the following formula:

$$S\left(\mathrm{g L}^{-1}\right) = (4.53\mathrm{A}_{280} - \mathrm{A}_{215})/300$$

derived from the simultaneous resolution of two equations, as given in Moreira-Vilar et al. (2014):

$$\mathrm{A}_{280} = 0.68F + 18S \,\mathrm{and}\, \mathrm{A}_{215} = 0.15F + 70S,$$

where A_{280} and A_{215} are the absorbance values at 280 and 215 nm, respectively; and *F* is the furfural concentration (g L^{-1}). Ash content was determined gravimetrically after combustion of the insoluble part of wood and bark at 550°C for 4 hr in a Muffle furnace.

3.4. Statistical analysis

Lignin content of each species from two types of forest was compared. Because our dataset is small (six replications for each species from each location), test of normality is inapplicable, and nonparametric tests are of low power. We therefore calculated the Bayesian credibility interval, equivalent to confidence intervals (Eberly & Casella, 2003), to estimate the significance of difference between means of lignin levels.

4. Results and discussion

The Klason lignin, acid soluble lignin, and ash contents of the wood and bark of 48 species are presented in Supplementary Table S1. The data corroborate the established fact (Novaes et al., 2010) that lignin content is fairly invariant within a species, but varies considerably between different species. The lignin content of certain species may vary, albeit slightly, with the sampling locale (Supplementary Table S1)

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Table 2. The six hardwood species showing Bayesian credible difference between means of wood and bark lignin contents from Odisha (OD) and West Bengal (WB) forests

		Mean Lignin Content (% w/w)		Difference between Means	
Species	Origin	Wood	Bark	Wood	Bark
Alstonia scholaris	OD	15.0	19.7		Credible
	WB	15.5	22.2		
Artocarpus integrifolia	OD	16.0	24.8	Credible	Credible
	WB	22.9	27.2		
Casearia tomentosa	OD	15.2	21.5	Credible	Credible
	WB	19.2	26.2		
Diospyros peregrine	OD	17.8	15.3	Credible	
	WB	14.0	12.8		
Holarrhena antidysenterica	OD	20.5	21.2	Credible	
	WB	22.5	25.0		
Shorea robusta	OD	21.1	21.8		Credible
	WB	19.9	24.8		
Tamarindus indica	OD	20.8	29.8		Credible
	WB	21.3	26.8		

with different edapho-climatic conditions. Lignin content of most of the species appear to be invariant. The difference between means of WL and BL contents from two habitats is within Bayesian credibility interval only for six species presented in Table 2. The difference in WL contents between two habitats is credible for four species, whereas the difference in BL contents is credible for five species.

The WL contents range from 10.8% (*Syzygium cumini*) to 28.2% (*Moringa oleifera*). Considerably high (>20%) lignin content is found in the wood samples of *Alangium salvifolium*, *Albizzia* spp., *Artocarpus heterophylla*, *Buchanania lanzan*, *Careya arborea*, *Cassia fistula*, *Diospyros melanoxylon*, *Ficus racemosa*, *Gmelia arborea, Holarrhena pubescens*, *Mytragyna parviflora*, *Pterocarpus marsupium*, *Tamarindus indica*, and *Terminalia* spp. The WL content in *S. robusta* is considerably less (19.9–21.1%) in our samples compared with the heartwood samples (29.3%) reported in Narayanamurti and Das (1955). BL contents of our samples range from 12.0 (*Haldina cordifolia*) to 36.8 (*Neolamarckia cadamba*). Pyrolysis of these high-lignin feedstock may produce better quality of biochar and resin than from other trees. Figure 2 shows the mean values of the wood and bark of the trees from two forest types.

The inner BL is chemically similar to the WL, but the outer (dead) bark contains different forms of it (Sjostrom, 1981). The BL content of hardwood trees is generally higher than WL (USDA, 1971). However, this contention, built primarily on the data from temperate, neotropical, and alpine trees, does not hold for some tropical deciduous trees. While the BL to WL ratio is >1.0 in 79% of our samples, 12 species have this ratio of <1.0. Kalita and Saikia (2004) also reported that the evergreen trees *Mimusops elengii* and *Plumeria alba* from northeastern India have BL < WL. In our study, the lowest ratio (0.54) is for *M. parviflora.*

The BL/WL ratio seems to be unrelated to the phylogenetic relatedness between taxa, with different BL/WL values for different species within the same genera, and is predictably, inversely related (slope $= -0.057$, $R^2 = 0.43$, $t = 1.98$) to the corresponding WL content (Figure 3). We further note that BL/WL < 1.0 corresponds to low quality (Class III) timber, (e.g., *D. malabarica*, *M. parviflora*, and *T. Chebula*), whereas trees with stronger and high quality (Classes I and II) timber (Sundararaj et al., 2015) tend to have a higher BL/WL ratio in our study. However, this apparent correspondence has a few

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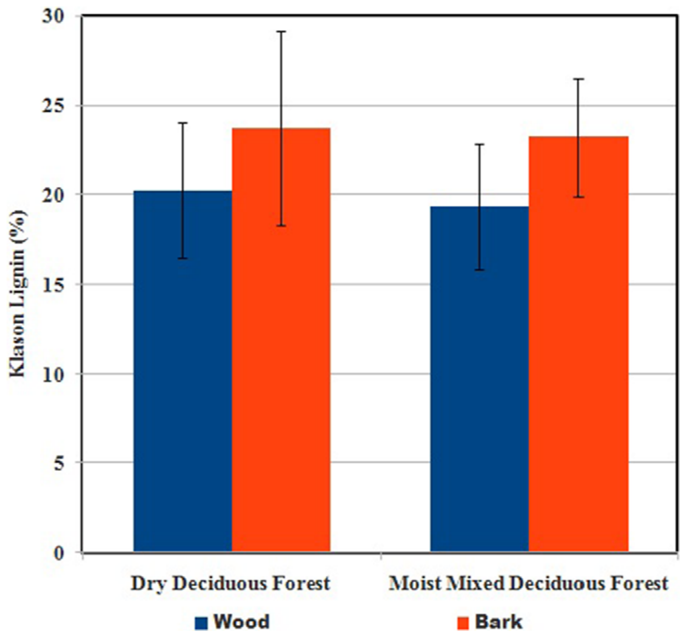

Figure 2. Mean % contents of Klason lignin in wood and bark of trees sampled from dry deciduous and moist deciduous forests.

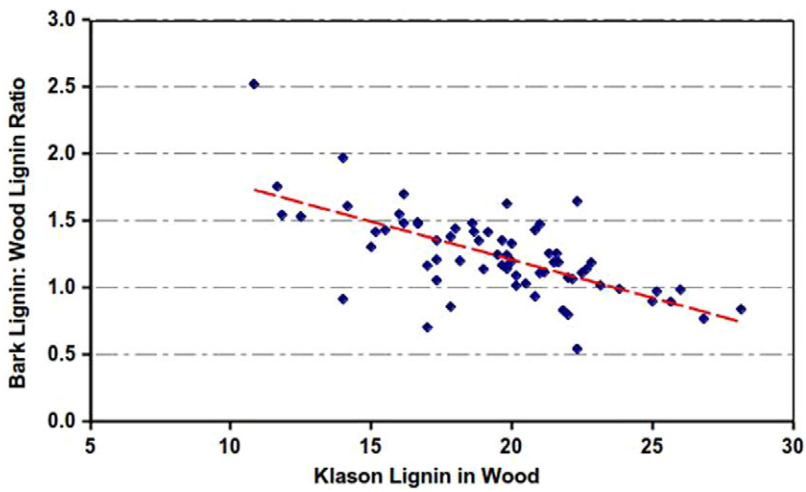

Figure 3. Regression of BL/WL ratio with wood lignin contents of 67 samples. Slope = −0.057, R^2 = 0.43, p < 0.001.

exceptions; *Anogeissus latifolia* and *N. Cadamba*, known to belong in Class III timber class (Sundararaj et al., 2015), have the value of BL/WL > 1.4 (Table S1).

5. Conclusion

Our study of 48 species reports a range of species-specific lignin contents, and reveal that some tropical trees are characterized by WL > BL, contrary to most hardwood trees. Further investigation is required to establish a predictive relationship between the mechanical properties of wood and their lignin profiles.

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Limitations of the study

1. With limited resources, we were unable to collect samples from more locations than two.
2. Owing to legal restrictions, and in conformity with conservation principles, we collected samples of tree branches instead of mature stem.

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Supplementary Materials. To view supplementary material for this article, please visit http://dx.doi.org/10.1017/exp.2021.18.

Data availability statement. All data are freely available from authors upon request.

Authorship contributions. Conceptualization, D.D.; Investigation, D.D.; Design, D.D.; Writing-original draft, D.D.; Chemical analyses, P.R.

Conflict of interest. The authors declare no conflict of interest.

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Peer Reviews

Reviewing editor: Dr. Richard Erickson

US Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd, La Crosse, Wisconsin, United States, 54603

This article has been accepted because it is deemed to be scientifically sound, has the correct controls, has appropriate methodology and is statistically valid, and has been sent for additional statistical evaluation and met required revisions.

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Review 1: Wood and Bark Lignin Contents of Trees from Deciduous Forests of Eastern India

Reviewer: Dr. Jamaludin Malik

Forest Products Research and Development Centre, Forest Products Utilisatiion, 5 Gunung Batu Street, Bogor City, West Java, Indonesia, 16144 and Forest Products R&D Centre

Date of review: 20 July 2021

Conflict of interest statement. Reviewer declares none

Comments to the Author: Because this manuscript presents a kind of basic research of wood science, presenting lignin content as many as wood species mentioned will be perfect. However, it is clear now after the authors added the limitation of the study.

Score Card

Presentation

5.0 /5

Is the article written in clear and proper English? (30%)	5/5
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Review 2: Wood and Bark Lignin Contents of Trees from Deciduous Forests of Eastern India

Reviewer: Dr. Richard Erickson

US Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd, La Crosse, Wisconsin, United States, 54603

Date of review: 28 July 2021

Conflict of interest statement. Reviewer declares none

Comments to the Author: Thank you for addressing the previous feedback and re-submitting.

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Analysis

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Does the discussion adequately interpret the results presented? (40%)	5/5
Is the conclusion consistent with the results and discussion? (40%)	5/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	5/5