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# LIGNIN AND CARBON ISOTOPES COMPOSITION OF SUSPENDED PARTICULATES BY CAPILLARY GAS CHROMATOGRAPHY OF CUPRIC OXIDE OXIDATION PRODUCTS

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## ABSTRACT

Lignin are high molecular weight phenolic polymers that occur as major constituents of vascular plants. As a result of their natural abundance, wide distribution, and resistance to microbial degradation, lignin are also commonly found in soil and sedimentary organic matter. The aim of this study was to identify the contribution of lignin to the suspended particulate fraction in the Losari Beach and Lae-lae Island, where indicate highly degraded lignin materials. Lignin was characterized by oxidative degradation, cupric oxide being chosen as the most suitable oxydizing agent to produce simple lignin-derived phenols that are extracted with hexane and analyzed by capillary gas chromatography on fused silica columns, provides the high sensitivity and precision required for the identification and quantitation of trace levels of lignin in seawater. A suite of up to 8 phenols is produced that reflects the relative concentration and plant tissue sources of lignin present in the suspended material.

**Keywords**: Lignin, CuO oxidation, Particulate matter

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## 1. INTRODUCTION

Organic matter in the marine environment, significantly affect the dynamics of microalgae through the increase of variabilitas turbidity[18,19]. Loads and types of organic materials entering the water, partly produced by some major producing organisms, such as phytoplankton, macroalgae and chemoautotrophic bacteria. This organic material will then experience degradation with different time[9]. Most terrestrial plants produce lignin, which is resistant to microbial degradation, as such lignin found in sediments is often used to explore the entry of terrestrial organic materials in aquatic and terrestrial vegetation[15].

Lignin, the second most abundant naturally occurring polymer after cellulose, is an exclusive and stable phenolic macromolecule found in vascular plants[20,28,29]. The lignin-derived phenols that was obtained through the CuO oxidation process[10] can be used to analyze lignin in plant tissues and environmental matrices[1,19,24]. Lignin analysis at the molecular level has been widely practiced in a variety of geochemical applications as a biomarker approach in investigating the source and processing of biogeochemistry or the reactivity of organic matter (OM) in sediment and soil, and at the study of limnology and oceanography[12,21].The $\delta^{13}C$ compositions of particulate organic carbon (POC) have been investigated in a range of sedimentary environments[5,8,19,25]; while carbon isotopic (especially $^{13}C$) data on lignin phenols in marine sediments remains sparse[3,23]. Therefore, any changes in environmental conditions and vegetation can be deduced from the composition of the organic compounds.

Identification and quantification of eight different lignin structural phenols were accomplished by comparison of mass spectral sample response to an internal ethyl vanillin standard and an external calibration curve of authentic lignin phenolic compounds[2]. Alkaline CuO oxidation of lignin yields a suite of phenols that are grouped based on plant taxonomy, and that are used to identify woody and nonwoody tissues of vascular plants[14,27]. The concentration of CuO lignin monomers relativized to the total of each variety were evaluated by principal components analysis to identify broad differences in lignin composition.

The objective of this study was to explain the behavior of transportation landfill into the sea using lignin phenol biomarkers combined with carbon isotopes ($\delta^{13}C$ values) of the suspended particulate fraction in to the Losari Beach and Lae-lae Island.

## 2. MATERIAL AND METHODS

### 2.1 Materials

Calibration compounds, CuO, glucose, $Fe(NH_4)_2(SO_4).6H_2O$, Ethyl vanillin, n-hexane, N,O-bis(trimethylsilyl)trifluoro acetamide (BSTFA) mixed with 1% trimethylchlorosilane (TMCS) and pyridine.

### 2.2 Study areas sampling and sample preparation

Suspended particulate matter was collected from the Losari Beach and Lae-lae Island, Makassar, South of Sulawesi (Fig. 1). Five liters of water were collected using a niskin bottle at the depth of 5 m below sea surface, refrigerated and transported to the laboratory where they were passed through pre-weighed GF/F 0.7 µm. The filters were dried (60 °C, ~24 h) and reweighed, yielding

**Figure 1.** Sampling locations in the Losari Beach (Red colour) and the Lae-lae Island (green colour)

200-500 mg of suspended particulate matter (SPM). This sample was analysed for stable carbon isotope composition and lignin content.

## 2.3 Elemental and carbon isotope analyses

Organic carbon (OC) and total nitrogen (TN) were measured using an elemental analyzer (Thermo Fisher Scientific) after acid treatment with 1 N HCl until no bubbles were observed. The sample pre-treatment steps for carbon isotope analysis. Aliquots of powdered suspended particulate matter from the acid treatment were assayed using a carbon analyzer and/or elemental analyzer. Analyses were carried out using an EA-IRMS (elemental analyzer–isotope ratio mass spectrometry). Carbon isotopic compositions are expressed as $\delta^{13}C$, in parts pr thousand (‰), relative to the VPDB standard.

## 2.4 Lignin phenol analysis

Eight lignin phenols were quantified for all samples and included three vanillyl phenols (vanillin, acetovanillone and vanillic acid) and three syringyl phenols (syringaldehyde, acetosyringone and syringic acid). Additionally, two cinnamyl phenols (p-coumaric acid and ferulic acid) were quantified solely for the freshwater samples.

Lignin analysis was carried out using the cupric oxide (CuO) oxidation method [10], with some modification as summarized in Table 1. Precisely 0.1 g sediment of SPM samples, 0.5 g $Fe(NH_4)_2(SO_4).6H_2O$ and 1.0 g CuO powder were added with 2 N NaOH into Teflon containers with stainless steel jackets at 175 °C in a drying oven for 3 h and manually shaken every hour. The oxidation products are cooled then added 10 ml of 1 N NaOH and centrifuged. This step was repeated twice. The supernatants were pooled and acidified to pH 1 with 6 N HCl. The supernatants were then extracted three times with 10 ml hexane.

Excess solvent dried off with a gentle stream of $N_2$. The product was diluted with equal volume of pyridine and the silylating reagent bis-trimethylsilyltrifluoroacetamide (BSTFA) with 1% trimethylchlosilane (TMCS) (as catalyst). The product, with

**Table 1.** Improvements in lignin phenol methodology from the original technique[10]

| Original Technique | Modification | Reference for modification | This Study | Notes |
|---|---|---|---|---|
| **N/A** | Addition of glucose to reaction vessel | Hernes *et al* (2002); Louchouarn *et al* (2010); Kaiser *et al* (2012); Jex *et al* (2014) | Glucose | Minimizes superoxidation effects in low organic matter |
| **Ethyl vanillin as quantification standard** | Cinnamic acid as quantification (internal standard) | Opsah et al (1999); Hernes and Benner (2002); Louchouarn *et al* (2010) | Ethyl vanillin | Eliminates steps, significant ethyl vanillin can be lost during laboratory processing in samples with limited matrix |
| **Oksidation at 150 °C (external reaction vessel temperature)** | Oksidation at 155 °C (internal reaction vessel temperature) | Goni and Hedges (1992); Louchouarn *et al* (2010); Loh *et al* (2012) | Oksidation at 175°C (external reaction bomb Teflon temp) | More accurate and representative temperature control |
| **Ethyl eter extraction** | Ethyl acetate extraction | Goni and Montgomery (2000); Louchouarn *et al* (2010); Spencer *et al* (2010); Jex *et al* (2014) | Hexan extraction | Substitutes highly explosive reagent for safer reagent, slightly higher extraction efficiency |
| **GC with Flame Ionization Detector** | GC with Mass Spectrometry Detector | Opsahl and Benner; Staniszewski *et al* (2001); Louchouarn *et al* (2010) | GC with Mass Spectrometry Detector | Increased sensitivity and eliminates co-elution problems |

silylating reagent, was derivatized by heating at 90 °C for 10 min and then allowed to cool. Immediately after cooling, the derivatized sample was analyzed for lignin-derived phenols using a gas chromatography mass spectrometer (GC–MS). Hewlett–Packard 5890 fitted with an SE30 capillary column (30 m, 0.25mm internal diameter, SUPELCO). The carrier gas was helium. The temperature of the GC was increased from 100 °C to 200 °C by 5 °C per minute and was held for 10 min. The temperature was then increased from 200 °C to 300 °C by 20°C per minute and was held at 300 °C for min. Both injector and detector temperatures at 300 °C. The equilibration time was 2 min, and the split ratio was 50:1. The average reproducibility of individual lignin phenols based on duplicate analyses of a same sample was 10% (coefficient of variation).

## 3. RESULTS AND DISCUSSIONS

### 3.1 Elemental and carbon

The magnitude of the particulate organic material from the landfill, has helped in tracking the extension of waste on the coast of Losari beach and Lae-lae islands. Percentage of Ctot and Ntot on suspense particle materials at Losari beaches is 0.602 and 0.033, while on Lae-lae island is 0.685 and 0.041. Ctot percentages are more abundant than Ntot, reflecting the situation of most terrestrial organic matter emptying into the ocean.

Spatial variation has implied that the distribution of organic matter from anthropogenic activities has more significant effect than natural processes. The value of C/N ratio in Losari beach and Lae-lae islands are 18.1 and 19.3, respectively, indicated that the organic material is derived from terrigenous. This is due to the inorganic particulate organic material that survived in transport to the observation distance, prolonging the pre-oxidized and more resistant to microbial degradation, thus providing the possibility in detecting the contribution of terrigenous organic matter.

Use of $\delta^{13}C$ values to distinguish sources of organic matter such as marine and significant differences were observed for the suspended samples. The $\delta^{13}C$ values of Losari Beach and the Lae-lae Island ranges from -7,861‰ to -9,658‰ and -8,762‰ to -10,231‰. The $\delta^{13}C$ values suggested that there is a conservative mixing between terrestrial and aquatic sources derived organic matter throughout the entire core. Therefore, the $\delta^{13}C$ values of SPM in the Losari beach and the Lae-lae Island indicate a small contribution of aquatic plant species.

### 3.2 Lignin-derived phenols

Eight “characteristic” lignin-derived phenolic monomers were detected in high concentrations in the suspended particulate fraction in the Losari Beach and Lae-lae Island, reflecting both the high abundance of lignin as a component of terrestrial plant biomass and the preferential accumulation of woody plant fragments (which have a high lignin content) from the estuary.

Contributions of three lignin index-phenols (V, S and C) indicating that influxes of the lignin. V-phenols were the most dominant CuO-oxidation products of lignin, followed by S-phenols and C-phenols in the lower parts of the core. In marine samples, the cinnamyl phenol concentrations are not usually reported because of significant non-lignin sources[13,26].

Lignin phenol ratios (S/V and C/V values) might be changed prior to deposition by a selective degradation of lignin structural units during the transfer of terrigenous organic matter from the water column to deep sediment deposits[16,17]. Syringyl phenols (S) are exclusively found in angiosperms and cinnamyl phenols (C) are solely found in non-woody tissue, so ratio values of these phenols to ubiquitos vanillyl phenols (V) can differentiate relative contribution OM sources from angiosperm and gymnosperm plant types (S:V) and non-woody and woody tissues (C:V)[26]. The C/V values higher than 0.20 and S/V values higher than 0.40 are indicative of non-woody and angiosperm tissues[6,7,11]. Hence, the S/V (0,14) and C/V (0.01) ratios in the Losari beach and the S/V values (0.13) and C/V (0.03) in the Lae-lae island, indicate the presence of woody and gimnosperm plant tissues.

The acid/aldehyde ratios of three lignin phenol groups have been used to characterize diagenetic alteration in a variety of geochemical samples. The acid:aldehyde ratios of V- and S-phenols (i.e. vanillic acid/vanillin (Ad/Al)v and syringic acid/syringaldehyde (Al/Ad)s) are indicators of the diagenetic alteration of lignin[4,10,11,22]. (Ad/Al)v is a better indicator of lignin diagenesis than (Ad/Al)s because there are usually higher levels of vanillyl oxidation products[11]. The values of (Ad/Al)v in the Losari Beach and the Lae-lae Island are 0.17 and 0.15, respectively.

## 4. CONCLUSIONS

Lignin phenol ratio in the Losari Beach and Lae-lae island explain the presence of woody and gimnosperm plant tissues. The value of C/N and $\delta^{13}$C ratios, spatially has implied that the distribution of organic matter from anthropogenic activities. The inclusion of organic matter in the coastal waters explains that the groundwater organic particulate matter that survived in transportation to the outermost distance, prolongs the pre-oxidized and more resistant to microbial degradation.

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