Recent Analysis of Carbon, Nitrogen, and Lignin Phenol Compositions in the Suspended Particulate Matters at Spermonde Archipelago, South Sulawesi, Indonesia

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ABSTRACT

This study analyzed the composition of monomer lignin phenols and its derivatives at the Spermonde Archipelago, South Sulawesi-Indonesia. Water samples were collected in the dry season (June 2017) and the rainy season (January 2018) from the estuaries of Tallo-Makassar, and Pangkep. Analysis of carbon and nitrogen contents was conducted by EA-IRMS (elemental analyzer-isotope ratio mass spectrometry), while lignin phenol was analyzed by Chromatography Gas-Mass Spectroscopy (GC-MS). Spatially, the six lignin phenols (A) content in the Tallo river estuary into several outermost islands is higher than that of the Pangkep river mouth. A values in the rainy season were higher (0.92-2.30) than in the dry season (0.62-2.07). In the dry season, the range of values for ratios of syringyl/vanillyl and cinnamyl/vanillyl was 0.35 to 1.12 and 0.39 to 0.57 indicating a low contribution of angiosperm plant tissue. In the rainy season, the values of ratios for syringyl/vanillyl and cinnamyl/vanillyl ranged from 0.37 to 1.18 and 0.32 to 0.62. The syringyl/vanillyl ratio indicates the contribution of plant tissue to angiosperms. The cinnamyl/vanillyl value is greater than 0.1, indicating a significant contribution of non-woody plant tissue. Spatially, the range of syringyl/vanillyl and cinnamyl/vanillyl ratios at the estuary of the Tallo river (0.37 to 1.12 and 0.32 to 0.57) were higher than at the Pangkep river estuary (0.35 to 1.18 and 0.39 to 0.62).

1. INTRODUCTION

The waters of the Spermonde Islands are located in the southwest of Sulawesi Province at 118°90'0"-119°30'0" E and 5°10'0"-5°50'0" S. These areas have an important economical and ecological role because it has a vast expanse of coral reefs, various types of sponges and enormous fisheries potential. Currently, the region received considerable pressure from a variety of activities (industry, logging and agricultural activities, and household activities) that occur in the mainland city of Makassar and Pangkep Regency (Nurdin et al., 2016). The magnitude of the land activity’s effect on the waters of the Spermonde islands can be done through organic matter analysis.

The composition of organic matter along coastal and marine areas is affected by solid materials which have undergone weathering, seasonal changes, and increased erosion (Louchouarn et al., 2010; Bayram et al., 2013; Qu and Kroeze, 2012). The organic materials in waters will be overhauled by essential nutrients through the process of decomposition. Organic materials are supplied by plants from land and water plants such as the production of phytoplankton, seaweed, and other marine organisms (Xing et al., 2011; Hedges and Oades, 1997; Li et al., 2015; Lagbas et al., 2017). The changing process of organic matter that occurs in the ocean can be identified through the stable isotopes of carbon (δ13C), nitrogen (δ15N), and the molecular
properties of sedimentary organic matter (SOM) (Onstad et al., 2000; Opsahl et al., 2001).

The ocean is the final destination for all activities on land and sea, naturally assimilating all foreign materials it receives. The ocean will lose the assimilation ability and pressure the ecosystem with pollution when the speed of assimilation is slower than the supply of the material. Input loads of dissolved and suspended materials that affect coastal and marine aquatic environments can cause eutrophication (Garnier et al., 2010), the possibility of the emergence of dangerous microalgal species (Gypens et al., 2009), and damage to coral reef ecosystems and biodiversity (Costa et al., 2008).

The characteristics of overflow from land in coastal waters can be determined by using the lignin biomarker method. Total lignin is the amount of vanillyl phenol, syringyl (S) and cinnamyl (C). In the angiosperm plants, the lignin content consists of syringyl and vanillyl phenols. Whereas gymnosperm tissue only produces vanillyl phenol, while only non-wood tissue produces cinnamyl phenol. Therefore, the S/V and C/V ratios are used to distinguish the source of angiosperms between wood and non-wood and gymnosperm plant tissue (Hansell and Carlson, 2001; Hedges and Oades, 1997). The ratio of vanillic acid to vanillyl (Ad/Al), and syringic acid for syringaldehyde (Ad/Al), are indicators from diagenesis lignin. The ratio of vanillic acid to vanillyl (Ad/Al), and syringic acid to syringaldehyde (Ad/Al), can be indicators of lignin diagenesis. Lignin biomarker has provided accurate predictions about water in the Amazon river system (Aufdenkampe et al., 2007), Bekanbeushi Moor, Northern Japan (Nagao et al., 2010) and Kapuas River, West Kalimantan (Loh et al., 2012).

Based on literature studies, until now, there has been no comprehensive research on the effects of organic runoff in the waters of the Spermonde Islands. The results of this study can be part of the pattern of development of coastal areas in the city of Makassar and Pangkep Regency that contribute to the waters of the Spermonde. This pattern of development will improve the quality of waters in spermonde and reduce damage to the diversity of animals and plants that exist around these waters. This study determined the effect of runoff of organic material from the mainland city of Makassar and Pangkep Regency into the waters of the Spermonde Islands using the composition of monomer lignin phenols.

2. METHODOLOGY

2.1 Site of study

The research was conducted in the dry season (June 2017) and the rainy season (January 2018). Spatially, the study location at the Tallo and Pangkep coastal waters areas were chosen perpendicular to the mainland. Determining the position/point of research stations during observations was done using Global Positioning System based on distance (Figure 1). This area was chosen because it is a productive area, in which there are mangrove ecosystems, seagrass beds and coral reefs (Spermonde Islands). The ecosystems are very important in sustaining the economic life of coastal communities and food security.

2.2 Methods

2.2.1 Samples preparation

Five liters of water were collected using a sample bottle at a depth of 1-2 m. The water samples were refrigerated and transported to the laboratory where they were passed through pre-weighed Whatman filter GF/F 0.7 μm assisted by vacuum pump (200 mmHg). The filters were dried (60 °C,~ 24 h) and re-weighed, yielding 500 mg of SPM.

2.2.2 Measurement of oceanographic parameters

Measurement of oceanographic parameter pH was determined by pH meter Orion 3 Star, temperature and dissolved oxygen were determined by dissolved oxygen meter YSI 550A, currents were determined by the current meter, salinity was determined by instrument WTW Multi 340iFitness, and brightness carried out by Secchi Disk.

2.2.3 Organic carbon content, nitrogen content, C/N ratio, stable isotope (δ13C and δ15N) analysis

Aliquots of powdered suspended particulate matter were assayed using a carbon analyzer. Stable isotope analyzes (δ13C and δ15N) were carried out using mass spectrometer (Delta V Advantage, IRMS) connected to the elements analysis (NA-2500, CE Instruments) with a percentage correction of 0.15 ‰. Stable isotope ratio values using conventional standards using the equation (Hoefs, 2009):

$$\delta^{13}C = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \text{ (‰)}$$
Where, $R_{\text{sample}}$ is the elements ratio of $^{13}\text{C}$ and $^{15}\text{N}$, while the $R_{\text{standard}}$ is a ratio of $^{12}\text{C}$ and $^{14}\text{N}$ based on Pee Dee Belemnite (PDB). Carbon standards ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ came from PDB, while nitrogen $\delta^{15}\text{N}$ used standard N2 atmospheric gas.

![Coastal Water of The Spermonde Archipelago, Indonesia](image)

**Figure 1.** Sampling location (■: sampling site)

### 2.2.4 Lignin phenol analysis

SPM Samples were composed of as much as 0.5 g of sediment, 1.0 g of CuO (99.9%, Sigma-Aldrich) powder was added together with 3 mL of 2 N NaOH into a mini bomb, and 10-15 mg of glucose (99.5%, Sigma-Aldrich) was added to eliminate the super oxidation effect. Samples were heated at room temperature to 175 °C with a ramping rate of 4.1°C/min, and allowed to continue at 175 °C for 3 h and manually shaken every hour. The oxidation product was cooled, then transferred by adding 10 mL of 1 N NaOH and centrifuged at 3,000 rpm for 10 min. The supernatant was collected from the centrifuge, acidified to pH 1 with 6 N HCl. Then extracted three times by using 10 mL of acetone (95%, Merck) (with 100 µL ethyl vanillin (98%, Merck) 0.5 mg/mL added as an internal standard). The excess solvent extract was evaporated by flowing N2, until a dry residue was obtained, then stored in a desiccator at 4 °C. Then a silylation method was carried out where the dry residue was diluted with 300 µL of standard pyridine (99.8%, Merck and 150 µL of silylating bis-trimethylsilyl trifluoroacetamide (BSTFA) (99%, Merck) solvent with trimethylchlorosilane (TMCS) (1%, Merck) (as a catalyst). Then the samples were derivatized by heating at 90 °C for 30 min in a closed glass bottle, allowed to cool, then concentrated by evaporation. The last step was analysis by Chromatography Gas-Mass Spectroscopy (GC-MS) (Shimadzu 2010 QP Ultra) on a 30 m DB-5 capillary column (0.25 µm i.d.) with a linear temperature program (Louchouarn et al., 2010; Juarez et al., 2011; Loh et al., 2012). The correlation between the parameters was tested by using t-test.

### 3. RESULTS AND DISCUSSION

#### 3.1 Aquatic conditions

Oceanographic parameters are pH(c): pH at coastal, pH(s): pH at sea, Temp(c): temperature at coastal, Temp(s): temperature at sea, Curr.(c): current at coastal, Curr.(s): current at sea, Salinity, Dissolved
Oxygen, and Brightness shown in Figure 2. The waters pH ranged from 7.36-7.76 during sampling in June 2017 (dry season) at the coast of the Tallo river estuary and the Makassar sea; temperatures ranged between 30.1-32.7 °C; the current speed range was 5.23-9.86 cm/s; the salinity range was 11.9-20.2‰; the dissolved oxygen range was between 5.51-5.97 mg/L, and the brightness range was 7.07-7.26%. While at the site estuary and seacoast in the area Pangkep have a pH range between 6.98-7.21; temperature range of 29.7-32.1 °C; current range 6.97-8.87 cm/s; salinity range 11.2-17.2‰; dissolved oxygen ranged from 4.92 to 5.76 mg/L and the brightness ranged from 31.6 to 72.4%.

Meanwhile, the oceanographic parameters range values in January 2018 (wet season) on the coastal estuary of the Tallo river and the Makassar sea, shows a pH range between 6.98-7.21; temperature range of 27.20-30.8 °C; current speed range 7.07-18.07 cm/s; salinity range 17.70-31.90‰; the dissolved oxygen range was 7.26-7.98 mg/L, and the brightness range was 18.40-65.40%. Observations for the coastal estuary of the Pangkep river and the sea, pH ranges from 6.89-7.18; temperature range of 27.5-30.5 °C; current speed range 11.15-16.22 cm/s; salinity range 14.8-24.5‰; the dissolved oxygen range was 7.14-7.26 mg/L, and the brightness range was 27.6-55.2%. Water quality parameters in all coastal observation seasons in the public domain and agricultural domains show the maximum pH and salinity range in the dry season due to the tendency of the conditions in the dry season to get the water from the land and rainwater to increase the salinity and pH range. DO and brightness obtained maximum values in the rainy season. The DO parameter value in the rainy season is higher than the dry season due to the mixing of seawater with fresh water which will increase DO value (Wulandari, 2008).

3.2 Elemental and isotopic composition

Analysis of organic matter source and biogeochemical changes can be done using element markers [Total Organic Carbon (TOC), Total Nitrogen (TN), and stable isotopes (δ13C and δ15N)]. The results of TOC measurements, TN magnitude, and C/N ratio of suspended particle material are shown in Table 1. The TOC was higher than TN and higher from the river rather than the sea area, showing low primary productivity and also reflecting the situation of most stored terrestrial organic materials before meeting the sea (Aufdenkampe et al., 2011). The magnitude and speed of runoff from land are strongly influenced by the seasons which have implications for TOC and TN. This result was due to an increase in organic production. TOC and TN values on the Tallo coast are 1.21-2.69% and 0.04-0.12% during the dry season: 0.68-1.87% and 0.04-0.11% respectively in the rainy season. TOC and TN value the Pangkep coastal 1.16-3.01% in the dry season, and 0.86-3.62% during the rainy season. The change in presentation is diminishing towards the sea, especially at a distance more than 11.46 km from the coast of Tallo and a distance more than 8.75 km on the coast of Pangkep (Figure 3).

Table 1. Composition of the organic matter in suspended particle material (Triplication).

| Sampling sites            | TOC (%) | TN (%) | [C/N]mol Ratio | δ13C (‰) | δ15N (‰) |
|---------------------------|---------|--------|----------------|----------|----------|
| **Dry season (June sampling)** |         |        |                |          |          |
| Tallo River Estuary       | 2.69    | 0.12   | 22.28          | -25.84   | 2.68     |
| Barrang Lompo Island      | 1.78    | 0.08   | 20.83          | -26.69   | 2.06     |
| Bone Tambung Island       | 1.62    | 0.08   | 18.85          | -21.85   | 3.38     |
| Langkai Island            | 1.21    | 0.06   | 20.71          | -24.88   | 2.22     |
| Lanjukang Island          | 1.35    | 0.04   | 35.30          | -23.66   | 2.06     |
Table 1. Composition of the organic matter in suspended particle material (Triplication) (cont.).

| Sampling sites            | TOC (%) | TN (%) | [C/N]mol Ratio | δ¹³C (‰) | δ¹⁵N (‰) |
|---------------------------|---------|--------|----------------|----------|----------|
| Pangkep River Estuary     | 3.01    | 0.16   | 18.31          | -29.98   | 2.62     |
| Laiya Island              | 1.79    | 0.08   | 21.25          | -22.88   | 2.46     |
| Sarappo Keke Island       | 1.39    | 0.05   | 28.73          | -24.29   | 2.32     |
| Kondong Bali Island       | 1.21    | 0.04   | 28.80          | -21.87   | 4.38     |
| Kapoposang Island         | 1.16    | 0.11   | 10.09          | -25.82   | 3.52     |

Rainy season (January sampling)

| Sampling sites            | TOC (%) | TN (%) | [C/N]mol Ratio | δ¹³C (‰) | δ¹⁵N (‰) |
|---------------------------|---------|--------|----------------|----------|----------|
| Tallo River Estuary       | 1.25    | 0.08   | 14.07          | -26.06   | 4.01     |
| Barrang Lompo Island      | 0.81    | 0.06   | 12.52          | -25.29   | 3.56     |
| Bone Tambung Island       | 0.68    | 0.05   | 13.34          | -22.33   | 3.38     |
| Langkai Island            | 0.72    | 0.04   | 15.14          | -27.11   | 3.54     |
| Lanjukang Island          | 1.87    | 0.11   | 16.75          | -23.34   | 2.64     |
| Pangkep River Estuary     | 1.52    | 0.11   | 21.79          | -23.04   | 2.75     |
| Laiya Island              | 1.31    | 0.02   | 69.79          | -22.92   | 2.99     |
| Sarappo Keke Island       | 0.86    | 0.05   | 16.67          | -22.12   | 2.61     |
| Kondong Bali Island       | 2.93    | 0.15   | 19.11          | -23.56   | 4.86     |
| Kapoposang Island         | 3.62    | 0.27   | 13.27          | -23.81   | 4.56     |

![Graphs showing %TOC, %TN, and C/N ratio value](image1.png)

**Figure 3.** %TOC, %TN, and C/N ratio value (Makassar dry season, Pangkep dry season); (Makassar rainy season, Pangkep rainy season)
Figure 3 shows that the C/N ratio value on the coast of Tallo and Pangkep decreased from terrestrial to the sea (on the dry season). While in the rainy season, there is an increase in the value of the C/N ratio at various sampling points, on the Tallo coast the value of the C/N ratio near the land (at a distance of 20.07 km) is lower than the point of the sea location in front of it. The value of the ratio increases at a distance of 39.85 km, then, the ratio value decreases at a distance of 43.27 km. On the other hand, on the coast of Pangkep, there has been a decline from terrestrial to sea. The value of the C/N ratio varies with the runoff distance from land-to-sea in the dry season and rain with a range of 15-68. The C/N ratio change is strongly influenced by the amount of runoff and the spread of anthropogenic inputs, local hydrographic, hydrodynamic regimes, and other environmental features (Horiuchi et al., 2000; Schubert and Calvert, 2001; Tani et al., 2002; Shen et al., 2005; Sutapa et al., 2018a, Wahab et al., 2019).

The average isotopic values and other chemical and environmental variables in the waters off the west coast of South Sulawesi from the two seasons of data collection are shown in Figure 4. Changes in the values of δ¹³C showed a systematic increase in the relative proportions of organic matter derived from C₃ and C₄ plants (δ¹³C in the dry season ranging from -27.22‰ to -20.37‰ and the rainy season ranged from -27.11‰ to -21.84‰). However, the corresponding C/N ratio value does not show a systematic increase in the relative proportions of organic carbon derived from C₃ plants. Thus, the variation of the values of δ¹³C observed is mostly due to thinning at ¹³C during primary production as a result of increased rainfall (Guo and Xie, 2006).

Figure 4 shows the change in the value of δ¹⁵N from 2.020‰ to 5.110‰ in the rainy season and 1.580‰ to 4.580‰ in the dry season. These results indicate differences of the value of δ¹⁵N in the rainy season and dry season. This is due to high rainfall in the rainy season changing biological production not only in coastal and marine waters but also on land. Therefore the supply of nutrients to coastal and marine waters is very high (Onstad et al., 2000; Ménot and Burns, 2001; Sharma et al., 2005; Kitagawa et al., 2007; Bianchi et al., 2011).

**3.3 Lignin phenols composition**

The composition profile of the lignin phenol contained in the SPM sample is shown in Figure 5. Based on the results, the SPM contained vanillin, vanillic acid, cinnamic acid, acetovanilins, syringic acid, syringaldheyde, and acetonsiringone.

Based on these results, further analysis was carried out to determine Λ, S/V, C/V, (Ad/Al)₅, and (Ad/Al)₆, as shown in Table 2. Table 2 shows the range of the Λ values (value of the normalized carbon of lignin/100 mg organic carbon) in the rainy season in all locations (0.92 to 2.30) are higher than in the dry season (0.62 to 2.07 (mg/100 mg OC). In addition, temporally, lignin phenol in the rainy season in Tallo waters is higher than in Pangkep waters, but the lignin phenol composition into the sea is getting smaller. Effect of seasonal factors on the composition of lignin phenol is very significant (p<0.05) (Figure 6), while
spatially is not significant (p>0.05) (Figure 7). The condition caused by the flow of freshwater along the watershed has brought various materials from the land which causes the magnitude of nutrient in the estuary to fluctuate (Grizzetti et al., 2012).

![GC-MS analysis profile of lignin phenol](image)

**Figure 5.** GC-MS analysis profile of lignin phenol

**Table 2.** Composition of lignin phenol from river inputs to ocean (Three time replication).

| Sampling sites              | A (mg/100 mg OC) | S/V | C/V | (Ad/Al)v | (Ad/Al)s |
|----------------------------|------------------|-----|-----|----------|----------|
| **Dry season (June sampling)** |                  |     |     |          |          |
| Tallo River Estuary        | 2.07             | 1.12| 0.57| 1.08     | 1.25     |
| Barrang Lompo Island       | 1.38             | 0.80| 0.52| 0.82     | 0.80     |
| Bone Tambung Island        | 1.15             | 0.71| 0.52| 0.83     | 0.74     |
| Langkai Island             | 0.94             | 0.62| 0.48| 0.76     | 0.72     |
| Lanjukang Island           | 0.72             | 0.45| -   | 0.62     | 0.43     |
| Pangkep River Estuary      | 1.94             | 0.87| 0.55| 0.82     | 0.87     |
| Laiya Island               | 1.46             | 0.72| 0.45| 0.80     | 0.73     |
| Sarappo Keke Island        | 1.11             | 0.69| 0.41| 0.72     | 0.68     |
| Kondong Bali Island        | 1.05             | 0.62| 0.39| 0.34     | 0.18     |
| Kapoposang Island          | 0.62             | 0.35| -   | 0.20     | 0.10     |
| **Rainy season (February sampling)** |                  |     |     |          |          |
| Tallo River Estuary        | 2.30             | 0.84| 0.43| 1.40     | 1.27     |
| Barrang Lompo Island       | 1.59             | 0.50| 0.39| 0.98     | 0.81     |
| Bone Tambung Island        | 1.53             | 0.80| 0.42| 0.72     | 0.87     |
| Langkai Island             | 1.15             | 0.61| 0.32| 0.65     | 0.74     |
| Lanjukang Island           | 0.92             | 0.37| 0.46| 0.92     | 0.71     |
| Pangkep River Estuary      | 1.95             | 1.18| 0.62| 1.19     | 1.30     |
| Laiya Island               | 1.87             | 0.87| 0.48| 1.01     | 1.18     |
| Sarappo Keke Island        | 1.76             | 0.62| 0.39| 1.04     | 0.97     |
| Kondong Bali Island        | 1.69             | 0.43| 0.43| 0.82     | 0.78     |
| Kapoposang Island          | 1.46             | 0.38| -   | 0.33     | 0.71     |
Based on Figure 5, reviewed by season (temporal), the S/V and C/V ratios range from 0.35 to 1.12 and 0.39 to 0.57, respectively, in the dry season sampling. These results indicate a low contribution of angiospermic plant tissue. While in the rainy season, the S/V and C/V ratios range from 0.37 to 1.18 and 0.32 to 0.62. This S/V value indicates the contribution of plant tissue to angiosperms, while the C/V value is greater than 0.1, indicating a significant contribution of non-woody plant tissue. The value of the S/V and C/V ratios based on the season shows that during the rainy season it is higher than the dry season, this is due to rainfall factors which accelerate the entry of runoff from the mainland into the river mouth and Spermonde sea waters. The difference of the runoff character is an implication of runoff along with the river flow. The activity along the Tallo river flow (urban and industrial) differs from activity along with the Pangkep river flow (aquaculture and agriculture) (Costa et al., 2008).

**Figure 6.** Box plots of the minimum and maximum values based on season

Spatially, S/V and C/V values were found to be relatively high at the estuary of the Tallo river (0.37 to 1.12 and 0.32 to 0.57) and the Pangkep river estuary (0.35 to 1.18 and 0.39 to 0.62) (Figure 6). A downward trend occurs when heading towards the outer islands. The C/V value in the dry season is not detected on Lanjukang Island and Kapoposang Island which is the outermost island and the farthest distance from the mainland, whereas in the rainy season it is not detected on Kapoposang Island. This shows that the presence of organic material in waters originating from three different types of plant tissue, including angiosperms, gymnosperms and non-woody gymnosperms is highly dependent on the speed of water flow carrying the material to deep waters (Dittmar and Lara, 2001). Furthermore, the ratio of S/V, C/V, vanillic acid to vanillin (Ad/Al)_v, and syringic acid to syringaldehyde (Ad/Al)_s based on sampling location is shown in Figure 7.
Spatially, the ratio value of \((\text{Ad/Al})_v\) at the location of the Tallo and Pangkep river mouths up to several outermost islands ranged from 0.62 to 1.40 and 0.20 to 1.19 respectively. While the value of the ratio \((\text{Ad/Al})_s\) in the estuary of the Tallo and Pangkep rivers ranges from 0.43 to 1.27 and 0.10 to 1.30. These results indicate that lignin (the most abundant content of humic acid) has undergone oxidative degradation and is associated with mineral from the soil (Goñi et al., 2000; Sutapa et al., 2018b).

4. CONCLUSION

The study shows the difference of the lignin phenol composition in Spermonde water (Makassar waters and Pangkep area) are influenced by seasonal factors. Makassar waters contain a higher average lignin phenol compared to Pangkep waters. Although the composition of lignin phenol content was greater in Makassar waters, the ratio of C/V lignin phenol not detected at the location of the outermost island sampling which was 43.27 km from the estuary of the Tallo river and 61.71 km from the Pangkep estuary. This condition explains that lignin has been degraded to several outermost islands.

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