Bioaccumulation of Lead (Pb) in the macroalgae *Padina australis* Hauck in Makassar Marine Waters, South Sulawesi, Indonesia

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Abstract. Marine pollution is a global problem that occurs in coastal areas. Pb lead pollutants derived from waste discharges and Makassar community activities can accumulate on the macroalgae *Padina australis* Hauck. This study determined the accumulation of Pb on *P. australis* and assessed the abundance of *P. australis* in three stations in Makassar marine waters of Loe-lae, Samalona and Barranglompo from November-April 2018. Sampling *P. australis* was done by purposive sampling method. Data of *P. australis* abundance was taken using the line transect method. Physico-chemical parameters measured were temperature, salinity, pH, light penetration, current, DO, and BOD. Pb analysis was done using the Atomic Absorption Spectrophotometer. The results showed difference of Pb content in *P. australis* was found from each research station. The content of Pb at Station I is in the range of 0.0964 - 0.1388 ppm, Station II 0.0496 - 0.1050 ppm, and station III 0.0597 - 0.1035 ppm which has exceeded the water limit specified (> 0.008) ppm. The average concentration of Pb at station I was compared to other stations because Station I was closer to the mainland, with potentially greater entry of heavy metal Pb. Abundance of *P. australis* at station I were 92 ind/m², station II 152 ind/m² and station III were 319 ind/m². Correlation analysis data showed no significant value \( r^2 = 0.384, p > 0.05 \) which meant that the Pb concentration did not affect *P. australis* abundance.

1. Introduction

Marine pollution is a global problem faced by countries in coastal areas. The pollution is caused by the increasing construction of settlements, agriculture and industries that produced waste or pollutant containing waste. The entry of waste into the marine ecosystem can lead to decreased water quality. Decreasing the water quality can change the community structure. One of the major pollutants contained in waste disposal is heavy metals such as lead (Pb) and copper (Cu) [11].

One of the pollutant materials that could potentially endanger the coastal and marine areas of Makassar City is heavy metal lead (Pb). These metals are generally produced from transportation and industrial activities. Heavy metal Pb can accumulate in the macroalgae of *Padina australis* Hauck (Bioaccumulation). Bioaccumulation of heavy metals Pb by *P. australis* is strongly influenced by the high concentration of Pb metal in marine waters.

The coastal area of Makassar City is a densely populated urban area. Pb pollutants contaminating the waters of Makassar City comes from industrial activities such as paint, plastics, and other industries, ship
building activities, ports, fisheries, hotels, marine tourism, and households producing marine discharges [22]. Furthermore, Hamzah (2007), stated that pollution in coastal waters of Makassar City is very high because there are two big rivers namely, Jeneberang and Tallo River, and canal or drainage from the city which flow to coastal waters of Makassar City, so that waters in Makassar potentially contain heavy metal such as lead (Pb).

The purpose of this study is to identify the level of accumulation of Pb on *P. australis* and to assess the abundance of macroalgae *P. australis* in Makassar marine waters. Naturally, heavy metal Pb can enter the water through Pb crystallization in the air with the help of rain, in addition it also comes from the erosion of many mineral rocks around the water [16]. Organisms exposed to heavy metals Pb with low concentrations usually do not experience death, but will experience sublethal influence, that is the effect that occurs on the organism without causing death in the organism. This sublethal influence can be distinguished into three types, namely *inhibiting* (example growth and reproduction), causing morphological changes, and altering the behaviour of organisms.

Macroalgae has been shown to be efficient in metal monitoring and absorption [15]. *Phaeophyceae* has been shown to be effective as a heavy metal bioindicator because it has a high carboxylic density present in alginate [18]. The results show the analysis of lead concentration (Pb) in thallus of *P. australis* Hauck in Totok Bay waters by 3.8 ppm and in Blongko waters at 1.1 ppm. The concentration of lead (Pb) in sediment in Teluk Totok Bay is 22.6 ppm and in Blongko waters is 4.4 ppm. The concentration of lead (Pb) on thallus *P. australis* in Totok Bay waters and Blongko waters is above the standard quality standard of SNI No. 7387 Year 2009 of 0.5 ppm. The detection of lead concentration (Pb) in the thallus *P. australis* indicates a lead absorption process (Pb) is accumulated in the thallus [20].

Macroalgae *P. australis* can be found on all stations to be observed, therefore in this study the concentration of heavy metal lead in *P. australis* on water conditions in the three islands was examined.

### 2. Materials and methods

The materials used in this research are the samples of macroalgae *P. australis*, sediment samples in marine waters, seawater samples, formalin, manganese sulphate (MnSO₄), NaOH, sulfuric acid (H₂SO₄), Na-thiosulfate, nitric acid (HNO₃), and aquadest. This research was conducted in November to April 2018. Observation and sampling were conducted in Makassar marine waters at three stations: station I in Lae-lae Island, II station in Samalona Island, and station III on Barrang Lompo Island. Sample preparation and analysis was conducted at Chemical Oceanography Laboratory, Faculty of Marine Science and Fishery, Hasanuddin University and Health Centre Laboratory, South Sulawesi Province using Atomic Absorption Spectrophotometer (AAS).

The subjects in this study included measurements of heavy metal lead (Pb) on samples *P. australis*, seawater and sediments on three water stations and the abundance of *P. australis* at three station in Makassar marine waters. Physico chemical parameters measured were temperature, salinity, light penetration, current, acidity degree, Biological Oxygen Demand (BOD) and Dissolved Oxygen (DO). The observations will be conducted on three islands; Lae-Lae Island, Samalona Island and Barrang Lompo Island to know the ratio of heavy metals in these three stations.
Figure 1. Sampling locations map.

Sampling of *P. australis* was done using the Purposive Sampling method. Each station is determined by 3 substations with sampling for analysis of 3 samples for 3 weeks with sampling interval 1 times a week. Number of samples of *P. australis* taken for metal analysis is 27 samples. Seawater and sediment samples were taken for each of 9 samples. Analysis of heavy metal Pb was done using the Atomic Absorption Spectrophotometer (AAS). The data collection of *P. australis* abundance was collected by line transect method. Each station was determined with 3 substations at intervals of 10 meters from each station. Sampling was conducted on each substratum with square plot measuring 1 x 1 m. The total plots sampled were 45 plots. Physico chemical parameters measured were temperature, salinity, light penetration, current, acidity degree, Biological Oxygen Demand (BOD) and Dissolved Oxygen (DO).

Preparation of metal samples in macroalgae samples of *P. australis* Hauck were washed using distilled water and then dried in an oven at 120 °C until they reached a constant weight. The dried sample is then crushed into powder and homogenized with mortar and then stored in aluminum foil until the analysis is carried out. Next, the sample was weighed to a total of 1 gram, then ignited with a Furnace at 400 °C for 20 hours until it became ash. Then, the ash is added with 4 ml concentrated HNO₃ + 1 ml concentrated HCl in a glass cup and heated using a hot plate. The filtrate is cooled at room temperature and placed in a 50 ml volumetric flask and dissolved with aquadest until the boundary mark, then filtered with Whatman 42 paper to obtain a clear yellowish solution ready for analysis.

Preparation of metal samples with 10 ml of sea water sample was put into Erlenmeyer flask, added with 0.5 mL concentrated HNO₃. The mixture is stirred until homogeneous, then heated using an electric bath until the solution dissolves. The dissolved solution was then put into a 100 mL measuring flask and added with distilled water. Samples are stored in plastic bottles ready for analysis. Preparation of metal samples in sediments were taken only on the surface of the substrate, namely the oxic part at a depth of 1-3 meters. Furthermore, in this section a measurement of lead metal concentration (Pb) will be carried out in the Laboratory.

Samples were also analysed to determine the Bioconcentration Factor (BCF) using the formula. Differences of Pb content in *P. australis* at three different stations were analysed using Two Way ANOVA with p < 0.05 indicating significance. Counting the population abundance of *P. australis*; Pearson
correlation analysis was performed on SPSS version 25 to determine correlation between metal concentration with *P. australis* abundance.

3. Results and discussion

3.1. Pb metal concentration in *Padina australis*

The result of the research indicate that there are differences in metal content of Pb at *P. australis* in every research station (figure 2). The content of metal Pb at Station I was in the range of 0.0964 - 0.1388 ppm, Station II 0.0496 - 0.1050 ppm, and station III 0.0597 – 0.1035 ppm which has exceeded the water limit specified that is > 0.008 ppm.

The average content of Pb concentration at station I was compared to other stations because Station I was closer to the mainland with potentially greater entry of heavy metal Pb in the sample. The high content of heavy metals in *P. australis* at station I is also caused by the low acidity of marine water with an average value of 7.25. The pH value is important to consider as it may affect the process and speed of chemical reactions in water. According to Phillips et al. (2009), *Phaeophyceae* has been shown to be effective as a heavy metal bioindicator because it has a high carboxylic density present in alginates (the main component of its cell wall). This can result in high levels of metal accumulation.

![Figure 2](image-url)  
*Figure 2. Pb metal concentration on Padina australis Hauck in each research station.*

3.2. Pb metal concentration in sea water

The value of Pb metal concentration in sea water at station I is in the range of 0.0105 - 0.0172 ppm, station II in the range of 0.0059 - 0.0089 ppm and in station III in the range of 0.0062 - 0.0094 ppm (figure 3). The concentration of Pb metal between research stations is significantly different (p < 0.05), this is because the waters of Lae-lae Island (Station I) are closer to the mainland and the level of turbidity is very high. The heavy metal Pb that enters the aquatic environment will undergo deposition, dilution, and disperse, possibly absorbed by the organisms living in the waters [12].
Figure 3. Pb metal concentration on sea water in each research station.

The average value of Pb metal concentration in the waters of Lae-la (Station I), Samalona Island (Station II), and Barranglompo Island (Station III) has a value of Pb metal concentration that has exceeded the standard threshold of Pb metal content in marine waters i.e. > 0.008 ppm [5]. The highest concentration of Pb metal in the waters of Lae-la (Station I), then Barranglompo Island (Station III) and next to Samalona Island (Station II). This is allegedly due to the close location of the waters of Pulau Lae-la with the mainland which contributes a lot of waste both industrial waste, household and oil extraction from ships that anchored. The high content of heavy metal Pb in the waters of Barranglompo Island (Station III) compared to the waters of Samalona Island (Station II) was because Barranglompo Island is an inhabited island that has the potential to contribute waste to the water bodies.

3.3. Concentration of Pb Metal in sediment
The value of Pb metal concentration in Station I was in the range of 0.0293 - 0.0682 ppm, Station II in the range of 0.0072 - 0.0148 ppm, and Station III in the range of 0.0116-0.0278 ppm (figure 4). The metal concentration of Pb between research stations was significantly different (P < 0.05). At station I obtained the data of high metal concentration of Pb compared to station II and III. This is because the station I is closer to the mainland of Makassar city, so it is more likely to exposure to metal Pb in the sediment. Disposal of industrial wastes, household waste, and others, shipbuilding / docking, as well as oil drainage activities of vessels into the water bodies will occur in the sediment deposition process.
Pb metal can naturally enter the aquatic bodies through the complexation of metal particles in the air due to rain around the waters and human activities with various forms in which the contaminants will enter into the body of water and settle in the sediment [16]. Pb metal entering the aquatic environment will undergo deposition on the bottom of the water (sediments) and then it will be absorbed by the living organisms in the marine water [12].

3.4. Bioconcentration Factor

Bioconcentration factor is an ability of organisms to accumulate heavy metals from the environment. Lead accumulated in Padina australis probably originated from the water column. This happens because the entire thallus Padina australis was in the water column and all parts of the thallus are able to absorb heavy metals. The data obtained show that the Pb metal was absorbed by Padina australis very high that indicates P. australis has the ability to accumulate Pb metals. The Pb metal concentration found in P. australis affected the ability of Pb metal accumulation in P. australis. The concentration of Pb metal in P. australis at Station I was higher than the other stations (Station II and III) in the range of 0.0964 - 0.1388 ppm, so that the BCF value was lower than Station II and III because P. australis has low capacity in absorbing heavy metal Pb in station I (figure 5). The morphology of P. australis thallus found at Station I looks imperfect, with black coloured thallus and its intact shape which affects the ability of Pb metal accumulation in P. australis. Station III has a high BCF value than stations I and II because at station III the environment is good with suitable and less polluted environmental conditions, which supports P. australis so that P. australis has the ability to accumulate heavy metals well in the thallus.

Figure 4. Pb metal concentration on sediments in each research station.

Figure 5. Bioconcentration factor value.
Janssen et al., (1997) stated that if the BCFs value > 1, it means that the organism has the ability to accumulate Pb metal in its body, whereas BCFs ≤ 1 means that the organism lacks or does not have the ability to accumulate metals in its body.

3.5. Abundance of macroalgae P.australis Hauck
Abundance of P. australis at Station I was 92 Ind / m², Station II 152 Ind / m², and Station III 319 Ind / m² (figure 6). The result of Pearson correlation analysis data showed value of 0.384, p > 0.05, that is there is no correlation between Pb concentration with abundance, but the direction of relation shows negative number (-0.331) which could indicate that if Pb concentration rises then P. australis abundance will decrease.

3.6. Physico-Chemical Parameters
From observation, mean value water temperature at the research station is 28.67 - 29.33 ºC (table 1). The value still support algae growth that is 15 - 30º C for the tropics. The average salinity value is 29 - 34 ‰ (table 1). The optimum salinity conditions for macroalgae plants range from 25 - 35 ‰. The pH measurement results in the three stations ranged from 7.25 - 7.36 (table 1). The pH value in the range of values is still feasible for algal growth. According to Odum (1971) a good pH for algal growth is 5 - 8. Average current velocity values range from 0.089 - 0.220 m/s (table 1). The average value of sea water light penetration ranges from 1.62 to 4.86 m. The standard of sea water quality according to the Minister of Environment No. 51 of 2004 is > 5 (table 1).

Table 1. Measurement of chemical physiological parameters based on seawater quality standards for marine biota (mean ± STDEV).

| No. | Physico-Chemical Parameters | UU nit | Station I (Lae-lae Island) | Station II (Samalona Island) | Station III (Barranglompo Island) | Quality standards* |
|-----|-----------------------------|--------|---------------------------|-----------------------------|---------------------------------|-------------------|
| 1   | Temperature                 | ºC     | 28.67 ± 0.01              | 29.33±0.01                  | 29±0.00                         | 28-30             |
| 2   | Salinity                    | %      | 29.00 ± 2.65              | 33.67±0.58                  | 34.00±1.00                      | 33-34             |
| 3   | pH                          | -      | 7.25 ± 0.03               | 7.33±0.03                   | 7.36±0.08                       | 7-8.5             |
| 4   | Current velocity            | m/s    | 0.0089 ± 0.012            | 0.160±0.016                 | 0.220±0.029                     | -                 |
| 5   | Light penetration           | m      | 1.62 ± 0.38               | 4.17±1.16                   | 4.86±0.23                       | >3                |
| 6   | DO                          | ppm    | 3.51 ± 0.53               | 6.42±0.25                   | 6.64±0.16                       | >5                |
| 7   | BOD                         | ppm    | 2.74 ± 0.42               | 1.38±0.42                   | 1.32±0.41                       | <10               |
The condition of the waters of Station I is classified as moderately contaminated with the value of 3.51 ppm, station II 6.42 ppm and station III is classified as light contaminated category, 6.64 ppm. The threshold value or seawater quality standard according to the Minister of Environment No. 51 of 2004 is DO > 5. The average value of BOD obtained was in the range of 1.32 to 2.74 ppm. The result of BOD5 obtained is <10 ppm, as according to Effendi (2003) if the waters are characterized by BOD5 of more than 10 ppm then this indicates the area is contaminated.

4. Conclusion

The content of metal Pb on macroalgae of P. australis Hauck at Station I is in the range 0.0964 - 0.1388 ppm, Station II 0.0496 - 0.1050 ppm, and station III 0.0597-0.1035 ppm which has exceeded standard defined water limit is > 0.008 ppm. The average height of Pb concentration at station I was compared to other stations because Station I was closer to the mainland with potentially greater entry of heavy metal Pb in the samples. Abundance of P. australis at station I were 92 individuals, station II 152 individuals and station III 319 individuals. The result of correlation analysis data showed no significant value ($\rho = 0.384$, $p > 0.05$) which mean that the Pb concentration did not affected P. australis abundance.

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