Analysis of heavy metals (Pb and Cd) in seagrasses *Thalassia hemprichii* and *Enhalus acoroides* from Pulau Sembilan, South Sulawesi Province, Indonesia

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Manuscript received: 1 March 2022. Revision accepted: 26 March 2022.

**Abstract.** Rosalina D, Rombe KH, Jamil K, Surachmat A. 2022. Analysis of heavy metals (Pb and Cd) in seagrasses *Thalassia hemprichii* and *Enhalus acoroides* from Pulau Sembilan, South Sulawesi Province, Indonesia. *Biodiversitas* 23: 2130-2136. Seagrasses are flowering plants (Angiospermae) with fruit, flowers, leaves, rhizomes and roots that grow in shallow marine waters. The seagrasses *Thalassia hemprichii* and *Enhalus acoroides* have been observed growing around Pulau Sembilan in Sinjai District, Indonesia, specifically Kambuno, Katindoang, and Liang-Liang Islands. Meanwhile, human activities such as seaweed cultivation, recreation, marine transportation, fishing boats and ports/piers, and human settlements produce heavy metals that affect these plants. Therefore, this research was conducted to measure the lead (Pb) and cadmium (Cd) content in *T. hemprichii* and *E. acoroides* (roots and leaves) as well as in the water column and sediment in Pulau Sembilan, Sinjai District, South Sulawesi Province, Indonesia. The research was conducted using a descriptive method and the sites were determined through purposive sampling. The Pb and Cd content were analyzed at the Laboratory of the Makassar Plantation Product Center using the AAS (Atomic Absorption Spectrophotometry) method. The results showed that both seagrasses accumulated Pb and Cd in leaves and roots. Seagrass Pb content was higher than Cd content: in *T. hemprichii*, the Pb content in roots and leaves was 1.485 mg/kg and 2.861 mg/kg, respectively; in *E. acoroides* they were 1.512 mg/kg and 8.343 mg/kg, respectively. Pb content was also higher than Cd content in the water column (1.083 mg/L) and sediment (7.753 mg/kg). Concentrations of both heavy metals exceeded the Indonesian environmental quality standard in the seagrasses and in the ambient environment.

**Keywords:** Cadmium, environmental quality standard, heavy metal content, lead

**INTRODUCTION**

Seagrass beds are complex shallow-water ecosystems with high biological productivity and have been discovered to be ecologically and economically important (Hitalnessy et al. 2015; Tchounwou et al. 2012; Rombe et al. 2020). However, previous research showed that they are one of the aquatic organisms directly affected by heavy metal pollution (Stankovic et al. 2015; Arisandy et al. 2012). It is important to note that coastal and marine pollution generally occurs due to high human population density. Moreover, coastal activities and infrastructure such as ports/piers, residential areas, marine transportation, and tourism as well as mining have the ability to cause environmental damage, due to the fact that they contain or release hazardous materials, including heavy metal elements (Ali et al. 2019; Lipi et al. 2021; Rosalina et al. 2012) such as lead (Pb) and cadmium (Cd). Household activities produce toxic materials such as Cd, while boats and ships using lead-containing diesel fuel and gasoline as well as the antifouling and other paint used on ship hulls are thought to have the potential to release Pb and other heavy metal pollutants (Rizkiana et al. 2017).

Arisandy et al. (2012) also showed that Cd and Pb are found in paints, including in paint pigments, wood preservatives, lubricating oils, gasoline components, and fuel oils which are widely used to support port-related activities. Meanwhile, Pb is a non-essential heavy metal that is toxic for living organisms above certain levels (Afzal et al. 2018; Khodijah et al. 2019; Zhuang et al. 2013). Seagrasses are marine plants with a high capacity to absorb metals because they interact directly with the water column through their leaves and with the sediments through their roots. This means the leaves and roots are good absorbents of metal ions (Ahmad et al. 2015a; Ismarti et al. 2017; Rosalina et al. 2018a; Tupan et al. 2014a). This is observed from the fact that the absorption of heavy metals in polluted waters can occur indirectly through the food chain (Sarong et al. 2013) with seagrass acting as a primary producer. The heavy metals in seawater and sediments enter the food chain system and affect seagrass (Kurniawan et al. 2013; Said et al. 2009; Tupan & Azrianingsih 2016). Meanwhile, Supriyantini et al. (2016) reported it is possible to detect pollution by examining the seagrass leaves and the same was also observed by Supriyantini et al. (2016). This means there is a need to study the accumulation of Pb and Cd in seagrasses in order to determine the content of heavy metals in the various parts of the plants and in the surrounding environment.

The activities and infrastructure of coastal communities in the Pulau Sembilan archipelago, Sinjai District, Indonesia, such as ports/piers, human settlements, marine
transportation, and tourism, can cause environmental damage due to the fact that they can release hazardous materials such as heavy metal elements into the environment. However, it was discovered that there had been no research on the presence of heavy metals such as Pb and Cd in seagrasses in Pulau Sembilan. Therefore, this research focused on analyzing the Pb and Cd content in the seagrasses Thalassia hemprichii and Enhalus acoroides in the Pulau Sembilan archipelago.

MATERIALS AND METHODS

Location and time of research

The research was conducted in June 2021 in the Pulau Sembilan archipelago, Sinjai District, South Sulawesi Province, Indonesia, as indicated in Figure 1. The survey sites within this location were determined purposively based on the survey method, with three observation stations. In order to determine the Cd and Pb content in the seagrasses, the water column, and the sediment, samples were collected at each station with three replicates. Station 1 (Kambuno Island) has the highest human population, Station 2 (Liang-Liang Island) is a port/marine transportation site, while Station 3 (Katindoang Island) is a tourist spot. The Pb and Cd samples included 72 samples of seagrass roots and leaves from two species (T. hemprichii and E. acoroides), 12 seawater samples from the water column, and 12 sediment samples, giving a total of 96 samples from the three stations combined.

The coastal communities in Pulau Sembilan, Sinjai District, have various activities and infrastructure such as settlements, ship docks, transportation routes, and domestic waste disposal. This led to a preliminary survey to determine the sites and stations within this research location based on the presence of seagrass and the land use by the community, in order to ensure the chosen sites were representative of the range of conditions in the seagrass beds. The methods used to collect primary data included direct observation which involved observing and directly measuring the condition of the seagrass ecosystem, as indicated in Figure 2.

Figure 1. Map of the research location in Pulau Sembilan, Sinjai District, Indonesia, showing the three sampling stations
Thalassia hemprichii and Enhalus acoroides identification

Station 1  Station 2  Station 3
Heavy metal waste (Pb and Cd)

Water  Seagrass  Sediment
Pb and Cd Content
Roots  Leaves

Observation:
- Pb and Cd content in water column and sediment
- Pb and Cd content in seagrasses Thalassia hemprichii and Enhalus acoroides (roots and leaves)

Quality standards
1. Minister of the Environment Decree No. 31/2004 (Water)
2. CCME, 2001 (Sediment)
3. Minister of the Environment Decree No. 31/2004 (Seagrass)

Sample analysis

Analysis of Pb and Cd content in the water column

Pb and Cd content in the water column was determined using the AAS (Atomic Absorption Spectrophotometer) flame test method in line with the Indonesian National Standard (2004). It was necessary to prepare the samples to be tested through the destruction method before the analysis, and the procedures used were as follows: (i) 100 ml sample was taken and 1 ml of nitric acid was added as a preservative and solvent. The preserved sample was heated to a volume of fewer than 10 ml, placed in a 100 ml volumetric flask and then distilled water was added up to 100 ml. (ii) The prepared solution was placed in the cuvette and tested with an AAS (Make, Model) using a Pb cathode (lamp) at a wavelength of 217 nm after which the absorbance and results were recorded to determine Pb content.

Analysis of Pb and Cd contents in sediment

Sediment samples were obtained and filtered using eight tiered sieves with mesh sizes of 8 mm, 4 mm, 2 mm, 1 mm, 500 µm, 250 µm, 125 µm, and 62.5 µm. Sediment fractionation was conducted to obtain particle size data to indicate the composition of the substrate at the research location. The sediment type was also determined based on grain size according to the Wentworth scale (Wibisono 2010). The residual water from the last filter was allowed to settle and the precipitate was dried in an oven at 80°C for 24 hours. After the dry weight had been determined, the sediment was burned again in a furnace to obtain the ash weight.

Analysis of Pb and Cd content in seagrass (roots and leaves)

The samples of T. hemprichii and E. acoroides seagrass leaves and roots were dried, weighed, and blended to a smooth paste. A 1 g aliquot of the blended sample was weighed and placed in a 25 ml glass beaker, after which 5 ml of 5 M HNO₃ was added; the mixture was stirred, heated on a hot plate until the contents dissolved, and then cooled for 15 minutes. The mixture was filtered through Whatman filter paper (pore size 20 mm) and the filtrate was placed in a 50 ml measuring cup; distilled water was added to the 50 ml mark, then the sample was ready to be analyzed.

A standard curve was made by inserting 10 ml of 1000 ppm lead solution into a 100 ml measuring cup and diluting with 1% HNO₃ solution to the 100 ml mark. This was followed by placing volumes of 1 ml, 2 ml, and 3 ml, respectively, of the solution into 100 ml glass beakers. Each of these solutions was diluted with 1% HNO₃ solution to 100 ml to produce the standard working solutions of 1 ppm, 2 ppm, and 3 ppm. The same procedure was followed for cadmium (Cd). The heavy metal content of the seaweed samples was determined by analyzing 2 ml of each prepared sample using the AAS at a wavelength of 283.3 nm.

RESULTS AND DISCUSSION

Pb and Cd Content in the Water Column and Sediment at Pulau Sembilan

The Pb and Cd content in the water column and sediment at the sampling sites in Pulau Sembilan are presented in Table 1 and Figures 3 and 4. The Pb content in the water column was highest (1.083 mg/L) at Station 3 and lowest (0.436 mg/L) at Station 1, while the Cd content was highest at Station 3 (0.051 mg/L) and lowest at Station 1 (0.034 mg/L). Meanwhile, the Pb content in the sediment was highest at Station 1 (7.753 mg/L) and lowest at Station 3 (6.373 mg/L) while Cd content was highest at Station 2 (4.677 mg/L) and lowest at Station 1 (3.456 mg/L).

The Pb and Cd content in the water column were in the range of 0.4359-1.0825 mg/kg and 0.0338-0.0512 mg/kg, respectively, while the ranges of these heavy metals in the sediment were 6.37255-7.7531 mg/kg and 3.456-4.6766 mg/kg, respectively. This shows that heavy metal content was higher in the sediment than in the water column. This was most likely associated with the fact that these heavy metals do not dissolve easily and tend to settle in sediments (Nasir et al. 2021), and a similar trend was also observed in
Sugiyanto et al. (2016). It is important to note that some of the heavy metals absorbed in the soil on land also enter the river flow system, are transported to the sea, and settle at the bottom to be deposited in the sediment. Moreover, the Pb content in both sediment and water exceeds the 0.008 mg/l quality standard stipulated in the Minister of Environment Decree N0.51 of 2004 concerning the quality standard of seawater for marine biota. The same was also observed with the Cd content which exceeded the 0.001 mg/l sets as the quality standard.

The high levels of the two heavy metals at all three sites were most likely caused by anthropogenic activities such as ship docking, which involves the painting, cleaning, welding, and building of vessels. It is suspected that the paints used contain metals, including Pb; Rizkiana et al. (2017) suggested that Pb may have been included to ensure the paint dries more quickly and to inhibit rust on metal surfaces. Contamination of sediments with heavy metals is an environmentally important issue with consequences for aquatic organisms and human health (Zahra et al. 2014). High content of these metals in waters usually negatively impacts aquatic biota. The distribution of heavy metals in the sediment is affected by the chemical composition of the sediment, grain size, and the total organic matter (TOM) content (Azadi et al. 2018; Zhao et al. 2014). Ship fuel is also one of the causes of lead in water due to the fact that Tetra Ethyl Lead (Pb(C₂H₅)₄) is added to increase the octane number; this produces exhaust gas containing lead which is toxic and damaging to the environment (Hidayati 2013; Fernandes et al. 2012; Sanyal et al. 2015). Moreover, Suryono (2016) reported that the higher content of heavy metals in sediments than in the water column could also be because sediment can absorb more metal particles, mainly because metals tend to bind to hydroxides and organic materials in the sediment. Sumekar et al. (2015) explained that heavy metals easily bind and settle at the bottom of the waters to unite with sediments. Meanwhile, there are some properties of sediments that work as a nutrient trap to attract the metals easily.

To conclude, two seagrasses (T. hemprichii and E. acoroides) present at the research stations were observed to have the ability to accumulate the heavy metals Pb and Cd. Pb content was higher than Cd content in the roots and leaves of both species as well as in the water column and sediment. The concentration of both heavy metals exceeded the Indonesian water quality standard in the leaves and roots of both seagrasses as well as in the ambient water and sediment.

**Pb and Cd content in roots and leaves of Thalassia hemprichii and Enhalus acoroides in Pulau Sembilan**

The data on Pb and Cd content in the roots and leaves of seagrass in Pulau Sembilan are presented in Table 2 and Figures 5 and 6. The mean Pb content in the roots of T. hemprichii was highest at Station 2 (1.485 mg/kg) and lowest at Station 1 (0.972 mg/kg), while the mean Cd content was highest at Station 3 (0.609 mg/kg) and lowest at Station 2 (0.037 mg/kg). In the leaves, the mean Pb content was highest at Station 2 (2.861 mg/kg) and lowest at Station 1 (0.431 mg/kg), while the mean Cd content was highest at Station 3 (0.462 mg/kg) and lowest at Station 1 (0.060 mg/kg). The mean Pb content in E. acoroides roots was highest at station 2 (1.512 mg/kg) and lowest at Station 3 (0.859 mg/kg) while mean Cd content was highest at Station 2 (1.127 mg/kg) and lowest at Station 3 (0.205 mg/kg). The mean Pb content in E. acoroides leaves was highest at Station 2 (8.343 mg/kg) and lowest at Station 3 (0.183 mg/kg) while the mean Cd content was highest at Station 2 (0.196 mg/kg) and lowest at Station 1 (0.066 mg/kg).

**Table 1. Mean Pb and Cd content in the water column and sediment at three sites in Pulau Sembilan**

| Plant part | Station | Pb       | Cd       |
|------------|---------|----------|----------|
| Water (mg/L) | ST. 1   | 0.44 ± 0.07 | 0.03 ± 0.02 |
|            | ST. 2   | 0.48 ± 0.07 | 0.05 ± 0.02 |
|            | ST. 3   | 1.08 ± 0.76  | 0.05 ± 0.01 |
| Sediment (mg/kg) | ST. 1   | 7.75 ± 0.64  | 3.46 ± 0.04 |
|            | ST. 2   | 6.45 ± 1.05  | 4.68 ± 0.07 |
|            | ST. 3   | 6.37 ± 0.09  | 3.67 ± 0.11 |

**Figure 3. Heavy metal content in seawater at three sites in Pulau Sembilan**

**Figure 4. Heavy metal content in the sediment at three sites in Pulau Sembilan**
Table 2. Mean Pb and Cd content in roots and leaves of Thalassia hemprichii and Enhalus acoroides

| Plant part | Station | Thalassia hemprichii | | Enhalus acoroides | |
|------------|---------|---------------------|----------------|------------------|------------------|
| Leaves (mg/kg) | ST. 1  | 0.97 ± 0.02 | 0.14 ± 0.02 | 1.31 ± 0.08 | 0.24 ± 0.02 |
| | ST. 2  | 1.49 ± 0.07 | 0.04 ± 0.00 | 1.51 ± 0.01 | 1.13 ± 0.14 |
| | ST. 3  | 1.02 ± 0.01 | 0.61 ± 0.05 | 0.86 ± 0.07 | 0.21 ± 0.01 |
| Roots (mg/kg) | ST. 1  | 0.43 ± 0.09 | 0.06 ± 0.00 | 1.17 ± 0.09 | 0.07 ± 0.02 |
| | ST. 2  | 2.86 ± 0.00 | 0.3 ± 0.02 | 8.34 ± 0.00 | 0.2 ± 0.00 |
| | ST. 3  | 1.08 ± 0.00 | 0.46 ± 0.06 | 0.18 ± 0.09 | 0.16 ± 0.00 |

Figure 5. Heavy metal contents in the roots of seagrasses from three stations in Pulau Sembilan

Figure 6. Heavy metal contents in seagrass leaves from three stations in Pulau Sembilan

The results show that Pb and Cd in seagrass leaves exceeded the maximum values stipulated in the Minister of Environment Decree No.51 of 2004 regarding seawater quality standards for marine biota, which are 0.008 mg/l and 0.001 mg/l, respectively. The heavy metal content observed in seagrass leaves is associated with the absorption of heavy metals in the form of ions which later dissolve in fat and have the ability to penetrate the cell membranes, thereby leading to the accumulation of the metal ions in cells and tissues (Jeong et al. 2021; Authman et al. 2015). In general, roots have a larger surface area than leaves and are the most effective plant part with respect to absorbing nutrients (Al-Saadi et al. 2013; Nasir et al. 2020; Bonanno & Di Martino 2016). This is in line with previous findings that seagrass has a high capacity to accumulate heavy metals because it interacts directly with the water column through leaves and also with the sediment through roots (Romero et al. 2006; Ralph et al. 2006; Rosalina et al. 2018b; Rosalina et al. 2019a; Rosalina et al. 2019b; Sidi et al. 2017; Ahmad et al. 2014). Llagostera et al. (2011) observed that these absorption processes appeared to be influenced by 3 factors: (i) the availability of metals in the water column and sediment in different quantities based on the location and sources of metals; (ii) the kinetics and passive absorption properties which can differ between leaves and roots; and (iii) the internal distribution of uptake metal through active and passive transport mechanisms. The high Pb and Cd content in the two seagrasses at the research location cannot be separated from human activities in the area, such as the ship painting activities using paints with these heavy metals as key ingredients. Moreover, it was found that the average Pb and Cd content were both greater in *E. acoroides* than in *T. hemprichii*, most likely due to differences in morphology, such as size. However, Govers et al. (2014) showed that body size does not always determine the bioaccumulation level of heavy metals in the seagrass. They also observed that the content of heavy metals in the environment, such as in the water column and substrate is not always correlated with the content in seagrass plants, but Alutoin et al. (2001) reported that the bioaccumulation of heavy metals in seagrass tends to be related to the bioaccumulation of environmental conditions in the waters and substrate, such as seawater and substrate pH, sediment size, temperature, as well as salinity and seawater nutrient concentrations.

The Pb content in the leaves of *E. acoroides* was also quite varied. Haryanti et al. (2012) and Tapan et al. (2014b) state that nutrients absorbed by the roots are normally transported to all parts of the plant through the transport network (xylem and phloem); heavy metals are usually accumulated in the leaf vacuoles and the accumulation rate increases with time because the leaves of older seagrass have a higher ability to absorb heavy metals. Ahmad et al. (2015b) also found that seagrass leaves can absorb water and dissolved substances, including heavy metals, through the stomata and cuticle. This is related to the presence of pectin in the cell wall, which plays an important role in ion absorption by these leaves; it is important to note that the level of the pectin also increases with the age of seagrass (Santana et al. 2019). This means
that the Pb content in leaves is due to both root activity and absorption processes in the leaves (Sugiyan et al. 2016).

ACKNOWLEDGEMENTS

This research was funded by the Politeknik Kelaautan dan Perikanan, Bone, Indonesia. The authors thank all who assisted in any way with the research and the manuscript. In particular, we recognize the valuable contribution of the volunteer students who assisted with fieldwork.

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