Macrozoobenthos Diversity as a Bioindicator of Heavy Metal Pollution in Segara Anakan Lagoon, Cilacap District, Indonesia

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Abstract. Various methods are available for monitoring pollution levels in surface water and sediments, including using organisms as bioindicators. Segara Anakan Lagoon is an estuarine ecosystem that could be affected by contaminants transported by rivers. Pollution sources include pesticides and fertilizers from upstream agricultural areas, domestic waste, and shipping traffic. Macrozoobenthos are among the organisms commonly used as bioindicators of environmental pollution. Macrozoobenthos live at the bottom of the water column, and as deposit feeders, they lend themselves to use as bioindicators. The structure of the macrozoobenthos community in Segara Anakan has changed, as evidenced by the diversity and abundance of benthic species. Furthermore, environmental pollution parameters, such as heavy metal concentrations in water and sediments, are relatively high in the lagoon. Concentrations of Hg, Pb, Cd, and Cr varied among collection sites, with average concentrations of 0.04, 4.4, 1.2, and 1.4 ppm, respectively. In general, the abundance of macrozoobenthos was low at collection stations with high heavy metal concentrations, demonstrating that heavy metal pollution has occurred in Segara Anakan. Correlation tests between macrozoobenthos and heavy metal concentrations in water and sediments yielded r-values of > 0.8225 and 0.7473, respectively.

1. Introduction
Segara Anakan, a lagoon surrounded by mangrove forests and muddy plains, is separated from the Indonesian Ocean by Nusakambangan Island. Substrates in lagoons are more heavily influenced by land than by the sea. Lagoons are also referred to as intertidal zones because salinity levels are influenced by both seawater and freshwater.

Segara Anakan has been subjected to numerous disturbances, and the rate of sediment deposition from the mainland is high. Lagoon waters continue to decrease in terms of both surface area and depth. Sediments deposited in Segara Anakan originate primarily from the Citanduy, Cibeureum, and Cikonde Rivers, with minor contributions from beach sediment [1]. Physico-chemical conditions in Segara Anakan are influenced by nutrients and pollutants in small rivers. Anthropogenic pollutants found in aquatic ecosystems include heavy metals, while anthropogenic pollution sources include agriculture, settlement, and transportation.
In the aquatic environment, heavy metals can bind with particulate matter and accumulate as sediments [2]. Sediment often contains very high metal concentrations, which is toxic to aquatic biota and humans [3]. Negative impacts of metal toxicity on macrozoobenthic communities include the disruption of physiological processes, which results in morphological irregularities and, ultimately, leads to changes in community structure and decreased biotic integrity [4]. Metals potentially contaminating Segara Anakan include mercury (Hg), lead (Pb), chromium (Cr), and cadmium (Cd), originating from upstream agricultural areas, domestic waste, and shipping traffic.

Macrozoobenthos are good bioindicators as they can adapt to environmental pollution; as such, the diversity of macrozoobenthos can be used as a proxy for environmental health. Macrozoobenthos are used as indicators of river water biology because they are generally sessile and have a relatively long life cycle; in addition, they are abundant and highly diverse and exhibit responses to water quality conditions from the cellular to the community level. Finally, they are easy to analyze, and collection is relatively straightforward. When the macrozoobenthos community is disturbed, then the ecosystem is also affected [5]. This is reflected in a decrease or exclusion of certain species or changes in dominance. Macrozoobenthos are gastropods that live at the bottom of the water column; as diggers and deposit feeders, they are often abundant in mud and soft sediments, which are typically rich in organic matter.

More research is required to better understand the potential for macrozoobenthos as bioindicators of metal pollution and early warning signals of pollution. The objectives of this study are to analyze heavy metal concentrations in the water and sediments of Segara Anakan, macrozoobenthos diversity in the lagoon, and the relationship between heavy metal concentrations and macrozoobenthos diversity.

2. Methods
The study area was Segara Anakan Lagoon, in Kampung Laut Sub-district, Cilacap District, Indonesia. Water, sediment, and macrozoobenthos samples were collected at six stations (Fig. 1), with three replicates collected per station. The collection stations were located in the middle and downstream reaches of the Cikonde, Cibeureum, and Cimeneng Rivers. Field data were collected in the period of June–August 2018. The equipment and materials used included an Ekman grab, cooler, Secchi disk, refractometer, thermometer, loupe, dissolved oxygen (DO) meter, 0.5 mm filter, and transparent plastic.

![Figure 1. Sampling locations](image-url)
2.1. Water Sampling
Water samples were collected in 1 L bottles in the middle of the river, at a single point at half the depth of the water column. Samples were stored in a cooler until analysis. Concentrations of Hg, Pb, Cr, and Cd were assessed in the laboratory using an atomic absorption spectrophotometer (AAS). Temperature, pH, and DO were assessed in-situ.

2.2. Sediment Sampling
Sediment samples were collected using an Ekman grab, and placed into transparent plastic. Samples were sundried initially, then heated in an oven at 105°C. Concentrations of Hg, Pb, Cr, and Cd were measured with the AAS.

2.3. Macrozoobenthos Sampling
Macrozoobenthos sampling was conducted using a grab sampler; three collections were performed at each station. Sediments were filtered through a 0.5 mm filter to separate organisms from the substrate; macrozoobenthos were then put into transparent plastic with 10% formalin, and labeled. Identification was conducted in the laboratory using taxonomic manuals [6–11] and other relevant reference materials. Macrozoobenthos diversity was represented by the Shannon-Wiener formula: $H' = -[(pA \ln pA) + (pB \ln pB) + (pC \ln pC) + ...........]$.

2.4. Statistical Analyses
We used descriptive statistics and correlation analysis to assess heavy metal concentrations and macrozoobenthos diversity.

3. Results
The highest heavy metal concentration in water was found at the downstream station of the Cimeneng station (Cd concentration of 4.437 mg/L). The lowest concentration recorded was 0.042 mg/L Hg at the Cibeureum middle stream station (Fig. 2).

![Figure 2. Heavy metal concentrations in water](image-url)
The highest heavy metal concentration in sediment was recorded at the Cimeneng middle stream station, for Cd (6.132 mg/L), while the lowest value was recorded at the Cibereum middle stream station, for Hg (0.042 mg/L) (Fig. 3).

![Figure 3. Heavy metal concentrations in sediment](image)

The highest $H'$ value was 0.95, recorded at the Cibereum middle stream station. No macrozoobenthos were found in the Cimeneng middle or downstream stations.

| Species          | Cikonde middle stream | Cikonde downstream | Cibeurem middle stream | Cibeurem downstream | Cimeneng middle stream | Cimeneng downstream |
|------------------|-----------------------|--------------------|------------------------|---------------------|------------------------|---------------------|
| Melanoides sp.   | 1111                  | 89                 | 756                    | 0                   | 0                      | 0                   |
| Hastula sp.      | 0                     | 44                 | 222                    | 133                 | 0                      | 0                   |
| Sermyla sp.      | 0                     | 133                | 311                    | 89                  | 0                      | 0                   |
| Abundance        | 1111                  | 266                | 1289                   | 222                 | 0                      | 0                   |
| $H'$             | 0.53                  | 0.61               | 0.95                   | 0.68                | 0                      | 0                   |

![Figure 4. Macrozoobenthos species in Segara Anakan: (a) Melanoides sp; (b) Hastula sp; and (c) Sermyla sp.](image)
The analysis of the correlation between heavy metal concentrations and macrozoobenthos diversity yielded r-values of > 0.8225 for water and 0.7473 for sediment (Fig. 5).

Figure 5. Correlations between heavy metal concentrations and macrozoobenthos diversity

4. Discussion

Our results suggest that heavy metal concentrations in the water in Segara Anakan exhibit an increasing trend between the middle and downstream reaches of rivers draining into the lagoon. Concentrations of Hg, Pb, Cr, and Cd differed among stations (Fig. 2), and concentrations of Pb, Cr, and Cd exceeded the standard values at all stations. The pattern of increasing concentrations in downstream reaches was not observed for sediment samples from the Cimeneng River. Concentrations were higher in sediment than in water (Figs. 2 and 3), as is typical for heavy metals. Sediment often contains very high heavy metal concentrations, causing symptoms of toxicity in aquatic biota [3,12]. The composition and abundance of macrozoobenthos may change according to the sensitivity of different species to the quality of the aquatic environment and disturbances such as heavy metal pollution. Shannon’s diversity index (H’) combines information on species richness, abundance, and evenness [13], and is therefore useful as an indicator of disturbance in aquatic ecosystems.

We found differences in the density and diversity of macrozoobenthos among stations. We detected three species, all of which are gastropods: *Melanoides* sp., *Hastula* sp., and *Sermyla* sp. All species were present at four of the six stations (middle and downstream stations in the Cikonde and Cibeureum Rivers), whereas no individuals were collected at either of the Cimeneng stations (Table 1); we suspect that this is because the Cimeneng River has been dredged. The highest H’ value was 0.95, recorded at the Cibeureum middle stream station; the lowest values were recorded at the Cimeneng stations (Table 1).

*Melanoides* sp. was dominant at the Cikonde and Cibeureum middle stream stations, *Hastula* sp. at the Cibeureum downstream station, and *Sermyla* sp. at the Cikonde downstream station. *Melanoides* is a genus of operculate freshwater snails in the family Thiaridae (Fig. 4a). *Hastula* is a genus of sea snails in the family Terebridae, also known as auger snails (Fig. 4b), and *Sermyla* is a genus of tropical operculate freshwater snails in the superfamily Certhioidea (Fig. 4c).

It is evident that heavy metal concentrations in both water and sediment affect the diversity of macrozoobenthos. Heavy metal concentrations in water and sediment are represented by the following regression equations: $y = -0.5221x + 1.8379$ and $y = -7205x + 2.5182$, respectively. Based on these equations, we conclude that macrozoobenthos diversity decreases as heavy metal concentrations in
water and sediment increase. Other environmental factors may also affect macrobenthos diversity. High heavy metal concentrations were shown to be associated with a low macrozoobenthos diversity index [3].

5. Conclusion

Heavy metal concentrations were found to be higher in sediment than water, and higher concentrations were correlated with lower macrozoobenthos diversity. Macrozoobenthos can thus be used as indicators of metal pollution in aquatic systems.

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