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Bioaccumulation of Lead (Pb) and Cadmium (Cd) in *Padina Australis* Hauck at Palang Beach, Tuban, East Java, Indonesia

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ABSTRACT

Waters polluted with Pb and Cd have a negative impact on the environment. *Padina australis* grows abundantly on the coast of Palang Subdistrict, Tuban, and the local community consumes it. Macroalgae as food must be free of metal contamination. This study aims to determine the impact of Pb and Cd bioaccumulation on *P. australis*. Sampling was conducted at two stations, including Station I, Panyuran Village, and Station II, Glodog Village. Analysis of Pb and Cd metal levels using Atomic Absorption Spectrophotometry (AAS). Analysis of protein content using the Kjeldahl method. The data obtained was analyzed with Principle Component Analysis (PCA). The results of the analysis of Pb and Cd levels in P. australis at station II, which are 0.200 ± 0.028 and 0.021 ± 0.004 $mg.kg^{-1}$ higher than station I, which are 0.194 ± 0.015 and 0.010 ± 0.001 $mg.kg^{-1}$. The protein content of P. australis at station I was 4.713 ± 0.508 $mg.kg^{-1}$, and at station II was 5.900 ± 0.928 $mg.kg^{-1}$. This shows that *P. australis* is still considered good for consumption even though it has been polluted and contains Pb and Cd metals. P. australis can tolerate and does not experience severe physiological damage so it has the potential as a heavy metal phytoremediator.

INTRODUCTION

Indonesia is a maritime country because it has a larger ocean area than a land area, with a coastline of ±95,000 km^2 (Sukamto 2017). Indonesian waters are rich in natural resources, including aquatic biological resources such as seaweed. Seaweed is widely used as a biomonitor for metal contamination levels. Some types of seaweed are known to have a high ability to accumulate metals so that they can be used as indicators of heavy metals in water (Sohrab & Alireza 2011). Macroalgae have a very important role in human life, both economically and ecologically. Macroalgae can be used as vegetables, traditional medicines, organic fertilizers, and animal feed (Ramdhan & Ratnasari 2021).

Padina australis is included in brown seaweed (Phaeophyta), which is widely found in Indonesian waters. In terms of nutrition, *P. australis* contains protein, fat, carbohydrates, water, ash (Maharany et al. 2017), and minerals such as calcium, magnesium, potassium, sodium, copper, zinc, and iron (Ramdhan & Ratnasari 2021). *P. australis* also contains various active compounds, such as steroids, terpenoids, flavonoids, tannins, and saponins (Hudaifah et al. 2020), which can be utilized as medicine (Priamanatha 2023). Kustantinah et al. (2022) revealed that *P. australis* from Kelapa Beach, Tuban Regency, has the potential to be utilized as animal feed, especially as a soluble carbohydrate and organic mineral source. Mantiri et al. (2019) in their research analyzed that *P. australis* can remain alive even though heavy metals have been found in its talus cell wall. The presence of heavy metal contents of As, Cu, Zn, and Mg in *P. australis* indicates that this macroalgae can be used as a heavy metal phytoremediator. According to Paz et al. (2019), Pb and Cd are listed as the most toxic metals found in edible seaweeds in Asia. *P. australis* can absorb Cd^{2+} ions in 3 h with an absorption presentation of 74.54% (Bijang et al. 2018). This is because the surface of its cell wall consists of functional groups, such as amino, hydroxyl, carboxyl sulfate imidazole, and sulfonate, that can bind to metal ions (Firdaus 2019).

Marine pollution is a global problem faced by countries located in coastal areas. Increased residential, agricultural, and industrial development produces discharges or wastes containing pollutants. One of the main pollutants is heavy metals, such as lead (Pb) and copper (Cu). Heavy metals are toxic if they enter the water in quantities exceeding the maximum limit that has been set (Nurhayati & Putri 2019). Heavy metals have high toxicity to the aquatic environment because they easily settle in sediments and marine biota for a long time (Nurfadhilla et al. 2020). These metals enter the bodies of marine organisms through the respiratory tract, are then absorbed by the digestive system, and accumulate in the liver and kidneys because they cannot be decomposed (Astuti et al. 2016). The entry of heavy metals into water bodies can cause a decrease in water quality both physically and biophysically.

Marine waters in Palang, Tuban Regency, are suspected to be polluted. According to Ummi et al. (2017), the average Pb level in seawater in Palang, Tuban Regency, was 0.18 ± 0.13 $mg.L^{-1}$, and Cd levels ranged from 0.008 to 0.04 on the North Tuban coast. This condition has exceeded the quality standard threshold based on the Decree of the Minister of Environment No. 51 Year 2004. Some seafood products from the Palang Sub-district of Tuban Regency, such as salted fish and shrimp paste, were also found to be positive for lead, with levels exceeding the threshold set by BSN. The lead level in shrimp paste was 8.84 ± 0.12 $mg.kg^{-1}$, and the lead level in salted fish was 6.72 ± 0.11 $mg.kg^{-1}$. Ummi et al. (2017) also found that tuna from the sea of Palang Subdistrict, Tuban Regency, was found to contain heavy metal lead (Pb) of 0.61 $mg.kg^{-1}$. Marine pollution in Palang, Tuban Regency, also occurs due to the offshore oil industry. This industrial waste is suspected to contain heavy metal compounds that are toxic and harmful to marine ecosystems and living organisms. Meanwhile, the possibility of Pb and Cd heavy metal contamination in macroalgae, including *P. australis,* is not yet known. *P. australis,* as a food ingredient, must meet food safety requirements. According to BPOM Regulation No. 21 of 2021, food safety is a condition and effort needed to prevent food from possible biological, chemical, and other contaminants that can interfere with and endanger human health, ensuring it is safe for consumption. One of the requirements for macroalgae food safety is to be free from heavy metal chemical contamination. The feasibility and food safety of macroalgae must be known and compared with the required quality standards.

Based on this background, it is necessary to analyze the levels of Pb and Cd metals in water, sediment, and *P. australis*, as well as protein levels in *P. australis* at Palang Subdistrict Beach, Tuban Regency, considering the consumption of *P. australis* by the local community and the absence of previous studies so that the potential of *P. australis* in accumulating heavy metals Pb and Cd and its food safety status is important to study.

MATERIALS AND METHODS

Study Site

This research was conducted from September to November 2023 at Palang Beach, Palang Subdistrict, Tuban Regency. Observations were made at two stations, namely Panyuran Village (Station I) and Glodog Village (Station II) (Fig. 1). Research samples included water, sediment, and P. australis, along with the measurement of physico-chemical parameters

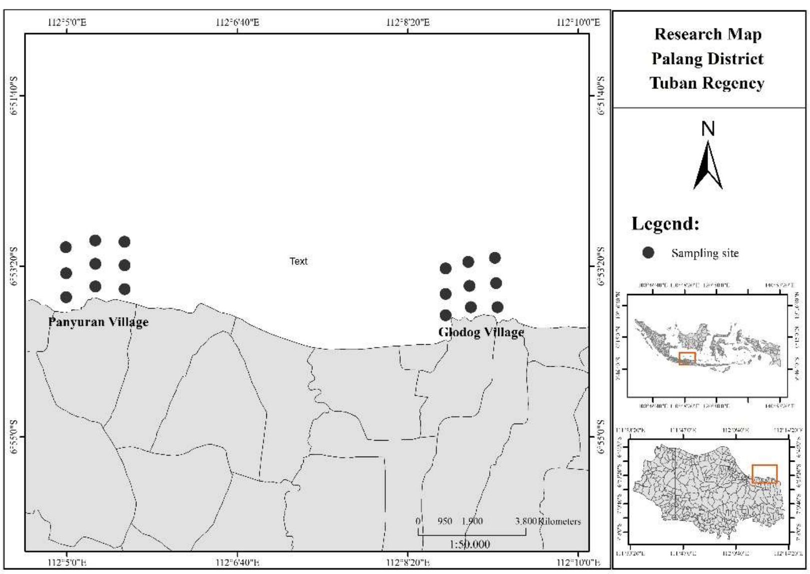

Fig. 1: Sampling location.

in the waters. Analysis of heavy metal levels of Pb, Cd, and protein levels was carried out at the Airlangga University Nutrition Laboratory.

Fieldwork

The equipment used to measure water quality included a Gea S-006 rod thermometer, a Milwaukee pH meter, an Atago S-28 refractometer, and an MW-600 Milwaukee DO meter. Materials needed in this study included macroalgae P. australis, seawater, marine sediments, clean water, distilled water, aquabides, Na_2SO_4-HgO (catalyst), ethanol 96%, HNO_3 65%, H_2O_2, HCl 0.2 N, NaOH 1%, H_3BO_4 4%, and H_2SO_4 98%.

Sampling of *P. australis* macroalgae for Pb and Cd metal content tests was conducted at two observation stations with two repetitions. The macroalgae samples taken had the same color and size. Samples of *P. australis* were stored in a cool box at a temperature of 2-4°C and then brought to the laboratory for analysis

Analysis of Lead and Cadmium Content

This analysis was carried out at the Nutrition Laboratory Airlangga University using the dry destruction method. 3.5-gram samples were weighed using an analytical balance, then placed in a porcelain dish, labeled, and incubated for 3 h at 800°C. The sample that has been kilned is put into a beaker glass and added 1 mL of concentrated HNO_3 and 10 mL of aquademin. Next, the sample was stirred until well mixed and filtered using filter paper. Furthermore, the sample is ready to be analyzed using the AAS method.

Analysis of Protein Content

In each treatment, 0.2 grams of *P. australis* was then mashed. After that, put it into a 30 mL Kjeldahl flask, then add Na_2SO_4-HgO (2:1) and 2 mL H_2SO_4. Boil the sample for 2.5 h until the liquid becomes clear. After the cold sample is added with 15 mL of distilled water, 10 mL of NaOH is added into the distillation tube, 10 mL of H_2BO_4 is added into Erlenmeyer, and then placed under the condenser. The sample was distilled until 15 mL of greenish distillate was collected in the Erlenmeyer. Next, titrate using 0.2 N HCL until the color changes to pink. Then do the blank determination and calculate the total N and protein percentage with the following formula.

$$\%\ N = \frac{ml\ HCl\ x\ N\ HCl \times 14.008}{Biomass\ (mg)} \times\ 100\%$$

$$\%\ Protein = \%\ N \times 6.25$$

Data Analysis

Water quality data from the measurement of physico-chemical parameters, including seawater Pb and Cd metal levels, were analyzed descriptively with reference to the quality standards of marine waters based on Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management. Data on sediment Pb and Cd metal levels were analyzed descriptively with reference to quality standards based on the 2002 Candian Council of Ministers for the Environment (CCME) Guidelines for Aquatic Sediment Quality. Data on macro algae Pb and Cd metal levels were analyzed descriptively with reference to quality standards based on the Decree of the Director General of Food and Drug Control No. 5 of 2018 50 concerning Maximum Thresholds for Pb and Cd Metal Contaminants in Food Ingredients. Protein content data were analyzed descriptively with reference to the Indonesian Food Composition Table (TKPI) of the Indonesian Ministry of Health 2017. The relationship between water quality, Pb and Cd levels of water, sediment, *P. australis*, and protein levels of *P. australis* were analyzed using Principal Component Analysis (PCA) using XLSTAT software for Excel.

RESULTS AND DISCUSSION

Physical and Chemical Water Quality

The physical and chemical conditions of the water also influence metal absorption. Based on the results of PCA analysis, station I is characterized by high pH and temperature, while station II is characterized by high salinity, dissolved oxygen, and current velocity. Pb metal levels are actually low at station I, which is known to have a higher temperature than station II (Table 1).

Based on the results of PCA analysis, it was known that temperature is negatively correlated with seawater Pb levels, sediment Pb levels, and Pb levels of *P. australis* (Fig. 2). This result is in accordance with the research of Paramita et al. (2017) which states that an increase in water temperature can reduce the adsorption of particulate heavy metal compounds, while a decrease in water temperature will increase the adsorption of heavy metals to particulates to settle to the bottom of the waters, these particulates will potentially become a secondary source of heavy metal pollution in the waters.

The pH at both stations is classified as alkaline because it is more than 7. According to Melsasail et al. (2018), macroalgae require an alkaline condition for their life so macroalgae tend to be found in alkaline waters. The high pH is due to the sampling time at the beginning of the rainy season. Yulis (2018) states that an increase in pH causes the

Table 1: Measurement results of physico-chemical parameters of water quality at Palang Beach, Tuban.

Parameter	Station	Average ± SD	Quality Standards (*)
Temperature (°C)	I	29,933 ± 1,576	28-30°C
	II	28,700 ± 1,236	
pH	I	7,993 ± 0,012	7-8,5
	II	7,867 ± 0,082	
Salinity (‰)	I	31,667 ± 0,471	33-34‰
	II	33,333 ± 0,471	
Dissolved Oxygen [$mg.L^{-1}$]	I	5,167 ± 0,590	>5 $mg.L^{-1}$
	II	5,473 ± 0,271	
Flow Velocity [$m.s^{-1}$]	I	0,263 ± 0,127	Low = 0,5 $m.s^{-1}$ Medium = 0,5 $m.s^{-1}$ High = >0,5 $m.s^{-1}$ (**)
	II	0,653 ± 0,052	

Note: (*) Government Regulation No. 22 Year 2021 (**) Yusuf et al. 2012. Bolded values are not in accordance with quality standards.

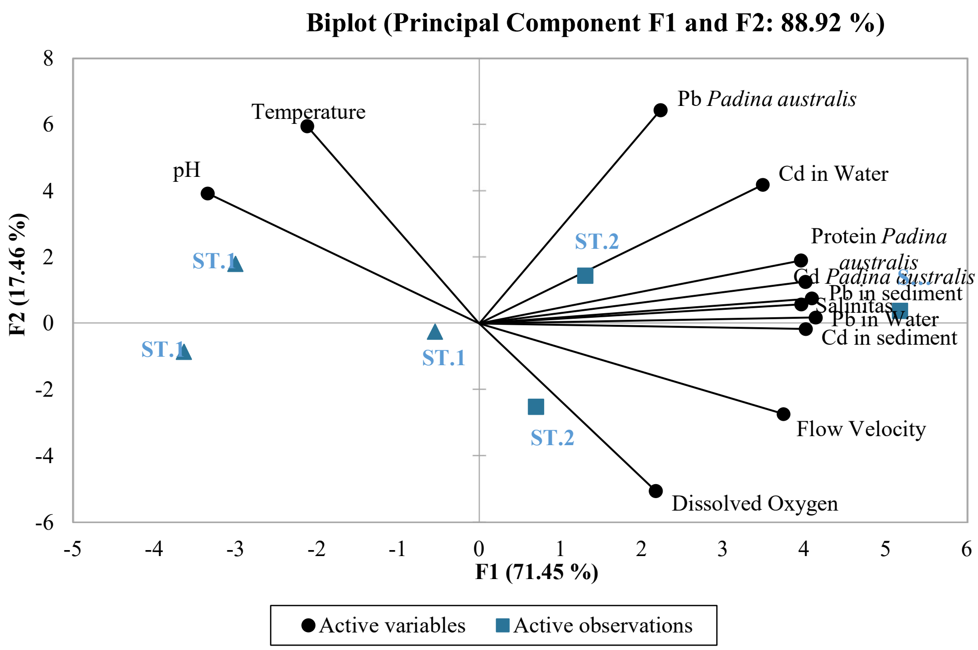

Fig. 2: PCA relationship of water physico-chemical parameters, Pb and Cd levels water, Pb and Cd levels sediments, *P. australis* Pb and Cd levels, *P. australis* protein levels at both stations.

solubility of heavy metals to decrease due to a change in the stability of the form of carbonate compounds to hydroxide compounds. The results of this study are similar to (George & Abowei 2018), who found that pH is strongly negatively correlated with water Pb levels (Fig. 2).

Salinity at station II is quite high but still meets the quality standards, while salinity at station I does not meet the quality standards based on Government Regulation No. 22 of 2021 (Table 1). Melsasail et al. (2018) in their research found that *P. australis* was able to survive in waters with a salinity of 26.3‰. A similar study (George & Abowei, 2018) also found salinity to be positively correlated with water Pb levels (Fig. 2). High salinity is associated with high electrical conductivity ions in the water that can increase the concentration of heavy metals in the water. According to Saraswati & Rachmadiarti (2021), decreasing salinity can lead to increased accumulation of heavy metals. This occurs due to the process of dilution and hydrodynamics that occur naturally in waters.

The current velocity at station II was higher than at station I (Table 1). Current speed is a parameter that influences the rate of metal sedimentation. Based on the results of PCA analysis, current velocity was most strongly positively correlated with seawater Pb levels, sediment Cd levels, and

P. australis Cd levels (Fig. 2). Current velocity causes stirring of particles in water, including metal ions. High current velocity causes metal ions to remain in the water body. Station II has a shallow water depth, so the water current tends to be stronger than station I, which has a deeper water depth. This is in line with Melsasail et al. (2018) which states that high current speed is influenced by water depth and the presence or absence of seagrass. The presence of seagrasses and sufficient water depth prevent strong currents.

Dissolved oxygen is classified as good at both stations because it meets the quality standards based on Government Regulation No. 22 of 2021 (Table 1). The influence of high current velocity can cause good dissolved oxygen at both stations. Based on the results of PCA analysis, dissolved oxygen is strongly positively correlated with current velocity (Fig. 2). According to Nurjanah et al. (2021), current speed increases water dissolved oxygen levels through oxygen exchange with the water surface. Sufficient oxygen in the waters can support maximum macroalgae growth. Metal levels with dissolved oxygen are most strongly positively correlated with sediment Pb (Fig. 2). Increased deposition of heavy metals in sediments causes a decrease in heavy metal levels in water bodies, so dissolved oxygen in the waters increases. According to WHO (1985), dissolved oxygen can affect the solubility and availability of nutrients that can be released from sediments in low dissolved oxygen conditions.

Pb and Cd levels

Based on the results of the analysis, seawater at Station I Panyuran Village and Station II Glodog Village has been polluted with Pb and Cd metals, where the levels have exceeded the maximum threshold of quality standards based on Indonesian Government Regulation No. 22 of 2021 (Table 2). The quality standard for Pb in seawater is 0.008 $mg.L^{-1}$. The quality standard for Cd metal levels is 0.001 $mg.L^{-1}$. The level of heavy metal Pb in seawater in this study is lower when compared to Ummi et al. (2017), who found the average Pb content of seawater in Palang Subdistrict, Tuban Regency was 0.18 ± 0.13 $mg.L^{-1}$. However, it is higher when compared to Azizah et al. (2018) in the Awur Bay Waters of Jepara, which found an average seawater Pb level of 0.003 $mg.L^{-1}$. This can be caused by differences in sampling location and time, as well as the physical and chemical conditions of the waters. Pb metal that enters the water has the potential to be deposited in sediments. Metal ions in waters are less stable, so they easily bind to organic substances and potentially settle in sediments.

Metal levels in sediments are contributed by water pollution. Based on the hydrological cycle, less than 0.1% of metals are dissolved in water, and more than 99.9% are stored in sediments (Pradit et al. 2013). Sediment is a good medium for heavy metal deposition. Metals in sediments continue to increase along with the increase in water metal levels and will persist for a long period. Based on the results of the analysis, the levels of Pb and Cd metals in the sediments of Palang District Beach still meet the quality standards based on the Candian Council of Ministers for the Environment (CCME) 2002 (Table 2). The quality standard for Pb in sediment is 30.2 $mg.kg^{-1}$. The quality standard for sediment Cd metal content is 0.7 $mg.kg^{-1}$. When viewed from the type of sediment, the two stations have different sediment characteristics. Station I is composed of sediments in the form of coarse sand, and there are few coral rocks, while station II is composed of sediments in the form of fine sandy clay, and there are quite a lot of coral rocks. This causes sediment metal levels at station II to be higher than at station I. According to Wardani et al. (2014), sediment type affects the absorption of heavy metals with the category that clay sediments more easily absorb heavy metals> sandy clay > sand.

Table 2: Pb and Cd levels in water, sediment, and *P. australis* in Palang Beach, Tuban.

Station	Heavy Metals	Seawater [$mg.L^{-1}$]	Sediment [$mg.kg^{-1}$]	*P. australis* [$mg.kg^{-1}$]
Station I	Pb	0,026 ± 0,004	2,235 ± 0,047	0,194 ± 0,015
	Cd	0,010 ± 0,003	0,052 ± 0,003	0,010 ± 0,001
Station II	Pb	0,040 ± 0,005	2,352 ± 0,073	0,200 ± 0,028
	Cd	0,015 ± 0,005	0,08 ± 0,007	0,021 ± 0,004

The results of this study show that although the water metal levels do not meet the quality standards, the sediment metal levels still meet the quality standards. This indicates that heavy metal pollutants entering the water are more in the water phase than settling into the sediment. This occurrence is caused by changes in the physical and chemical conditions of waters, such as pH, temperature, salinity, and organic substances that can allow the decomposition of metals from the solid phase, decomposition and degradation of organic matter in an oxidized state (Rumhayati & Retnaningdyah 2018), the influence of current velocity, dilution of heavy metals in waters due to rain, and the characteristics of the sediment fraction itself. The Pb and Cd levels of Palang Subdistrict Beach sediments are known to be lower when compared to the results of Syaifullah et al. (2018), which found the Pb metal content of Tuban Sea sediments to be 2.944 ± 0.360 $mg.kg^{-1}$ and the Cd metal content to be 2.978 ± 0.224 $mg.kg^{-1}$. Metals contained in sediments can be absorbed by organisms that live attached to sediments, one of which is *Padina australis* seaweed.

P. australis at both stations is known to contain metals Pb and Cd. *P. australis* lives submerged in water and attached to the sediment so that it can absorb Pb and Cd metals. Based on the analysis results, the Cd level of *P. australis* at both stations is still below the quality standard threshold according to the Decree of the Directorate General of Food and Drug Control (BPOM) No. 5 of 2018, which is 0.05 $mg.kg^{-1}$, while the Pb level of *P. australis* at both stations has approached the quality standard threshold, which is 0.20 $mg.kg^{-1}$ (Table 2). The Pb and Cd levels of *P. australis* at the Palang sub-district beach were higher than those of *P. australis* in South Sumatra, which contained Pb metals of 0.0496 - 0.1050 ppm to 0.0964 - 0.1388 ppm (Supardi & Nugroho 2019). However, it is lower than *P. australis* in Sanur Beach Bali, which contains a Pb metal of 0.368 $mg.L^{-1}$ and Cd of 0.212 $mg.L^{-1}$ (Rosiana et al. 2022). Based on the levels of heavy metals contained (Naw et al. 2020), *P. australis* from Palang District Beach is not too toxic and can be recommended to be applied and utilized by the food and medicine industries. In addition, the utilization of *P. australis* for animal feed is also highly recommended. However, the utilization of *P. australis* must still pay attention to the levels of heavy metals contained.

Seawater, sediment, and *P. australis* at station II (Glodog Village) had higher levels of heavy metals Pb and Cd than station I (Panyuran Village) (Table 2). Station II of Glodog Village is an anchorage for fishing boats located close to the Palang Fish Auction Place (TPI). The loading and unloading activities at the TPI and the traffic of fishing boats contribute Pb and Cd heavy metal pollutants to the waters. In addition, station II is a residential area that is also close to the river estuary that carries contaminants from the settlement. Tosepu & Effendy (2016) mentioned that ship traffic and ship corrosion, organic insecticides, pesticides, and agricultural fertilizers are sources of metal pollution in waters. Lee et al. (2021) added that residential areas directly affect metal concentrations in water, sediments, and organisms. This is worrying for local people who consume marine organisms because Cd and Pb are highly toxic and harmful to the body. Meanwhile, station I is a residential area located far from TPI Palang but quite close to the river mouth. This shows that TPI Palang is the biggest source of pollution.

Based on the type of heavy metal, the Pb metal level in Palang Subdistrict Beach, Tuban Regency, is higher than the Cd metal level. This is because there are more sources of Pb metal in Palang Beach than Cd metal. According to Mamboya (2007), the availability of heavy metals in water affects the bioavailability of the heavy metals themselves. Pb heavy metal is widely used by industry and is contained in many industrial products, so its pollution in the environment is found to be quite high. Syaifullah et al. (2018) mentioned that the source of damage and pollution of the Tuban Sea comes from the waste of residents in the fishing village area along Tuban Beach. Based on its ability to precipitate, Pb can precipitate better than Cd. According to Hutagalung (1991), the ability of some heavy metals to settle in sediments in order is Hg > Cu > Ni > Pb > Co > Cd. Bayu et al. (2022) explained that the accumulation and solubility of heavy metals are also related to the characteristics of each heavy metal. The relative density/atomic weight of each heavy metal can affect its solubility in waters and sediments. The higher the atomic weight, the easier it is to precipitate.

The results of this study showed that an increase in seawater metal levels was followed by an increase in

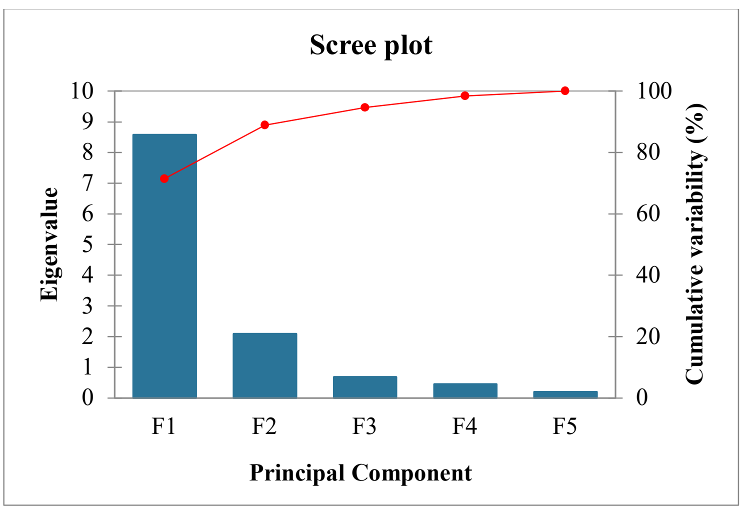

Fig. 3: Scree plot Principal Component Analysis (PCA) and Cumulative variability.

(a) (b)

Fig. 4: *P. australis* in Palang sub-district beach, Tuban Regency. (a) Morphology of *P. australis*. (b) *P. australis* grows attached to the coral sediment (Personal Documentation).

sediment metal levels and *P. australis* metal levels. Based on the results of PCA analysis, all three were positively correlated, meaning that an increase in one variable was followed by an increase in the other (Fig. 3). Pb and Cd metal levels in the sediment correlated more strongly with *P. australis* metal levels than with seawater metal levels. This is because the roots attached to the sediment can absorb metals, spreading metals from the roots to other organs (Sholikah & Rachmadiarti 2019). Plants can localize metals in a particular organ, for example, in the roots themselves (Irhamni 2017). In addition, the deposition and solidification of metal ions in sediments causes metal ions that have solidified in sediments to be difficult to release, so sediments will continue to contain heavy metals even though the waters are not polluted with metals. Metal levels in sediments will increase when there is metal pollution in the waters. This affects the roots of *P. australis* which will absorb metals from the sediment all the time. According to Amini et al. (2013), the positive correlation between sediment metal content and algae indicates that this algae species can absorb metals from sediments and can be used as an object of biomonitoring pollution.

Protein Content of *P. Australis*

Based on the observation of *P. australis* samples morphologically from Palang Subdistrict Beach, it has brown talus with sizes varying from small to large that live attached to the coral substrate (Fig. 4). *P. australis* is a phytoremediator that can be used as a bioindicator of metal pollution (Al-Awlaqi et al. 2019) because it includes brown seaweed as a source of alginate. Alginate is a polysaccharide organic polymer composed of 2 monomeric units of D-sour guluronate and L-sour manuronate or alternating both. Carboxyl and hydroxyl groups are the active parts of alginate that absorb metals. Heavy metals accumulated in the talus of *P. australis* can cause morphological and physiological disorders.

In this study, the crude protein content of *P. australis* containing heavy metals Pb and Cd was analyzed to determine the effect of heavy metals on the physiology of *P. australis* and to determine the safety of utilizing *P. australis* for industrial applications. Physiologically, heavy metals affect the process of photosynthesis and protein in algae (Zamani-Ahmadmahmoodi et al. 2020). Brown seaweed has the lowest protein content of about 15% of dry weight when compared to red and green seaweed protein (Sahri 2023). Based on the results of the analysis (Table 3), it is known that the protein content of *P. australis* at station I amounted to 4.713 ± 0.508 $mg.kg^{-1}$ and at station II amounted to 5.900 ± 0.928 $mg.kg^{-1}$. The protein content of *P. australis* from both stations has exceeded the minimum quality standard of commercial protein content of 2.80% (Murdinah 2009). *P. australis* from Palang Sub-district Beach is classified as good and has the potential to be a commercially valuable protein source for industry use. However, the utilization of *P. australis* from Palang District Beach must consider the levels of heavy metals it contains. The protein content of *P. australis* from Palang Subdistrict Beach, Tuban, is higher than that of *P. australis* from Karimun Jawa, with 3.9% protein (Siahaan et al. 2018). However, it is lower than that of *P. australis* from Pantai Kelapa Tuban, which has a crude protein level

Table 3: Protein content in *P. australis*.

Station	Protein Content [$mg.kg^{-1}$]	Quality Standards [$mg.kg^{-1}$]*
Station I (Panyuran Village)	4.713 ± 0.508	2.8
Station II (Glodog Village)	5.900 ± 0.928	2.8

Note: (*) Murdinah 2009.

of 12.57% (Hidayah et al. 2022), and P. australis from Pantai Kelapa Lima Teluk Kupang, with a crude protein level of 13.89%. This difference can be attributed to variations in the age of *P. australis*, weather conditions, and habitat (Salosso et al. 2020). In this study, the age of *P. australis* was unknown, but only those with talus of relatively similar size and color were selected. Sampling coincided with the beginning of the rainy season. According to Denis et al. (2010), the protein content of *P. australis* varies and is influenced by the season. The highest concentration of seaweed protein occurs during early spring and winter, while the minimum concentration is during early fall and summer.

The results of this study indicate that the protein content of *P. australis* is still relatively good even though *P. australis* has been contaminated with metals. These results differ from some previous studies that metal levels in seaweed were negatively correlated with protein levels. *P. australis* seaweed containing heavy metals has low protein levels. Anggreani & Rachmadiarti (2021) in Sendang Biru Beach Malang found that *P. australis* with an average Cd metal content of 0.019 ± 0.001 $mg.kg^{-1}$ to 0.027 ± 0.001 $mg.kg^{-1}$ contains protein levels of 0.0002 $mg.L^{-1}$ to 0.0004 $mg.L^{-1}$. The Cd metal content and protein levels are the same. Cd metal levels and protein levels are negatively correlated, which means that the higher the levels of Cd metal, the lower the protein content in seaweed *P. australis*. Based on the results of Pearson correlation analysis in this study, it is known that the levels of Cd metal *P. australis* and protein levels *P. australis* have a very strong positive correlation (0.890) (Fig. 3). The higher the Cd metal content of *P. australis*, the higher the protein content of *P. australis*. The correlation between Pb metal and protein content of *P. australis* was strong and lower than Cd. The higher the Pb metal content of *P. australis*, the higher the protein content of *P. australis*. The correlation between the two was not significant. The results of this study are similar to Carfagna et al. (2013) who found that the effect of Cd metal on *Chlorella sorokiniana* microalgae was greater than Pb metal. This may be because Cd has greater toxicity than Pb. The difference between this study and Carfagna et al. (2013) is that in the experimental study, Carfagna et al. (2013) found that Cd and Pb metals had a negative impact on microalgae. Microalgae exposed to Cd for 24 h had very low total protein levels, which were only 48 and 35% of the control cells. These protein levels were lower than microalgae exposed to Pb at the same time. According to research by Bavi et al. (2011), protein synthesis is strongly influenced by cadmium. The higher the cadmium concentration, the greater the decrease in protein levels. The reduction in protein levels can be caused by a decrease in protein synthesis or an increase in the rate of protein degradation (Balestrasse et al. 2003). Cadmium will react with SH groups and result in protein denaturation (Fuhrer 1982). Cadmium causes the production of reactive oxygen species (ROS) in plant cells (Haider et al. 2021). Cd metal that enters the body of organisms can interfere with photosynthesis and growth (Vo et al. 2020).

P. australis has a high tolerance to Pb and Cd metals, so in this study, the metal levels contained in *P. australis* can still be tolerated and do not cause significant physiological damage. Algae have various binding groups such as COO^-, SH^-, OH^-, RNH_2^-, RS^-, and RO^- for biosorption of metal ions. These binding groups exist on the cell surface and in the cytoplasm, especially within the vacuole (Salama et al. 2019). Carboxyl functional groups (COO^-) are the most abundant acidic functional groups in the brown cell walls of algae. Excretion and exclusion of metals from cells, production of proteins such as proline and other binding compounds such as metallothioneins (MTs) and glutathione (GSH) are among the mechanisms used by algae to prevent metal-induced damage (Aude-Garcia et al. 2016). If a plant experiences abiotic stress, protein is used to meet energy needs (Wang et al. 2004). An increase in total protein content indicates an increase in antioxidant enzyme levels (Kovtun et al. 2000). Antioxidant enzymes present in cells scavenge reactive oxygen species and eliminate oxidative stress. Superoxide dismutase is essential in converting more reactive and harmful oxygen radicals into less reactive hydrogen peroxide (Foyer 2018).

CONCLUSION

This study showed that the physico-chemical quality of seawater at Palang Subdistrict Beach, Tuban Regency, influenced the levels of Pb and Cd in water and sediment. The increase in metal levels in seawater was followed by an increase in sediment metal levels and metal levels in *P. australis*. Pb and Cd levels at station I, which amounted to 0.194 ± 0.015 and 0.010 ± 0.001 $mg.kg^{-1}$, while at station II, which is 0.200 ± 0.028 and 0.021 ± 0.004 $mg.kg^{-1}$. Pb metal levels in Palang Subdistrict Beach, Tuban, are higher than Cd metal levels because there are more Pb metal pollutant sources than Cd metal pollutant sources. Station II (Glodog Village) has higher levels of heavy metals Pb and Cd than station I (Panyuran Village) because station II is close to the fish auction and residential areas that cause higher levels of pollution. Protein levels of *P. australis* at stations I and II, namely 4,713 ± 0.508 and 5,900 ± 0.928 $mg.kg^{-1}$. This shows that heavy metal levels in *P. australis* affect the protein content of *P. australis*. The protein content of *P. australis* is still classified as good according to quality standards even though *P. australis* has been polluted and contains Pb and Cd metals, so it is still safe for consumption. In addition, *P. australis* can tolerate heavy metals because it does not experience significant physiological damage so it can be used as a bioindicator of Pb and Cd heavy metal pollution in seawater.

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