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Lead Level Analysis in Roots and Leaves of Mangrove Plants *Ceriops tagal* and *Rhizophora apiculata* in Clungup Mangrove Conservation

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Abstract

Indonesia is an archipelago with more water than land, which is battling water and land pollution. One form of pollution is lead which is commonly caused by battery waste, fishing boat activities, diesel waste. One solution is phy-toremediation using hyperaccumulator plants such as mangroves. Mangroves can absorb heavy metals and maintain ecosystem stability. The dominant species in Clungup Mangrove Conservation are *Ceriops tagal* and *Rhizophora apiculata*. The objectives of this study were: 1) To determine the level of lead metal (Pb) pollution in mangrove sediments in the area. 2) To determine the levels of lead metal (Pb) in the roots and leaves of mangrove species *Ceriops tagal* and Rhi-zophora apiculata. 3) To determine the ability of lead metal (Pb) absorption in mangrove plants *Rhizophora apiculata* and *Ceriops tagal*. Lead test results in sediments in this area are classified as very low. Lead levels in the roots of *Ceriops tagal* were higher than *Rhizophora apiculata*. Pb levels in the leaves of *Ceriops tagal* were also higher than the leaves of *Rhizophora apiculata*. Bioconcentration Factor (BCF) values were higher than *Rhizophora apiculata*, but both were categorized as lead removal plants (BCF < 1). The Transfer Factor (TF) value showed a lower value for *Ceriops tagal* than *Rhizophora apiculata*, indicating that *Rhizophora apiculata* has better translocation ability. However, both are categorized as unstable plants (TF < 1). The results of the phytoremedia-tion calculations showed that *Ceriops tagal* had superior absorption potential compared to *Rhizophora apiculata.*

Keywords: mangrove, lead, phytoremediation

1. Introduction

Indonesia is an island country with a larger water area than its land, making water and soil pollution a problem that is often encountered in Indonesia. Water amendment could be threatened by human activities, such as garbage disposal, washing, and fishing. These activities produce waste, including Toxic Hazardous Material (HAZMAT). Nursabrina et al. (2021) [1]: Hazardous waste generated from industrial activities in Indonesia reached 44,939,612.36 tons of which 99.80% were managed and unmanaged waste up to 0.2%. The ecological balance of water, air, and soil can be disrupted by hazardous waste carried by rivers to the coast, affecting living organisms and posing a serious threat to marine ecosystems and public health. The impact of water pollution by heavy metal elements, in addition to disrupting marine ecosystems, can also indirectly damage marine biota, phytoplankton death by inhibiting enzyme activity in cell division, inhibiting growth rates and photosynthesis processes, changing or reducing cell volume, and can cause the death of living organisms that live in these waters [2]. Heavy metals from hazardous waste disposal, which are toxic and carcinogenic substances that endanger living things and ecosystems if their concentration is too high, are easily absorbed into the marine environment and pollute it. Heavy metals that have a negative impact on water quality include cadmium (Cd), copper (Cu), and lead (Pb) [3]. The Ministry of State for Population and Environment of Indonesia with Dalhousie University, Canada, states that the maximum limit of heavy metals in soil is as follows: Lead (Pb) at 100 ppm ± SE [4]. Lead is a heavy metal usually found in battery waste, fishing boats, and diesel waste. Which is one of the lead metal pollution due to ship ballast water discharge averaging 0.16 kg [5]. Pb is a heavy metal that has many adverse effects on living organisms, and if marine biota contaminated with heavy metals are eaten by humans, it has a negative impact on humans, especially when the amount exceeds the threshold [6].

Phytoremediation is an environmentally friendly and cost-effective biotechnological approach that uses heavy metal-binding plants (hyperaccumulators) to bind heavy metals in soil. Phytoremediation is a technology used to improve land-use plants [7]. Phytoremediation uses many mechanisms, including degradation, accumulation, dissipation, and immobilization, to degrade, remove, and immobilize pollutants [8]. Hyperaccumulator plants, such as mangroves, can absorb or detoxify harmful contaminants, such as heavy metals, and reduce their concentration in the soil, acting as biofilters that filter, bind, and trap pollution in the wild. Mangrove plants that can phytoremediate the environment will maintain ecosystem stability in the future [9,10]. Mangroves are included in hyperacular plants because they function as natural bioremediation agents and can absorb heavy metals in nature; this function is referred to as biosorption [11]. Examples of physical aspects include keeping the coastline stable, protecting beaches and rivers from erosion and abrasion, and managing toxic waste [12].

Clungup Mangrove Conservation in Tambakrejo Village, managed by the local Sendang Biru community, is possibly affected by carried garbage from river currents, nearby settlements, small harbors, and possibly metal pollution sources. Based on previous literature, it was found to contain Pb in water at a concentration of 0.0015 mg/l in 2022, as well as several other metal elements [13]. Heavy metals can bind to organic matter easily, settle to the bottom of the water, and blend with sediments, so that the heavy metal content in sediments can be higher than that in water [14]. In this study, we used two dominant plant species, *Ceriops tagal* and *Rhizophora apiculata*, to determine their phytoremediation capabilities in the Clungup Mangrove Conservation Area, where no similar research has been conducted to improve the management and awareness of lead pollution. Based on the existing problems, the aims and objectives of this research are as follows: 1) To determine the level of lead metal (Pb) pollution in mangrove sediments in the Clungup Mangrove Conservation area. 2) To determine the levels of lead metal (Pb) in the roots and leaves of the mangrove species *Ceriops tagal* and *Rhizophora apiculata* in the Clungup Mangrove Conservation area. 3) To determine the ability of lead metal (Pb) uptake ability of the mangrove plant species *Rhizophora apiculata* and *Ceriops tagal*.

2. Research Methodology

2.1 Time and Location

The research was carried out for two months, from February 20th to March 31st, in the Clungup Mangrove Conservation with an area of 77.7 ha, in Tambakrejo Village, Sumbermanjing Wetan District, Malang (**Figure 1**). The sample testing and analysis of the research results were performed at the Unisma Integrated Laboratory.

Figure 1. Research sampling points on Clungup Mangrove Conservation (CMC)

2.2 Examples of good map for the manuscript

The sampling method used a Line Transect with a plot area of 10 × 10 m for trees separated by 20m between plots along the transect line, and the distance between transect lines was 100m.

$$\Sigma plots = \frac{Total\ areal \times sampling\ intensity}{Total\ plots \times 100\%} \tag{1}$$

$$\Sigma plots = \frac{77.7 \times 1\%}{0.01 \times 100\%} \tag{2}$$

$$\Sigma plots = 77{,}7 \tag{3}$$

The sampling intensity is the ratio of the number of units of the measuring plots to the number of population units. Therefore, from the needed plot 77,7 is rounded up to 78 sample plots. The determination and length of the transect line is adjusted to the length of the mangrove area from the shoreline to the deepest limit of the area or the lowest tidal area [15].

Each plot used purposive sampling for sampling by considering the research based on the samples needed (sediment, root, and leaf). Based on the transect plot line used from each plot, it was necessary to collect some of the necessary samples, including sediment, root, and leaf samples. The shape and technique of plotting are shown in Figure 2, Line Transect Design.

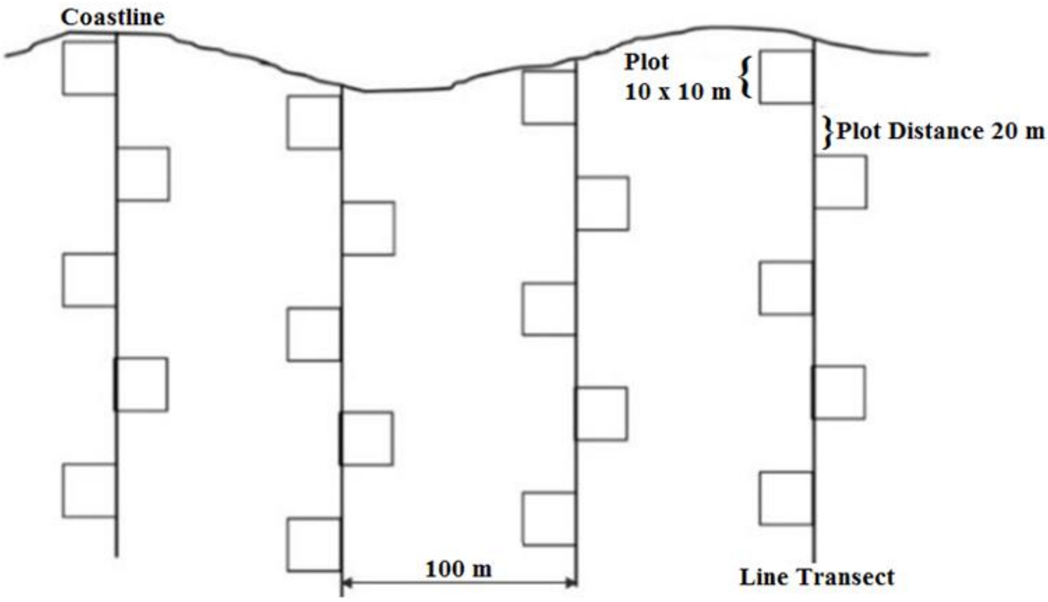

Figure 2. Line transect design

Based on the transect plot line used in each plot, the samples used were as follows.

a. Sediment samples

Composite sediment samples were obtained by combining samples from each sampling plot of ±5 g at a depth of 10 cm. Surface samples are considered less representative because they always fluctuate and are easily eroded during high and low tides; therefore, samples taken from a certain depth are considered more representative at each observation point [16].

b. Root samples

Three root samples were taken 3 pieces per plot under the condition that the roots were submerged in the soil, so in total, there were 3 × 78 samples per species. Mangrove tree roots can absorb heavy metals in seawater because they are submerged in water at high tide [17].

Leaf samples were collected from 10 old leaves from each plot for each species. The period in question is when older leaves grow perfectly so that they have a higher absorption rate than younger leaves [18].

Sample analysis was performed using atomic absorption spectrophotometry (AAS). Stages that need to be done before analyzing the samples included:

a. Sample preparation

The sediment samples were prepared by drying at 105°C for 24 h. The roots and leaves were chopped and then baked at the same temperature for 4 h. The dried samples were pulverized into powder. The purpose of drying is to remove water until the weight is constant [19].

b. Measurement of Pb concentration of the sample

A dried and sieved sample was taken as much as 0.5 grams and then put into a beaker glass, 5 ml of concentrated HNO3 was added then homogenized. The beaker glass was then closed and slowly boiled using a hot plate at a temperature of 95 degrees C, and the volume of the solution when 15 to 20 ml remains. After cooling, 10 ml of HNO3 and concentrated HCLO4 homogenize the solution again, heat the solution until the HCLO4 vapor disappears, and then wait until it cools and add 50 ml of distilled water. Filter the solution while rinsing with distilled water until the filtrate volume is 100 ml then homogenize [20]. The absorbance of the sample filtrate was measured using AAS (Atomic Absorption Spectrophotometry) at a specific wavelength of λ 283.3 nm for lead metal (Pb) [21].

2.3 Phytoremediation

Indicators that could be used to determine the phytoremediation ability of these types were calculated according to Rachmawati (2018) [22] and Tangahu et al. (2011) [23], with the observed variables:

a. The bioconcentration factor (BCF) is used to measure the ability of plants to absorb or accumulate heavy metals from a substrate.

$$BCF = \frac{Heavy\ metal\ concentration\ in\ roots}{Sediment\ heavy\ metal\ concentration} \quad (4)$$

Criteria: BCF > 1: Accumulator; BCF = 1: Indikator; BCF < 1: Excluder

b. The translocation Factor (TF) indicates the ability of plants to translocate heavy metals from the roots to the upper parts of the plant (stems or leaves).

$$TF = \frac{Heavy\ metal\ concentration\ in\ leaves}{Heavy\ metal\ concentration\ in\ roots} \quad (5)$$

Criteria: TF < 1: Phytostabilization; TF > 1: Phytoextraction

c. Phytoremediation (FTD) is the difference between BCF and TF, and is used as an indicator of the plant absorption ability of heavy metals [24].

The analysis used in this study was qualitative, and descriptive analysis was used to analyze data from Atomic Absorption Spectrophotometry (AAS) and phytore-mediation calculation data (TF, BCF, FTD). Data from measurements of lead metal levels in the roots and leaves of *Ceriops tagal* and *Rhizophora apiculata* were analyzed.

Table 1. Caption of a table (Word style: MK Caption of tables). The font used was Cambria (size: 9 pt. Please refrain from manually creating a figure caption; kindly utilize the "Insert Caption" tool available in Microsoft Word.

No.	Tree species	Family	Group	Feed distance (m)
1	*Ficus elastica*	Moraceae	A	0.0
2	*Sterculia macrophylla*	Malvaceae	C	9.0
3	*Octomeles sumatrana*	Moraceae	C	10.0
4	*Artocarpus elasticus*	Tetramelaceae	C	0.7
5	*Sterculia oblongata*	Malvaceae	A	0.0
6	*Pterospermum javanicum*	Malvaceae	A	7.0
7	*Schima walichii*	Theaceae	D	0.5
8	*Shorea* sp.*	Dipterocarpaceae	D	8.0
9	*Artocarpus elasticus*	Moraceae	A	0.0
10	*Ficus microcarpa*	Moraceae	B	0.0
11	*Sterculia macrophylla*	Malvaceae	K	0.0
12	*Pterospermum javanicum*	Malvaceae	K	4.0
13	*Dillenia sumatrana*	Dilleniaceae	J	0.0
14	*Litsea* sp.*	Lauraceae	J	4.5

* Note can be added, if necessary, to the bottom of the table. Consider adding note if you need to emphasize something in the table (Calibri 8pt)

3. Results

3.1 Lead contamination level

The Clungup Mangrove Conservation Area is directly adjacent to the fishing port of Sendang Biru Beach and is downstream from the river that passes through the settlement of Tambakrejo Village. It can be assumed that there is a high possibility of Pb content originating from plastic waste and ship fuel from fishing boats that can pollute the Clungup Mangrove Conservation area. According to previous studies, Clungup Mangrove Conservation has a lead content in water of 0.0015 mg/l in 2022 [13]. Based on sample testing, Pb levels in soil sediments in the Clungup Mangrove Conservation area conducted with three repetitions are shown in Table 2.

Table 2. Pb levels in soil sediments at Clungup Mangrove Conservation

Samples name (10th May 2023)	Replicated	Pb concentration (ppm ± SE)
Sediment	1	5,7675 ± 0.079
	2	5,8602 ± 0.079
	3	6,0365 ± 0.079
Average		5,8881 ± 0.079

* Note can be added, if necessary, to the bottom of the table. Consider adding note if you need to emphasize something in the table (Calibri 8pt)

The average Pb concentration in the sediment was 5.8881 ppm ± 0,079. When compared to previous research by Retnaningdyah et al. (2022) [13], the lead level of 0,0015 ppm ± 0,079 in water was lower than the lead levels in the tested sediments. According to Nurbarasamuma et al. (2022) [25], heavy metals easily bind to organic matter and settle to the bottom of the waters together with sediments. This results in a higher heavy metal content in the sediment than that in wa-ter.

According to the Law of the Republic of Indonesia (2009), quality standards aim as a basis for critical limits or values of living things, substances, energy, components, or pollutants whose presence in certain natural resources can be interpreted as environmental elements. Lead is carcinogenic and toxic; therefore, if it exceeds the quality standard, it can have a direct impact on living things. The quality standard in Indonesia was based on a review by Brooks (1992) [4]. This indicates that the maximum limit of Pb in soil is 100 ppm ± SE in a joint study by the Ministry of State for Population and Environment of Indonesia and Dalhousie University, Canada. The standard limits of Pb levels in soil from several countries are presented in Table 3.

Table 3. Pb levels in soil sediments at Clungup Mangrove Conservation

	Standard limits	
Country	Years	ppm (mg/kg)
Australia	2012	300
Canada	2009	200
China	2015	80
Germany	2007	1000
Tanzania	2007	200
Netherlands	2007	530
New Zealand	2012	160
USA	2014	200

*note: EPAA 2012, CME 2009, EPMC 2015, EEA 2007, TMS 2007, NZME 2012 & US EPA 2014 [26]

Based on the analysis of soil sediment sample testing in the Clungup Mangrove Conservation area, Pb levels are relatively low from the threshold or quality standards of several major countries in the world and Indonesia's own quality standards that were carried out in 1997. The lead level in the Clungup Mangrove Conservation area is only 5.8881 ppm ± 0,079, which is about 0.5-6% of the quality standards owned by several countries, which means that lead metal has not polluted the Clungup Mangrove Conservation area and the level of pollution is said to be very low.

3.1. Lead levels in *Ceriops tagal* and *Rhizophora apiculate*

Based on sample testing, Pb levels in the roots and leaves of *Rhizophora apiculata* and *Ceriops tagal* mangrove plants in the Clungup Mangrove Conservation area conducted with three replicates are presented in Table 4.

Table 4. Pb levels in soil sediments at Clungup Mangrove Conservation

Repetition	Pb samples concentration (ppm ± SE)			
	Rhizophora apiculata		*Ceriops tagal*	
	Root	Leaf	Root	Leaf
1	1.4096 ± 0.0913	0.6345 ± 0.098	2.3521 ± 0.1675	0.7799 ± 0.0303
2	1.7135 ± 0.0913	0.4268 ± 0.098	2.6026 ± 0.1675	0.7187 ± 0.0303
3	1.6379 ± 0.0913	0.7632 ± 0.098	2.9307 ± 0.1675	0.6756 ± 0.0303
Average	1.5870 ± 0.0913	0.6081 ± 0.098	2.6285 ± 0.1675	0.7247 ± 0.0303

Based on the test results, lead levels in the roots of *Ceriops tagal* averaged 2.6285 ppm ± 0.1675 higher than those in the roots of *Rhizophora apiculata*, which only absorbed 1.587 ± 0.0913. *Ceriops tagal* is suspected to be an accumulator plant that can absorb metals from the soil at high concentrations. Pb levels in *Ceriops tagal* leaves had an average value of 0.7247 ppm ± 0.0303, then by *Rhizophora apiculata* leaves with an average Pb level of 0.6081 ppm ± 0.098. When compared, the Pb levels in both plants are known to be higher in *Ceriops tagal* leaves.

Mangrove plants can absorb and stabilize heavy metal elements that exist in the vicinity. Based on the testing of lead levels that have been carried out on leaf root samples of mangrove plants, *Rhizophora apiculata* and *Ceriops tagal* species have elemental contents in each part of the leaves and roots, as shown in Table 4.3 which has been presented. The presence of Pb in the roots and leaves of *Ceriops tagal* and *Rhizophora apiculata* proves that these species possess accumulator ability. The ability to absorb metal concentrations is different in each type of mangrove, so mangroves act as metal accumulators in coastal seas [9].

In mangrove plants, absorption occurs in the roots; therefore, it is inevitable that the lead content in the roots will be higher than that in the leaves. Mangroves absorb heavy metals in water via their roots, and the absorption process is called rhizofiltration. Mangrove roots are directly exposed to pollutants and are in direct contact with water and soil sediments [27].

3.2. Phytoremediation

BCF and TF calculations were conducted to determine the ability of *Rhizophora apiculata* and *Ceriops tagal* to absorb and translocate lead metals in their bodies. The results of the BCF and TF calculations are presented in Table 5.

Table 5. Pb levels in soil sediments at Clungup Mangrove Conservation

Parameters	*Rhizophora apiculata*	*Ceriops tagal*
Bio Concentration Factor (BCF)	0.2695 ppm ± 0.0064	0.4464 ppm ± 0.0297
Translocation Factor (TF)	0.3832 ppm ± 0.0106	0.2757 ppm ± 0.0172
Phytoremediation (FTD)	-0.1137 ppm ± 0.0166	0.1707 ppm ± 0.0461

According to Manikasari et al. (2018) [28] plants 0that have phytoremediation ability of plants can be determined using the calculation of bioconcentration factors (BCF) and Translocation Factors (TF). Based on the BCF calculation that has been done, the BCF result of *Ceriops tagal* is higher than that of *Rhizophora apiculata* with BCF value of *Ceriops tagal* of 0.4464 ppm ± 0.0297and *Rhizophora apiculata* of 0.2695 ppm ± 0.0064. However, both BCF values (BCF < 1) indicate that *Ceriops tagal* and *Rhizophora apiculata* fall into the category of excluder plants for Pb. The nature of plants that limits the absorption of heavy metals in the environment, both sediment and water, is an excluder, but when entering the plant body, heavy metals can be easily translocated to other parts of the plant's growth or biomass [22]. According to Manikasari et al. (2018) [28], mangrove vegetation density affects the concentration of Pb metal in the water and shows a positive coefficient value. This means that the higher the absolute density of mangrove vegetation, the higher the concentration of heavy metals in the water. Because the water trapped in the roots is surface water, the particulate form of heavy metals causes them to be trapped in root sections. Therefore, the denser the root conditions, the higher the metal content trapped in the plant area. This can be seen in Figure 3, where the root conditions in *Ceriops tagal* are denser than *those in Rhizophora apiculata*, so it cannot be denied that the BCF of *Ceriops tagal* roots is higher.

Figure 3. Research sampling points on Clungup Mangrove Conservation (CMC)

TF calculations that have been done obtained TF value *Ceriops tagal* lower than *Rhizophora apiculata* with TF value *Ceriops tagal* of 0.2757 ppm ± 0.0172 and *Rhizophora apiculata* of 0.3832 ppm ± 0.0106, meaning that the transloca-tion ability of *Rhizophora apiculata* better than *Ceriops tagal*. However, both TF values (TF < 1) indicate that *Ceriops tagal* and *Rhizophora apiculata* belong to the category of phytostabilized plants. Phytostabilization is the termination of heavy metals in soil and water through absorption and accumulation by roots (rhizosphere) so that heavy metals do not enter other parts of the plant stem. The assumption is that plants do not translocate heavy metals from the roots to other tissues, such as leaves and stems, in large quantities [9]. In addition, Analuddin et al. (2017) [29], R. apiculata leaves can absorb and store heavy metals. The accumulation of heavy metals in the leaves of *Rhizophora apiculata* is a form of high TF that shows the adaptability of the plant in overcoming the toxicity of Pb in its body.

This means that *Rhizophora apiculata* and *Ceriops tagal* work by inhibiting metal mobility in sediment. After measuring BCF and TF, the phytore-mediation (FTD) of the two species can be determined to determine which one is better used for phytoremediation. A comparison of the measurement results of the phytoremediation values of *Rhizophora apiculata* and *Ceriops tagal* plants is presented in Figure 4.

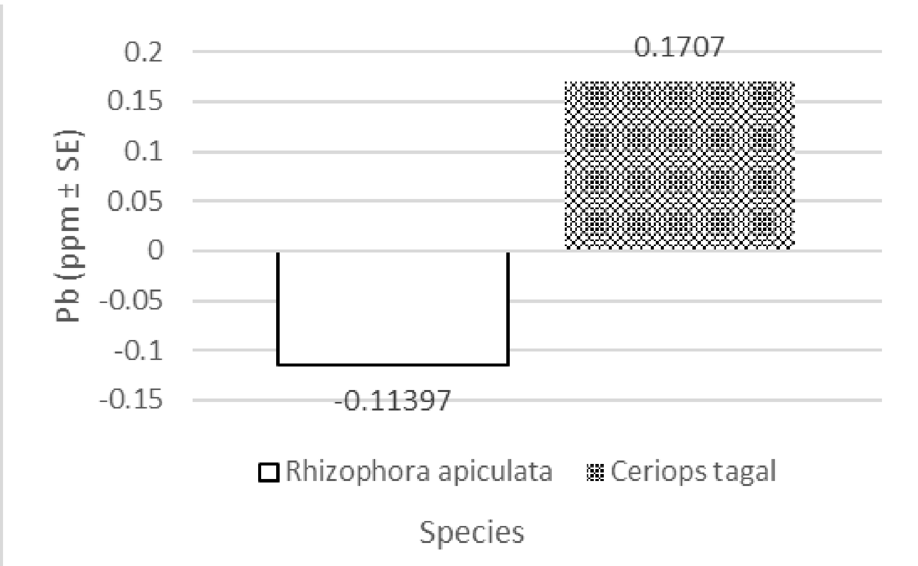

Figure 4. Research sampling points on Clungup Mangrove Conservation (CMC)

Physiologically, when there are high concentrations of heavy metals in sediment, mangroves reduce the uptake of heavy metals by dilution or dilution of toxins (Wulandari et al., 2018). For example, Khairuddin et al. (2018) [10], *Rhizophora apiculata* can be used as a toxic countermeasure by weakening the toxic effect through dilution; however, in the calculation of phytoremediation type, *Ceriops tagal* 0.1707 ppm ± 0.0461 was greater than that of *Rhizophora apiculata*(-0.1137 ppm ± 0.0166). The calculation shows that the superior absorption potential is owned by *Ceriops tagal*, while the *Rhizophora apiculata* species is better at neutralizing heavy metals in aquatic sediments.

4. Conclusion

The level of lead in the sedimentation of the Clungup Mangrove Conservation area is only 5.8881 ppm ± 0,079, this means that lead metal has not polluted the Clungup Mangrove Conservation area, and the level of pollution is said to be very low compared to the quality standards of several countries. Measurement of lead levels in the roots of *Ceriops tagal* was 2.6285 ppm ± 0,1675 higher than that in the roots of *Rhizophora apiculata* plants, which only absorbed 1.587 ppm ± 0,0913. Pb levels in *Ceriops tagal* leaves had an average value of 0.7247 ppm ± 0,0303, then in *Rhizophora apiculata* leaves had an average Pb level of 0.6081 ppm ± 0,098. The Bio Concentration Factor (BCF) value of *Ceriops tagal* is higher than that of *Rhizophora apiculata* with a BCF value of 0.4464 ppm ± 0.0297 for Ceri-ops tagal and 0.2695 ppm ± 0.0064 for *Rhizophora apiculata*. However, both BCF values (BCF < 1) indicated that the excluder plants were exposed to lead metal. The value of Translocation Factors (TF) that has been done obtained the value of TF *Ceriops tagal* lower than *Rhizophora apiculata* with TF *Ceriops tagal* value of 0. 2757 ± 0.0172 and *Rhizophora apiculata* of 0. 3832 ± 0.0106. However, TF values in both species (TF < 1) indicate the phytostabilized plant category. Phytoremediation (FTD) type *Ceriops tagal* 0.1707 ppm ± 0.0461 greater than the type of *Rhizophora apiculata* -0.1137 ppm ± 0.0166, indicating better potential for use as a phytoremediation plant in the Clungup Mangrove Conser-vation mangrove type *Ceriops tagal*.

Author Contributions

FAC: Writing - Review & Editing, Conceptualization, Supervision: **MC:** Review & Editing, Conceptualization, Supervision; and **SIP**: Conceptualization, Methodology, Software, Investigation, Writing - Review & Editing.

Conflicts of interest

There are no conflicts to declare.

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