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Bioaccumulation of Lead Metal (Pb) by Mangrove Plants (*Rhizopora apiculata*) in the Waters of Poka Village, Inner Bay of Ambon

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Abstract

This research aims to study the extent of bioaccumulation of the heavy metal Pb by mangrove plants (*Rhizopora apiculata*) in the waters of Poka Village, Ambon Bay. Mangrove plants were chosen because they can naturally accumulate heavy metals around their roots. In this study, sediment grains were measured using a Sieve Shaker, the content of Pb metal in sediment and parts of mangrove plants was determined using AAS, while the bioaccumulation value of Lead metal (Pb) by mangrove plants was calculated using the BCF and TF formulas. The results showed that the particle size in gravel ranged between 0.00-0.13%, sand 20.44-66.35%, and mud 33.65-79.56%. Lead (Pb) levels in Poka Village waters in sediment ranged from 10,925-16,925 mg/kg, roots ranged from 0.5-21.35 mg/kg, and leaves ranged from 0-4,675 mg/kg. Meanwhile, the BCF value at sampling point 1 = 0.03, sampling point 2 = 1.40 and sampling point 3 = 0.56, the TF value at sampling point 1 = 7.8, sampling point 2 = 0.03 and sampling point 3 = 0.09. This shows that mangrove plants function as phytoextraction and can be used as phytoremediation agents to accumulate the heavy metal lead (Pd) from waters.

Keywords: Mangrove, Pb metal, Poka village, sieve shaker, AAS

INTRODUCTION

The development of industrial and commercial areas and urbanization have contributed greatly to anthropogenic heavy metals in the biosphere (Wilda, Hamdan, & Rahmi, 2021). Human activities in these sectors, as well as other domestic activities, have contributed greatly to the increase in heavy metal concentrations in air, soil, and water (Lotfinasabasl, 2012).

One of the water areas in Maluku Province that also experiences this is the inner Ambon Bay area. This area is residential, a power plant center, and various shipping activities both modern and traditional. This directly affects the condition of the inner Ambon Bay (Male et al., 2017). The bay has also become a container for various types of waste, including plastics, organics, and chemicals, as well as waste from households and industrial activities in the vicinity. Several toxic heavy metals such as Cd, Pb, As, and Hg accumulate in sediments in Ambon Bay (Male, Seumahu, & Malle, 2020).

One of the villages located on the inner coast of Ambon Bay is Poka Village. This village is located in the center of the Poka Diesel Power Plant (PLTD), trade area, culinary, and dense population have an impact on the amount of waste that pollutes the surrounding waters, both waste containing organic and inorganic materials. One of the inorganic waste that affects environmental quality and pollutes the waters of the inner Ambon Bay is lead metal (Selanno, Tuahatu, Tuhumury, & Hatulesila, 2014).

Lead metal enters the aquatic environment accumulates in sediments and is then absorbed by the organisms that live in it (Yona, Andira, & Sari, 2016). Heavy metal lead (Pb) is then absorbed by aquatic biota from seawater. Inhaled lead enters the respiratory tract and spreads to tissues and organs. More than 90% of lead absorbed in the blood binds to red blood cells, disrupting the hemoglobin synthesis process (Rosita & Mustika, 2019). Humans who consume marine biota contaminated with lead metal will have adverse health effects such as biosynthesis disorders, anemia, neurological diseases, brain disorders, and behavioral changes in children, namely impulsive and hyperactive conditions (Yona et al., 2016).

To prevent the adverse effects of lead metal contamination, many studies have been conducted to reduce the metal concentration by utilizing mangrove plants as phytoremediators (Gupta & Chakrabarti, 2013; Lotfinasabasl, 2012). Mangrove plants are used as phytoremediators because they can accumulate heavy metals around their roots naturally, which is referred to as biosorption. Mangroves also filter, capture, and bind pollution in the environment such as sediment overflow due to anthropogenic activities and various types of household waste, and contribute greatly to improving water quality (Lufthansa, Titah, & Pratikno, 2021; Utami, Rismawati, & Sapanli, 2018).

The purpose of this research is to study the content of Pb metal in sediments and parts of the roots and leaves of mangrove plants including the ability of these plants to accumulate Pb metal from the waters of Poka Village, inner Ambon Bay.

METHODOLOGY

Materials and Instrumentals

The materials used in this study include concentrated HNO_3, concentrated HCl, roots and leaves of Mangrove plants (*Rhizopora apiculata*), sediment, Whatman filter paper no 42, and distilled water.

The instruments used in this study include Tools used in this study are GPS (Garmin), Refractometer (ATC), pH Meter (ATC), Thermometer (Omron Digital Thermometer MC-341a), Atomic Absorption Spectrophotometer (SSA, 7000 SHIMADZU), Sive shaker (Sieving machine AS 200 badic), Analytical Balance (Cyberscan CON 1110), Oven (Memert), Mortal and pestle, Glassware (Pyrex), Hot plate (Cimarec).

Methods

Temperature, Salinity, and pH Measurements

Seawater samples were put into a 100 mL beaker, the temperature was measured using a thermometer. The measured temperature is then recorded.

The refractometer glass is rinsed with distilled water and then dried, then drop the seawater sample on the glass at the bottom front of the refractometer, carefully observing the number which is the salinity level indicated by the boundary between white and blue colors.

The seawater sample was put into a 100 mL beaker, measured using a pH meter into a beaker containing seawater for 5 minutes, then the measured pH value was recorded.

Sediment Sampling

Samples were taken with a PVC pipe at a depth of 10 cm. The samples were then put into labeled sample bags.

Mangrove Roots and Leaves Sampling

Samples taken are roots and leaves, roots and leaves are taken on the same tree at each point. The roots taken are roots that are submerged directly in seawater, while the leaves are taken as many as ± 50 sheets (200 grams). Then the samples were put into a bag and labeled.

Determination of Sediment Particle Grain Size

The sediment grain size analysis procedure uses a wet sieving system (dry sieving) with a sieve shaker. Sediment samples were dried at 70 °C-80 °C for 24 hours, then the dry samples were weighed and the dry weight numbers obtained were recorded as dry weight. Soak the samples that have been weighed for 5 hours with distilled water to release the granules then arrange the sieve according to the order of size from bottom to top, 0.032, 0.063, 0.090, 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mm. Put the sample that has been soaked at the top of the sieve sequence, namely the size of 4.00 mm after that wash the sample by flushing brought running water while stirring with a brush that releases the grains of one another. Each sediment fraction left on the sieve was transferred to a 100 mL aluminum tray, and then each sample fraction was dried in the oven at 70 °C-80 °C for 2 hours. Weigh each sediment fraction and record the weight, the weight of each fraction obtained is recorded as the weight of the grain size fraction.

Sediment Sample Preparation

The samples were put into a Petri dish and then heated in an oven at 105 °C until they dried. The samples were pulverized and sieved using a 200 mesh sieve and weighed. The next stage is the process of packing samples in labeled sample bags for the next stage of the analysis process.

Mangrove sample preparation

Mangrove plant samples (roots and leaves) were cut into small parts and then heated in an oven at 105 °C to dry. The samples were then mashed using a mortar, sieved using a 200 mesh sieve, and weighed. The next stage is the process of packing samples in labeled sample bags for further analysis.

Sediment and Mangrove Sample Destruction Process

Samples of sediment, roots, and mangrove leaves were weighed as much as 4 g and put into 3 100 mL beakers, into each beaker was added concentrated HNO_3 solution (65%) as much as 4 mL and concentrated HCl solution as much as 12 mL. The sample was heated with a hotplate at 200 °C for ± 10 minutes. The deconstructed solution is then filtered and distilled water is added to the limit mark. The sample of the deconstructed solution is ready for the analysis stage using AAS.

Preparation of Pb Standard Solution

From the 1000 ppm Pb mother liquor, 1 mL of Pb solution was pipetted and then diluted in a 100 mL volumetric flask. The next step is to make a standard solution of 0.2; 0.4; 0.6; 0.8; and 1.0 ppm which is done by taking with a pipette as much as 2; 4; 6; 8 and 10 mL of Pb solution from 10 ppm standard solution that has been made before then dilute with distilled water until the limit mark. Then the absorbance was measured using SSA at a wavelength of 217 nm. Then from the data obtained, a relationship curve between absorbance (A) versus concentration (C) was made so that a straight-line standard curve was obtained.

Calculating the Bioconcentration Factor (BCF) Value

The purpose of this calculation is to determine the amount of Pb metal accumulation by mangrove plants which is calculated by comparing the concentration of Pb metal in sediments and roots using the BCF formula. BCF calculation formula is as follows:

$$BCF = \frac{Pb\ metal\ in\ the\ root}{Pb\ metal\ in\ sediment} \quad (1)$$

According to Baker (1981 in Mariwy et al, 2021) 3 categories of BCF values are as follows:

1. Accumulator: BCF > 1
2. Indicator: BCF = 1
3. Excluder : BCF < 1

Calculating the Translocation Factor (TF) Value

The purpose of calculating the TF value is to determine the amount of Pb metal transfer from roots to leaves of mangrove plants (translocation process). TF value can be calculated by the formula:

$$TF = \frac{Pb\ metal\ in\ the\ leaves}{Pb\ metal\ in\ the\ root} \quad (2)$$

Two categories of TF values according to Yoon, et al 2006 cited by (Mariwy, et al 2021), namely:

TF> 1: Phytoextraction mechanism

TF < 1: Phytostabilization mechanism

RESULTS AND DISCUSSION

Description of Sampling Process

Research samples of sediment, roots, and mangrove leaves were taken at 08.00-09.48 a.m. and at that time the seawater conditions receded, this condition facilitated the sampling process. Samples of sediment, roots, and leaves of mangrove (*Rhizopora apiculata*), were taken at 3 different stations. The sampling location can be seen in Figure 1.

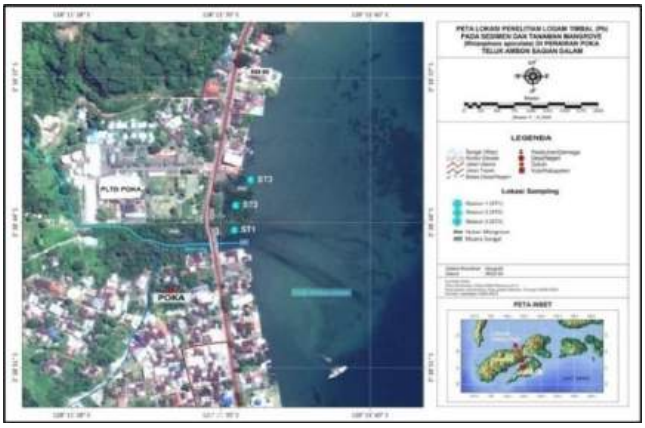

Figure 1. Sampling Location

Seawater Quality Measurements

Seawater quality measurement results are shown in Table 1.

Table 1. Measurement Results of Physical and Chemical Parameters

Parameter	Station 1	Station 2	Station 3
pH	6.5	6.6	6.6
Temperature (°C)	29.5	29.5	30
Salinity (‰)	26	26	25

The data in Table 1 shows that the water temperature range in Poka Village is 29.5-30 °C. Based on the Decree of the Minister of Environment No. 51 of 2004 year, the appropriate temperature standard for waters is 28-30 °C, so it can be concluded that the temperature of the waters of Poka Village in the inner bay of Ambon is still in normal and safe conditions for marine biota (Tuahatu, Tubalawony, & Kalay, 2023). According to Mariwy, Manuhutu, & Frans (2021) the higher the water temperature, the higher the solubility of heavy metals. While the pH in the waters of Poka

Village ranges from 6.5 to 6.6. When compared to the Decree of the Minister of Environment No. 51 of 2004 year concerning seawater quality standards, where the appropriate pH for marine biota life is 7.0-8.5, so pH value of this location is classified as unsafe for marine biotals because a decrease in pH in waters causes greater heavy metal toxicity (Mariwy, Male, & Manuhutu, 2019).

The data in Table 1 also shows that the salinity in the waters of Poka Village ranges from 25-26‰. When compared with the quality standard value of salinity in waters issued by the Indonesian Ministry of Environment No. 51 of 2004 year it ranges from 33-34‰. Then the salinity in the waters of Poka Village is lower than the required quality standards for marine biota. The low salinity in the waters of Poka Village is caused by the flow of the river that empties into the waters so it contributes to the abundant supply of fresh water to reduce salinity. This is in line with the opinion that salinity levels in estuarine areas will decrease due to the abundant supply of fresh water and also by tidal events in these waters (Haidar, Handoyo, & Indrayanti, 2021).

Determination of Sediment Particle Grain Size

The particle size classification of sediments from the 3 research stations measured with a sieve shaker can be seen in Figure 2.

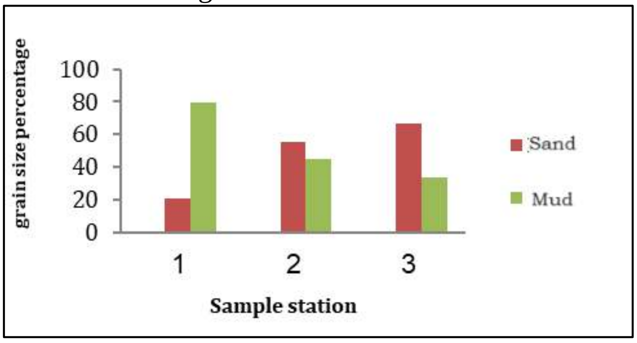

Figure 2. Classification of grain sediment size

The sediment particle size data as shown in Figure 2 shows that the sediment texture is mud and lumpy sand with different percentages of sediment particle size. At station 1, the percentage of mud is greater than sand so it is classified into the mud substrate type. High concentrations of heavy metals in waters are strongly influenced by sediment texture with mud substrate types compared to sand substrates because mud substrates with fine textures have a large surface area with a more stable ion density to bind heavy metals (Male et al., 2017). This data corresponds to the Pb metal content at station 1 which is higher when compared to the Pb metal content at stations 2 and 3. At station 2, the substrate type is classified into the type of passive mud substrate, and sediments at station 3 are dominated by sand substrates, this also follows the Pb metal content at stations 2 and 3 which is lower than station 1.

Destruction of Sediment Samples, Mangrove Roots, and Leaves

Samples of sediment, roots, and leaves of mangrove (*Rhizopora apiculata*) were dried at 105 °C. Furthermore, the samples were wetly deconstructed using concentrated HNO_3 and concentrated HCl (1: 3) often called aquaregia, a mixture of these two acid solutions is widely used to accelerate the deconstruction process, because these two acids are strong oxidizers. The addition of acid has its purpose, in the deconstruction method with the addition of HNO_3 as an oxidizer because HNO_3 is a good metal solvent.

Standard Curve Preparation

The standard curve is the most important part of analyzing the concentration of an element. The preparation of the calibration curve begins with the preparation of a series of lead (Pb) standard solutions with concentrations of 0.2; 0.4; 0.6; 0.8 and 1.0 ppm. Then the absorbance is measured using an Atomic Absorption Spectrometer (SSA). The absorbance measurement results of the standard solution can be seen in Table 3 below.

Table 3. Absorbance of Standard Solution

Concentration (ppm)	Absorbance
0.2	0.0036
0.4	0.0076
0.6	0.0113
0,8	0.0147
1.0	0.0188

The absorbance of the measurement results was then plotted against the concentration of the standard solution to obtain a standard curve. The standard curve in Figure 3 shows that the absorbance of the standard solution increases as the concentration of the standard solution increases, indicating that there is a linear relationship between concentration and absorbance. This fact is evidenced by the correlation coefficient which is close to 1, namely 0.998, and the regression equation obtained in this measurement is y = 0.019x - 0.000 where y is absorbance and x is concentration.

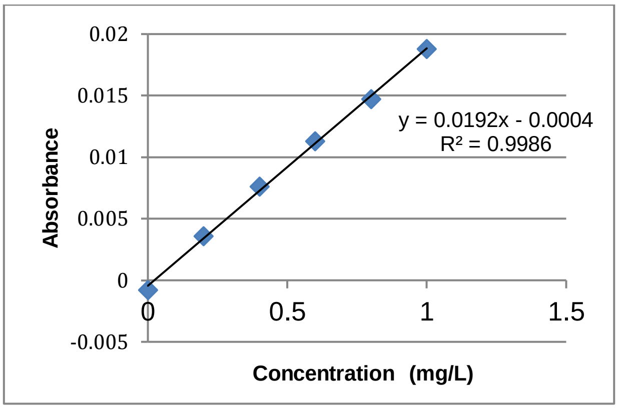

Figure 3. Standard Curve of Pb

Pb Levels in Mangrove Sediment, Root and Leaf Samples

After the Pb metal standard curve is made, the absorbance of the sample solution is measured using Atomic Absorption Spectrophotometry. From the measurement results data on Pb levels at each station. Data on Pb levels in mangrove sediment, root, and leaf samples at station 1 are shown in Table 4 below.

Tabel 4. Pb levels at station 1

Sample	Absorbance	Pb concentration (mg/L)	Pb levels (mg/kg)
Sediment	0.0126	0.676	16.925
Root	0.0000	0.024	0.534
Leaf	0.0032	0.187	4.675

The data in Table 4 shows that Pb levels in sediments at station 1 are quite high. The high Pd levels at this station are related to the sediment grain size as shown in Figure 2 where the sediment grain size at station 1 is dominated by mud substrate. Metals found in mangrove ecosystems are adsorbed on the surface of fine mud substrates, this is because sediments with fine mud substrates have greater absorption of heavy metals when compared to sediments that have larger substrate sizes (Kaewtubtim, Meeinkuirt, Seepom, & Pichtel, 2016). The results of the analysis of Pb levels shown in Table 4 also show that the levels of lead metal in sediments at station 1 are still below the standard set by the National Sediment Quality Survey (US EPA) which is 47.82-161.06 ppm.

While the measurement results of Pb levels in plant root tissues at station 1 showed very low values. This can be caused by two things: first, the translocation process runs smoothly so that the Pb metal absorbed by the root tissue is directly translocated to the top of the leaves and second, the Pb metal has not been absorbed by the plant root tissue.

The data in Table 4 shows that Pb levels in mangrove leaves at station 1 are also high. The high level of Pb in the leaves is the process of accumulation of Pb metal by mangrove plants through the translocation mechanism (Chowdhury et al., 2015). The location of station 1, which is located right in front of the Poka PLTD and land transportation routes, contributes to Pb metal that occurs through the attachment of Pb particles to the surface of mangrove plant leaves and enters the leaf tissue through stomata. The graph of the measurement results of Pb levels in each sample at station 1 is shown in Figure 4 below.

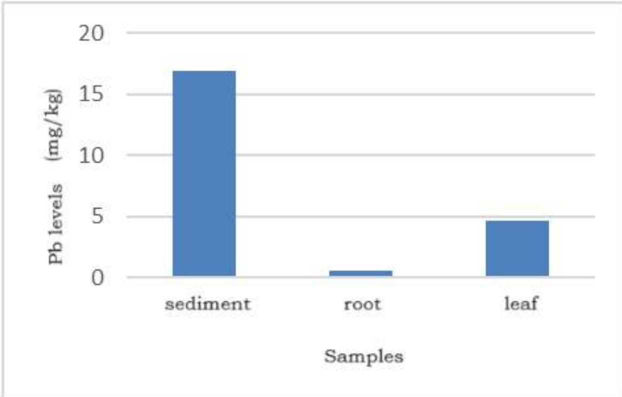

Figure 4. Graph of Pb levels at station 1

Pb Metal Levels in Samples at Station 2

Data on Pb levels in mangrove sediment, root, and leaf samples at station 2 are shown in Table 5 below.

Table 5. Pb levels at station 2

Sample	Absorbance	Pb concentration (mg/L)	Pb levels (mg/kg)
Sediment	0.0113	0.606	15.225
Root	0.0160	0.854	21.353
Leaf	-0.0002	0.012	0.254

The data in Table 5 shows that Pb levels in sediments at station 2 are also quite high. This is related to the size of the sediment grains as shown in Figure 2 where the size of the sediment grains at station 2 is dominated by a mixture of sand and mud which is called sands-mud. This is in accordance with the results of research by Male et al., (2017) that sediments characterized by sands-mud can also absorb heavy metals although their absorption ability is not as good as sediments with mud texture (Male et al., 2017). The results of the analysis of Pb levels shown in Table 5 also show that lead metal levels at station 2 are still below the standards set by the National Sediment Quality Survey (US EPA 2022) which is 47.82-161.06 ppm.

The measurement of Pb levels in the root tissue of mangrove plants at station 2 showed a high value. This happens because in the roots there are endodermis cells that function as filters in the process of absorption of heavy metals (Selanno et al., 2014). The data in Table 5 also shows that Pb levels in mangrove leaves at station 2 are very low. This can be caused by two things, namely: The translocation mechanism has not been running because most of the heavy metal content is still centered on the roots and the location of station 2 which is located some distance from the Poka power plant so that it has an impact on the low content of Pb in leaf samples. The graph of the measurement results of Pb levels in each sample at station 2 is shown in Figure 5 below.

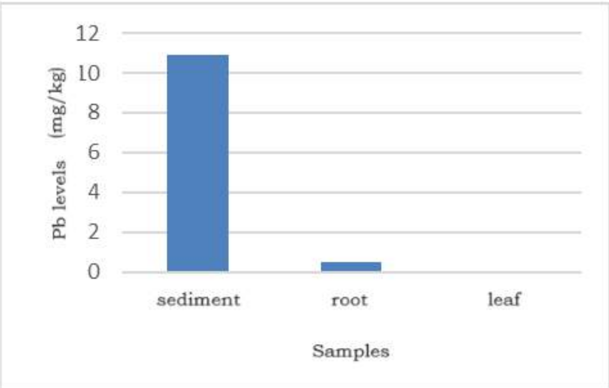

Figure 5. Graph of Pb levels at station 2

Pb Metal Levels in Samples at Station 3

Data on Pb levels in mangrove sediment, root, and leaf samples at station 3 are shown in Table 6 below.

Table 6**.** Pb levels at station 3

Sample	Absorbance	Pb concentration (mg/L)	Pb levels (mg/kg)
Sediment	0.0080	0.435	10.925
Root	0.0000	0.024	0.523
Leaf	0.0004	0.002	0

Data on Pb levels in each sample at station 3 as shown in Table 6 shows that Pb levels in sediments at station 3 are lower than Pb levels at stations 1 and 2. This phenomenon is also caused by the size of sediment grains as shown in Figure 2 where the size of sediment grains at station 3 is dominated by sand substrates. Compared to mud substrate, which has a large uptake of heavy metals, the texture of sand substrate type is very difficult to bind metals due to the coarser sediment size so other organic materials and metals are difficult to settle (Male et al., 2017).

While the measurement of Pb levels in the root tissue and mangrove leaves at station 3 showed a very low value. This is due to two things: the mechanism of absorption of Pb metal by plant root tissue has not occurred so that the translocation process does not run and the location of station 3 which is located some distance from the Poka power plant and the highway so that it has an impact on the low content of Pb metal in leaf samples. The graph of the measurement results of Pb levels in each sample at station 3 is shown in Figure 6 below.

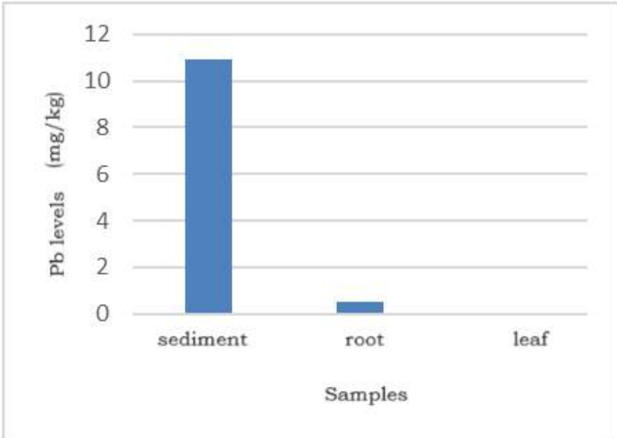

Figure 6. Graph of Pb levels at station 3

Value of Bioconcentration Factor (BCF) and Translocation (TF) of Pb in Mangrove (*Rhizopora apiculate*)

Calculation of the value of biocentrentation factor (BCF) and translocation factor (TF) is done to determine how much the ability of mangrove in absorbing Pb metal. BCF and TF values can be seen in Table 7.

Table 7**.** CF and TF value

No.	Station	BCF	TF
1.	1	0.03	7.78
2.	2	1.40	0.03
3.	3	0.56	0.09

Data in Table. 7 shows that the BCF value greater than 1 is at Station 2. this indicates that mangrove plants function as accumulators because the BCF value> 1 (1.40). According to Baker (1981) that there are 3 categories of BCF values, namely accumulators, indicators, and excluders. While the TF value greater than 1 means that mangrove plants are able to translocate Pb metal from the roots to other plant organs (Baker, 1981).

There are 2 categories of TF values, namely phytoextraction and phytostabilization (Tangahu et al., 2011). In this study, the TF value of more than 1 is at station 1, this phenomenon is due to the concentration of Pb in mangrove leaf samples at station 1 is higher when compared to stations 2 and 3. This indicates that Rhizopora apiculata mangrove serves as phytoextraction in translocating Pb metal because the TF value > 1 (7.78). Phytoextraction is a process in which pollutants are absorbed by roots and translocated into other plant tissues. An important stage of this process is that heavy metals that have been absorbed by the roots are then translocated to the crown to be re-treated or removed when the plant leaves fall or are harvested (Paz-Alberto & Sigua, 2013).

CONCLUSION

Based on the results of the research that has been done, it can be concluded that: Pb metal levels in sediments at research stations 1, 2 and 3 in the waters of Poka Village, inner Bay of Ambon are still below the standards set by the National Sediment Quality Survey (US EPA) in 2022 where sediments with fine mud substrates have a greater absorption of heavy metals when compared to sediments that have a larger substrate size.

While the calculation of BCF value shows that Rhizopora apiculata mangrove plants function as accumulators because BCF value > 1 (1.40) and TF value more than 1 indicates that mangrove plants function as phytoextraction because TF value > 1 (7.78). The results of this study prove that Rhizopora apiculata mangrove plants can be used as a phytoremeditoring agent to reduce lead metal (Pb) content in waters.

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