EFFECTIVITY OF BIOACCUMULATION AND TRANSLOCATION OF HEAVY METALS (CD, ZN, AND PB) IN AVICENNIA MARINA GROWING AT WONOREJO MANGROVE ECOSYSTEM, EAST SURABAYA

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ABSTRACT

Avicennia marina is a species of mangrove that has been studied extensively for its capability to uptake and accumulate heavy metals. Since *Avicennia marina* was found in the Wonorejo Mangrove ecosystem, which confirmed receiving anthropogenic inputs from Surabaya as the second largest city in Indonesia, the effectivity of bioaccumulation and translocation metals Cd, Zn, and Pb was studied. The sediments, roots, stems and leaves of *Avicennia marina* were collected in three sites at in the Wonorejo Mangrove Ecosystem, East Surabaya. The measurement of Cd, Zn and Pb was conducted by ICP-MS. To assess the effectiveness of accumulation and translocation capability, Bioconcentration Factor (BCF) and Translocation Factor (TF) were calculated. The results showed that the distribution of the heavy metals Cd and Pb in the organs of the mangrove *Avicennia marina* exhibited distinct patterns. Although both of them are considered non-essential elements, Cd tends to distribute evenly until leaf parts, while Pb tends to retain in the roots. In the case of Zn, as an essential element, the uptake was more intense compared to non-essential elements and mobilised until the leaf parts. Interestingly, once all metals reach the stem, they might transfer to the leaf as shown by TF values more than 1. Therefore, *Avicennia marina* has an effective defense mechanism in the presence of metals by regulating the accumulation and translocation of metals into detoxifying organs.

Keywords: *Avicennia marina*, heavy metals, bioaccumulation, translocation, detoxification mechanisms

1. INTRODUCTION

The city of Surabaya is one of the cities that is experiencing very rapid industrial growth. Based on data from the Surabaya Government, in 2012 there were 7,721 industrial units. This data experienced an increase to 16,395 units in 2020, consisting of several industries such as the chemical, agriculture, forest products, metals, machinery, and electronics industries (Wijaya & Sanjaya, 2021). The growth of sector units has positive impacts, such as increasing employment opportunities and economic growth. However, on the other hand, industrial development can also have a negative impact on the environment, especially in coastal areas (Soheti *et al*., 2020).

One of the coastal areas in Surabaya that may have high levels of accumulated pollution is Wonorejo, located in Rungkut District. This area receives water discharge from three large rivers: the Kali Jagir Wonokromo, Wonorejo, and Gunung Anyar watersheds. The pollutants that are the main focus in big cities are heavy metals. Heavy metals in waters that accumulate excessively will have a negative impact on life. The entry of lead (Pb) and cadmium (Cd) into waters is caused by the impact of human activities (Sari *et al*., 2017). Based on research conducted in June 2019, the heavy metal content found in the sediments of the Wonorejo area was as follows: the heavy metal content Pb reached 0.304 ppm, the heavy metal Cd reached the highest level of 0.047 ppm, and the metal Cd reached the highest level of 0.024 ppm (Wijaya & Sanjaya, 2021). The heavy metal content of Zn in sediments in the Wonorejo area was also detected at 0.201 ppm (Amin *et al*., 2019). Increased accumulation of Pb in plants can cause problems in leaf tissue, such as chlorosis (change in leaf color to pale yellow), necrosis (tissue death), and the appearance of black spots (Testi *et al*., 2019).

Excessive accumulation of the heavy metal Cd can result in stunted plant growth and accelerate plant death (Dharmaningtyas *et al*., 2021).

The mangrove ecosystem dominates coastal areas and supports the sustainability of the ecosystem at the Wonorejo River estuary. One type of mangrove that can survive in muddy areas with high salt levels and is often found in various mangrove ecosystems is *Avicennia marina*. This type of mangrove is included in the pioneer mangrove category, which can adapt to these conditions (Utami *et al*., 2018). *Avicennia marina* has a vital role as an absorber of heavy metals through its roots. Based on this role, the mangrove type *Avicennia sp.* can be used as a phytoremediation agent (Amin *et al*., 2019).

Research on the absorption of heavy metals in the mangrove *Avicennia sp.* has been carried out in various waters in Indonesia. One of the studies in Muara Kali Wonorejo conducted by Rachmawati in 2018 showed that the Pb metal content in the roots of *Avicennia sp.* reached 4.71 ppm, while the Pb content in the leaves reached 7.09 ppm (Rachmawati *et al*., 2018). The same research was also carried out by Hamzah and Setiawan (2010) in Muara Angke Jakarta, which showed that the roots and leaves of *Avicennia sp.* have a Pb metal content of 57.52 – 59.16 ppm and 61.93 – 64.32 ppm. Research in Muara Angke also shows that the average BCF and TF for Pb is > 1.

Previous studies have shown that the *Avicennia marina* mangrove has the ability to absorb heavy metals in aquatic areas. The process of absorption of heavy metals by *Avicennia marina* mainly occurs through its complex root system (Rachmawati *et al*., 2018). Based on the background above, it is crucial to conduct further research to examine the content of heavy metals Pb, Cd, and Zn in the sediment, roots, stems, and leaves of the *Avicennia marina* mangrove. This research was also carried out to determine the bioaccumulation and translocation capacity of heavy metals in the *Avicennia marina* mangrove, especially the heavy metals Pb, Cd and Zn in the Wonorejo Mangrove Area. The BCF was calculated to evaluate metal accumulation in the root, while TF to indicate metal movement to upper part of the plant (Chowdhury *et al*., 2017).

2. METHOD

This research was carried out in June – August 2023 in Wonorejo, East Surabaya (Figure 1). Preparation for heavy metal content analysis will be carried out at the Fisheries and Marine Resources Exploration Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University. Analysis of heavy metal content in sediment, root, stem and leaves samples was carried out at the Fisheries and Marine Resources Exploration Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University.

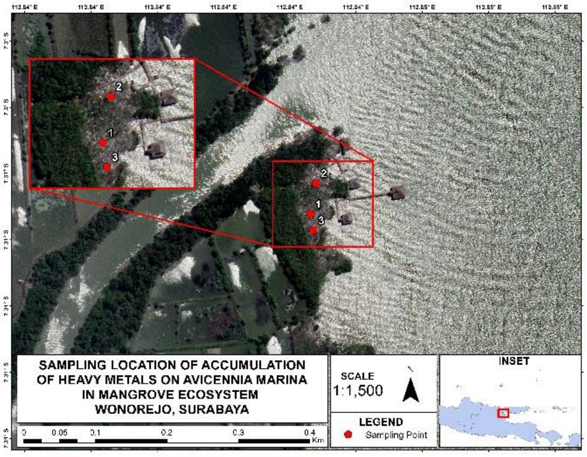

Figure 1. Sampling Location in Mangrove Ecosystem Wonorejo, Surabaya

Sampling was carried out on three individual *Avicennia marina* trees located at coordinates S 07°18.853 E 112°50.670. Trees were selected according to the criteria of a height of >1.5 m and a diameter of more than 20 cm (Sholiqin *et al*., 2022). For each individual mangrove tree sampled, the surrounding surface sediment, roots, stems and leaves were taken to be used as samples. The initial step in taking sediment samples is to insert the prepared spatula into the sediment to a depth of 30 cm to obtain samples in certain layers of sediment depth (Supriyantini and Soenardjo, 2016). It is assumed that at this depth the sample can represent pollutants vertically. The sample taken was approximately 1 kg with 3 repetitions around the mangrove. The sediment that has been taken is then put into plastic and labeled (Jupriyati *et al*., 2013).

The part of the mangrove root taken was in the form of a pencil root (the part that was submerged in sludge because there was a possibility of accumulation of heavy metals in this part more than in other parts). The mangrove roots taken were

±1cm in diameter and 20cm - 30cm long. Sampling was carried out directly with a composition of 8 composite samples taken from various sides of each tree's root and carried out on three different trees of the same species. The sample is put in plastic and labeled, after which the sample is put in a cool box (Fadhilah *et al*., 2018).

Stem sampling is carried out by cutting a sample of the bark on the main stem of a plant in the tree category which has a diameter of >30 cm. Composite skin samples were taken of 500 grams from various sides of each tree and taken from 3 trees of the same species in one area. Sampling from each tree was carried out using a stainless steel knife to avoid aggravating the wound on the tree trunk so that it was hoped that the plant could recover (Supriatna *et al.,* 2019).

The way to take mangrove leaf samples is to take dark green leaves. This is because it is assumed that dark green leaves are old leaves and have accumulated heavy metals over a certain period of time. The size of the leaves taken is about 4-8 cm long and 4 cm wide. 5-7 leaves were collected in a composite manner from various sides of each tree and carried out on three different trees of the same species. Next, the sample is put in plastic that has been labeled and put in a cool box for research in the laboratory (Amin *et al*., 2019).

Pre-treatment of samples was conducted by chemical destruction using 65 % of HNO3 in 1200C during 2 hours for root, stem and leaves mangrove (Sari et al., 2023). The sediment was pre-treated by using HCl and H2O2 solution. The samples was measured for Cd, Zn and Pb Concentration using ICP-AES refer to APHA 3120B method. Furthermore, the data was assessed for Bioconcentration Factor (BCF) and Translocation Factor (TF). The statical analysis was conducted by using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Concentration of metals in the surface sediment

The heavy metals being investigated in the sediment and the organ of mangrove *A. marina* in the present study comprises Cd and Pb as representative of non-essential elements and Zn as a essential elements. As predicted, concentrations of Zn in the surface sediment of *A. marina* found as the highest number compared to Cd and Pb concentrations. The average of Zn, Cd and Pb Concentration in the surface sediment sourronding *A. marina* plant was approximately 51, 0.92, and 7.84 µg/g, repectively (Table 1). This numbers is similar to the order series concentrations of same metals (Zn>Pb>Cd) assessed in sediment shore of Erongo Region, Western Namibia (Ameh Sylvanus *et al*., 2016).

Table 1. Concentration of metals in the surface sediments

	Concentration (µg/g)		
Metals	Cd	Zn	Pb
Present study	0.924	50.833	7.839
EPA Guidelines for Sediment (µg/g)	-	<90	<40
Status*	Not polluted	Not polluted	Not polluted

*(Ameh Sylvanus et al., 2016)

Table 2. The status of metals concentration in the mangrove sediments citing from previous studies

Location	Concentration	Reference
Wonorejo, East Surabaya	0.924 µg/g (Cd) 7.839 µg/g (Pb) 50.883 µg/g (Zn)	This present study
Lamong, East Java	0.11 mg/L(Pb) 34.84 mg/L (Cu)	Awaliyah et al., 2018
Coast of Red Sea	1.23 mg/kg (Cd) 45.2 mg/kg (Pb) 47.2 mg/kg (Zn)	Usman et al., 2013
Zhanjiang, China	20.95 mg/kg (Cu) 76.60 mg/kg (Zn) 71.53 mg/kg (Cr)	Shi et al., 2019
Klang estuary, Malaysia	9.38 mg/kg (Cu) 37.39 mg/kg (Pb) 22.46 mg/kg (Zn)	ELTurk et al., 2019
Muara Porong, Surabaya	0.3723 mg/kg (Cu) 0.584 mg/kg (Zn) 0.068 mg/kg (Pb)	S. H.J. Sari et al., 2018

Based on Table 2, the concentrations of Cd in Wonorejo Mangrove Ecosystem, East Surabaya was lower than in the Lamong, East Java. Pb concentration in present study was higher compared to those in Lamong, and Muara Porong, Surabaya. However, Pb concentration is this study was found around 6 and 5 fold lower than in those in the Coast of Red Sea and in Klang estuary, Malaysia, respectively. Furthermore, Concentration of Zn in present study was similar to Zn concentration in Coast of Red Sea, while the Zn concentration in Zhanjiang, China found higher than those in Wonorejo, East Java. The variation of metals concentration in the surface sediment of Mangrove ecosystems is believed due to different rate of anthropogenic inputs. Sediments in Mangrove ecosystems are considered rich organic matter with anaerobic conditions. These characteristics lead to significant pollutant sinks since they own substantial capacity to accumulate pollutants including heavy metals (Ram et al., 2018). The spatial variation of heavy metals are dependent on intensity of anthropogenic activities (Shi *et al*., 2019).

3.2. The concentration of metals in the mangrove's organ

The Concentration of metals (Cd, Pb and Zn) was determined in roots, stems and leaves of Avicennia marine growing at Wonorejo Mangrove Ecosystem (Figure 2). The average Cd content found in the roots of *Avicennia marina* growing at Wonorejo Mangrove Ecosystem was 0.34 ± 0.02 µg/g, while in the stems, Cd content in the stem and leaves were 0.27 ± 0.008 µg/g and 0.32 ± 0.04 µg/g, respectively. Statistically, there were no significant differences in Cd concentrations in *Avicennia marina*'s roots, stems, and leaves. Moreover, the Concentration of Pb accumulated in the roots of *Avicennia marina* was 0.30 ± 0.10 µg/g. However, Pb concentrations in stems (0.12 ± 0.006 µg/g) and leaves (0.14 ± 0.006 µg/g) showed almost the same values. Generally, Pb concentrations in mangrove organs tend to accumulate in the roots, inhibiting Pb translocation to the stems and leaves. The content of non-essential heavy metals such as Cd and Pb in mangrove species *Avicennia marina* under controlled conditions is higher in the roots than in the leaves (MacFarlane *et al*., 2003).

The distribution of heavy metals Cd and Pb in the organs of the mangrove *Avicennia marina* exhibited distinct patterns. The amount of Cd and Pb uptake by the roots revealed almost a similar value. However, for the heavy metal Cd, the average Concentration in the roots, stems, and leaves showed no different values, indicating that Cd was distributed equally through the mangrove's organ. Cd was transferred upward and reached the leaves parts. An excretion process in the leaves will occur actively through glands in the canopy or passively, marked by the fall of old leaves (Riyanti *et al*., 2019).

In contrast to Cd, the accumulation of the heavy metal Pb in stems and leaves is much lower than in its roots, suggesting that *Avicennia marina* is a species of mangrove that is very strict in absorbing Pb or even does not absorb it at all. Based on physiological mechanisms, mangroves actively restrict the absorption of heavy metals when the Concentration or toxicity of metals in the sediment is high. Still, absorption will occur in limited amounts and accumulate in the roots. Apart from that, there are endodermal cells in the roots that act as filters in the process of absorbing heavy metals. From the roots, heavy metals will be translocated to other tissues such as stems and leaves and undergo complexation with other substances such as phytochelatin (Sari *et al*., 2023).

The Zn content in the *Avicennia marina* species revealed that Zn accumulates highest in the roots which reached 31.21 ± 8.43 µg/g. The stems and leaves of the *Avicennia marina* mangrove contained Zn of 25.32 ± 3.24 µg/g and 21.35 ± 4.15 µg/g, respectively. The high Zn content in the roots is caused by extracellular localization efforts in mangrove roots (Subiandono *et al*., 2013). The accumulation of Zn obtained in the *Avicennia marina* species is evenly distributed in the roots, stems, and leaves of mangroves because Zn is an essential metal needed by a plant so that, in general, more Zn will accumulate in the roots and then be transferred to other parts such as stems and leaves (Hamzah & Pancawati, 2013).

Generally, based on the distribution of Cd, Pn, and Zn di the organs of Avicennia marina (Figure 2), it can be concluded that those metals accumulated more in the root than in other organs. However, statistically, there was no significant difference in Cd and Zn concentration in the root, stem, and leaf. Yet, these results confirmed the role of roots to restrict mobility of metals to the upper part of the plant.

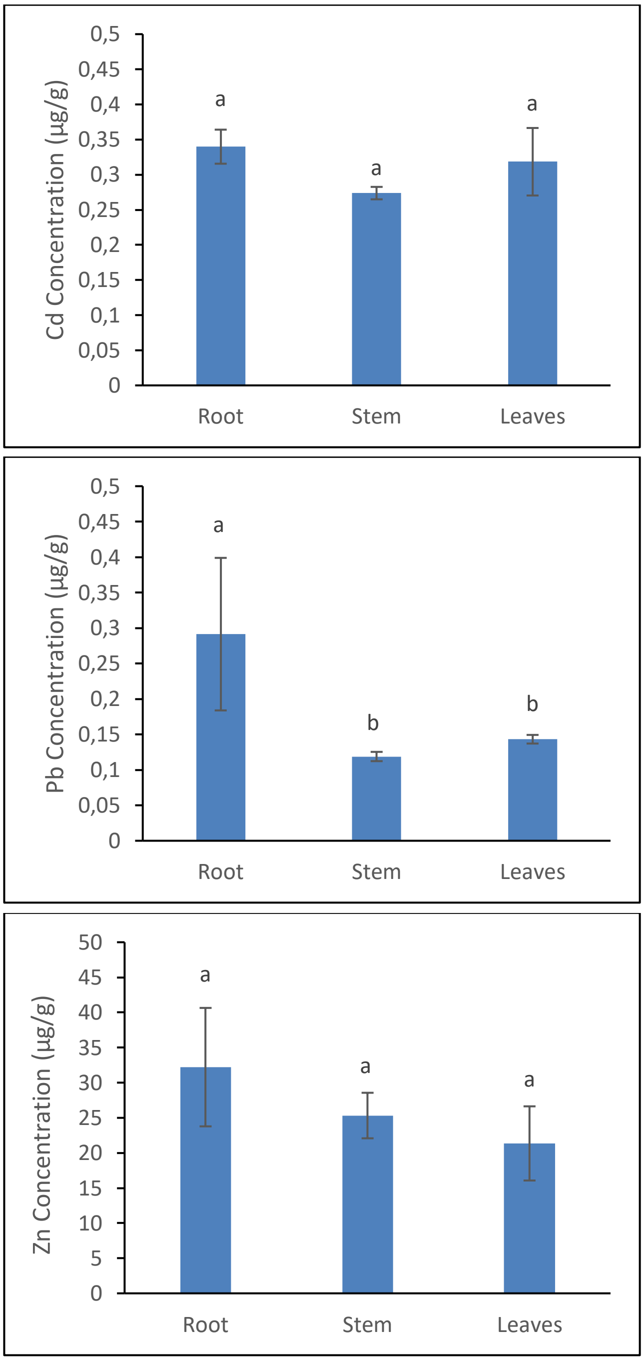

Figure 2. The average concentrations (µg/g) of Cd, Pb and Zn in roots, stems and leaves of *Avicennia marina*. The different letters showed significant differences in metal concentrations among mangrove organs.

A barrier of transport was performed by root epidermis. The endodermal casparian strips located in root tissue have a role in restricting the movement of molecules due to impermeable waxy substance, and also the availability of lignin and suberin in wetland plants, including *Avicennia marina* (Weis & Weis, 2004) .

3.3. Bioaccumulation and translocation of metals in mangrove *Avicennia marina*

Bioaccumulation factor (BCF) can be defined as a ratio between metal content in roots part of plants to metal level in their surrounding medium (soil, sediment or water). This is an essential parameter to demonstrate absorption of metals by mangrove plants from their sediments. However, translocation factor (TF) highlights mobilization of metals via different part of plants. The TF is calculated to comprehend the process of metal absorption and accumulation from roots to aerial tissues. BCF and TF are necessary to measure a plant's potential for metal accumulation. If BCF dan TF are more than 1 indicating the plants' phytoremediation capacity (Bhatti et al., 2018). Therefore, the effectiveness of bioaccumulation and translocation conducted by *Avicennia marina* was evaluated by bioaccumulation factor (BCF) and translocation factor (TF) and presented in Table 3.

Table 3. The BCF and TF of Cd, Pb and Zn of mangrove *Avicennia marina*

	BCFsediment-root	TFroot-stem	TFstem-leaf
Cd	0.37	0.81	1.16
Pb	0.04	0.41	1.21
Zn	0.63	0.79	1.04

The BCF and TF of Cd, Pb and Zn were presented in Table 3. It can be seen that all BCF values were less than. However, BCF of non-essential metal such as Cd and Pb was lower than BCF of Zn as an essential metal. Particularly, BCF of Pb was remarkably low, indicating *Avicennia marina* inhibit to uptake this metal. Pb confirmed as the second-most toxic metals, behind As and it has no function in living organisms, including mangrove (Zulfiqar *et al*., 2019). The BCF of Zn in *Avicennia marina* was the highest compared to Cd and Pb. As essential element for biological system, Zn playing an important roles not only in growth but also in development and defense mechanisms (Cabot *et al*., 2019). Therefore, the BCF values of metals suggest a preference for mangroves to uptake metals from surrounding sediments.

Translocation capability was evaluated from TF root to stem, and TF stems to leaves, as seen in Table 3. The TF root to stem of Cd, Pb and Zn was 0.81, 0.41 and 0.79, respectively. All the TF root to stem was less than 1 indicating most of metals contents was retained more in the roots. In contrast, TF stem to leaves for all metals showed values more than 1. Interestingly, although Cd is high toxic elements for plants, their TF stem to leaves were more than 1 indicating these metals once they accumulate in stems, they mobilized intensively to the leaves. Cd has high mobility element in plants system, therefore Cd once being uptake by root plant, Cd easily to transferred to aerial parts of plants (Saraswat & Rai, 2011). In the case of Pb, the mobility of Pb from stem to leaves determines the detoxification mechanisms occurred in leaves parts. The plants including mangrove undergo particular defense mechanisms on presence of amount of metals that have been reached their leaf's cells. In leaves, metals can be sequestrate into less metabolic parts such as trichome (Gao *et al.*, 2021). The mature leaves then subsequently dropping into the ground (Upadhyay, 2014). Moreover, the zinc content in leaves related to Zn content in the sediments sugesting the high Concentration of Zn in mangrove sediment, the more Zn translocates in leaves parts (Weis & Weis, 2004).

It can be concluded that mangroves *Avicennia marina* tend to limit Cd and Pb uptake, while the uptake of Zn confirmed more intensively although all the BCF values of these metals less than 1. Regardless of type of metals as essential and non-essential metal, more portion of these metals were compartmentalization at root parts and the less remaining metals tend to translocate into leaves parts as shown in TF values more than 1 in all metals.

4. CONCLUSION

The effectivity of bioaccumulation and translocation of metals Cd, Pb and Zn was evaluated in mangrove *Avicennia marina* found in Wonorejo Mangrove ecosystem. The status of these metals content in sediments of mangrove confirmed as not polluted. However, the portion of metals Cd, Pb and Zn still found in root, stem and leaves of *Avicennia marina* indicating this plant capable to uptake metals from its sediment. Although most of Cd portion was accumulated in roots, but due to its highly mobility characteristics, Cd was distributed evenly in all organs of *Avicennia marina*. In contrast, Pb was preferred to be accumulated in roots, but once it reached stem, Pb mostly relocated into leaves parts indicating detoxification mechanisms might be occurred in this parts. In the case of Zn, mangroves tend to uptake more according to Zn availability in sediments surrounding of *Avicennia marina* and transferred into stems and leaves almost equally. Interestingly, Cd and Zn distribution in root, stem and leaves seemed to have similar pattern while there was different with Pb distribution in mangrove organs. However, translocation factor of all metals from stem to leaves showed general pattern indicating remaining of metals might accumulated and detoxified in leaves parts.

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