# Anatomical Adaptation of Grey Mangrove (*Avicennia marina*) Leaf in the Pond and Coast Located in Mangunharjo, Semarang, Central Java

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**Abstract.** Mangrove is a brackish plant that can live in habitats with extreme environmental conditions. For instance, the grey mangrove (*Avicennia marina*) is a mangrove species that can adapt anatomically in habitats with a wide range of salinity, such as ponds and coasts. This study examines the forms of anatomical adaptation of *A. marina* by comparing the anatomical structure of *A. marina* leaves that live in two different habitats, namely pond, and coast in the Mangunharjo area, Semarang, Central Java. The third leaf from the tip of the tree branches was used as samples and taken from three different trees in each habitat. Cross-section prepared microscope slides of the leaves were made using the embedding method and observed using a photomicrograph. The quantitative data were analyzed using the T-test at a 90% confidence level, while the qualitative data were analyzed descriptively. The results showed that different environmental conditions do not cause differences in the anatomy of *A. marina* leaves, but there are some histological modifications as a form of adaptation. These modifications are differences in the thickness of the cuticle, adaxial epidermis, parenchymal palisade tissue, spongy parenchyma, and the number of xylem cells in one vascular bundle. This research might initiate more advanced studies regarding the correlation between mangroves' anatomical structure and anatomical structure of *A. marina* leaves that live in two habitats with different environmental conditions.

Key words: anatomical adaptation, leaf, mangrove, osmotic, salinity

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## **INTRODUCTION**

Mangroves are trees that live in communities between the sea and the land. Thus, mangroves are affected by tides. Mangroves can be found in 105 countries worldwide, and 52% of the mangroves in the world are only found in 10 countries. In Indonesia, the area of mangrove forests is 23,324 km<sup>2</sup> and contains 26% to 29% of all the mangroves globally (Hamilton and Casey, 2016). Physically, mangrove plants provide a physical barrier for tidal dynamics and a canopy for the ecosystem beneath them (Krauss et al., 2014). The habitats of mangroves are generally found at the border between river estuaries and seawater, which allows them to protect the land from large ocean waves. Several mangrove species are found in the saline zone, while the others are in the low-salinity freshwater zone (Hastuti & Budihastuti, 2017).

Mangroves can live in habitats with various environmental conditions, such as ponds and coasts. Ponds and coasts have different environmental conditions, especially salinity levels. The salinity in coastal areas is relatively higher than in river or pond areas. The nutrient content found in ponds and coasts also shows differences. Generally, mangrove cultivation activities are carried out in the pond area.

Mangroves are halophyte plants resistant to high salt and can adapt to extreme habitat conditions, for example, on coasts. Most mangroves also have various tolerance capacities to environment quality changes (Hastuti & Budihastuti, 2016). The changes in environmental quality are usually driven by seasonal variables (Agunbiade et al., 2010). Mangroves can adapt to high salinity habitats because they have a salt filtering system and a complex root system, which has a function to overcome immersion in high salinity water as well as large waves in mangrove habitats. Mangroves can also adapt to low oxygen conditions in waterlogged mud (Flowers and Colmer, 2015).

Temperature and light intensity affect the rate of transpiration. The higher the temperature and

light intensity, the higher the transpiration rate. As a result, the salinity level will also increase. temperature Salinity and high can be environmental stressors for plants. Mangroves can adapt morphologically, physiologically, and anatomically to habitat conditions with environmental stresses such as high salinity in coastal areas and polluted environments. Tihurua et al. (2020) stated that one form of adaptation observed is anatomical structure. The anatomical structure of plants will directly or indirectly play a role in physiological and anatomical adaptations.

Anatomical adaptations between one plant and another are different based on the environmental conditions in their habitat. Kumar et al. (2011) stated that the Avicennia marina is a mangrove species that can tolerate and have high resistance to environmental stresses to adapt to a wide range of salinity, from high to low salinity. A. marina generally lives in the front zone of the mangrove, zonation, which has high temperature and salinity close to the coast or on the shoreline. However, A. marina can also live in brackish water areas such as ponds with lower salinity and temperature. Robert et al. (2011) stated that the leaves of A. marina have several unique anatomical parts, namely salt glands and water-storage tissue (hypodermis), which play an important role in adaptation mechanisms to extreme habitats.

Research about the influence of environmental conditions in various habitats on the anatomical structure of several mangrove species has been frequently carried out. Still, research on the influence of environmental conditions in Mangunharjo pond and coast on the anatomical structure of *A. marina* has never been carried out. This study aims to analyze the anatomical structure of *A. marina* leaves that live in Mangunharjo pond and coast as a form of anatomical adaptation in both habitats.

# **METHODS**

The tools used were Water Quality Checker Multi-Parameter Horiba Series U50, and lux meter for measuring environmental conditions and rotary microtome and photomicrograph to make and observe the cross-section prepared microscope slides of *A. marina* leaves. The materials used for making the prepared microscope slides of *A. marina* leaves were alcohol, xylol, paraffin, glycerin, safranin, and Canada balsam.

# The Measurement of Environmental Parameters

The environmental parameters measured were air temperature, light intensity, water temperature, water pH, dissolved oxygen, and salinity in the Mangunharjo pond and coast. Environmental parameters were measured in the morning. The leaf samples used were the third leaves from the tip of the tree branches, which were taken from 3 different trees in each habitat. The leaves were cut, sectioned in the middle through the leaf bone with a 1 x 1 cm size, and fixed in 70% alcohol until the cross-section prepared microscope slides.

# The Making of Cross-Section Prepared

Microscope Slides of A. marina leaves

Cross-section prepared microscope slides of A. *marina* leaves were made by using the paraffin embedding method, following the method used by Ruzin (2000). The stages of making prepared microscope slides are as follows: (1) Dehydration with 70%, 80%, 90%, and 100% alcohol for 30 minutes, respectively; (2) Dealcoholization or clearing using graded xylol, namely absolute alcohol:xylol = 3:1; absolute alcohol:xylol = 1:1; absolute alcohol: xylol = 1:3; pure xylol, for 30 minutes each; (3) Infiltration with graded paraffin in an oven with a temperature of 60°C, namely: xylol:paraffin = 1:9 for 24 hours followed by pure paraffin for 1 hour. This infiltration stage is carried out in an oven; (4) Embedding in pure paraffin for 24 hours; (5) Trimming and sticking to an objectglass. The paraffin pieces that have been attached to the holder were then sliced or trimmed using a rotary microtome with a thickness of 10 µm and attached to an object-glass; (6) Deparaffinization with xylol; (7) Staining with safranin in 70% alcohol; (8) Dealcoholization with graded xylol; (9) Mounting or closing with Canada balsam. The anatomical structure and measurement of the thickness of the cuticle, adaxial epidermis, hypodermis tissue, palisade parenchyma tissue, spongy parenchyma tissue, and the number and diameter of xylem cells in one vascular bundle were carried out using a photomicrograph.

The qualitative data were analyzed by describing the components and the location of the cell or tissue components from the cross-section of the leaf. The quantitative data were analyzed using the T-test at a 90% confidence level.

# **RESULTS AND DISCUSSION**

The measurement and analysis of environmental data in Mangunharjo pond and coast are presented in Table 1 below.

unfavorable environmental conditions. Reef and Lovelock (2015) stated that several adaptation mechanisms of mangroves related to salinity mainly contribute to reducing the vapor pressure

Parameter	LocationAverage		Level of Significance [Sig. (2- <i>tailed</i> )]	Difference [Sig. (2- <i>tailed</i> )<0.10]
Salinity	Coast	31,07 ppt	0.061	Significant
	Pond	30,40 ppt		
Light Intensity	Coast	96.957 Lux	0.076	Significant
	Pond	78.215 Lux		
Air Temperature	Coast	35.3 − 36.3°C	0.161	Non-significant
	Pond	33.2 – 35.8°C		
Water Temperature	Coast	33.47 – 34.21 ℃	0.004	Significant
	Pond	30.91 − 32.02°C		
Dissolved Oxygen (DO)	Coast	4.94 ppm	0.997	Non-significant
	Pond	4.95 ppm		
Water pH	Coast	7.60	0.006	Significant
	Pond	7.21	0.000	Significant

Table 1. Environmental parameters of Mangunharjo pond and coast

Environmental parameters that show differences between Mangunharjo pond and coast are salinity, light intensity, water temperature, and water pH. However, air temperature and dissolved oxygen between Mangunharjo pond and coast do not differ significantly. The coast has a higher light intensity and salinity level than the pond. Light intensity and salinity are the main between differentiators the environmental conditions of the pond and the coast of Mangunharjo, Semarang. Freshwater and seawater have different properties and mixtures, affecting environmental parameters (Hastuti et al., 2020). The level of light intensity, salinity, and temperature on the coast, which is higher than that of the pond, is the cause of the differences in the anatomy of A. marina leaves between those living on the coast and those living in the pond. This is a form of adaptation to be able to survive in their habitat. Light intensity, salinity, and high temperature cause the water potential in the growing medium to below. As a result, it is difficult for the roots to absorb water while the transpiration rate is high. These conditions cause the plants to suffer from physiological drought and have the potential to interfere with the physiological mechanisms of A. marina.

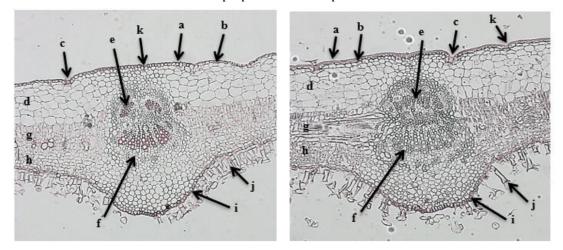
*A. marina* has several adaptation mechanisms to prevent physiological disturbances due to

deficit (VPD) in their leaves to the surrounding air. Examples of adaptation include small leaf size, trichomes on leaves, excretion of salt crystals from the leaf surface, and cryptophore stomata. The form of adaptation to reduce leaf temperature is the arrangement of leaf location and succulence. VPD is the most important marker of water loss through transpiration in leaves. Species that are more tolerant to salt, such as *A. marina*, are more sensitive to changes in VPD. Consequently, adaptations will be carried out by *A. marina* plants.

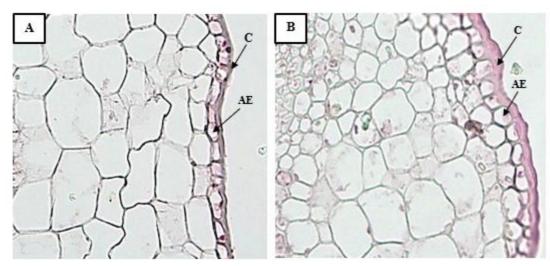
The observation of cross-section prepared microscope slides of *A. marina* leaves showed that from the top surface of the leaf downwards, consist of the cuticle, adaxial epidermis with salt glands as epidermal derivatives, hypodermis tissue, mesophyll tissue, and the lowest part is the abaxial epidermis with epidermal derivatives in the form of non-glandular trichomes and stomata. Mesophyll tissue consists of palisade parenchyma tissue at the top and spongy parenchyma tissue at the bottom. Several vascular bundles between the mesophyll tissue consist of xylem and phloem (Figure 1). The available collateral vascular bundles are found on the leaf bone, where the xylem and phloem are located side by side where

there is cambium between the two of them. The type of stomata is diacytic stomata that have kidney-shaped guard cells and are surrounded by two subsidiary cells perpendicular to the long axis epidermis of *A. marina* leaves on the coast are thicker than those in the pond (Figure 2). The diameter of the epidermal cells on the leaves of *A. marina* that live on the coast is larger than those in

The anatomical structures of *A. marina* leaf in Mangunharjo pond and coast are shown in cross-section prepared microscope slides below



**Figure 2.** Cross-section of *A. marina* leaf that lives in Mangunharjo pond (A) and coast (B): cuticle (a), the adaxial epidermis (b), salt gland (c), hypodermis tissue (d), xylem (e), floem (f), palisade parenchyma tissue (g), spongy parenchyma tissue (h), the abaxial epidermis (i), non-glandular trichome (j), and stomata (k). (x100)



**Figure 1.** Cross-section of *A. marina* leaf that lives in Mangunharjo pond (A) and coast (B): cuticle (C), the adaxial epidermis (AE). (x1000)

of the guard cells. Based on the location of the stomata, the type of stomata is cryptophore stomata. The guard cells are deep below the epidermal cells' surface or are often referred to as sunken stomata (Samsuri, 2013; Tambaru et al., 2019).

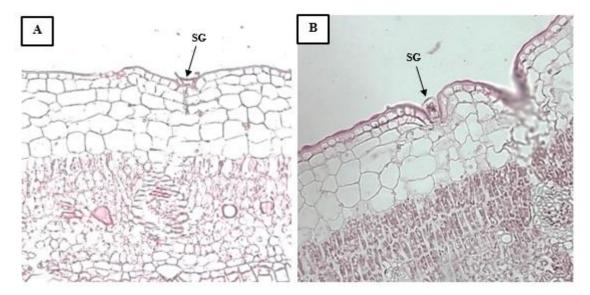
The histological differences between *A. marina* leave that life on the coast and those in the pond of Mangunharjo, Semarang are the cuticle, adaxial epidermis, and salt glands. The cuticle and adaxial

the pond. This is due to differences in environmental conditions between the pond and the coast. The cuticle is a thick and dense wax layer located outside the adaxial epidermis and serves as a barrier to limit transpiration (Surya and Hari, 2017). The cuticle consists of a waxy layer called the epicuticle, an inhibitor for excessive water evaporation. The presence of cuticles on the leaf surface is an adaptive feature of mangroves. The adaxial epidermis is the outer part of the upper leaf, which functions to protect the leaf tissues underneath it from environmental stresses. According to Crang et al. (2018), the epidermis is the outermost cell layer in plants that provides physical protection against water loss and mechanical damage. Salt glands remove excess salt in mangroves. Therefore, salt glands have an important role in the mechanisms of anatomical adaptation of

A. marina lives in high-salinity habitats, namely excreting excessive salt through the leaf's surface (Tan et al., 2013). The salt glands of A. marina leaves in the pond are rectangular shaped with two long and curved left and right ends. In contrast, the salt glands of A. marina leave that life on the coast are round and located on the surface of the adaxial epidermis that has undergone modifications (Figure 3). following the opinion of Naidoo et al. (2011), who stated that in

A. marina, the thickness of the cuticle increases as the salinity level increases. The cuticle plays a role in minimizing water loss in leaves or inhibiting excessive transpiration in coastal habitats with high temperatures. The cuticle will experience thickening due to high salinity, temperature, and light intensity. Epidermal cells will synthesize the cuticle in response to environmental stresses. This is a mechanism for the thickening of the cuticles. Anatomically, plants with thicker cuticle layers will have relatively more resistant leaves than plants with thin cuticle layers (Yeats and Rose, 2013).

#### The thickness of the adaxial epidermis



**Figure 3.** Salt gland (SG) on the cross-section of *A. marina* leaf that lives in Mangunharjo pond (A) and coast (B). (x400)

The quantitative data of *A. marina* leaves that live in Mangunharjo pond and coast are presented in Table 2 below.

The differences in the anatomy of *A. marina* leaves that life on the coast and those that live in the pond are the thickness of the cuticle, the thickness of the adaxial epidermis, the number of xylem cells in one vascular bundle, the thickness of the palisade parenchyma tissue, and the thickness of the spongy parenchyma tissue, while other parameters such as the thickness of the hypodermis tissue and the diameter of xylem cells show no significant difference.

#### The thickness of the cuticle

The cuticle of *A. marina* leaves that live on the coast is thicker than those in the pond. This is

The adaxial epidermis of *A. marina* leaves that live on the coast is thicker than those in the pond. This is due to the cells that make up the adaxial epidermis of A. marina leaves that live on the coast, larger than those of A. marina leaves that live in the pond. One factor that influences the thickness of epidermal cells is light intensity. This follows the opinion of Sundari and Atmaja (2011). They claimed that the size of epidermal cells increases in length and the decrease in light intensity received by plants. Pantilu et al. (2012) also reported that plants could adapt to high light intensity by thickening the epidermis. Apart from light intensity, the composition of the air in the plant's habitat also affects the thickening of epidermal cells.

# The number of xylem cells in one vascular bundle

The number of xylem cells in one vascular bundle of *A. marina* leaves that live on the coast is

pressure. Cavitation occurs in the xylem of vascular plants when the tension of water within the xylem exceeds atmospheric pressure. The sap vaporizes locally so that either the vessel elements

The quantitative data of *A. marina* leaves that live in Mangunharjo pond and coast are presented in Table 2 below

			Level of	Difference
Parameter	Location	Average	Significance [Sig.	[Sig. (2-
			(2-tailed)]	<i>tailed</i> )<0.10]
Thickness of Cuticle	Coast	9.400 µm	0.039	Significant
	Pond	5.333 µm		
The thickness of Adaxial Epidermis	Coast	12.767µm	0.098	Significant
	Pond	9.867 µm		
The thickness of Hypodermis Tissue	Coast	182.367 µm	0.140	Non-significant
	Pond	169.467 μm		
Number of Xylem Cell in One	Coast	7,67	0.047	Significant
Vascular Bundle	Pond	6,33		
Diameter of Xylem Cell	Coast Pond	12.950 μm 14.333 μm	0.296	Non-significant
The thickness of Palisade Parenchyma Tissue	Coast Pond	144.867 μm 100.800 μm	0.026	Significant
The thickness of Spongy Parenchyma Tissue	Coast Pond	78.267 μm 101.833 μm	0.000	Significant

<b>Table 2.</b> Quantitative data of <i>A. marina</i> leaves that live in Mangunharjo pond and coast	Table 2. (	Ouantitative data	of A. marina leaves	that live in Mangunhar	io pond and coast
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higher than in the pond. This is related to the water potential in the habitat of A. marina. If the water potential is high, the anatomical adaptation mechanism increases the number of xylem cells and shrinks the xylem cells. Changes that occur in the xylem facilitate transporting water and nutrients by plants. The tolerance of mangroves towards salinity is associated with less efficient water transport and more conservation of water usage. Okello et al. (2017) stated that water availability for plants is influenced by several factors, namely drought, soil texture and structure, and salinity. Sedimentation in mangrove forests can also result in low water availability, triggered by the increase of difficulty in water absorption by the roots. Reducing water uptake due to sedimentation can also be equated with physiological drought.

Most plants experience stresses on the xylem close to the xylem cavitation threshold. Cavitation is defined as the phenomenon of vapor-bubble formation of a flowing liquid in a region where the pressure of the liquid falls below its vapor or tracheid are filled with water vapor. Xylem cavitation diminishes a plant's capacity to transport water from the soil to the leaves (Vilagrosa et al., 2012).

#### The thickness of palisade parenchyma tissue

The palisade parenchyma tissue of A. marina leaves that live on the coast is thicker than those in the pond. Palisade parenchyma tissue has an important role in photosynthesis because it contains a lot of chloroplasts. Therefore, high light intensity and temperature result in the thickening of the palisade parenchyma tissue to increase the efficiency of photosynthesis in leaves. This is a form of anatomical adaptation of *A. marina* to high light intensity, salinity, and temperature. This follows the opinion of Corrêa et al. (2017), who reported that palisade parenchyma is a plant tissue closely related to the photosynthetic process. Shade reduces the thickness of the leaf mesophyll, particularly the palisade parenchyma tissue. Leaf thickening occurs when plants are exposed to high light intensity in a habitat.

## The thickness of spongy parenchyma tissue

The spongy parenchyma tissue of A. marina leaves that live on the coast is thinner than those in the pond. Spongy parenchyma tissue plays an important role in photosynthesis, namely as a temporary storage place for photosynthetic products in the form of gas (O<sub>2</sub>) and a storage place for CO<sub>2</sub> before being used in the photosynthesis process. Therefore, light intensity and high temperature result in the thinning of the spongy parenchyma tissue, which aims to increase the efficiency of photosynthesis in leaves. This is a form of anatomical adaptation of A. marina to high light intensity, salinity, and temperature. Starzecki (2015) stated that spongy parenchyma tissue is part of the mesophyll that forms a layer adjacent to palisade cells. In addition, spongy parenchyma tissue is the main assimilation tissue. Well-developed spongy parenchyma is characterized by higher photosynthetic activity, better supply of carbon dioxide, more efficient migration of assimilation, better water supply, and optimal light conditions.

This research initiated a research method to achieve a deeper understanding of the anatomical structure of mangroves since it is still rarely analyzed in Indonesia. The results of this study are expected to provide information to researchers about the anatomical structure of A. marina leaves that live in two habitats with different environmental conditions (e.g., different levels of salinity and light intensity). Moreover, this research was done to trigger society to conduct mangrove conservations after knowing the importance of these plants in our environment. Thus, researchers should continue exploring mangroves to educate people about these plants and their role in preserving water quality and reducing environmental pollution.

# CONCLUSION

The anatomical structure of *A. marina* leaves that live in Mangunharjo pond and coast are similar. Still, there are some histological modifications to adapt to different environmental conditions. These modifications are thicker cuticle, adaxial epidermis, parenchymal palisade tissue, and a higher number of xylem cells in one vascular bundle in *A. marina* leaves that live on the coast than those that live in the pond. Meanwhile, the spongy parenchyma tissue of *A. marina* leaves in the pond is thicker than those on the coast.

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