Investigated The Presence of Oil in the Xylem Vessels of Mangrove Stems from Oil-contaminated Beaches

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Abstract. Mangroves are highly susceptible to pollution caused by oil spills in marine waters, leading to potential mortality. Research on the anatomical impact of oil contamination on mangrove trunks, particularly in the coastal areas around Jakarta, remains limited. This study aimed to assess the presence of oil in the xylem vessels of mangrove stems in oil-contaminated locations and quantify the number of oil-filled xylem vessels to estimate their physiological functionality. Tissue analysis was conducted using Adobe Photoshop and ImageJ software to calculate the number, area, and percentage of oil-filled xylem vessels. The results revealed a significant difference in xylem vessel density between the oil-exposed treatment and the control group. Among the three observed mangrove species, *Rhizophora mucronata* exhibited the lowest percentage of xylem vessels presumed to be filled with oil, in contrast to *Avicennia marina* and *Avicennia alba*.

Keywords: Anatomy, mangrove, oil spill, stem, xylem vessel.

1 Introduction

Indonesia has a vast mangrove forest area covering 3.31 million hectares. This figure is equivalent to 21% of the world's mangrove ecosystems. The mangrove areas are distributed along the coastal regions of 257 cities and districts in Indonesia [1]. According to Rahmanto, the mangrove ecosystem in Indonesia is currently under pressure, with a high degradation rate of up to 52,000 hectares per year [2]. Out of the 3.31 million hectares of mangrove forests in Indonesia, 637,000 hectares or 19% are in a critical condition, as reported by the Ministry of Environment and Forestry (KLHK) in 2017 [3]. The deteriorating condition of mangroves in Indonesia is attributed to various factors, including land conversion, illegal logging, high rates of erosion, and waste pollution. One of the hazardous pollutants affecting the marine environment is crude oil. Oil pollution in coastal waters can lead to a reduction in mangrove

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areas [4]. Marine waters can become contaminated with crude oil due to spills during offshore drilling and oil refinery operations, as well as transportation, loading and unloading activities in ports, and tanker ship waste [5].

Mangroves are woody plants that grow in the vicinity of estuaries, tidal areas, or coastal regions [6]. The habitat of mangroves falls within the ecotone between marine and terrestrial communities, which results in mangroves having specialized anatomical and physiological adaptations for survival. Mangroves serve various physical, biological, and economic functions. Physically, they act as a natural buffer against coastal erosion, abrasion, and the intrusion of seawater. Biologically, mangroves provide a habitat and a source of food for marine life. Economically, mangroves can be used for tourism, as a source of timber, and for the production of medicinal resources [7]. Despite the numerous benefits offered by mangroves, their quantity has not kept pace with the increasing demand. Currently, it is known that the remaining mangroves are concentrated around estuaries with mangrove forest thickness ranging from 10 to 100 meters, predominantly consisting of *Avicennia, Rhizophora*, and *Sonneratia* species [8].

Mangroves are ecosystems with a high ecological sensitivity to the environment [9], especially when it comes to oil spills [10]. The oil well leakage incident in the Pertamina Hulu Energi Offshore Block in Northern West Java in 2019 resulted in the contamination of hundreds of thousands of mangrove trees along the coast of Jakarta and its surroundings. Mangrove ecosystems affected by oil pollution undergo severe damage and require an extended recovery period [11]. Oil contamination has negative impacts on mangroves as it can cause both physical and physiological harm [12]. Furthermore, oil contamination can become a source of pollutants for the surrounding water bodies. Oil-contaminated water is absorbed by the roots and travels to the leaves through the xylem vessels in the stem. The movement of water from the roots to the leaves is influenced by several factors, including root pressure, capillarity in the stem's xylem vessels, and transpiration pull. The capillary force in the stem's xylem vessels is disrupted when oil contamination enters the xylem vessels, obstructing the water transport system in mangrove trees [13]. However, it is noted that water movement in oil-contaminated mangrove stem xylem can still occur, presumably due to the various adaptive mechanisms that mangroves employ. These adaptive mechanisms vary greatly among different mangrove species, depending on their genetic factors and the specific environmental conditions in which they grow [14].

Based on the provided background, this research aims to estimate the presence of oil within the xylem vessel cells of mangrove trees growing in oil-contaminated areas along the northern coast of Jakarta and its surroundings. It also intends to quantify the number of xylem vessels filled with oil to infer their physiological function. The study is expected to provide scientific insights into mangrove species' resilience to oil stress, thereby offering data that can be used to identify suitable mangrove species for potentially oil-polluted locations.

2 Experimental Method

2.1 Time and Location

The research was conducted from November 2020 to February 2021. Samples were collected from the coastal areas in Kepulauan Seribu, Bekasi, and Karawang. The preparation of microscopic specimens and sample analysis took place in the Plant Physiology and Genetics

Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), IPB University.

2.2 Equipment and Materials

The main equipment used in the research included a sliding microtome (G.S.L.I WSL Bismensdorft, Switzerland), an Olympus CX23 compound microscope, an Indomicro microscope, a stereo microscope, a Dino-Lite microscope, ImageJ 1.8.0 software, Adobe Photoshop CC 2019, and IBM SPSS version 26. The research materials used consisted of samples of mangrove tree stems (*Rhizophora mucronata, Avicennia alba, Avicennia marina*), 70% and 96% alcohol, clear nail polish, 20% glycerin, safranin:alcian blue stain (0.65%:0.35% w/v), and distilled water.

2.3 Samples Preparation

The stem samples were randomly collected and grouped into control and exposed categories based on the presence or absence of oil contamination along the coastal areas. The samples were preserved in 70% alcohol. The sample codes used in the research are presented in **Table 1**.

Table 1. Mangrove Stem Samples Used in the Research

Mangrove Type	Source	Sample Code	Treatment	Region
Avicennia alba	Mekarpohaci village	C59	C	Karawang
Avicennia alba	Mekarpohaci village	E46	OE	Karawang
Avicennia marina	Pantai bahagia village	C14	C	Bekasi
Avicennia marina	Pantai bahagia village	E18	OE	Bekasi
Avicennia marina	Pantai bahagia village	E32	OE	Bekasi
Rhizophora mucronata	Pantai bahagia village	C11	C	Bekasi
Rhizophora mucronata	Untung jawa island	C70	C	Kep. Seribu
Rhizophora mucronata	Pusaka jaya utama village	E38	OE	Karawang
Rhizophora mucronata	Sedari village	E40	OE	Karawang
Rhizophora mucronata	Lancang island	E63	OE	Kep. Seribu
Rhizophora mucronata	Untung jawa island	E68	OE	Kep. Seribu

Note: C: control, OE: oil-exposed

The samples stored in 70% alcohol were transferred to tubes filled with distilled water and soaked for 3 days to remove the alcohol from the stems. Soaking in the Gifford solution was carried out for mangrove stems with a tough texture that is difficult to section. The samples were cross-sectioned (transversely) using a sliding microtome with a thickness of 5-10 µm. Next, they were stained with safranin:alcian blue stain (0.65%:0.35%) for 3 minutes. The samples were then rinsed with distilled water until free from remaining stains, immersed in 96% alcohol for one minute, and placed on glass slides. The subsequent process involved mounting

using 20% glycerin, and the specimens were covered with glass coverslips and sealed with clear nail polish. Microscopic specimens were then photographed using an Indomicro camera (see **Figure 1**).

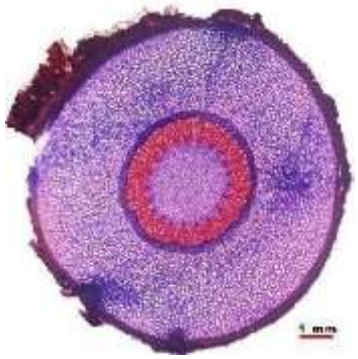

Fig. 1. Example of cross-sectional microscopic specimen photographs

2.4 Tissue Analysis

The specimen images were analyzed using Adobe Photoshop and ImageJ software. Adobe Photoshop was used to isolate the xylem vessel area from the bark, cortex, and pith of the stem using the polygon lasso tools. Oil and other materials covering the xylem vessels were removed using the eraser tool and quick selection tool. After obtaining the stem cross-section image with empty xylem lumens, contrast adjustment (contrast value: -80) and brightness adjustment (brightness value: -80) were performed. The image sharpness of the cross-section was adjusted using the unsharp mask with values of 125 for intensity and 20 for radius to convert the image to black and white for analysis using ImageJ.

ImageJ software was used to obtain numerical data from the xylem vessels. The first step before analysis was calibrating the scale according to the image magnification in ImageJ. Afterward, the black and white cross-section images of the xylem vessels were processed to measure the diameter and area of the xylem vessels using the wand tool. The images were processed by adjusting the threshold and background color to detect the xylem vessels. Particle analysis was then performed, resulting in an outline image that contained the number and area of each numbered xylem vessel cross-section (see **Figure 2**). All measurement results were stored in Excel format (xlsx and csv documents).

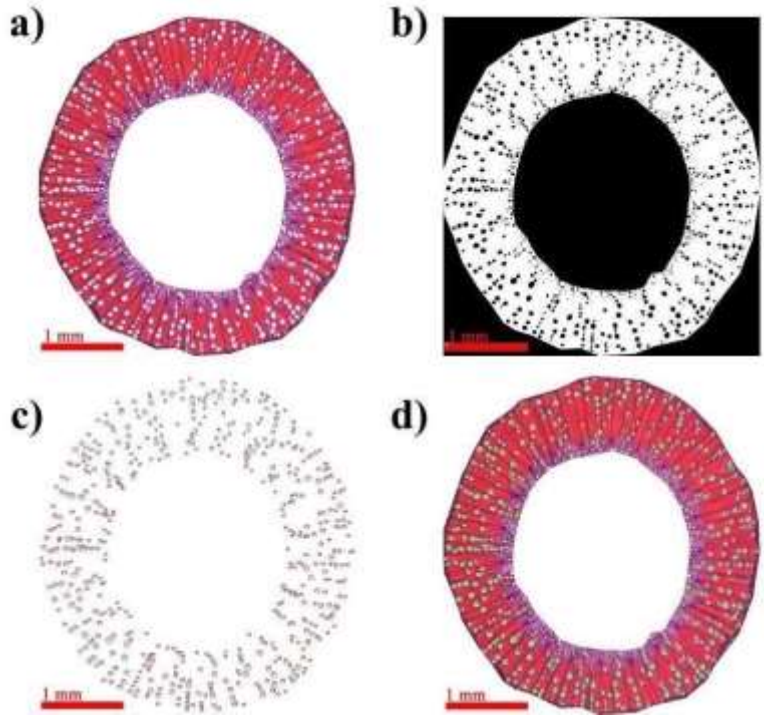

Fig. 2. The process of analyzing cross-sectional images of stem xylem vessels using Adobe Photoshop and ImageJ software: (a) the xylem area that has been separated from other tissue components, (b) the image with threshold and background color adjustments, (c) the outline image, (d) the merging of images (a) and (c).

2.5 Data Analysis

The data on the number of xylem vessels and the cross-sectional area of the stem were used to calculate xylem vessel density for each species and treatment. The percentage of oil-filled xylem vessels was calculated using the following formula:

$$Percentage\ of\ oil-filled\ sylem\ vessels = \frac{Number\ of\ oil\text{-}filled\ xylem\ vessels}{Total\ number\ of\ xylem\ vessels} \times 100\% \quad (1)$$

The data on the number of oil-filled xylem vessels in *R. mucronata* were analyzed using a Ttest to determine the difference between the control and oil-exposed treatments. Statistical analysis was performed using IBM SPSS version 26. However, for samples of *A. alba* and *A. marina*, no statistical tests were conducted because the field data did not meet the required criteria for statistical analysis. Therefore, the analysis for these two species was carried out descriptively.

3 Discussion

3.1 Anatomy of Mangrove Stem

The arrangement of mangrove stem tissues consists of the epidermis, cortex, phloem, xylem, and pith (see Figure 3). The cross-section of *A. marina*'s stem displays a layered cortex structure with air-filled parenchyma (see Figure 4). The epidermal structure in *A. marina* is starting to diminish and is being replaced by a periderm layer that forms the bark. Periderm is a secondary protective tissue that grows from the cortex layer beneath the epidermis and is formed by cork cambium. Periderm forms a structure rich in lignin or suberin, which serves to protect the stem [15]. *Avicennia* and *Rhizophora* belong to the category of mangroves with diffuse-porous xylem [16]. A characteristic of these pores is their even distribution of xylem vessels and relatively uniform vessel sizes in each growth ring [17].

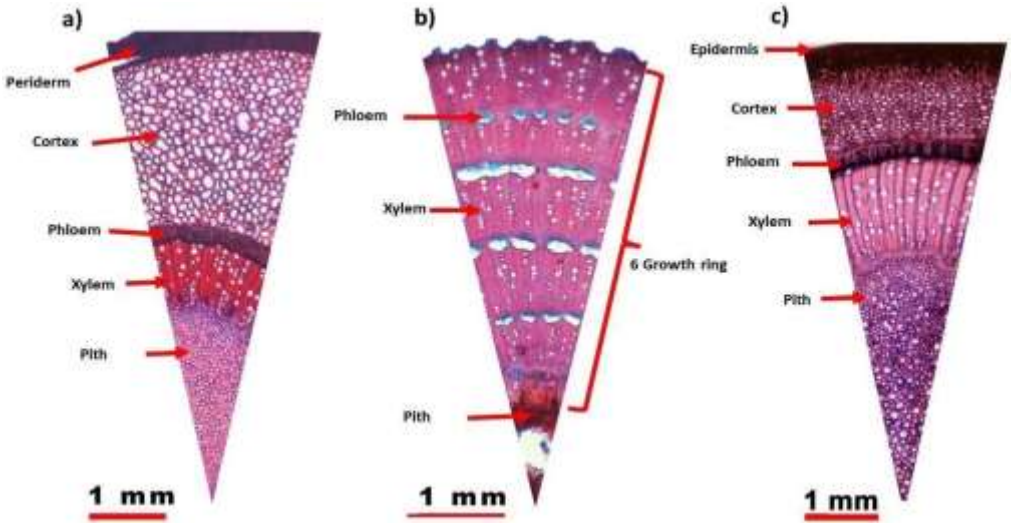

Fig.3. Cross-section of *A. marina* (a), *A. alba* (b), and *R. mucronata* (c) mangrove stems.

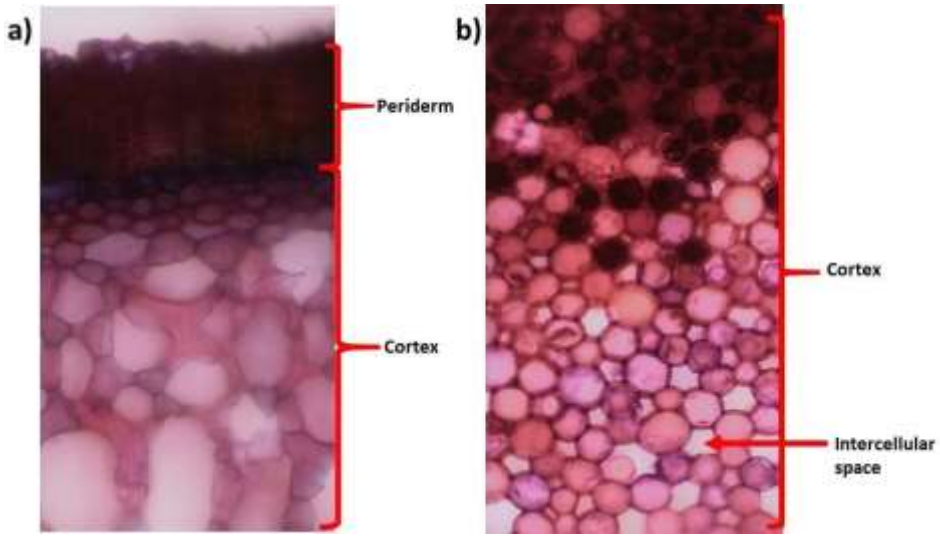

Fig.4. Cortex structure of the stem magnified 40x10: (a) *Avicennia marina*, (b) *Rhizophora mucronata*.

The cross-section of *A. alba* stem reveals that this species has undergone secondary growth with the presence of annual growth rings in the stem tissue. Annual growth rings are concentric layers of secondary xylem influenced by seasonal variations in stem growth. Abundant water early in the growing season results in larger vessels with thinner walls (earlywood). As the growing season progresses and water becomes scarcer, smaller vessels with thicker walls (latewood) are formed [18]. Annual growth rings can be identified by the darker latewood layers and the lighter earlywood layers [19]. In this study, there is a different annual growth ring structure than usual, where each annual ring consists of both xylem and phloem (**Figure 3**). *Avicennia* exhibits a phloem structure located within the secondary xylem (included phloem) [20]. Included phloem is one type of secondary anomalous growth. In this case, a complex secondary phloem structure composed of sieve elements, companion cells, meristematic tissues, and fibers is formed by the inward growth of cambium [21].

The anatomy of the *R. mucronata* stem features a denser cortex, unlike the cortex in *A. marina*, which has many air cavities (see **Figure 4**). This is believed to be related to the mangrove's habitat. *Rhizophora* typically grows in areas that are only inundated with water during high tides [22]. *Avicennia*, on the other hand, is a mangrove type that grows in areas closest to the sea, where its habitat is consistently submerged in water [23]. Mangroves inundated with water experience low oxygen stress (hypoxia) [24]. In response to waterlogging, mangroves form air cavities (aerenchyma) in their roots and stems [25]. Aerenchyma serves as an internal air system to facilitate oxygen diffusion [26].

3.2 Xylem Vessel Density

The cross-sectional area of the stem and the number of xylem vessels were used to calculate xylem vessel density between species (**Table 2**). Xylem vessel density was calculated based on the number of xylem vessels within a unit area of the stem [27]. The observations revealed that the xylem vessel density in oil-exposed *R. mucronata* was significantly higher ($p<0.05$)

compared to the control. Similar results were also found in samples of *A. alba* and *A. marina*. Oil-exposed *A. alba* exhibited a higher xylem vessel density (45.48 mm-2) compared to the control (31.02 mm-2). The xylem vessel density in oil-exposed and control *A. marina* were 11.63 mm-2 and 10.49 mm-2, respectively.

Table 2. Cross-sectional area and the number of xylem vessels in the stems of *R. mucronata*, *A. alba*, and *A. marina* under control and oil-exposed conditions.

Species	Treatment	Stem Cross-Sectional Area (mm2)	Number of Xylem Vessels	Xylem Vessel Density (mm-2)
A. alba	C	17.76	551	31.02
	OE	50.95	2317	45.48
A. *marina*	C	48.54	509	10.49
	OE	687.57	7996	45.48
R. *mucronata*	C	245.28	420	1.71a
	OE	288.22	1628	5.84b

Note: C: control, OE: oil-exposed, values followed by different letters in the same species column indicate statistically significant differences in the T-test (α=5%). Statistical analysis was not conducted for *A. alba* and *A. marina* samples due to the lack of replicates.

The high xylem vessel density in the oil-exposed conditions for each species is believed to be an adaptation to the conditions faced by the observed plants. Xylem vessel characteristics can be used as an indicator to predict the plant's response to stress. This is supported by the findings of Robert *et al.*, which showed an increase in xylem vessel density in mangroves experiencing embolism due to salinity stress [28].

The stem sections of the mangrove plants showed cells filled with a yellow-brown material suspected to be oil contamination filling the xylem vessels (**Figure 4**). The microtome blade surface used to section the mangrove stems also showed black residues with a smooth, oily texture. According to Lehman *et al.*, oil that enters mangrove plant tissues can appear slightly yellow to brown or black [29]. The color of the oil may change due to the presence of tannin compounds from the mangrove that dissolve in the oil [30]. Oil exposure can be absorbed into the tissues through sediments, roots, stems, epidermis, and pneumatophores [31]. Oil exposure can be absorbed by mangroves because the oil within sediments is naturally dispersed by microbes associated with mangrove roots [32]. Microbes can be a source of surfactants that aid in oil degradation and dissolution in water [33, 34]. Oil absorbed into xylem vessels can block the water transport pathways. The closure of xylem vessels by oil can lead to the risk of cavitation in plants [35].

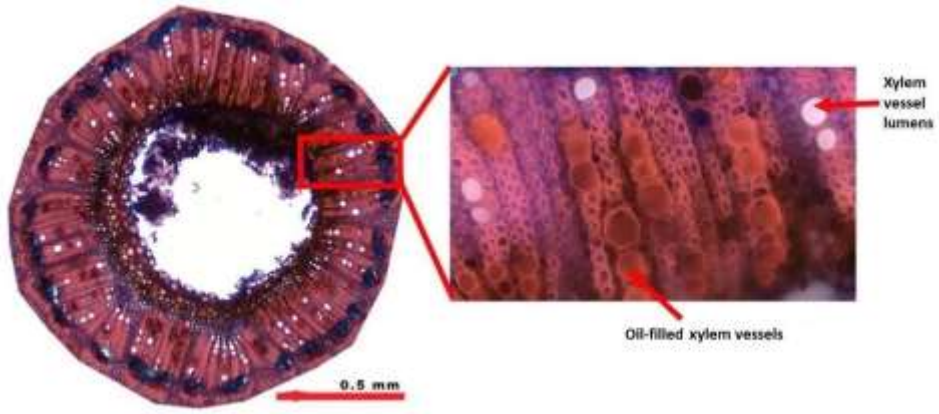

Fig.5. Presence of oil in the xylem vessels. Cross-sectional view of the stem of *A. alba*.

The stress induced by oil exposure results in low water potential in the stem, creating high tension or negative pressure in the xylem vessels [36]. This tension leads to mangroves experiencing cavitation [37, 38]. The cavitation risk faced by mangroves involves the formation of embolisms. Embolism occurs when xylem vessels are blocked by substances or materials that impede water movement in the xylem [39]. Embolism significantly affects the functionality of the hydraulic system in plants [40]. Oil trapped in xylem vessels forces mangroves to protect their water transport system from cavitation and hydraulic conductivity [41, 42]. Xylem hydraulic conductivity is the ability of xylem vessels to facilitate the movement of water from the roots to the canopy [43]. High xylem vessel density is one of the adaptive mechanisms employed to avoid a reduction in hydraulic conductivity after mangroves experience embolism.

Some mangrove species like *R. mucronata* can cope with embolism by increasing their xylem vessel density [44]. Similar occurrences were observed in the mangrove species Laguncularia racemosa in Mexico [45]. The results of this study show that xylem vessel density increased in the stems of *R. mucronata*, *A. alba*, and *A. marina* exposed to oil compared to the control. High xylem vessel density not only enhances hydraulic efficiency but also helps maintain the water transport system's proper functionality [46]. The increased vessel density elevates the water transport pathways, allowing mangroves to continue the water movement process even if some xylem vessels are blocked by oil [47].

3.3 Percentage of Xylem Vessels Filled with Oil

Based on the presence of yellowish material suspected to be oil exposure, this study found no oil covering the xylem vessels in the control and exposed *R. mucronata* stems. On the other hand, in the exposed oil treatments of *A. alba* and *A. marina*, the percentage of xylem vessels filled with oil was 41.3% and 1.36%, respectively. The presence of oil in the xylem vessels was determined from the total number of xylem vessels counted in the first annual ring, which is equivalent to 64.77% (*A. alba*) and 2.35% *(A. marina*) of the total area of the xylem lumen (**Table 3**).

Table 3. Percentage of xylem vessels filled with oil

Species	Treatmen t	Number of xylem vessel		Number of xylem vessels filled with oil (%)	Area of Xylem Lumen (mm2)		Area of Xylem Lumen Filled with Oil (%)
		Total	Filled with oil		Total	Filled with oil	
A. alba	C	551	0	0	0.21	0	0
	OE	247	102	41.30	0.08	0.05	64.77
A. *marina*	C	509	0	0	0.82	0	0
	OE	405	6	1.36	0.43	0.01	2.35
R. *mucronata*	C	420	0	0	1	0	0
	OE	1682	0	0	6.87	0	0

Note: C: control, OE: oil-exposed

The sensitivity of mangroves to oil is believed to be related to the same mechanisms when mangroves face salt stress symptoms (salt injury) [48]. Suprayogi and Murray stated that saltexcluders like *R. mucronata* have the ability not to absorb salt and some organic compounds, including hydrocarbons, which enables them to avoid oil stress and minimize biochemical pathway damage [49]. Salt-extruders like *Avicennia* have some ability not to absorb salt and hydrocarbons, making them more vulnerable to the greater absorption of hydrocarbons by the roots compared to *R. mucronata*. Another factor that could explain why no oil was found in the xylem vessels of *R. mucronata* is that this genus has more efficient capabilities to accelerate the degradation of certain hydrocarbon fractions [50]. The results of the study by Hidayati *et al.* stated that *Rhizophora* sp. can reduce total petroleum hydrocarbons (TPH) in the environment and have a higher survival rate compared to Bruguiera sp. and *Avicennia* sp [51].

The presence of oil in the xylem vessels of *Avicennia* is due to the ability of this mangrove genus to absorb organic substances like oil contaminants from the sediment [52]. According to Bernard *et al.*, sediments can contain hydrocarbon pollution after an oil spill, even when there is no evidence of crude oil contamination in the trees or in the surrounding water samples [53]. Sediments contaminated with oil continue to store oil residues. Various types of aromatic hydrocarbons can remain in plant tissues for 5-8 years, or at least 20 years to ensure that oil toxicity has disappeared [54, 55]. Oil can penetrate soft sediments and coat the roots [56], causing plants to lack oxygen, wilt, exhibit irregular growth, and, in the long term, lead to death [57, 58]. Getter *et al.* stated that the main mechanism of oil toxicity is due to oil entering through the roots and being transported to the stem and leaves, disrupting the physiological processes of mangroves [59]. Plant organs covered by oil exposure undergo stress [60, 61]. Oil covering the stem inhibits gas exchange at the lenticels, disrupting transpiration processes in mangroves [62, 63].

4 Conclusion

Yellow to brownish material suspected to be oil was detected covering the xylem vessel lumens in the stems of *A. alba* and *A. marina*. The percentage of xylem vessels suspected to be filled

with oil in *A. alba* was 41.3%, and in *A. marina*, it was 1.36%. The xylem vessel density in the oil-exposed treatment had higher values compared to the control.

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