

Study of Laccase Activity as a Biosensor for Peatland Degradation in Oil Palm Plantations in Pesisir Selatan of West Sumatra

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Abstract - The aim of the research was to study enzyme activity as biosensors for peatland degradation in oil palm plantations. The study was conducted in Pesisir Selatan, West Sumatra, on two peatlands with different thicknesses and location coordinates, namely peat with a thickness of <3 m S: 02°18'45.5", 101°00'37.3" and peat with a thickness >3 m S: 02°20'07.5", E: 101°00'22". The oil palm in these two locations is 11 years old (planting year of 2007). Observations and sampling of peatlands were carried out on the plantation blocks using the transect method. The transect was set perpendicular to the drainage canal. Peat samples were collected outside the roots (non-rhizosphere) of oil palm. Observation sites were at a distance of 5, 15, 25, 50, 75, 100, 150 m from the edge of the drainage canal and at the thickness of the root layer of 0-25 and 25-50 cm. Peat characteristics observed were water table level, laccase activity, water content, pH, total Fe, and Cu. The water table level in one transect ranged from 60-80 cm and was still within tolerable limits. The laccase activity as a peat degradation biosensor in oil palm plantations in Pesisir Selatan peatland was higher in the 0-25cm layer with an average of <0.5 $\mu\text{mol/g}$. The increase in water content decreased the laccase activity along with increasing of the distance from the drainage canal and the thickness of the peat layer. The increase in Fe and Cu resulted from increased levels of ash, particularly in peat with a thickness of <3 m, may suppress laccase activity. Peatland in the oil palm plantation of Pesisir Selatan is still relatively stable despite the decomposition processes characterized by laccase activity as a biosensor for peat degradation.

Keywords: *Laccase activity, moisture content, oil palm, peatland*

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1. Introduction

The conversion of peatland into agricultural land, especially oil palm, is carried out by making drainage canals. Drainage canals are attempts to remove a portion of water from peatlands to create space for the development of plant roots. Drainage activities can accelerate the degradation of peatlands if done excessively (over drainage), which triggers the decomposition of organic matter in peatlands. Peatlands are vulnerable to degradation because the majority of the peat constituent materials are organic materials that are easily decomposed. [13] Explains that water loss due to peat drainage causes the maturation of the peat material physically, so that the peat material shrinks, and the entry of air into the peat layer changes the condition of the peatland to be more aerobic. The process of decomposition and the rate of subsidence (decrease in surface) on peatland are influenced by the regulation of water management (drainage thickness) [6]. The lower the groundwater table, the higher the rate of subsidence in peatland [8].

Peat degradation is characterized by the decomposition of phenylpropan from the lignin component, which dominates tropical peat. One enzyme that marks the decomposition of lignin is laccase activity as a biosensor for the decomposition of tropical peat. Laccase is one of the enzymes produced by degrading lignin microbes such as white rot fungi, with its main carbon source being lignocellulase produced from the breakdown of lignin compounds. These microbes secrete an amount of enzymes that can break down the complex structures of lignin compounds [3]. Laccase is an enzyme that works by oxidizing diverse aromatic and non-aromatic compounds and is used as a hydrogen donor [3]. The breakdown is carried out by several extracellular enzymes that work together to oxidize lignin molecules, such as lignin peroxidase, manganese peroxidase, and Cu-laccase (benzenediol: oxygen oxidoreductase) [6].

Laccase activity has a significantly negative correlation with peat water content and micronutrient content (Fe and Cu). This shows that the increase in water content and total Fe & Cu content of peat can suppress enzyme activity [5], thereby inhibiting the decomposition rate. This, be inhibited which in turn, can improve the stability of peat. The Pesisir Selatan area is dominated by extensive peatland, which covers around 94,893 hectares or approximately 45% of the total peat area in West Sumatra [11]. Pesisir Selatan of West Sumatra is one of the centers of palm oil production in West Sumatra with an area of 70,000 ha. The breakdown of lignin by microorganisms is closely related to peat decomposition, which ultimately results in CO₂ emissions. It is feared that CO₂ emissions from peat will cause damage to peat vulnerability due to agricultural processes. A majority of the peatland has been cleared for oil palm plantations by making drainage canals. It is not impossible that peatlands that have been developed for oil palm may experience continuous damage due to excessive drainage. The presence of high-valence metal cations provides stability to peat because they can form organo-metal complexes which can keep peat from decomposing. Based on the background above, this study aimed to investigate laccase activity as a biosensor for peatland degradation in oil palm plantations.

2. Method

Time and Place

The study was conducted by collecting peat samples in oil palm plantations of Pesisir Selatan as the representative of the largest tropical peat in West Sumatra. Observations were performed on two peat thicknesses, namely thick peat at the coordinate point of S: 02°20'07.5", E: 101°00'22" and thin peat at the coordinate point of S: 02°18'45.5", 101°00'37.3". Oil palm at these two locations was 11 years old (planting year of 2007). The location of oil palm plantations with peat thickness close to large river flows, while peat with >3 meters is located some distance from the river body. The study was conducted from April 2018 to September 2018.

Research method

Observation and sampling of peat were carried out on plantation blocks using the transect method. The transect was set perpendicular to the drainage canal (collection drain) and positioned in the middle of the block. The observation site on the transect was set at a distance of 5, 15, 25, 50, 75, 100, 150 m from the edge of the canal. Peat sampling was carried out outside the oil palm canopy or in non-rhizosphere area at the thickness of the 0-25 cm, 25-50 cm peat layer depending on the water table level. Peat sampling was carried out by drilling and composite from three sampling sites at that distance. Three subsamples were taken to the laboratory, and enzyme samples were carried with styrofoam at the same temperature as the field conditions. Soil analysis was performed at the Laboratory of Chemical and Soil Fertility, Department of Soil Science, Andalas University.

Peat Materials Analysis

Determination of the total nutrient content of peat includes analysis of water content by the % weight of peat samples carried out using the gravimetric method and ash content with the dry ashing method [9]. pH measurement of 1: 2 peat material (10 g of material: 20 ml ion-free water) was carried

out using a pH meter 2700 Autech Instrument (BPPT, 2005). The total micronutrients (Fe, Cu) were analyzed by the wet destruction method (extractor 60% HClO₄ and HNO₃), and the extraction was measured by Atomic Absorption Spectrophotometer (AAS) Shimadzu AA-6300. Laccase activity was measured to determine the activity of lignin-degrading enzymes using the ABTS method, modified from [2]. The activity of extracellular laccase enzymes was determined by a spectrophotometer using a substrate of 0.5 mM ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulphonate)). Oxidation of ABTS was monitored by determination of a spectrophotometer at a wavelength of 420 nm.

Data processing

Data obtained in the form of peat lignocellulose composition, nutrient status, and enzyme activity from peat material are presented in the form of descriptive analysis. Data processing was performed using Microsoft Excel to see differences based on standard deviations from observed environmental factors. In order to see the correlation between parameters, the Microsoft Excel program was used.

3. Result and Discussion:

Peat characteristics in oil palm plantations of Pesisir Selatan

a. Observation of water table level

Groundwater table on peatlands is the dominant factor that determines changes in the properties and characteristics of peat under oil palm stands. In Figure 1, we can see peat water for peat with a thickness of less than 3 m (<3 m) and more than 3 m (>3 m). The water table level in oil palm blocks with peat with a thickness of <3 m fluctuated from 60-80 cm. In plantation blocks of <3 m thickness, it tended to decrease as the distance from the canal increased, ranging from 40-100 cm. The difference in water table level in these two peat thicknesses indicates that in peat more than 3m the water table level can be maintained at around 40-80 cm except for those near the canal. This means that the water table level in the field is still within the tolerable limit of 60-80 cm. According to decrease as the layer of peat material should always be below the surface of the water because peat easily shrinks. Therefore, the groundwater table in general should be maintained at a thickness of between 60-100 cm from the canal.

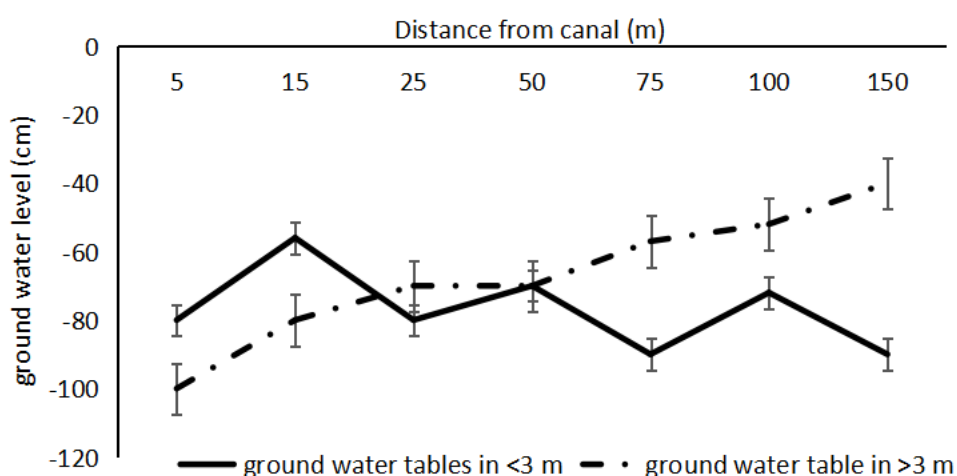


Figure 1. Ground water level on two different peat thicknesses based on distance from the canal.

b. pH value and ash content

Determination of pH and ash content of peat is an indicator of peat mineralization as a result of

peat decomposition. The speed of the biochemical reaction is controlled by pH, where enzyme activity is usually higher at the optimum pH and decreases at a lower pH. Figure 2 shows the pH of peat under oil palm plantation ranging from 4-5, both at <3 m and >3 m peat thickness. Based on the distance from the canal (Figure 2), the pH gradually changes. This is due to the groundwater level, which increases along with the increasing distance from the canal.

The increase in water table level dissolves most of the organic acids, affecting that it affects the decrease in peat pH. A low pH affects enzyme activity. The acidity of peat soil is strongly influenced by the presence of organic acids. The H⁺ ion in peat soil is in the form of functional groups of organic acids, mainly in the form of carboxylic groups (-COOH) and hydroxyl groups of phenolics (-OH).

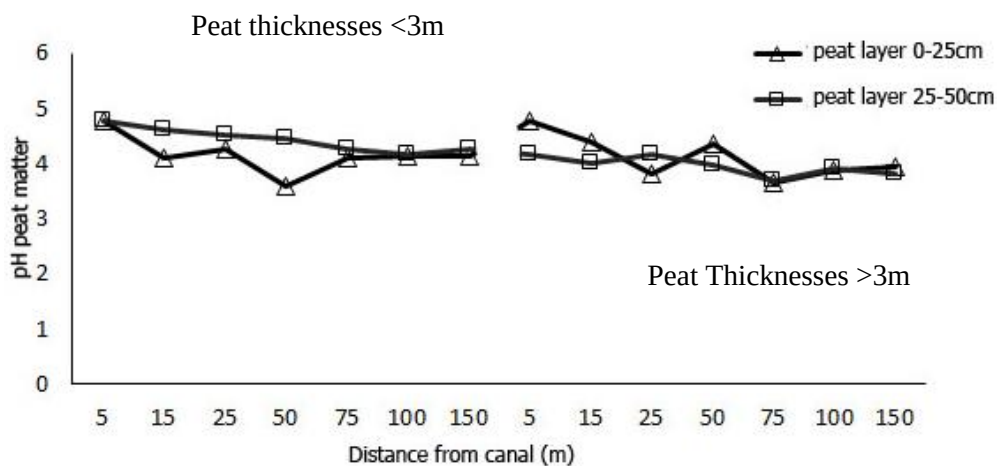


Figure 2. Peat pH at different thicknesses based on distance from the canal

Organic acid functional groups are weak acids that can dissociate to produce H⁺ ions and are able to maintain soil reactions to changes in soil acidity. The pH value of peat in the 0-25 cm layer was lower than that of the 25-50 cm layer in peat with a thickness of <3 m, and vice versa. Peat with a thickness of >3 m in the 0-25 cm layer had a higher pH value than that of the 25-50 cm layer. This is related to the level of peat ash, where the layer of 0-25 cm was lower than the 25-50 cm layer (Figure 3).

The ash level of peat is also an indicator of peat mineralization in addition to pH. Figure 3 shows that the ash content of peat with a thickness of <3 m was higher than peat with a thickness of >3 m. Peat with a thickness of <3 m had an ash content of > 20%, and vice versa, the average ash content in peat with a thickness of >3 m was less than 20%. The high ash content in <3 m peat is caused by the addition of mineral materials derived from flood runoff from rivers that are not far from the plantation block. The high ash content in the 25-50 cm layer in peat with a thickness of <3 m indicates a high mineral content under the peat layer. This relates to the enrichment of peat material with mineral materials derived from flood runoff from nearby rivers.

The activity of enzymes serves as biosensors for peatland degradation.

Changes in the water table level will cause changes in biochemical reactions on peatlands. The decrease in the groundwater table gives rise to an oxidative layer that activates microorganisms in producing enzymes. Enzyme activity is an indicator of peat decomposition. The content of peat water is an important factor that influences enzyme activity. Figure 4 shows a very significant exponential relationship ($R = -0.912$) between water content and laccase activity in oil palm plantations on drained peatlands. There is a negative correlation between water table level and laccase activity, which degrades lignin.

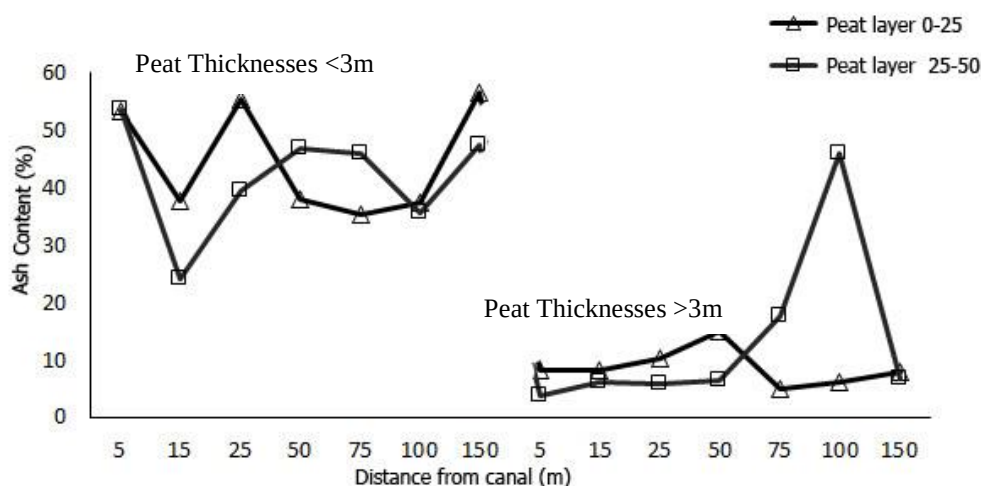


Figure 3. Peat ash content at different thicknesses based on distance from the canal.

Increased water content of peat shows a significant decrease in enzyme activity. According to [4], water content has a significantly negative correlation with phosphatase, β -glucosidase, and laccase. High water content suppresses enzyme activity. A decrease in laccase activity with an increase in peat water content (% by weight) indicates a low rate of decomposition of peat at high water content. The environment of peat is characterized by high hydration, which can suppress enzyme activity in the soil through changes in the amount of microbes [4].

According to [11], the large water binding capacity of peat material determines its stability, making peat so that peat is not easily degraded because peat is metastable. Hence, peat is easily damaged by the decomposition process when there is a decrease in the groundwater table. The relatively high content decreases microbial activity as a lignin decomposer in producing laccase, resulting in low laccase activity. The activity of laccase can be accelerated by the presence of supporting oxidation and pH conditions. This condition can be achieved when there is lower water content in peatlands.

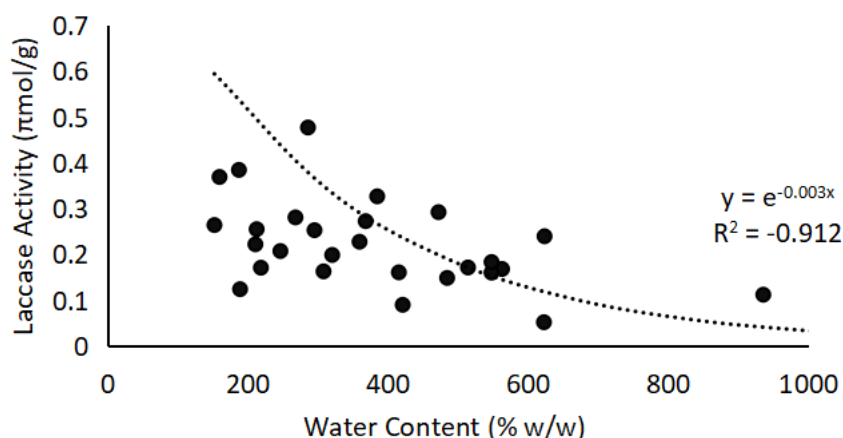


Figure 4. Relationship between water content and enzyme activity in the peatland of Pesisir Selatan oil palm plantations.

A. Enzyme activity in peatlands with different thicknesses

Figure 6 shows enzyme activity on peatland with a thickness of <3 m, which is <0.4 μmol/g peat material with a moisture content of <60%. Based on peat distance from the canal, there was an increase in enzyme activity with decreasing water content. Also, fluctuations in water content

determine the rate of decomposition of peat. Laccase activity in the 0-25cm layer was higher with lower water content ranging from 5m, 75 and 100 m from the canal. In contrast, laccase activity in the 25-50cm layer was lower with higher water content, particularly at a distance of 100 m from the canal. If associated with peat ash content, peat with a thickness of <3 m had a much higher ash content than that of peat with a thickness of >3 m. This triggers an increase in laccase activity, particularly in the 0-25 cm layer. Laccase is an enzyme that works by oxidizing organic molecules to release nutrients and increase ash content ([4].

In peat with a thickness >3 m, laccase activity was lower than that of peat with a thickness <3 m. Ground water tabel had an average of more than 400%, thus reducing enzyme activity significantly below 0.2 $\mu\text{mol/g}$ of peat, as shown in Figure 5. At a distance of 150 m from the canal, there was a significant increase in laccase activity in the 0-25 cm layer due to a decrease in water content to below 300%. However, laccase activity in the layer of 25-50 cm decreased dramatically with water content increasing to > 1000%. In the layer of 0-25 cm, laccase activity was higher than that in the 25-50cm layer.

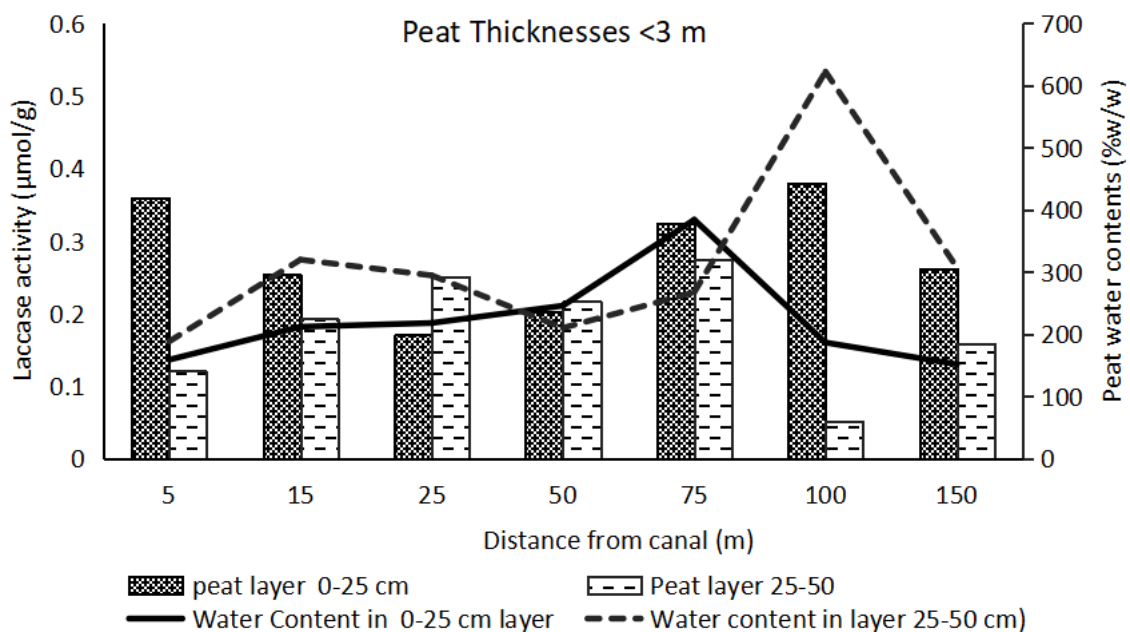


Figure 5. Laccase activity on peatland with a thickness of <3 m in Pesisir Selatan oil palm plantation.

The peat decomposition process occurs more in the surface layer because the surface layer is more oxidative than the layer below it. Layers 25-50 cm are still influenced by the moving groundwater table capillary. The laccase activity outside the root area (<0.4 $\mu\text{mol/g}$ is lower than the rhizosphere area, > 2 $\mu\text{mol/g}$ peat [5]. This proves that laccase activity in the rhizosphere area is higher than non-rhizosphere because in the rhizosphere, there is an energy source for microorganisms from root exudates. Additionally, the atmosphere in the rhizosphere is more oxidative than in the non-rhizosphere, triggering that this triggers an increase in enzyme activity.

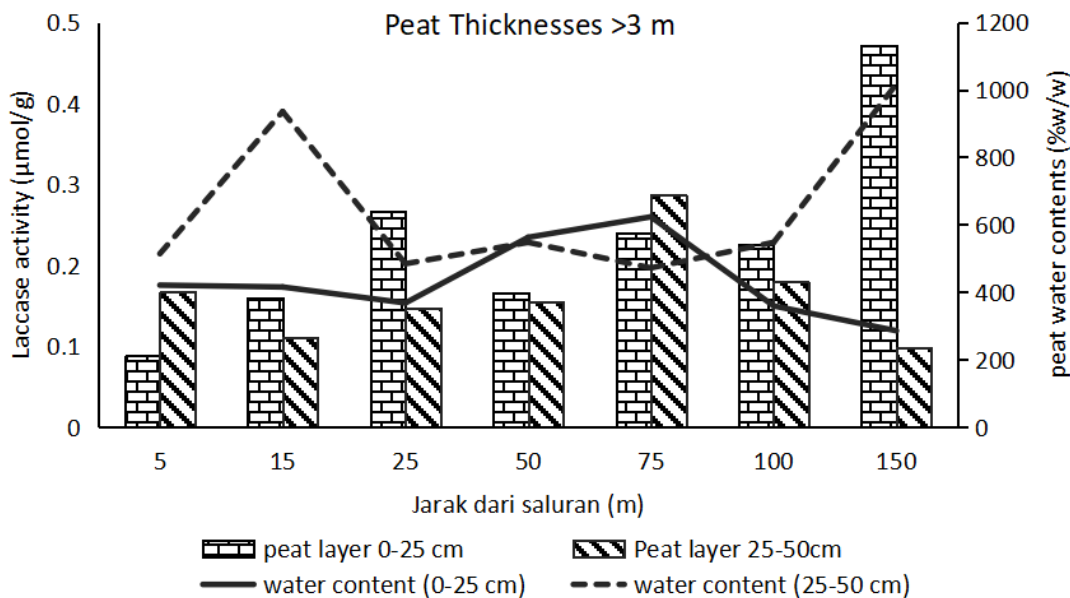


Figure 6. Laccase activity on peatland with a thickness of > 3m in Pesisir Selatan oil palm plantation

B. Total peat Fe and Cu levels and their relationship to enzyme activity.

Fe and Cu cations are highly reactive in binding to organic acids, which can make organic compounds in peat more stable, preventing that it is not easy to lose carbon through decomposition and bleaching. The increase in total Fe and Cu levels in peat can suppress enzyme activity [5], especially laccase activity. Fe content in peat with thickness <3m was higher (50-200 mg/L) than peat with thickness >3m (100 mg/L) as seen in Figure 8. Fe content in layer 0-25 cm was lower than that in layers 25-50 cm and tended to increase with increasing distance from the canal.

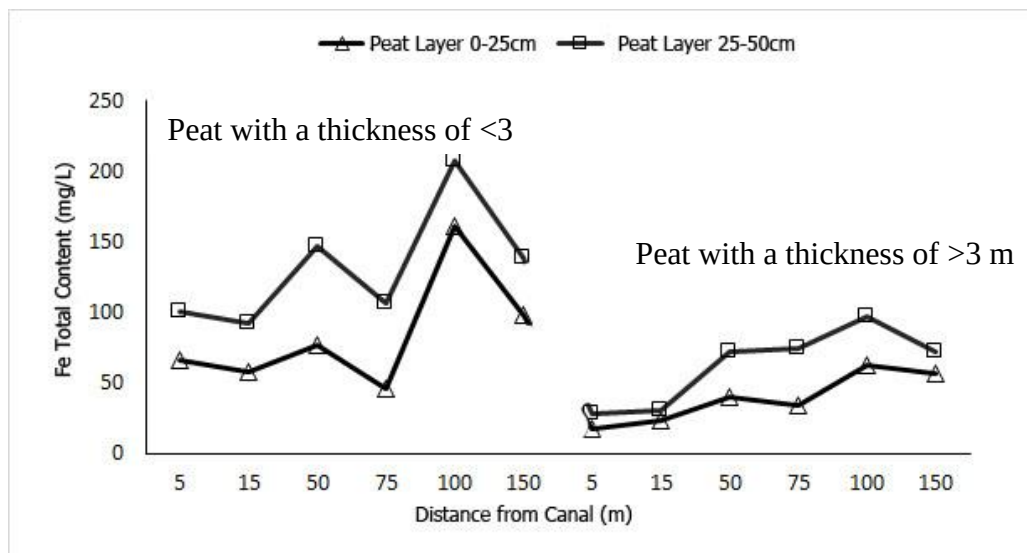


Figure 7. Total Fe content in peatland with a thickness of <3 meters and >3 meters in the Pesisir Selatan oil palm plantation.

Fe content increases with increasing thickness compared to the surface layer. This is triggered by dissolved Fe from the substratum of peat so that the deeper and closer to the substrate, the total Fe content increases. When compared with the results of research by [5], the Fe content of peat with a thickness of > 3m in Riau was higher, ranging from 300-4500 mg/kg, compared to peat in Pesisir Selatan (<200 mg/L). Fe cation is more active if it binds to organic acids and is carried by drainage

water so that there is more in the bottom layer. In addition, many Fe cations are donated by minerals originating from flood deposits of nearby rivers. Increasing Fe content based on distance from the drainage canal indicates that peat farther from the canal is relatively more stable than the decomposition and degradation processes because Fe cations play a role in maintaining the stability of organic compounds in peat from further humification processes [7].

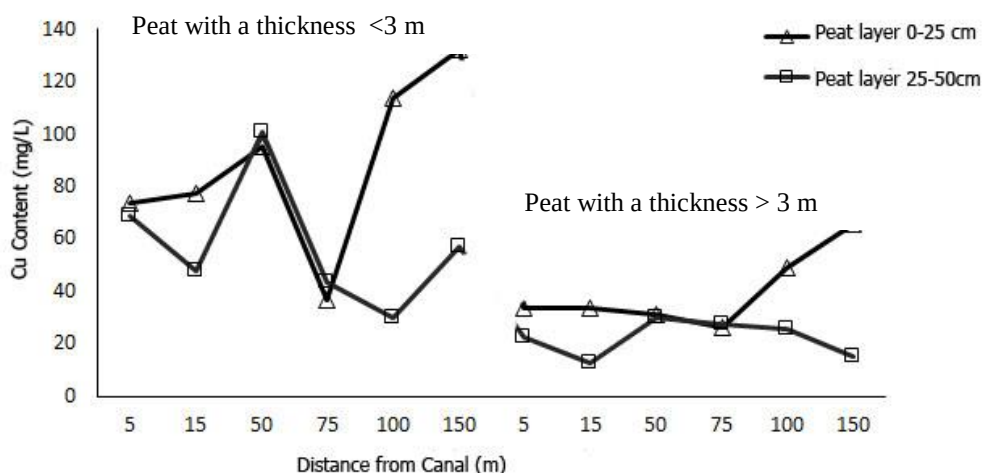


Figure 8. Total Cu content in peatland with a thickness of <3 m and >3 m in the Pesisir Selatan oil palm plantation.

Cu content in peat with a thickness <3 m was higher than that in peat with a thickness >3 m (Figure 8). This is due to the enrichment of minerals from flood overflows from rivers around the peat location, which is not far from the river's position. Based on the distance from the canal, Cu level increased particularly in 0-25cm layers at a distance of 50, 75, and 150m. The farther distance from the canal, the Cu level increased more, and when seen from the enzyme data, the enzyme activity decreased. This is influenced by decreased water content. When the Cu content in the oil palm rhizosphere aged > 15 years, was measured, a Cu content of <20 mg/kg, which was lower than the results of this study, which was > 20 mg/L (Figure 8). This is due to the absorption of Cu in the rhizosphere, whereas in this study, the Cu content analyzed was outside the root area, so Cu absorption was minimized.

As seen from the enzyme analysis, laccase activity in Pesisir Selatan peatlands was lower than in Riau peatlands, which was <0.5 $\mu\text{mol/g}$. The laccase activity in Riau's peatland had average 5 $\mu\text{mol/g}$, even up to 14 $\mu\text{mol/g}$ [5]. It was relevant with good management system of peat water in Pesisir Selatan oil palm plantations, which applies one collection drain on one side of the block and makes a water reservoir on the other side of the block in oil palm plantation. The water reservoir functions as water conservation to keep peat water contents always high and moist, thus the oxidative layer on the peat surface is not formed. With high water contents, oil palm productivity was achieved by good management of the mechanical system of fertilizer applications.

Oil palm production of peatland in Pesisir Selatan was 22 ton/acre/year on average of TBS, and the higher one was 28 ton/acre/year. Oil palm productivity in peatland and peat stability were supported by higher contents of Fe and Cu from mineral materials as polyvalent cations that react to bind organic matter in peat matter. Fe and Cu elements are a few of the enzyme activators, yet the high levels of total Fe and Cu can suppress enzyme activity because the stability of peat increases due to the formation of organic-metal complexes so that the decomposition of peat is inhibited. Fe and Cu bond with aliphatic or aromatic organic compounds to form bonds that are not easily hydrolyzed because ligand/complex bonds are

stronger than hydrogen bonds or water bridges [12]. [9] showed that Fe cations have a very high reactivity towards ferulic acid. Increasing the stability of chelated compounds (Fe-organic acids) will result in a decrease in phenolic acids and a reduction in the amount of dissolved Fe.

4. Conclusion

The laccase activity as a peat degradation in oil palm plantations in Pesisir Selatan, West Sumatra peatlands was higher in the 0-25cm layer, which is $<0.5 \mu\text{mol/g}$, than 25-50cm layers. The increasing water content decreased the laccase activity along with the increasing distance from the drainage canal and the thickness of the peat layer. This is triggered by an increase in Fe and Cu levels derived from an increase in peat ash levels, especially in peat with thicknesses of $<3 \text{ m}$, which suppresses laccase activity. Peat in Pesisir Selatan of oil palm plantations is still relatively stable from the decomposition processes, which is characterized by low laccase activity as a biosensor of peat decomposition. Maintaining water content, like water reservoirs in every block of the plantation, and enriching peat with mineral materials could reduce laccase activity as a biosensor for peat degradation in oil palm plantations in Pesisir Selatan.

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